This is to certify that the

dissertation entitled

Genetic Relationships Among Bean Cultivars
as Evaluated by Cluster and Other
Multivariate Analyses of Disease Reactions
Isozyme Mobility Patterns and
Agrophysiological Traits.

presented by

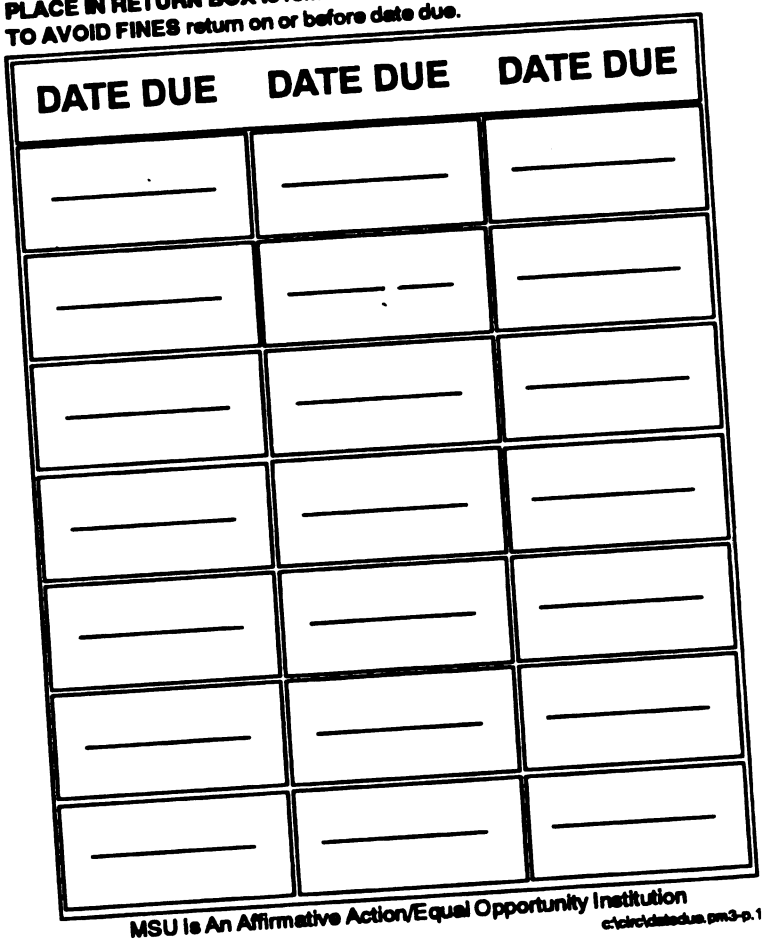
Imru Assefa

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Crop and Soil Sciences

M. W. Adams
Major professor

Date April 1995



GENETIC RELATIONSHIPS AMONG BEAN CULTIVARS AS EVALUATED BY CLUSTER AND OTHER MULTIVARIATE ANALYSES OF DISEASE REACTIONS ISOZYME MOBILITY PATTERNS AND AGROPHYSIOLOGICAL TRAITS

By

Imru Assefa

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Crop and Soil Sciences

1995

ABSTRACT

GENETIC RELATIONSHIPS AMONG BEAN CULTIVARS
AS EVALUATED BY CLUSTER AND OTHER
MULTIVARIATE ANALYSES OF DISEASE REACTIONS
ISOZYME MOBILITY PATTERNS AND
AGROPHYSIOLOGICAL TRAITS

By

Imru Assefa

Several clustering algorithms in different system programs (SAS, SPSS-X and CLUSTAN) along with other multivariate methods (e.g., PCA) were applied to disease reaction data in the field and uniform test conditions, data from isozyme mobility, and agrophysiological scores. Cultivar relationships were also examined by pedigree analysis and indices of similarity based on the above traits. Finally, Mendelian genetic analysis of F_2 data provided insights to cultivar interrelationships on the basis of reactions to particular described rust isolates.

The patterns of clustering of bean lines in field test conditions in the 1975, 1976 and 1977 IBRNs resulted in major patterns leading to the following postulates: (i) Bean cultivars assigned to a cluster under a given set of test conditions are postulated to possess a set of similar genes or genic complexes for reaction to rust isolates; (ii) Clustering of bean lines regardless of test conditions or cluster method used on the basis of similar reaction response patterns is attributed to possession of a broad genetic base with presumably several genes for resistance to multiple races that enable them to behave consistently from season to season; and

(iii) The presence of new dominant pathotypes eliciting similar reactions on cultivars possessing corresponding genes for reaction to these races.

Testing under uniform conditions improved the efficiency of clustering. Cultivars and/or rust isolates were clearly separated into a few groups that express correct classification by precise reaction phenotypes or virulence/pathogenicity classes that indicate similar genes or genic complexes in a host–parasite interaction system.

Two major clusters were obtained based on isozyme mobility patterns as fast and slow using Ward's method and on the basis of Nei's genetic identities/distance calculated from allele frequency of enzyme loci using the UWPGMA method. The clustering pattern derived on the basis of scores for six agrophysiological traits were influenced by certain undefined variables, which resulted in commercial class clusters being associated with a preponderance of a single reaction phenotype as a class.

Coefficient of parentage (r) values indicated the lack of significant pedigree relationships for the majority of bean lines tested.

Fifteen percent greater genetic identity or similarity for cultivars within clusters as compared to cultivars between clusters, as judged from Mendelian genetic tests of F_2 data to four rust isolates, provided support to substantiate the position taken in this study that cultivars within–clusters were genetically more similar than cultivars between–clusters. F_2 data indicated that monogenic, dominant factors were important for resistance in several cultivars. Two–gene and three–gene differences for reaction in most cultivars to the four races also suggested that oligogenes account for a substantial proportion of resistance to these races.

DEDICATION

To my younger brother, Mulugetta Assefa, who to me is the epitome of fortitude and perseverance.

To my wife Shitaye Moges and our children, Negash, Azeb, Tizita, Fassil and Benyam.

ACKNOWLEDGEMENTS

"Time brings all things to pass." Aeschylus

I have waited for so long to simply say thank you to so many individuals who have been very helpful and kind to me and my family.

I wish to express my appreciation to members of my guidance committee, Drs. M.W. Adams, J.D. Kelly and C. Cress for serving in that capacity. I also thank Dr. Pat Hart, Pesticide Research Center, MSU, for agreeing to represent the plant pathology department on my guidance committee on short notice and without any hesitation. With deep gratitude I wish to remember Dr. A. W. Saettler who had served on my guidance committee. He will be missed very much by all of us who knew him as a warm and caring human being.

I express my gratitude to three persons who have made this thesis research worth pursuing. Dr. M. W. Adams, my major professor, who was not only instrumental in bringing me here to Michigan State University, but through my association with him over the years provided me with the opportunity to broaden my understanding and quest for sound scientific thinking and learning. Through persuasive discussion usually conducted in a friendly manner, I have been made aware of several concepts in plant genetics and breeding and in particular, to opening the door to view the vast gene-for-gene relationships in host-parasite systems that I intend to pursue further. I am particularly grateful for his confidence in my perseverance and his due regard for the well being of myself and my family.

Dr. J. D. Kelly, a member of my guidance committee, for critical review and useful suggestions of the research proposal in the beginning, and an equally important suggestion on

the arrangement and writing of the literature review. I have particularly enjoyed the association with Dr. Kelly and learned a lot through his casual but challenging discussions in the bean fields, joyful exchanges in the bar and the many holiday occasions at his home for me and my family. His concerns to my family and his help is gratefully appreciated. I have been most fortunate to have been trained/apprenticed under Dr. R. J. Stavely, research plant pathologist, USDA, Beltsville, Maryland, on rust disease methodologies. I have felt most welcome by Dr. Stavely, who kept his door open for me, including home phone calls on questions of bean rust. Dr. Stavely kindly provided pertinent literature, the described rust races included in the genetic studies, and most of the pureline bean cultivars used for this thesis research. Disease reaction data for several described rust races were also kindly provided by Dr. Stavely for cluster and other multivariate analyses. In addition to his critical suggestion in the planning stage of the proposal, Dr. Stavely has provided a critical review of the draft thesis in its entirety. I am most grateful to him and I say, "Thank you, Dr. Stavely."

Special thanks to the following people who have responded to me through unreserved encouragement and through providing me with useful literature and seed material of certain bean cultivars for this study:

Dr. G. Freytag, research geneticist, USDA, TARS, Mayaguez, Puerto Rico, for encouragement in my work and providing me with invaluable pedigree information for several bean cultivars;

Dr. Phillip McClean, North Dakota State University, for encouragement and providing me with information on pedigree of several bean cultivars;

Dr. Dermot P. Coyne, University of Nebraska, Lincoln, Nebraska, for an encouraging letter while on flight and for providing me with pedigree sources for several bean cultivars;

Dr. M. J. Silbernagel, research plant pathologist, USDA, IAREC, Prosser, Washington, and my former guidance committee member, for encouragement and his friendly visits to me

and my family every time he stops at Michigan State University. My family and I were honored by a surprise visit from Dr. and Mrs. Silbernagel during the summer of 1990 at our home in Spartan Village. Information provided on pedigree and seed material is highly appreciated;

Dr. Joe Tohme, CIAT, Cali, Colombia, for providing me with several sources of pedigree information on beans; and

Dr. Art van Schoonhoven (formerly of CIAT, Cali, Colombia), program leader, ICARDA, ALEPPO, Syria, for encouragement in my studies and for providing me with seeds of various bean cultivars for my research.

Special thanks go to Dr. Susan L. Sprecher for being my first tutor in the art and science of electrophoresis techniques and for cordial friendship of her and her husband, Dr. Steven Sprecher, to me and my family over the years.

I express my thanks to the following: Dr. C. Ramm, Forestry, MSU, for allowing me to visit his multivariate statistics class and the several unappointed meetings to discuss multivariate statistical methods appropriate for my research.

Dr. Thomas G. Isleib for encouragement and unreserved help in genetic analysis and interpretations.

Dr. Clay Sneller for providing and helping set up his pedigree program to suit my data. I have benefitted and learned a lot more by my association with Clay Sneller and through several hours of enthusiastic discussions on the subject matter of each other's thesis.

Dr. Eunice Foster for encouraging and giving me the opportunity to fulfill my requirements as a teaching assistant.

Luica Afanador and Dr. Mireille Khairallah for cordial friendship to me and my family and for all the help that was there all the time.

To my sponsors, the Food and Agriculture Organization (FAO) of the United Nations (UN), Rome and the Institute of Agricultural Research (IAR), Addis Ababa, Ethiopia, for giving me this second chance to pursue a doctoral degree in crops and soil sciences and for financing my studies through the contract period of thirty-nine months at MSU, I am very grateful.

To the International Studies and Scholar's Office (ISSO) at MSU for picking up the remainder of the financial burden at periods when I needed the additional time to finish my studies. To all the wonderful people at ISSO, MSU and, in particular, the personal help and encouragement from Dr. David Horner, director, ISSO, MSU, Dr. Kenneth Ebert, and Mrs. Elda Keaton, I thank you all. To the Thoman Foundation, for honoring me as a Thoman fellow and to the SAGE Foundation for defraying the cost of thesis preparation, I express my gratitude.

Luck was with me and I was privileged to work for the Michigan Department of Public Health (MDPH) in the Biochemistry Unit of the Quality Control section. I have learned tremendously from partaking in various activities in the section as an intern.

I wish to express my special thanks to the following: Dr. Robert Meyer, chief, BLES, for giving me the opportunity at a juncture when I was financially strapped;

Dr. Leigh Charamella, chief, Blood Derivatives, for writing that critical letter that summoned me to his section and his support and constant encouragement to "get that degree now."

Mr. Jake Eckenrode for his support and confidence in me and encouragement at work.

Mr. Graylon Copedge, chief, Biochemistry Section, for his support, his understanding and friendship to me and my family, and encouragement to "hang in there" during hard times.

Finally, I am most indebted to my wife and friend, Shitaye Moges, who was with me in the quiet hours of early morning and late nights in the greenhouse hauling clay plots and

soil, inoculating plants, making crosses and moreover, keeping the family adequately fed and cared for. To my children here Tizita Assefa, Fassil Assefa, and Benyam Assefa who always wondered, "When is he gonna be done with this work?" and never complained as kids for not taking them to the movies. To Negash Assefa and Azeb Assefa, who never made it here with us for the last several years and who are particularly, anxiously awaiting our safe return home, thank you for your fortitude, and, God willing, we shall all be together.

TABLE OF CONTENTS

LIST OF TABLES

55 M
m
k

59 M
p

510 T
l

511 C
o

512 M
p

513 T
l

514 C
o

515 M
p

516 D

517 C
g

518 C
g

519 M
p

520 C
to

521 M
pa

522 C
to

523 C
in

LIST OF FIGURES

GENERAL INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is considered a major food crop with world-wide distribution. It is an important protein and fiber source for both urban and rural populations in many countries of the world.

Many high-yielding and improved cultivars of diverse types are grown in many countries both for domestic and external markets. These improved bean cultivars are products of breeding efforts in which attention has been given to a sometimes fickle market and to consumer demands for specific seed types of uniform quality. This catering to market and concurrent demands for specific seed types entails the use of elite breeding lines and/or already improved cultivars as recurrent parents that leads eventually to a narrowing of the genetic base. The outcome tends toward groups of genetically related and uniform cultivars that because of their homogeneity tend to be more vulnerable to the hazards of pest outbreaks than would be the case if heterogeneous landraces were in use (NAS, 1972).

The view that genetic uniformity predisposes a widely grown crop to the high risk of disease and insect epidemics and that it is the basis for vulnerability to disease for many crops has been substantiated (NAS, 1972).

To the extent that breeding to a type results in genetic uniformity for factors affecting disease, insect or stress susceptibility, concerns exist about the variety and the production region exposed to possible epidemics of a new virulent race of the pest (Adams, 1977; Browning et al., 1969; NAS, 1972).

The extent to which common parentage or shared germplasm among cultivars exists within commercial production regions has been investigated by Adams (1977) who postulated that regions that grow a single major market class of beans are more vulnerable to hazards of disease epidemics than those regions that grow several different market classes (Adams, 1977; Browning et al., 1969).

Levels of protection against impending epidemics are known to exist in the form of crop distribution throughout the various bean-growing regions, the diverse spectrum of commercial classes grown in different regions, and the diversity of cultivars grown within a region (Adams, 1977).

Alternatives to breeding for specific type that broaden the genetic base of germplasm has been suggested to counteract genetic uniformity (Browning et al., 1969; Coyne and Schuster, 1975; Stavely, 1984a, 1984b).

On the other hand, the exchange of germplasm has become common. Germplasm materials are used either in various breeding programs, or even directly for commercial production. This is particularly the case for those genotypes having the attributes of wide adaptability and/or high, stable yield. A possible consequence of such practices is the generation of cultivars with similar genetic background grown over wide production areas. This, in turn, may lead to the development of similar races of pathogens in varied environments that are capable of infecting many of the same cultivars (Adams, 1977; Van der Plank, 1968). The threat of such infection is probably much more menacing in pathogens such as the bean rust fungus that behave as an obligate parasite and where the infective agent consists of airborne spores derived from multiple inocula sources (Van der Plank, 1968).

The bean rust fungus (*Uromyces appendiculatus* (Pers.) Unger Var. *appendiculatus* (= *U. Phaseoli* (Reben) Wint.) has worldwide distribution, and causes widespread and destructive losses of both dry and snap bean crops in the tropics (Coyne & Schuster, 1975). It is

considered a major production problem in humid tropical and subtropical areas, and causes periodic severe epidemics in humid, temperate regions (Stavely and Pastor-Corales, 1989). Major losses occur in Latin America, east and southern Africa, and severe epidemics have occurred in Australia, China, the United States and some areas of Europe (Stavely and Pastor-Corales, 1989). Severe epidemics of cyclic nature occur in some regions of the world while in other regions rust is endemic, causing substantial annual losses. Although estimates vary depending on the season and locality, severe yield losses result when infections occur during the pre-flowering and flowering stages of development. Yield losses may range from a low of 18 percent to as high as 100 percent on a plant dry-weight basis (Stavely and Pastor-Corales, 1989).

Cultural, chemical and biological control mechanisms have been suggested to control bean rust, none of which has proven completely adequate singly, but which when used to augment each other and as an integral part of a control system that includes host resistance, has provided effective control (Stavely and Pastor-Corales, 1989).

Several types of host resistance have been indicated by many investigators including monogenic, dominant factors in many cultivars effective against multiple pathogenic races, which can occur independently and can occur in linkage groups, one for each race (Stavely, 1984b; Stavely, 1985). Other forms of protection through host resistance include decreased spore production, or reduced intensity of uredinia per unit leaf area, leaf hairiness, and tolerant reactions that may constitute different forms of horizontal resistance. However, the predominant resistance type in the bean host-rust pathogen system appears to be race specific host responses or the vertical resistance type that can be explained in terms of the gene-for-gene hypothesis of Flor (1955, 1971). Based on Flor's (1955) hypothesis that for each specific locus in the host determining susceptibility and resistance there is a specific and related locus in the parasite that determines virulence and avirulence, respectively, Person (1959) asserted

that these relationships should occur as a general rule in host–parasite systems rather than as the exception. The existence of the gene–for–gene system in bean–rust parasite interactions has long been recognized by bean researchers (Harter and Zaumeyer, 1941; Christ and Groth, 1982a, 1982b; Stavely, 1984a). Use of a new analytical method proposed by Person (1959) allows treatment of the host–parasite system as a complete and integral unit to explain this relationship for which raw data can be collected and accumulated in routine race surveys.

Pest monitoring using several host plant differentials in diverse geographic locations each year is a necessary and routine practice in helping researchers assess pest incidence. These nurseries not only provide the means for monitoring and tracking pathogen incidences and race composition but also help in identifying resistant lines. A spectrum of reaction patterns that give an indication of the type of resistance genes that exist in these lines is revealed. The method also reveals the units that are interacting within a system, it identifies gene similarities as well as gene differences, and it can lead to interpretations that can readily be treated by genetic methods (Person, 1959) including appropriate quantitative statistical techniques (Person, 1959; Seal, 1964).

The main overall purpose of the present study was to examine genetic diversity among bean cultivars through assessment of genetic similarities (or dissimilarities) by disease reaction scores, agronomic and morphological traits, biochemical (isozyme patterns) and pedigree data. In particular, the objectives of this investigation were the following:

1. Observe the reactions of 13 parental bean cultivars to four races of bean rust in the greenhouse in East Lansing, and to examine the disease reaction data of several bean cultivars to 26 races in Beltsville, Maryland, and to classify the cultivars into reaction response patterns or clusters based on their reaction responses to the different rust races used in the tests.

2. Observe the reaction of parental bean cultivars, and their F_1 and F_2 progenies to simultaneous inoculation with rust suspensions of four different single spore isolates (pathotypes) in the greenhouse.

3. Observe the segregation pattern and determine the number of genetic factors involved in resistance of 13 parental bean cultivars to the four described races of rust.

4. Estimate coefficients of similarity (S), from agrophysiological, disease and isozyme data and coefficients of relationship (R) from pedigree information of parental bean cultivars to help support the outcomes of cluster inter-relationships.

5. Assay allozyme variation of parental bean cultivars to compare genetic inter-relationships among and within these cultivar clusters.

6. Using various clustering algorithms (SLINK, CLINK, AVERAGE, WARDS and CENTROID methods) and other appropriate multivariate statistical methods (PCA and Mahalanobis distance) on data from reaction grades to rust isolates, agrophysiological traits, and biochemical data to establish genetic relationships within and between the various bean cultivars included in the test.

7. Using F_2 segregation data of each cross, determine genetic linkage relationships by examining paired segregation data and testing for independence by Chi-square analysis.

LIST OF REFERENCES

Adams, M.W. 1977. An estimation of homogeneity in crop plants, with special reference to genetic vulnerability in the dry bean, *Phaseolus vulgaris*. Euphytica 26:665–79.

Browning, J.A., M.D. Simons, K.J. Frey and H.C. Murphy. 1969. Regional deployment for conservation of oat crown–rust resistance genes. In: Disease consequences of intensive and extensive culture of field crops, pp. 49–56. Iowa Agric. Home Econ. Exp. Stn. Spl. Rep. 64, 55 pp.

Christ, B.J. and J.V. Groth. 1982a. Inheritance of resistance in three cultivars of beans to the bean rust pathogen and the interaction of virulence and resistance genes. Phytopathology 72:771–773.

Christ, B.J. and J.V. Groth. 1982b. Inheritance of virulence to three bean cultivars in three isolates of the bean rust pathogen. Phytopathology 72:760–770.

Coyne, D.P. and M.L. Schuster. 1975. Genetic and breeding strategy for resistance to rust (*Uromyces phaseoli* Reben (Wint.) in beans (*Phaseolus vulgaris* L.). Euphytica 24:795–803.

Flor, H.H. 1971. Current status of the gene–for–gene concept. An. Rev. Phytopath. 9:275–296.

Flor, H.H. 1955. Host–parasite interaction in flax rust: Its genetics and other implications. Phytopathology 45:680–685.

Ghaderi, A., M.W. Adams and A.W. Saettler. 1984. A quantitative analysis of host–pathogen–environment in International Bean Nurseries (IBRN). Ann. Rept. Bean Imp. Coop., vol. 27.

Harter, L.L. and W.J. Zaumeyer. 1941. Differentiation of physiologic races of *Uromyces phaseoli typica* on bean. J. Agr. Res. 62:717–731.

National Academy of Sciences. 1972. Genetic vulnerability of major crops. NAS, Washington, D.C.

Person, C. 1959. Gene–for–gene relationships in host–parasite systems. Can. J. Bot. 37:1101–1130.

Seal, H. 1964. Multivariate statistical analysis for biologists. Methuen and Co. Ltd., London, 2098.

Stavely, J.R. 1985. The modified Cobb Scale for estimating bean rust intensity. Ann. Rept. Bean Improv. Coop. 28:31-32.

Stavely, J.R. 1984a. Pathogenic specialization in *Uromyces phaseoli* in the United States and rust resistance in beans. Plant Dis. 68:95-99.

Stavely, J.R. 1984b. Genetics of resistance to *Uromyces phaseoli* in a *Phaseolus vulgaris* line resistant to most races of the pathogen. Phytopathology 74:339-344.

Stavely, J.R. and M.A. Pastor-Corales. 1989. Rust. chap. in: Bean production problems in the tropics. H.F. Schwartz and M.A. Pastor-Morales, ed. CIAT, CALI, Columbia.

Van der Plank, J.E. 1968. Disease resistance in plants. Acad. Press, New York, NY. 206p.

GENERAL MATERIALS AND METHODS

Cultivars and plant propagation

Thirteen parental bean cultivars were randomly selected for this study from five different cluster groups of a previous study. Cultivars were grouped according to their reaction response patterns to the bean rust pathogene in the field conducted by CIAT and reported in the 1975–76 International Bean Rust Nursery (Ghaderi et al., 1984). The number of cultivars to include was predetermined to be three from each original cluster owing to the greater number of clusters and cultivar members in each cluster. All the members of the original clustering in cluster V (three cultivars) and cluster VIII (two cultivars) were used for the study. Two cultivars in cluster IV and three cultivars each were selected at random from cluster groups III and VII, which contained 11 and 31 members, respectively. The original cluster grouping and cultivars selected within each cluster are shown in Table 1.1. Bulked seed from the progeny of all plants following several generations of single-plant selfing for each cultivar listed in Table 1.1 were also provided by Drs. J.R. Stavely, A.W. Saettler and Art Van Schoonhoven for host reaction and genetic studies. Field reaction data for cultivars

Table 1.1: Number of clusters and cultivars selected from original cluster grouping.

III	IV	V	VII	VIII
LaVega	CNC-2	Cuilapa-72	Ecuador-299	ICA-Pijao
Mexico—235	C-49-242	Rico Bajo-1014	Nep-2	KW-780
CNC-3		Mexico-309	Aurora	

uniformly tested in the 1975, 1976 and 1977 IBRN were also selected for cluster and other multivariate analysis.

Seedlings for testing were raised by planting one seed per pot in a 12.5 cm pot for check plants intended for inoculation with a single isolate/plant and two seeds/pot in a 15 cm pot for plants intended for simultaneous inoculation with four races per plant. A commercial soil medium (BACTO Professional Soil Mix) was used throughout the entire testing period. Plants were kept in rust-free greenhouse sections at 25-28° C until needed for inoculation or hybridization.

Rust races (pathotypes) and inoculum preparation

Urediniospores from *U. appendiculatus* races 41 (from Michigan), 46 and 53 (from Florida) and 49 (from Nebraska), supplied by Dr. J.R. Stavely, were used for reaction studies of parental bean cultivars and genetic studies in the greenhouse in East Lansing, Michigan. The same parental bean lines and several other cultivars were also tested for their reactions against 26 races in Beltsville, Maryland.

Three scoops of a thin, stainless-steel spatula or approximately 0.03 grams of frozen urediniospores of each race were used to give approximately 40,000 to 60,000 spores/ml of inoculum concentration for each inoculation as determined by a Hemacytometer count. Urediniospores were measured out and placed in 50 ml of a 0.01 percent Tween 20 and tap-water mixture in a 250-ml Erlenmeyer flask. The mixture was stirred at a speed of 800 rpm on a Fisher flexa-mix stirrer for about two minutes while adding another 50 ml of the Tween-20 water suspension to wet and disperse spores.

Inoculation, incubation and reaction grade scoring

Two types of inoculations were made. In the first, single isolates of the rust fungus were applied on individual plants intended as check plants using an unmodified sprayer, while in the second type simultaneous inoculation of four races per plant on target primary leaves of plants with primaries 35 percent to 45 percent fully expanded (7–9 day old plants) were used. In the multiple race inoculation method, each half of the abaxial surface of the primary leaf was inoculated with one isolate according to the techniques and spraying equipment used by Stavely (1983). After inoculation, plants were transferred to a greenhouse mist chamber of 100 percent R.H. (free running water) for 16–24 hours in darkness and later transferred to a greenhouse section at 22–25° C for 12–15 days until reactions were recorded.

Reaction grades were read 12–14 days after inoculation. To assign plants as either immune (I), highly resistant (HR), resistant (R), moderately susceptible (MS) or susceptible (S), both criteria of pustule size and intensity (percent leaf area covered) were used according to the Uniform Bean Rust Grading Scale adopted by the Bean Rust Workshop in Mayaguez, Puerto Rico, in 1983.

CHAPTER I

EVALUATION OF DISEASE REACTION RESPONSE PATTERNS IN BEAN CULTIVARS (*P. Vulgaris* L.) TO MULTIPLE PATHOTYPE INOCULATIONS OF THE BEAN RUST FUNGUS (*U. appendiculatus*)

INTRODUCTION

Disease reaction data collected from greenhouse or small, uniform field nurseries and accumulated over several environments provide the raw material that when used appropriately reveal the nature of existing diversity of host resistance genes and pathogen variability. There is an obvious advantage in facilitating such an understanding of host resistance genes and the composition of pathogen virulence by testing pureline cultivars along with described pathogenes in controlled environments over field test conditions using non-pureline cultivars. On the other hand, complications of data interpretations that could otherwise arise from seed mixtures, race mixtures, and the confounding effect of uncontrolled environment is avoided by testing in controlled test conditions. The availability of rapid test techniques with possibilities of multiple-pathotype inoculations per plant allows for rapid screening of many cultivars in such environments.

The objective of this study was to observe disease reaction of parental and non-parental bean cultivars (a subset of the 1976 International Bean Rust Nursery, IBRN) that were maintained as purelines against four isolates (in East Lansing, Michigan) and nine and 26 isolates (in Beltsville, Maryland) in the greenhouse.

LITERATURE REVIEW

Uromyces appendiculatus (Pers.) Unger var. *appendiculatus* (= *Uromyces phaseoli* (Reben) Wint) is an obligate parasite that belongs to the Basidiomycotina subdivision of fungi with an autoecious, macrocytic life cycle that is completed entirely on the bean host (Andrus, 1931; Cummins, 1978). The life cycle commences when overwintering or resting teliospores germinate with provision of appropriate stimuli to produce structures called basidia that bear the basidiospores. These spores infect the host leaf and develop sexual structures known as pycnia in which pycniospores are produced. Upon cross-fertilization with pycniospores of opposite mating types, an aecium is produced that bears aeceospores that infect to produce uredinia. Urediniospores are capable of causing repeated infections that take place throughout the growing season. The uredinia eventually mature and age to produce thick-walled teliospores (Stavely and Pastor-Corales, 1989).

Prolonged periods of moisture (10-18 hours) of greater than 95 percent R.H. and moderate temperature (17-24° C) favor infection by *U. appendiculatus* (Augustin et al., 1972; Harter et al., 1935). Optimal temperature for uredeospore germination is between 16 and 24° C, where temperatures greater than 32° C kill the fungus (Imhoff et al., 1982; Crispin, et al., 1976) while temperatures less than 15° C retard fungal development (Crispin, et al., 1976; Imhoff et al., 1981 and 1982).

Day length and light intensity are important epidemiological factors and Augustine et al. (1972) reported that infection is favored by incubations in low light intensity for 18 hours. The latent period (inoculation to 50 percent open uredinia) for uredinium development ranged

from 7 days at 24° C to 9 days at 16° C constant canopy level air temperature (Imhoff et al., 1982). Constant air temperature of 27° C inhibits an infection from developing to the sporulation stage. Moisture and temperature also influence production and release of urediniospores, with the greatest number of spores released during temperate, dry (60 percent R.H.) days preceded by a long dew period or rain the previous night (Imhoff et al., 1982; Nasser, 1976). Yarwood (1961) reported that *U. appendiculatus* can produce 10^6 urediospores/cm^2 on beans bearing 2 to 100 uredinia/cm^2. Sporulation per unit leaf area varies inversely with uredinium density (Imhoff et al., 1982) with dense infection in turn reducing uredinium size (Harter and Zaumeyer, 1941; Stavely, 1984a). Survival of urediospores in the field lasts for more than 60 days (Zambolim and Chavez, 1974). Teliospores overwinter on bean debris and wooden trellises and supports (Davison & Vaughan, 1963b). Urediospores can be transported long distances by wind currents, animals, reptiles, man and on seeds and provide primary and secondary inoculum sources of infection. Bean rust infection incidences are known to be influenced by many factors including cropping systems and microclimate.

During infection, a germ tube emerges from the spore and develops an appressorium upon physical contact with the stoma. Infection is more efficient on younger leaves while older leaves have fewer appressoria, less necrosis, fewer and smaller uredinia (Schein, 1965; Stavely, 1987; Shaik and Steadman, 1986; Alten, 1983; Kolmer et al., 1984; Zulu and Wheeler, 1982). An infection peg develops from the appressorium and pushes between the guard cells until fungal cytoplasm is transferred into the substomatal vesicle. Infection hyphae emerge from the substomatal vesicle at the tip of which a haustorium mother cell is formed upon contact with the parenchymatous cell layer (Mendegen, 1978a). The host cell at this time is penetrated transferring nutrients from host to haustorium and invasion intercellularly until a young uredinium is formed (Pring, 1980; Sziraki et al., 1984). This situation leads to alteration of host physiology and biochemistry affecting respiration and photosynthesis (Raggi,

1980). Deposition of tannins and death of affected cells occurs soon after infection in non-sporulating, hypersensitive type reactions. Infection eventually inhibits transfer of metabolic by-products (Zaki and Durbin, 1965), with the infection lesions acting as "sinks."

The effect of such invasion is manifested in different plant parts, mostly on leaves and pods but rarely on stems and branches. Symptoms occur on the lower leaf surface as minute, whitish, slightly raised spots 5 to 6 days from inoculation that enlarge to form a reddish-brown mature uredinial pustule that ruptures the epidermis. Sporulation peaks 10 to 12 days after inoculation depending upon temperature, followed by development of secondary and tertiary uredinia around the primary uredinia (Harter and Zaumeyer, 1941). The entire infection cycle occurs within 10 to 15 days. Later, black teliospores may form in the uredinia as infection progresses and teliospores replace urediospores.

U. appendiculatus is considered among the most pathologically variable of plant pathogens (Stavely, 1983; Stavely et al., 1983; Groth and Roelfs, 1982a). Pathogenic races were first reported for this autoecious, macrocyclic member of the *Puccinieae* by Harter et al. (1935). Having described the existence of variation in pathogenicity of *U. appendiculatus* in 1935, Harter and Zaumeyer (1941) characterized 20 races of the rust fungus based upon reactions of seven differential bean cultivars to inoculation with different isolates.

Variability in *U. appendiculatus* has been reported from many regions of the world including Australia, Brazil, Central America, Colombia, East Africa, Jamaica, Mexico, New Zealand, Peru, Portugal, Puerto Rico, Taiwan and the United States. Eighty, 65, 31, 29, 21, 18 and 15 races were reported, respectively, from Brazil (Augustin and Da Costa, 1971; Barbosa and Chavez, 1975; Carrijo et al., 1980; Dias, and Da Costa, 1968), United States (Fisher, 1952; Groth and Shrum, 1977; Harter and Zaumeyer, 1941; Stavely, 1984a; Stavely et al., 1989; Zuniga de Rodriguez and Victoria, 1957), Mexico (Crispin and Dongo, 1962), Australia (Ballantyne, 1978; Ogle and Johnson, 1974), Jamaica (Shaik, 1985b), Puerto Rico (Lopez,

1976; Ruiz et al., 1972) and Taiwan (Yeh, 1983). From 2 to 8 races were frequently found in single-field collections from a susceptible cultivar (Ballantyne, 1978; Barbosa and Chavez, 1975; Groth and Roelfs, 1982a; Stavely, 1984a).

Results from studies on pathogen variability in the U.S. were reported by Stavely (1984). The author reported on twenty previously undescribed pathogenic races of *U. appendiculatus* isolated from collections in the continental U.S. These newly described races were identified and numbered from races 38 through 57, which included two commonly found races and 18 other races that were minor components in field collections. These races were defined and identified from single uredinial isolation by the reaction of 19 differential bean cultivars. The author pointed out the existence of the high degree of variability and great potential for *U. appendiculatus* races to break host resistance. Bean cultivars with broad resistances were also noted with the cultivar CNC having resistance to all 20 races at the time.

Stavely et al. (1989) reported on identification of races of *U. appendiculatus* possessing new patterns of virulence on the most broadly resistant germplasm represented in the standard bean differential cultivars. The authors reported the identification of new virulence combinations in 13 single-uredinium isolates described as races 58 through 70. Some of these new races are noted as the first to contain certain important combinations of virulence on such differential cultivars as Early Gallatin, Mexico-309, Nep-2, Aurora and Olathe. The new race 67 is the first such race virulent to cultivar CNC, which previously had resistance to all 20 races.

The implication of these findings on the development of comprehensive and stable rust resistance in the common bean is considerable. The accumulation of pathogenic variability data and continued research on the genetics of resistance from various sources will in the long run yield valuable information on genetic similarities and differences.

MATERIALS AND METHODS

Greenhouse tests in East Lansing, Michigan

Plant propagation, inoculum preparation, inoculation procedures and disease reaction rating has been mentioned in the General Materials and Methods section. Thirteen parental bean cultivars were included for the study against four rust races (41, 46, 49 and 53) in East Lansing, Michigan. A total of eight inoculation cycles were scheduled with the parental cultivars tested as inoculated controls along with their F_1 and F_2 progenies tested at each cycle.

Reaction grades were assigned according to the scale of Davison and Vaughn (1963) as modified and adopted at the 1983 Bean Rust Workshop meeting in Mayaguez, Puerto Rico (Stavely et al., 1983). The grades were later converted to a convenient scale from 1 to 7 corresponding to the original scores (Tables 1.2 and 1.3) for purposes of computational ease in statistical and mendelian genetic analysis.

Greenhouse tests in Beltsville, Maryland

Seeds from thirteen parental bean and ten check cultivars were tested by Dr. J.R. Stavely against 26 rust isolates in Beltsville, Maryland. Where seed availability permitted, at least 5 plants were planted and tested for each cultivar. Plants were raised in 12-inch pots and simultaneously inoculated to at least four isolates/plant and repeated at least one more time to verify symptom expressions to each race.

Table 1.2: **Bean rust reaction grades, definition and designated symbols for degree of resistance/susceptibility**

Grade	Definition	Symbol
1	Immune, no visible symptoms	I
2	Necrotic or chlorotic spots, without sporulation, and less than 0.3mm diameter	HR
2^{+}	Spots, without sporulation, 0.3–1.0mm diameter	HR
2^{++}	Spots, without sporulation, 1.0–3.0mm diameter	HR
2^{+++}	Spots, without sporulation, greater than 3.0 diameter	HR
3	Uredinia (sporulating pustules), less than 0.3mm diameter	R
4	Uredinia 0.3–0.5mm diameter	MR
5	Uredinia 0.5–0.8mm diameter	MS
6	Uredinia greater than 0.8mm diameter	S

Range of reactions that can be encountered and response designations

Grade or grades	Designation symbol and meaning
1	I = Immune
2, 2^{+}, 2^{++}, 2^{+++}	HR = Hypersensitively or highly resistant
3, 34, 23, 32 or 2^{+}, 3	R = Resistant, 3s present and if 4s 3s predominant
4 or 43	MR = Moderately resistant, none larger than 0.5mm
345,45,435,54, etc.	MS = Moderately susceptible, none larger than 0.8mm
456,546,3456,4356, etc.	S = Susceptible, uredinia larger than 0.8mm present
65, 654	VS = Uredinia larger than 0.8mm predominant

Table 1.3: Conventional bean rust reaction grades, new rust reaction scoring scale for computational purposes, symbols and resistance categories for genetic studies

Pustule size	Conventional reaction grade scale	Reaction grades encountered during testing	New Score	Symbol**	Resistance categories for genetic analysis
No pustule	1	1	1	I	R
Necrotic spots < 0.3mm diameter	2	2	2	HR	R
Necrotic spots predominantly < 0.3 to 0.3 – 1.0 mm diameter	2,2'	2,2'	2	HR	R
Necrotic spots predominantly 0.3–1.0mm diameter with some < 0.3 mm diameter	2',2	2',2	2	HR	R
		2,2'	2	HR	R
Necrotic spots 1.0–3.0mm diameter	2''	2'',2	3	HR	R
		2,2''	3	HR	R
Necrotic spots > 3.0 mm diameter	2'''	2'',2'''	3	HR	R
		2''',2''	3	HR	R
		2''',2	3	HR	R
		2'',2	3	HR	R
Sporulating uredinia < 0.3 mm diameter	3	3	4	R	R
		3,2	4	R	R
		2,3	4	R	R
		2',3	4	R	R
		2',2,3	4	R	R
		3,4	4	R	R
Sporulating uredinia 0.3–0.5mm diameter	4	4	5	MR	R
		4,3	5	MR	R
Sporulating uredinia 0.5–0.8mm diameter	5	5,4,453,345	6	MS	S
		4,5	6	MS	S
Uredinia > 0.8mm	6	56,4356,3456	7	S	S
		5463,5643	7	S	S
		65,635,6435	7	S	S

* New arbitrary scale used for computation purposes

** I = Immune; HR = Hypersensitive resistance; R = Resistant; MR = Moderately resistant; MS = Moderately susceptible; S = Susceptible

RESULTS AND DISCUSSION

Reaction categories and percent resistant and susceptible parental bean cultivars

Thirty-five to forty seeds were used for each parental cultivar tested as inoculated and uninoculated controls along with their F_1 and F_2 progenies over eight cycles of testing in the greenhouse. The results of reaction categories, percent resistant and susceptible plants from simultaneous inoculation to four races (41, 46, 49 and 53) per plant are summarized in Table 1.4.

Reaction to race 41 for eleven out of thirteen parental bean cultivars was identical percentage-wise with 100 percent resistant plants in each. Cultivars Kentucky Wonder 780 and ICA-Pijao had 100 percent susceptible plants while the black-seeded cultivar C-49-242 produced 8 percent susceptible plants from a total of 36 seeds tested. This indicates that C-49-242 is not a pureline cultivar. Segregation for reaction was also indicated by Stavely (1984) on four cultivars when he was comparing reactions to the Mexican races and on three bean differentials as well as Australian differentials to seven races.

Four parental bean cultivars (Mexico-235, Mexico-309, Rico Bajo-1014 and Ecuador-299) showed 100 percent resistant plants for race 46 while three others (Nep-2, Aurora and Kentucky Wonder 780) produced plants that were 100 percent susceptible to the same race. Of a total of 29, 7, 27, 28, 27 and 28 plants tested for cultivars Lavega, Compuesto Negro Chimaltenango-3 (CNC-3), Compuesto Negro Chimaltenango-2 (CNC-2), C-49-242, Cuilapa-72 and ICA-Pijao, respectively 10.3 percent, 14.3 percent, 3.7 percent, 89.3 percent, 22.2 percent and 21.4 percent susceptible plants were produced by each cultivar.

Table 1.4: The reaction of 13 parental bean cultivars (HR, R and S) and percent (%) R and S plants to each of four races of the bean rust fungus (*Uromyces appendiculatus*) over 8 cycles of testing in East Lansing, Michigan

Cultivar/host reaction		percent											
		41	R	S	46	R	S	49	R	S	53	R	S
LaVega	HR	0			0			0			0		
	R	38	100.0	0.0	26	89.7	10.3	0	0.0	100.0	30	96.9	3.1
	S	0			3			30			1		
Mexico-235	HR	9			0			9			9		
	R	0	100.0	0.0	9	100.0	0.0	0	100.0	0.0	0	100.0	0.0
	S	0			0			0			0		
CNC-3	HR	2			1			0			0		
	R	5	100.0	0.0	5	85.7	14.3	7	100.0	0.0	7	100.0	0.0
	S	0			1			0			0		
CNC-2	HR	33			2			0			32		
	R	4	100.0	0.0	24	96.3	3.7	0	0.0	100.0	4	100.0	0.0
	S	0			1			37			0		
C-49-242	HR	0			0			0			0		
	R	33	91.7	8.3	3	10.7	89.3	2	5.4	94.6	30	85.7	14.3
	S	3			25			35			5		
Mexico-309	HR	2			0			0			2		
	R	36	100.0	0.0	26	100.0	0.0	0	0.0	100.0	36	100.0	0.0
	S	0			0			35			0		
RB-1014	HR	1			0			0			3		
	R	32	100.0	0.0	31	100.0	0.0	30	100.0	0.0	24	100.0	0.0
	S	0			0			0			0		
Cuilapa-72	HR	30			0			0			31		
	R	0	100.0	0.0	21	77.8	22.2	1	96.8	3.2	0	100.0	0.0
	S	0			6			30			0		
Ecuador-299	HR	5			0			0			5		
	R	0	100.0	0.0	5	100.0	0.0	5	100.0	0.0	0	100.0	0.0
	S	0			0			0			0		
Nep-2	HR	33			0			0			34		
	R	0	100.0	0.0	0	0.0	100.0	0	0.0	100.0	0	100.0	0.0
	S	0			33			32			0		
Aurora	HR	33			0			0			34		
	R	0	100.0	0.0	0	0.0	100.0	0	0.0	100.0	0	100.0	0.0
	S	0			29			32			0		
KW-780	HR	0			0			25			0		
	R	0	0.0	100.0	0	0.0	100.0	0	100.0	0.0	0	0.0	100.0
	S	25			21			0			25		
ICA-Pijao	HR	0			0			0			0		
	R	0	0.0	100.0	22	78.6	21.4	5	13.9	86.1	0	0.0	100.0
	S	37			6			31			37		

For race 46, the number of host plants in each cultivar that produced both resistant (R) and susceptible (S) reactions is by far greater than for the other three rust races. This may be due either to inoculum impurity in race 46 or race 46 more sensitive to minor environmental variations or cultivar impurities.

For race 49, five cultivars (Mexico-235, CNC-3, Rico Bajo-1014, Ecuador-299, and KW-780) produced all resistant plants (100 percent R) while five others (Lavega, CNC-2, Mexico-309, Nep-2 and Aurora) produced plants that were 100 percent susceptible. Three cultivars (C-49-242, Cuilapa-72 and ICA-Pijao) produced variable numbers of both susceptible and resistant plants. Cuilapa-72 had predominantly resistant (R) plants at 96.8 percent while C-49-242 and ICA-Pijao had predominantly susceptible (S) plants at 94.6 percent and 86.1 percent respectively. This again indicates that these three cultivars were heterogeneous and not pureline for this trait as expected. Incidentally, it was observed that one race revealed a set of cultivars as non-true breeding where another race did not, which may also indicate the use of such isolates to detect purity and homogeneity.

For reaction to race 53, nine out of 13 parental cultivars had 100 percent resistant (R) plants while two cultivars (LaVega and C-49-242) produced predominantly resistant plants at 96.9 percent and 85.7 percent respectively. KW-780 and ICA-Pijao produced plants that were all susceptible (100 percent S).

Although the number of plants for each cultivar was categorized as resistant (R) or susceptible (S) for convenience, the classification in Table 4 included three distinct symptom expressions that are recognizable by their pustule types: 1) hypersensitive resistance (HR), 2) resistant (R), and 3 susceptible (S). The intergrades such as moderately resistant and/or moderately susceptible are excluded so as not to introduce unnecessary confusion.

The presence of plants with both resistant and susceptible reactions has been encountered for a few of the parental cultivars. This is particularly true for cultivar C-49-

242, which produced resistant and susceptible plants for all four races, Cuilapa-72 (for races 46 and 49), CNC-2 and CNC-3 (for race 46) and LaVega for races 46 and 53. It cannot be determined precisely whether the cause was due to heterogeneity of seed material or mechanical seed mixture, pathogenic mixture or contamination of races. However, it is very important to establish at the outset the precise behavior of the cultivar to the races and avoid unnecessary complications that could arise from contaminations and mechanical mixtures if a meaningful interpretation of the data is to be made or used for subsequent work, such as inheritance studies. It is prudent to assume here that purity of the cultivars may have been less than desirable to be accepted as purelines.

The reaction of twenty-three bean cultivars to nine rust isolates

Table 1.5 summarizes the reaction of 13 parental bean cultivars along with 10 others that were uniformly tested (and a subset of those tested against 26 races in Beltsville, Maryland) against 9 races in the greenhouse in Beltsville, MD. Initially, reaction grades were assigned using the conventional scale (Table 1.2) of Davison and Vaughn (1963), and later converted to a new scale from 1 to 7 (summarized in Table 1.3) for computational convenience.

Admittedly, while computational convenience and simplicity are attained by adopting this new scale, detail and clarity may have been sacrificed. Nevertheless, the ability to distinguish the hosts based on their reaction to the rust isolates and the pathogens by the reaction they elicit on these hosts in a gene-for-gene system is not diminished. In reality, the new scale separates the former HR reactions that are now known to be under a different gene control (Stavely, 1984) and renders distinct cultivar characterization easier.

Comparison of disease reaction response patterns of parental and non-parental bean cultivars against all others across a spectrum of rust races does reveal existing relationships

Table 1.5: Disease reaction grades of 23 bean cultivars to 9 races of the bean rust fungus (*U. appendiculatus*) tested in the greenhouse at Beltsville, Maryland

Cultivar	Races								
	38	39	40	41	43	46	49	52	53
1 LaVega	4.0	4.0	7.0	2.0	4.0	5.0	7.0	4.0	2.0
2 Mexico-235	1.0	1.0	2.0	4.0	4.0	4.0	2.0	2.0	2.0
3 CNC-3	1.0	1.0	7.0	4.0	1.0	7.0	4.0	4.0	4.0
4 CNC-2	1.0	1.0	4.0	4.0	2.0	4.0	2.0	4.0	4.0
5 C-49-242	5.0	5.0	7.0	6.0	7.0	6.0	6.0	4.0	6.0
6 Mexico-309	2.0	2.0	4.0	4.0	4.0	4.0	7.0	3.0	3.0
7 Rico-Bajo-1014	2.0	2.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
8 Cuilapa-72	2.0	1.0	2.0	2.0	7.0	4.0	6.0	2.0	2.0
9 Ecuador-299	1.0	1.0	2.0	2.0	4.0	4.0	4.0	2.0	2.0
10 Nep-2	2.0	2.0	2.0	2.0	2.0	7.0	7.0	2.0	2.0
11 Aurora	2.0	2.0	2.0	2.0	7.0	7.0	7.0	2.0	2.0
12 KW-780	3.0	3.0	3.0	7.0	7.0	7.0	3.0	7.0	7.0
13 ICA-Pijao	2.0	2.0	7.0	7.0	7.0	7.0	4.0	7.0	7.0
14 CNC	1.0	1.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
15 B-190	2.0	2.0	4.0	4.0	4.0	4.0	7.0	4.0	4.0
16 Olathe	2.0	2.0	2.0	2.0	2.0	4.0	4.0	7.0	7.0
17 Pindak	2.0	2.0	7.0	7.0	4.0	6.0	6.0	7.0	7.0
18 UI-111	2.0	2.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
19 M/WhfRnr	3.0	3.0	3.0	7.0	7.0	7.0	3.0	7.0	7.0
20 GN-1140	2.0	1.0	7.0	4.0	6.0	4.0	4.0	7.0	7.0
21 Seafarer	2.0	2.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
22 C-20	2.0	2.0	4.0	4.0	7.0	7.0	7.0	2.0	2.0
23 51051	2.0	2.0	2.0	2.0	7.0	4.0	7.0	2.0	2.0

among these cultivars. A simple matching coefficient using the reaction of a host cultivar to an array of rust isolates (Table 1.6) was computed from the relationship S = K'/K where the cultivars had the same reaction phenotype for K' out of K (K=9 for nine reaction phenotypes to the 9 races) loci assuming a one-locus control of the character. These computed coefficients of similarity, ranging from 0.00 to 1.00, represent similarity indices (SI) among these cultivars, where SI = 0.00 indicates no relationship and an SI = 1.00 indicates strong relationship. It allows a better assessment of relationship between cultivars with a single value to compare than just an array of host reactions to several races. On the basis of this similarity index, the cultivar LaVega was compared to twenty-two other cultivars. The number of identical matches with a number of cultivars was in general low, ranging from SI = 0.00 to SI = 0.18 with eleven other cultivars. The highest value of SI at 0.44 for LaVega was with the cultivar Ecuador-299. Mexico-235 had SI = 0.00, 0.11, 0.22, 0.33, 0.44 and 0.56 with 5, 1, 1, 8, 1 and 3 cultivars, respectively. The highest SI value of Mexico-235 was at 0.78 with Ecuador-299. The cultivar Ecuador-299 has been equated with Mexico-235 (Stavely et al., 1989; Freytag, 1989, personal communication).

Cultivar Compuesto Negro Chimaltenango-3 (CNC-3) and CNC-2 are selections from landrace variety CNC. CNC-3 produced SI = 0.56 with CNC-2 and a high SI = 0.67 with its parent cultivar CNC. CNC-2, a selection from the same parent (CNC) as CNC-3, behaved comparably. It produced the highest SI = 0.78 with its progenitor CNC.

Mexico-309 produced SI = 0.44 with two cultivars (CNC, and 51051) and SI = 0.56 and .067 with C-20 and Rico-Bajo-1014, respectively. The highest SI = 0.78 was with cultivar B-190, of which it is a parent. The cultivar Rico-Bajo-1014 produced high values of coefficient of similarity at SI = 0.78 with CNC and SI = 0.89 with B-190. Cuilapa-72 had SI = 0.56, 0.67 and 0.67 for reaction response with cultivars Mexico-235, Nep-2 and Aurora, respectively. The highest value of SI = 0.78 was with the cultivar 51051. Cuilapa-72 was

Table 1.6: Similarity indices (SI) of 23 bean cultivars on the basis of their reactions to 9 races of the bean rust fungus (*U. appendiculatus*)

	LaVega	M-235	CNC-3	CNC-2	C-49 242	M-309	RB1014	Cuilapa 72	E-299	Nep-2	Aurora	KW780	ICA Pijao	CNC	B-190	Olathe	Pindak	U.I. 111	M/WhfRnr	GN-1140	Seafarer	C-20	51051
LaVega	X	0.22	0.33	0.11	0.22	0.11	0.33	0.22	0.44	0.22	0.22	0.00	0.22	0.33	0.22	0.22	0.22	0.11	0.00	0.22	0.11	0.11	0.22
Mexico-235		X	0.33	0.56	0.00	0.33	0.33	0.56	0.78	0.33	0.33	0.00	0.00	0.56	0.33	0.22	0.11	0.00	0.00	0.33	0.00	0.33	0.44
CNC-3			X	0.56	0.22	0.11	0.44	0.11	0.33	0.11	0.11	0.11	0.33	0.67	0.33	0.11	0.11	0.22	0.11	0.44	0.22	0.22	0.00
CNC-2				X	0.11	0.33	0.56	0.22	0.33	0.11	0.00	0.00	0.00	0.78	0.56	0.22	0.00	0.00	0.00	0.33	0.00	0.22	0.11
C-49-242					X	0.00	0.11	0.22	0.00	0.00	0.11	0.11	0.22	0.11	0.11	0.00	0.33	0.22	0.11	0.11	0.22	0.11	0.11
Mexico-309						X	0.67	0.22	0.22	0.33	0.33	0.00	0.22	0.44	0.78	0.33	0.33	0.33	0.00	0.33	0.33	0.56	0.44
RB-1014							X	0.22	0.33	0.22	0.22	0.00	0.33	0.78	0.89	0.44	0.33	0.22	0.00	0.44	0.22	0.44	0.33
Cuilapa-72								X	0.67	0.56	0.67	0.11	0.22	0.22	0.22	0.44	0.22	0.22	0.11	0.33	0.22	0.44	0.78
Ecuador-299									X	0.44	0.44	0.00	0.11	0.56	0.22	0.44	0.11	0.00	0.00	0.33	0.00	0.22	0.56
Nep-2										X	0.89	0.11	0.33	0.00	0.33	0.56	0.22	0.44	0.11	0.11	0.44	0.67	0.78
Aurora											X	0.22	0.44	0.00	0.33	0.44	0.22	0.56	0.22	0.11	0.56	0.78	0.89
KW-780												X	0.56	0.00	0.00	0.22	0.33	0.56	1.00	0.22	0.56	0.22	0.11
ICA-Pijao													X	0.11	0.22	0.56	0.67	0.89	0.56	0.56	0.89	0.44	0.33
CNC														X	0.67	0.22	0.11	0.00	0.00	0.44	0.00	0.22	0.11
B-190															X	0.33	0.33	0.33	0.00	0.33	0.33	0.56	0.44
Olathe																X	0.44	0.44	0.22	0.56	0.44	0.22	0.56
Pindak																	X	0.67	0.33	0.44	0.67	0.22	0.22
U.I.-111																		X	0.56	0.44	1.00	0.56	0.44
M/WhfRnr																			X	0.22	0.56	0.22	0.11
GN-1140																				X	0.44	0.22	0.22
Seafarer																					X	0.56	0.44
C-20																						X	0.67
51051																							X

released in Guatemala from a line in Costa Rica known as 51051 (Joe Tohme, personal communication).

Cultivars Nep-2 and Aurora had a near-perfect match with similar reaction responses to 8 races out of 9 (SI = 0.89). Both cultivars reacted almost identically and had comparable similarity index values with other cultivars against which they were matched.

There was a one-to-one match for reaction response to the 9 isolates (SI = 1.00) between cultivars Kentucky Wonder-780 and Mountain White Half Runner (M/WhfRnr). Stavely (1984) also noted the identical reaction between KW-780 and M/WhfRnr to all races they were tested against. The cultivar ICA-Pijao produced a high similarity index value at SI = 0.89 with UI-111 and Seafarer, both of which showed susceptibility to 7 out of 9 isolates.

Reaction of 19 bean cultivars to 26 rust isolates

Comparison of reaction response of 19 (10 parental and 9 other cultivars) bean cultivars (Table 1.7) was also submitted to the same formula for computing a simple matching coefficient between pairs of cultivars based on their reaction responses to 26 rust isolates (Table 1.8). The inclusion of more rust isolates to compare similarity of reaction response patterns has advantages over using few such races since it allows one to assess the extent of similarity on more races, and the value of similarity based on several variables is obviously more reliable than similarity values based on few variables or races. Such values of indices of similarity between any two cultivars matched reaction for reaction to each race may indicate stronger and closer affinity that reflects fundamental genetic relationships. While higher values of coefficients of similarity do reflect closer relationship, identical values of coefficients of similarity may not necessarily be for matches for the same array of races. It is therefore important that these values may be examined carefully. A total of 171 pairwise comparisons (matches) have been made. Of these, only 35 percent of the matches, those having at least 10

Table 1.7: Disease reactions of 19 bean cultivars to 26 races of the bean rust fungus (*U. appendiculatus*) tested in the greenhouse in Beltsville, MD

	38	39	40	41	42	43	45	46	47	48	49	50	51	52	53	56	57	58	59	60	61	63	64	65	66	67
LaVega	4.0	4.0	7.0	2.0	2.0	4.0	7.0	5.0	7.0	6.0	7.0	7.0	7.0	2.0	2.0	4.0	4.0	7.0	7.0	4.0	7.0	7.0	7.0	7.0	7.0	7.0
Mexico-235	1.0	1.0	2.0	4.0	2.0	4.0	4.0	4.0	4.0	4.0	2.0	2.0	7.0	2.0	2.0	2.0	2.0	4.0	2.0	2.0	2.0	7.0	4.0	4.0	7.0	4.0
CNC-2	1.0	1.0	4.0	4.0	4.0	2.0	4.0	4.0	2.0	1.0	2.0	6.0	4.0	4.0	4.0	2.0	4.0	1.0	7.0	2.0	4.0	4.0	4.0	4.0	4.0	6.0
C-49-242	5.0	5.0	7.0	6.0	4.0	7.0	4.0	6.0	7.0	6.0	6.0	6.0	7.0	4.0	6.0	4.0	4.0	7.0	4.0	6.0	4.0	6.0	7.0	7.0	6.0	6.0
Mexico-309	2.0	2.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	7.0	7.0	7.0	4.0	4.0	4.0	4.0	7.0	4.0	4.0	4.0	4.0	4.0	7.0	4.0	6.0
Cullapa-72	2.0	1.0	2.0	2.0	2.0	7.0	4.0	4.0	4.0	4.0	6.0	6.0	6.0	2.0	2.0	2.0	2.0	6.0	2.0	2.0	2.0	4.0	4.0	7.0	4.0	6.0
Ecuador-299	1.0	1.0	2.0	2.0	2.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	7.0	2.0	2.0	2.0	2.0	4.0	2.0	2.0	2.0	7.0	7.0	4.0	7.0	4.0
Nep-2	2.0	2.0	2.0	2.0	2.0	2.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	2.0	2.0	2.0	2.0	7.0	2.0	2.0	2.0	4.0	4.0	7.0	4.0	7.0
Aurora	2.0	2.0	2.0	2.0	2.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	2.0	2.0	2.0	2.0	7.0	2.0	2.0	7.0	7.0	7.0	7.0	7.0	7.0
KW-780	3.0	3.0	3.0	7.0	7.0	7.0	7.0	7.0	7.0	3.0	3.0	3.0	3.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	3.0	3.0	3.0	3.0	7.0
CNC	1.0	1.0	4.0	4.0	4.0	4.0	4.0	4.0	1.0	1.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	2.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	6.0
B-190	2.0	2.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	7.0	7.0	7.0	4.0	4.0	4.0	4.0	7.0	4.0	4.0	4.0	4.0	4.0	7.0	4.0	7.0
Olathe	2.0	2.0	2.0	2.0	4.0	2.0	4.0	4.0	2.0	4.0	4.0	4.0	2.0	7.0	7.0	7.0	7.0	6.0	2.0	2.0	2.0	2.0	2.0	4.0	2.0	4.0
Pindak	2.0	2.0	7.0	7.0	7.0	4.0	6.0	6.0	7.0	6.0	6.0	6.0	7.0	7.0	7.0	7.0	7.0	7.0	2.0	7.0	2.0	7.0	7.0	7.0	7.0	7.0
UI-111	2.0	2.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
M/WhfRnr	3.0	3.0	3.0	7.0	7.0	7.0	7.0	7.0	7.0	3.0	3.0	3.0	3.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	3.0	3.0	3.0	3.0	7.0
Seafarer	2.0	2.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
C-20	2.0	2.0	4.0	4.0	2.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	4.0	2.0	2.0	2.0	2.0	7.0	2.0	2.0	2.0	4.0	4.0	7.0	7.0	7.0
51051	2.0	2.0	2.0	2.0	2.0	7.0	4.0	4.0	4.0	4.0	7.0	7.0	7.0	2.0	2.0	4.0	2.0	4.0	2.0	2.0	2.0	1.0	4.0	7.0	4.0	7.0

Table 1.8: Similarity indices (SI) of 19 bean cultivars on the basis of their reactions to 26 races of the bean rust fungus (*U. appendiculatus*)

	LaVega	M-235	CNC-2	C-49 242	M-309	Cuilapa 72	E-299	Nep-2	Aurora	KW780	CNC	B-190	Olathe	Pindak	U.I. 111	M/WhRfr	Seafarer	C-20	51051
LaVega	X	0.23	0.27	0.46	0.54	0.12	0.27	0.38	0.50	0.15	0.31	0.54	0.08	0.42	0.42	0.15	0.42	0.42	0.23
Mexico-235		X	0.42	0.19	0.31	0.58	0.85	0.42	0.46	0.00	0.31	0.31	0.35	0.23	0.12	0.00	0.12	0.42	0.58
CNC-2			X	0.35	0.50	0.35	0.27	0.23	0.08	0.00	0.73	0.30	0.27	0.00	0.00	0.00	0.00	0.27	0.19
C-49-242				X	0.46	0.31	0.19	0.19	0.27	0.12	0.38	0.46	0.12	0.35	0.27	0.12	0.27	0.23	0.27
Mexico-309					X	0.35	0.23	0.42	0.31	0.08	0.62	1.00	0.23	0.27	0.31	0.08	0.31	0.42	0.54
Cuilapa-72						X	0.58	0.58	0.50	0.04	0.27	0.35	0.38	0.23	0.12	0.04	0.12	0.50	0.69
Ecuador-299							X	0.42	0.54	0.00	0.31	0.23	0.46	0.27	0.15	0.00	0.15	0.35	0.58
Nep-2								X	0.85	0.19	0.12	0.42	0.31	0.35	0.46	0.19	0.46	0.81	0.69
Aurora									X	0.23	0.00	0.31	0.27	0.46	0.62	0.23	0.62	0.81	0.65
KW 780										X	0.00	0.08	0.15	0.38	0.58	1.00	0.58	0.23	0.08
CNC											X	0.62	0.23	0.04	0.00	0.00	0.00	0.19	0.19
B-190												X	0.23	0.27	0.31	0.08	0.31	0.42	0.54
Olathe													X	0.31	0.23	0.15	0.23	0.19	0.38
Pindak														X	0.69	0.38	0.69	0.35	0.27
UI-111															X	0.58	1.00	0.50	0.31
M/WhfRnr																X	0.58	0.23	0.08
Seafarer																	X	0.50	0.31
C-20																		X	0.58
51051																			X

similar matches out of the 26 possible matches for any pair, are considered for further discussion.

The cultivar LaVega had SI = 0.46 with C-49-242, SI = 0.54 with cultivars Mexico-309 and B-190, SI = 0.50 with Aurora, and SI = 0.42 with cultivars Pindak, Seafarer, UI-111 and C-20.

Mexico-235, which is reported to be highly related to Ecuador-299, produced a high SI = 0.85 with that cultivar, SI = 0.58 with Cuilapa-72 and 51051, SI = 0.46 with Aurora, and SI = 0.42 with Nep-2 and CNC-2. CNC-2 produced the highest similarity index value of 0.73 with CNC; SI = 0.50 with Mexico-309 and B-190. Cornell 49-242, produced SI = 0.46, with the pedigree-related cultivars Mexico-309 and B-190. Mexico-309 and its progeny B-190 produced a perfect match with a similarity index value of 1.00; SI = 0.62 with CNC; SI = 0.54 with 51051, and SI = 0.42 with cultivar Nep-2 and C-20. Cuilapa-72 and 51051 gave a similarity index of 0.69, SI = 0.58 with cultivars Ecuador-299 and Nep-2 and SI = 0.50 with Aurora and C-20. Ecuador-299 had SI = 0.58 with 51051, SI = 0.54 with Aurora and SI = 0.42 with Nep-2.

SI = 0.85 was recorded between Nep-2 and Aurora, followed by Nep-2 and C-20 at SI = 0.81, Nep-2 and 51051 at SI = 0.69, Nep-2 with UI-111 and Seafarer at SI = 0.46 and Nep-2 with B-190 at SI = 0.42. Aurora, which has a high value of similarity with Nep-2, was matched to the same cultivars as was Nep-2 with almost identical values. A perfect match was obtained for cultivars KW-780 and M/WhfRnr at SI = 1.00. CNC and B-190 were matched with KW-780 at an SI value of 0.58.

UI-111 and Seafarer were also matched with a perfect SI = 1.00. Other than their resistant reactions to races 28 and 39, both were susceptible to 24 other races. These two cultivars are not otherwise genetically related. SI as applied here deals only with rust reactions and no other traits. Inferences or interpretations of genetic identity from high values of

coef

Wh

reac

gen

gen

pre

Rel
hea

isol

am

des

me

for

1.0

ref

alp

ref

Ab

ref

Ab

coefficients of similarity for reaction to rust isolation should therefore be treated with caution. While high SI values may indicate genetic relationship among bean cultivars exhibiting R reactions to the rust isolates, the same logic may not be extended for S reactions to infer genetic relationships. This is because SI values for R reactions indicate presence of similar genes for reaction in the cultivar pairs, while SI for S reaction may be for reasons other than presence of similar genes for that reaction.

Relationships of bean rust isolates based on their ability to elicit similar reaction responses on bean cultivars

The 19 cultivar x 26 isolate disease reaction data set was transposed to produce a 26 isolate x 19 cultivar raw data matrix for purposes of assessing the extent of interrelationships among the bean rust races on the basis of their ability to elicit similar disease reactions on these cultivars. The 26 x 19 raw data matrix is summarized in Table 1.9. Three hundred twenty–five pairwise comparisons (matches) have been computed employing the same formula for computing a simple matching coefficient to represent a value of similarity index (Table 1.10).

Race 38 was compared for its ability to produce similar reactions on 19 bean cultivars with 25 other bean rust races. Race 38 and Race 39 had similar reactions elicited on 18 cultivars out of 19 with an SI value of 0.95. Race 38 also produced SI = 0.42, 0.42 and 0.47 with Races 40, 59 and 61 respectively. It had SI = 0.00 with Race 67, indicating distant or no relationship and SI = 0.37 with several other races.

Race 40 showed higher coefficients ranging from SI = 0.42 to SI = 0.74 with 15 out of 26 races. The highest similarity was with Races 41, 42, 52, 53, 57, 60 and 61 at SI = 0.74.

High similarity index values were also produced by Race 41 that indicated close relationships with 14 out of 21 races it was compared against. It produced the highest values

Table 1.9: Reactions elicited by 26 bean rust races on 19 bean cultivars

Rust Races	LaVega	M-235	CNC-2	C-49 242	M-309	Cuilapa 72	E-299	Nep-2	Aurora	KW780	CNC	B-190	Olathe	Pindak	U.I. 111	M/WhfRnr	Seafarer	C-20	51051
R38	4	1	1	4	2	2	1	2	2	3	1	2	2	2	2	3	2	2	2
R39	4	1	1	4	2	1	1	2	2	3	1	2	2	2	2	3	2	2	2
R40	4	2	4	7	4	2	2	2	2	3	4	4	2	7	7	7	7	4	2
R41	5	4	4	4	4	2	2	2	2	7	4	4	4	7	7	7	7	2	2
R42	4	2	4	4	4	2	2	2	2	7	4	4	4	7	7	7	7	2	2
R43	4	4	2	7	4	7	4	2	7	7	4	4	2	4	7	7	7	7	7
R45	7	4	4	4	4	4	4	7	7	7	4	4	4	6	7	7	7	7	4
R46	5	4	4	4	4	4	4	7	7	7	4	4	4	6	7	7	7	7	4
R47	7	4	2	7	4	4	4	7	7	7	1	4	2	7	7	7	7	7	4
R48	6	4	1	6	4	4	4	7	7	3	1	4	4	6	7	3	7	7	4
R49	7	2	2	6	7	6	4	7	7	3	4	7	4	6	7	3	7	7	7
R50	7	3	3	6	7	5	4	7	7	3	4	7	4	6	7	3	7	7	7
R51	7	7	4	7	7	6	7	7	7	3	4	7	2	7	7	3	7	4	7
R52	4	2	4	2	4	2	2	2	2	7	4	4	7	7	7	7	7	2	2
R53	4	2	4	4	4	2	2	2	2	7	4	4	7	7	7	7	7	2	2
R56	2	2	2	4	4	2	2	2	2	7	4	4	7	7	7	7	7	2	4
R57	4	2	4	4	4	2	2	2	2	7	4	4	7	7	7	7	7	2	2
R58	7	4	1	7	7	6	4	7	7	7	2	7	6	7	7	7	7	7	4
R59	2	2	4	4	4	2	2	2	2	7	4	4	2	2	7	7	7	2	2
R60	4	2	2	6	4	2	2	2	2	7	4	4	2	7	7	7	7	2	2
R61	4	2	4	4	4	2	2	2	7	4	4	2	2	7	7	7	7	2	2
R63	7	7	4	6	4	4	7	4	7	3	4	4	2	7	7	3	7	4	1
R64	7	4	4	7	4	4	7	4	7	3	4	4	2	7	7	3	7	4	4
R65	7	4	4	7	7	7	4	7	7	3	4	7	4	7	7	3	7	7	7
R66	7	7	4	6	4	4	7	4	7	3	4	4	2	7	7	3	7	7	4
R67	7	4	6	6	7	5	4	7	7	7	6	7	4	7	7	7	7	7	7

Table 1.10 Similarity indices (SI) of 26 races of the bean rust fungus based on their ability to elicit similar reactions on 19 bean cultivars

	R38	R39	R40	R41	R42	R43	R45	R46	R47	R48	R49	R50	R51	R52	R53	R56	R57	R58	R59	R60	R61	R63	R64	R65	R66	R67
R38	X	0.95	0.42	0.32	0.37	0.16	0.05	0.05	0.11	0.21	0.11	0.11	0.16	0.32	0.37	0.26	0.37	0.05	0.42	0.37	0.47	0.16	0.16	0.11	0.16	0.00
R39		X	0.37	0.26	0.32	0.16	0.05	0.05	0.11	0.21	0.11	0.11	0.16	0.26	0.32	0.21	0.32	0.05	0.37	0.37	0.42	0.16	0.16	0.11	0.16	0.00
R40			X	0.74	0.74	0.47	0.32	0.32	0.37	0.32	0.32	0.32	0.53	0.74	0.74	0.58	0.74	0.21	0.68	0.74	0.74	0.58	0.63	0.42	0.53	0.16
R41				X	0.79	0.53	0.53	0.58	0.47	0.26	0.16	0.16	0.37	0.74	0.79	0.68	0.79	0.32	0.79	0.74	0.79	0.47	0.53	0.32	0.42	0.32
R42					X	0.47	0.53	0.53	0.37	0.26	0.26	0.26	0.26	0.89	0.95	0.79	0.95	0.26	0.84	0.84	0.89	0.37	0.37	0.32	0.37	0.32
R43						X	0.58	0.58	0.68	0.42	0.42	0.42	0.37	0.47	0.47	0.47	0.47	0.47	0.47	0.58	0.53	0.37	0.47	0.53	0.42	0.47
R45							X	0.95	0.74	0.68	0.33	0.53	0.37	0.42	0.47	0.47	0.47	0.58	0.47	0.37	0.47	0.47	0.58	0.58	0.58	0.58
R46								X	0.68	0.68	0.47	0.47	0.32	0.42	0.47	0.47	0.47	0.53	0.47	0.37	0.47	0.42	0.53	0.53	0.53	0.53
R47									X	0.63	0.42	0.42	0.42	0.37	0.37	0.47	0.37	0.68	0.37	0.47	0.37	0.47	0.63	0.53	0.58	0.58
R48										X	0.58	0.58	0.32	0.21	0.21	0.26	0.21	0.47	0.21	0.26	0.21	0.47	0.53	0.53	0.58	0.47
R49											X	1.00	0.63	0.21	0.21	0.26	0.21	0.53	0.21	0.32	0.21	0.42	0.37	0.74	0.47	0.68
R50												X	0.26	0.21	0.21	0.26	0.21	0.53	0.21	0.32	0.21	0.42	0.37	0.74	0.47	0.68
R51													X	0.26	0.26	0.21	0.26	0.53	0.26	0.26	0.26	0.68	0.68	0.74	0.63	0.53
R52														X	0.95	0.79	0.95	0.26	0.79	0.84	0.84	0.37	0.37	0.26	0.37	0.26
R53															X	0.84	1.00	0.26	0.84	0.84	0.89	0.37	0.37	0.26	0.37	0.26
R56																X	0.84	0.32	0.79	0.79	0.74	0.32	0.37	0.21	0.37	0.26
R57																	X	0.26	0.84	0.84	0.89	0.37	0.37	0.26	0.37	0.26
R59																		X	0.21	0.26	0.21	0.26	0.42	0.63	0.37	0.74
R60																			X	0.79	0.95	0.37	0.37	0.21	0.37	0.21
R61																				X	0.84	0.42	0.37	0.21	0.42	0.32
R63																					X	0.37	0.37	0.21	0.32	0.21
R64																						X	0.84	0.47	0.89	0.32
R65																							X	0.58	0.84	0.32
R66																								X	0.53	0.68
R67																									X	0.37
																										X

of SI = 0.79 with five races (42, 53, 57, 59 and 61). Relatively high SI values of 0.74, 0.74 and 0.68 respectively were recorded for Race 41 with Races 52, 56 and 60, respectively.

High SI values were produced for Race 42 with 10 other races. The highest value at SI = 0.95 were recorded for Race 42 with Races 53 and 57. Race 42 also produced high SI = 0.89 with Races 52 and 61, SI = 0.84 with Races 59 and 60 and SI = 0.79 with Race 56.

Moderately high similarity index values were recorded for Race 43 with eighteen other races. The highest SI value at 0.68 was with Race 47. Similarity values with the remainder of the races ranged from SI = 0.42 to SI = 0.58. Identical reactions were produced on 11 cultivars with Races 45 and 46.

Race 45 also produced moderate (SI = 0.42) to very high (SI = 0.95) with 17 other races. The highest coefficient of similarity was between Race 45 and Race 46 at SI = 0.95. It had SI = 0.68 and 0.74 with Races 47 and 48, respectively. With five of the races (58, 64, 65, 66 and 67), it produced an SI value of 0.58.

The highest value of coefficient of similarity for Race 46 was at 0.68 with Races 47 and 48. Race 47 also had a similar coefficient of similarity value at 0.68 with Race 58. The highest matching coefficient for Race 48 was with Races 49, 50 and 66 at SI = 0.58.

The reaction matches between Race 49 and Race 50 was perfect at an SI value of 1.00. High SI were also recorded for Race 49 with Race 65 (SI = 0.65), Race 67 (SI = 0.68). Race 50, other than its perfect match with Race 49, produced high coefficients of similarity with Race 65 (SI = 0.75), Race 67 (SI = 0.68) and Race 51 (SI = 0.83). High SI were recorded for Race 51 with Races 65 (SI = 0.74), 63 and 64 (SI = 0.68) and Race 66 (SI = 0.63).

Race 52 produced SI = 0.95 with Races 53 and 57, SI = 0.84 with Races 60 and 61 and SI = 0.79 with Races 56 and 59. The reaction elicited by Race 53 on 19 cultivars perfectly matched that of Race 57.

High coefficients of similarity for reaction were also recorded for Race 53 with Races 61 (SI = 0.89), 56, 59 and 60 (SI = 0.84) and a perfect match (SI = 1.00) with Race 57.

Race 56 had identical reactions with Race 57 (SI = 0.84) and produced relatively high matching coefficients with Races 59 and 60 (SI = 0.78) and 61 (SI = 0.74). Race 57 produced a coefficient of similarity value of 0.89, with Race 61 and 0.84 with Races 59 and 60.

SI = 0.74 was recorded for Race 58 and Race 67 and SI = 0.95 for Race 59 with Race 61, indicating a high degree of similarity. Races 60 and 61 were matched at an SI value of 0.84.

High degrees of relatedness were also indicated for several races, including Race 63 and Race 66 (SI = 0.89), and Races 63 and 64 (SI = 0.84), Race 64 and Race 66 (SI = 0.84) and Race 65 with Race 67 (SI = 0.68).

Comparison of degree of resistance to 9 races (Table 1.5) indicated that Mexico–235 and Ecuador–299, with no susceptibility reaction to any of the 9 races, are the most resistant, followed by CNC–2, which had resistance to all 9 races, but with resistance of small uredinia reaction type to 4 out of 9 races. CNC was also resistant to all 9 races, but with resistant reaction of the small uredinia type, to 7 out of 9 races. UI–111 and Seafarer were the most susceptible having resistance only of the large necrotic spot type to Races 38 and 39.

The comparison for degrees of resistance of the bean cultivars to 26 races (Table 1.7) were similar to the comparison against 9 races, with CNC–2 and CNC being the most resistant cultivars, having resistance to 25 of the 26 races. Mexico–309 and Ecuador–299 were susceptible to only 3 and 4 races, respectively, and were the second and third most resistant cultivars. In both tests, there were no cultivars that would be considered universally resistant to all races nor cultivars that were universally susceptible.

The comparison for degree of virulence of the rust races revealed that Race 67 (collected in Homeland, Florida, in 1985) was the most virulent, with Mexico–235 being the

only cultivar that has some degree of resistance to it, followed by Race 58 and Race 51 having 14 and 15 cultivars, respectively, which are susceptible to each. Race 39 and Race 38 were the least virulent, with all cultivars showing resistant reaction grades.

Highly variable pathogenicity of the bean rust fungus, *U. appendiculatus*, has been recognized with frequent occurrence of mixed collections indicative of a high degree of natural diversity (Stavely, 1984; Stavely et al., 1989). This diversity in the pathogen is known to be related to cultivar (host) susceptibility to a wide range of races that permit the occurrences of multiple virulence genes in the pathogen (Stavely, 1984). The variability that is found in the pathogen is also correspondingly matched by a range of host resistance reaction that has its origin in cultivar resistance genes in accordance with the gene-for-gene system (Stavely, 1984).

Person (1959) suggested that specific gene-for-gene relationships may well occur as a rule rather than the exception in host-parasite systems.

Indeed, the *P. vulgaris/U. appendiculatus* host-parasite relationship has existed since, perhaps, several millennia. Resistance in beans to the rust fungus is expressed variably, a clear indication in evolutionary terms of the long association in a cycle of dynamic competition in the bean host and rust fungus. Several single dominant genes controlling reaction grades that can be characterized in the gene-for-gene relationship have been shown to occur in *P. vulgaris/U. appendiculatus* host-parasite system (Stavely, et al., 1989). Closely linked single, dominant genes, one per race, conditioning reaction of the small uridinium type to several races have been reported by Stavely (1984) on cultivars Mexico-309 and its progeny B-190. Five of the differential cultivars (Aurora, Ecuador-299, Mexico-235, Nep-2 and 51051) develop small necrotic spots or flecks in response to 22 races from the U.S. and Tanzania (Stavely, 1989). The same reaction response has been reported to occur with all of the Australian races by a resistance gene designed as Ur-3 (Ballantyne, 1978). A single gene

control of necrotic reaction (HR) to at least six races (Kardin and Groth, 1985) and a different gene or locus conditioning necrotic reaction to Races 38 through 70 and Tanzanian races T1 through T9 in KW-780 and Early Gallatin and most bush snap beans has been reported (Stavely, et al., 1989). The presence of such broadly effective genes strongly suggests that these cultivars contain the same gene or genic complexes conditioning the reactions to these races.

There is no doubt that the continued exposure of bean cultivars to the selective pressure of the rust fungus is the basis for the occurrence of several reaction phenotypes. The presence of broadly effective genes or genic complexes that behave as single genes in transmission and giving the appearance of relatedness in many of these cultivars is a result of a group of contiguous and tightly linked genes. It is theorized that these component genes may be related functionally to form an adaptive gene combination, and segregate as a single unit in inheritance (Anderson 1949).

SUMMARY AND CONCLUSION

Reaction of parental and non-parental bean cultivars were evaluated against four described rust isolates in the greenhouse in East Lansing, Michigan, and against 26 isolates in Beltsville, Maryland. The reaction data was converted into a single index of similarity (SI) for pairs of cultivars or rust isolates for easy comparison. The data revealed basic similarities and differences among the cultivars or isolates, which indicated underlying genetic similarities and differences.

On the basis of reaction phenotype classification to each rust isolate, the cultivars could be grouped into categories as R or S. By this criterion, 11 cultivars were R to Races 41 and 53, while two cultivars (KW-780 and ICA-Pijao) were S to these same isolates. For Races 46 and 49, cultivars were either predominantly R or S due to the presence of either R or S reactions on plants belonging to a cultivar. This is attributed to the heterogeneity of the bean cultivars used in the study.

Similarity indices (SI) computed from pairwise comparison of cultivar or rust isolate provided a single value for easy comparison in each group. On the basis of SI values, cultivars or rust isolates could be categorized into those with high SI (SI = 0.75 – 1.00) and those with low SI (SI = 0.00 – 0.08). The following pairs of bean cultivars have high SI: B-190/Mexico-309, C-20/Nep-2, Aurora/Nep-2, Mexico-235/Ecuador-299, CNC/CNC-2 and Mountain White Half-Runner/KW-780. Examples of cultivars with low SI include Aurora/CNC-2, KW-780/Mexico-235, CNC/UI-114, and Olathe/Lavega. Similarly, rust isolates producing high SI (SI = 0.74–1.00) for eliciting similar reaction on an array of bean

cultivars are the following: R49/R50, R53/R57, R38/R39, R45/R46, R41/R53, R42/R53, R52/R53, R42/R57, R52/R57 and R63/R66. Examples of rust isolates with low SI (SI = 0.00 - 0.11) include the pairs between the mainly snap bean Races 38 and 39 with many of the rust isolates included in the study.

High SI values for cultivars could be for either R or S reactions to an array of rust isolates as high SI value for rust isolates are for eliciting similar R or S reaction to an array of bean cultivars. However, high SI between pairs of bean cultivars or pairs of rust isolates should be viewed cautiously. While high SI values for R reaction indicate presence of similar genes for reaction in the cultivar pairs, high SI for S reaction may be for reasons other than presence of similar genes for the reaction.

LIST OF REFERENCES

Alten, H. von. 1983. The effect of temperature, light and leaf age on the frequency of appressoria formation and infection with *Uromyces phaseoli* (Pers.) Wint. Phytopath Z. 107: 327–335.

Anderson, G. 1949. Introgeressive hybridization. John Wiley, New York.

Andrus, C.F. 1931. The mechanism of sex in *Uromyces* appendiculatus and *U. vignae*. J. Agr. Res. 42: 559–587.

Augustine, E. and J.C. DaCosta. 1971. Nova raca fisiologica de *Uromyces phaseoli typica* no Sul do Brasil. Agropec. Bras., Ser. Agron. 6:137–138.

Augustine, E., D.P. Coyne and M.L. Schuster. 1972. Inheritance of resistance in *Phaseolus vulgaris* to *uromyces phaseoli typica* Brazilian rust race B 11 and of plant habit. J. Amer. Soc. Hort. Sci. 96:526–529.

Ballantyne, B.J. 1978. The genetic bases of resistance to rust, caused by *Uromyces appendiculatus* in bean (Phaseolus *vulgaris*). Ph.D. Thesis, Univ. of Sydney, Australia, 262 p.

Barbosa, C., R.S. and G.M. Chaves. 1975. Comparacao de dois metodos de amostragem na identificacao de racas de *Uromyces phaseoli typica* Arth. experentiae 19: 149–186.

Carrijo, I.V., G.M. Chaves and A.A. Pereira. 1980. Reacao de vinte cinco variedades de *Phaseolus vulgaris* a trinta e nove racas fisiologicas de *Uromyces phaseoli* var. *typica* Arth., em condicoes de casa–de–vegetacao. Fitopatologia Brasieira 5: 245–255.

Crispin, M.A., J.A. Sifuentes A. and J. Campos Avila. 1976. Enfermedades y plagas del Frijol en Mexico. pp. 6–9. Inst. Nac. Invest. Agr., SAG, Foll. Tec. de Divulgacion No. 39.

Crispin, A. and S. Dongo D. 1962. New physiologic races of bean rust, *Uromyces phaseoli typica*, from Mexico. Plant Dis. Reptr. 46: 411–413.

Cummins, G.B. 1978. Rust fungi on legumes and composites in North America. Univ. of Arizona Press, Tucson, 424 p.

Davison, A.D. and E.K. Vaughan. 1963a. A simplified method for identification of races of *Uromyces phaseoli* var. *Phaseoli*. Phytopathology 53: 456–459.

Davison, A.D. and E.K. Vaughan. 1963b. Longevity of urediospores of race 33 of *Uromyces phaseoli* var. *phaseoli* in storage. Phytopathology 53: 736–737.

Dias, F., I.R. and J.C. Da Costa. 1968. Indetificacao de racas fisiologicas da ferrugem (*Uromyces phaseoli typica* Arth.) do feijoeiro (*Phaseolus vulgaris* L.) em duas regioes fisiograficas do Rio Grande do Sul, Brazil. Pesq. Agropec. Bras., Ser. Agron. 3: 165–170.

Fisher, H.H. 1952. New physiologic races of bean rust (*Uromyces phaseoli typica*). Plant Dis. Reptr. 36: 103–105.

Groth, J.V. and R.D. Shrum. 1977. Virulence in Minnesota and Wisconsin bean rust collections. Plant Dis. Reptr. 61: 756–760.

Groth, J.V. and A.P. Roelfs. 1982a. Genetic diversity for virulence in bean rust collections. Phytopathology 72: 982–983.

Harter, L.L. and W.J. Zaumeyer. 1941. Differentiation of physiologic races of *Uromyces phaseoli typica* of bean. Jour. Agr. Res. 62(12):717–731.

Harter, L.L., C.F. Andrus and W.J. Zaumeyer. 1935. Studies on bean rust caused by *Uromyces phaseoli typica*. Jour. Agr. Res. 50(19):737–759.

Imhoff, M.W.; C.E. Main and K.J. Leonard. 1981. Effect of temperature, dew period, and age of leaves, spores and source pustules on germination of bean rust urediospores. Phytopathology 71:577–583.

Imhoff, M.W., K.J. Leonard and C.E. Main. 1982. Patterns of bean rust lesion size increase and spore production. Phytopathology 72:441–446.

Kardin, M.K. and J.V. Groth. 1985. The inheritance of resistance in two white seeded dry bean cultivars to seven bean rust isolates. Phytopathology 75: 1310 (Abstr.).

Kolmer, J.A., B.J. Christ and J.V. Groth. 1984. Comparative virulence of monokaryotic and dikaryotic stages of five isolates of *Uromyces appendiculatus*. Phytopathology 74:111–113.

Lopez, G. 1976. Identificacion de razas fisiologicas de la roya (*Uromyces appendiculatus* (Pers.) Unger) de frijol (*Phaseolus vulgaris* L.) en Puerto Rico. Ph.D. Dissert. Univer. de Puerto Rico, Mayaguez, 50 p.

Mendgen, K. 1978a. Attachment of bean rust cell wall material to host and non-host plant tissue. Arch. Microbiol. 119: 113–117.

Nasser, L.C.B. 1976. Efeito da ferrugem em diferentes estadios de desenvolvimiento do feijoeiro e dispersao de esporos de *Uromyces phaseoli* var. *typica* Arth. Tesis M.S., Univer. Federal de Vicosa, Minas Gerais, Brazil, 79 p.

Ogle, H.J. and J.C. Johnson. 1974. Physiologic specialization and control of bean rust (*Uromyces appendiculatus*) in Queensland. Queensland J. Agr. and Animal Sci. 31: 71–82.

Pring, R.J. 1980. A fine-structural study of the infection of leaves of *Phaseolus vulgaris* by urediospores of *Uromyces phaseoli*. Physiol. Plant Path. 17: 269–276.

Raggi, V. 1980. Correlation of CO_2 compensation point with photosynthesis and respiration and CO_2-sensitive compensation point in rust-affected bean leaves. Physiol. Plant Path. 16: 19–24.

Ruiz, G.H., P.L. Melendez and R.P. Rodrigues. 1982. Physiological races of *Uromyces* rust of beans in Puerto Rico. Phytopathology 72: 1973 (Abstr.).

Schein, R.D. 1965. Age-correlated changes in susceptibility of bean leaves to *Uromyces phaseoli* and tobacco mosaic virus. Phytopathology 55: 454–457.

Shaik, M. and J.R. Steadman. 1986. Variation in a rust-resistance reaction of *Phaseolus vulgaris* L. due to leaf age. Phytopathology 76: (in press) (Abstr.).

Shaik, M. 1985b. Races of the bean rust fungus *Uromyces appendiculatus* var. *appendiculatus* from Jamaica. Ann. Rept. Bean Improv. Coop. 28:20–21.

Stavely, J.R. and Pastor-Corales, M.A. 1989. Rust. Chap. 7 in Bean production problems in the tropics. H.F. Schwartz and M.A. Pastor-Corales, eds. CIA T, Cali.: Columbia (in press).

Stavely, J.R., Steadman, J.R. and McMillan, R.T., Jr. 1989. New pathogenic varability in *Uromyces appendiculatus* in North America. Plant Disease 73: 428–432.

Stavely, J.R. 1984a. Pathogenic specialization in *Uromyces phaseoli* in the United States and rust resistance in beans. Plant Disease 68:95–99.

Stavely, J.R. 1983. A rapid technique for inoculation of *Phaseolus vulgaris* with multiple pathotypes of *Uromyces phaseoli*. Phytopathology 73:676–679.

Stavely, J.R., G.F. Freytag, J.R. Steadman and H.F. Schwartz. 1983. The 1983 bean rust workshop. Ann. Rept. Bean Improv. Coop. 26:iv–vi.

Sziraki, I., L.A. Mustardy, A. Faludi-Daniel and Z. Kiraly. 1984. Alterations in chloroplast ultrastructure and chlorophyll content in rust-infected pinto beans at different stages of disease development. Phytopathology 74:77–84.

Yarwood, C.E. 1961. Urediospore production by *Uromyes phaseoli*. Phytopathology 51:22–27.

Yeh, C.C. 1983. Screening of common beans for rust resistance and physiological races of bean rust fungus in Taiwan. J. Agr. Res. China 32: 259–269.

Zaki, A.I. and R.D. Durbin. 1965. The effect of bean rust on the translocation of photosynthetic products from diseased leaves. Phytopathology 55:528–529.

Zambolim, L. and G.M. Chaves. 1974. Efeito de baizas temperatura–umidale relativa sobre a viabilidad dos urediospotos de *Hemileia vastatrix* Berk. & Br. e *Uromyces phaseoli typica* Arth. Experientiae (Brazil) 17:151–184.

Zulu, J.N. and B.E.J. Wheeler. 1982. The importance of host factors of bean (*Phaseolus vulgaris*) on the control of rust (*Uromyces appendiculatus*). Trop. Agric. (Trinidad) 59(3):235–238.

Zuniga de Rodriquez, J.E. and J.I. Victoria K. 1975. Determinacion de las razas fisiologicas de la roya del frijol (*Uromyces phaseoli* var. *typica*) Arth. en el Valle de Cauca. Acta Agron. 25: 75–85.

CHAPTER II

GENETIC RELATIONSHIPS OF BEAN CULTIVARS AS EVALUATED BY ISOZYME ELECTROPHORETIC PATTERNS AND AGROPHYSIOLOGICAL TRAITS

INTRODUCTION

Molecular techniques that combine electrophoresis with histochemical staining methods that allow detection of specific activity of enzymes (isozymes) are being used extensively to study genetic variations in a wide array of living organisms.

Traditional methods that rely on morphological traits are less reliable as yardsticks for characterization and identification of crop cultivars due to the large influence of the environment on their expression unlike allozymes, which are not so affected. Allozymes also exhibit co-dominant expression of the alleles that allow easy observation of such alleles.

In beans, seeds, roots and young trifoliate leaves can be used for enzyme assays. In the present case, isozyme data involving twelve enzyme systems have been obtained on twenty bean cultivars from three types of seedling tissues. The purposes of this study were: 1) to assess genetic relationships among and between parental and non-parental bean cultivars using their isozyme banding patterns from 12 enzyme systems assayed on leaf, root, and seed tissue; and 2) to compare the results of isozyme banding with disease, agrophysiological and genetic data.

LITERATURE REVIEW

Interest in the characterization of genetic diversity within and among elite breeding materials and cultivars is important in providing information regarding genotypic purity, estimates of genetic relationships and comparative levels of diversity among elite, exotic and wild germplasm (Adams 1977). Recently, the combined ability of electrophoretic and histochemical staining techniques to reveal large amounts of variation in the form of isozymes or allozymes has led to its application in many fields of research, including numerical taxonomy and related cluster and other multivariate techniques (Smith et al., 1984). Particularly, the easily understood co-dominant genetic control of isozyme loci in several crops has allowed direct interpretation of allelic frequencies from electrophoretic banding patterns that are also amenable to analysis and interpretation of genetic interrelationships using multivariate statistical techniques.

Smith et al. (1984) presented results of an extensive allozyme survey using 19 enzyme loci to compare variation patterns among 79 accessions of teosinte (*Zea mexicana*) from Mexico and Guatemala. Analysis of isozyme allele frequencies at 19 loci using principal component analysis based on the covariance matrix of allele frequencies revealed 133 electrophoretic variants. In addition to revealing the extent of distribution of the various alleles in the accessions tested, genetic relationships were inferred between some of the same accessions and the extent of diversity of the material assessed.

An electrophoretic survey of isozyme variation among widely grown maize hybrids of the US was carried out by Smith (1984) in order to assess genetic diversity, to determine the

potential for using isozyme data to identify and characterize hybrid cultivars and to reveal relationships among hybrids. Isozymes coded by 21 loci for 111 US hybrid cultivars of maize were surveyed. PCA was used based on the covariance matrix of allele frequencies with each hybrid treated as an individual unit. The author found that elite material showed a reduction in number of polymorphic alleles and an increase in number of monomorphic loci when compared to exotic and wild germplasm. PCA also revealed that approximately 90 percent of the hybrids had different allele frequencies. The author suggested that isozyme data can be used to characterize inbred lines and hybrids and that sufficient variability exists among isozymes to allow for rapid checking for purity of US hybrid maize.

Genetic variability in historically important lines of maize within the US maize germplasm pool was assessed by Smith et al. (1985). Principal component analysis was performed on the covariance matrix of allele frequencies from isozyme data for 21 loci in 72 historically important US Corn Belt and Southern lines of maize in order to compare relationships with those expected from known pedigree or phylogenetic data. Isozyme data tended to group lines of similar backgrounds together through tight clustering of related lines was not found in their studies. The study also revealed the germplasm base of US maize was broad and diverse.

Decker (1985), in an attempt to clarify the systematics of *Cucurbita pepo* cultivars, assayed allozyme variation among 50 accessions representing 14 commercial cultivars using six enzyme systems representing 12 loci, seven of which were polymorphic. Statistical treatment of allozyme data revealed a biochemical basis for characterizing cultivars that agreed with morphology. A cluster analysis of the matrix of coefficients of genetic identity using the Unweighted Pair Group Method (UPGM) using arithmetic averages and PCA based on cultivar allelic frequencies corroborated patterns observed in the analysis of variance. Homogeneity of accessions within cultivar groups and close clustering of cultivars within groups was noted.

Estimates of genetic similarity (or genetic distances among populations) can be based on biochemical, morphological, quantitative or pedigree data. Cox et al. (1985a) compared similarity coefficients (s) based on polyacrylamide gel electrophoresis patterns with coefficients of parentage (r) computed from pedigree analysis for all pairwise combinations of 43 US hard red winter wheat cultivars to determine whether there were genetic clusters of cultivars within the gene pool of US hard red winter wheat. Each index varied from zero for two unrelated cultivars to unity for two identical cultivars. Cluster analysis performed using the UPGM method of clustering based on the r and s matrices revealed dissimilar patterns of relationships in the hard red winter wheat gene pool. The authors suggested that a composite index which includes both coefficient of parentage (r) and coefficients of similarity (s) based on zymogram patterns of several enzymes be used as an estimate of genetic relationships.

Cox et al. (1985b) found close agreement between estimates of genetic similarity indices (S, S_z, and S_m) and pedigree data coefficients of parentage (r) for combinations of 115 soybean cultivars and ancestral introductions. Pedigree data (r) were analyzed after Delannay et al. (1983) while similarity indices were computed from a combination of biochemical and morphological data representing 20 genetic loci (S, K=20), biochemical data only (S_z, K=13) and morphological data only (S_m, K=7). Similarity between two cultivars or introductions was defined as S = K'/K where the cultivar or introduction had the same genotype for K' out of K (=20, 13 or 7) loci. Rank correlation coefficients were calculated for each group between r and each of the similarity indices (S, S_z, S_m). The authors noted correlations for r and s were higher where higher numbers of loci were considered and for groups of cultivars released in the 1970s than for earlier released cultivars because of the greater importance of identity by descent values relative to identity in phenotype in determining s. The usefulness of an estimate of genetic relationship of a composite index that includes both r and s was emphasized by the authors in helping form decisions for selecting diverse parents.

Bassiri and Adams (1978b) assayed the same three enzyme systems used in a previous study (Bassiri & Adams, 1978a) to distinguish between 34 bean cultivars belonging to 9 commercial classes grown in the United States. They noted no class was defined by only one enzyme pattern and that homology of total isozyme banding pattern for three enzymes was often high for cultivars in the same commercial class. In the same study, grouping of cultivars by number of polymorphic isozyme bands in common produced clusters whose members were known to share pedigree relationships.

Sprecher (1988) assayed six enzyme systems in leaf, root and seed tissues of 375 Malawian bean landrace accessions. She reported a limited amount of variability among the isozymes surveyed, which was also correlated to the seed size gene pool groups known in common beans. Fewer than ten of the theoretically possible 64 combinations of alleles (2^6 = 64) were observed, the majority of which fell into two patterns designated as pattern 1 for large-seeded beans and pattern 7 for small-seeded beans (Sprecher, 1988).

Gepts et al. (1986) used one-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and two-dimensional isoelectric focusing to examine variability of the major seed storage protein (phaseolin) of the common bean in a group of 136 wild bean accessions and 118 landraces from Mexico, Central and South America. The authors reported in all regions of Latin America that cultivars with T or C phaseolin tended to have large seeds and cultivars with the S phaseolin tended to have smaller seeds. Based on distinct phaseolin banding patterns, they suggested independent domestication of the common bean with Mesoamerican and Andean germplasm.

Singh et al. (1991) used starch gel electrophoresis to assess patterns of diversity at nine polymorphic allozyme loci of 227 cultivated landraces of the common bean representing a geographic distribution from Mexico to Argentina and Chile. The study confirmed the existence of two major groups, Mesoamerican and Andean American, in the cultivated and

wild beans by cluster analysis based on Nei's genetic distance (D) and the unpaired group method of clustering. Their results also suggested at least five subgroups within Mesoamerican and four within Andean cultivar groups. The authors identified within the Mesoamerican and Andean cultivated germplasm clusters of landraces that share a common allozyme and can presumably be traced to a common ancestor. Landraces that represent hybrids between the Andean and Mesoamerican group were identified. Indications of cultivars within the same allozyme genotypes that have undergone further evolutionary diversification for morphological traits (seed traits mainly) but not for molecular markers was noted by the authors.

MATERIALS AND METHODS

Plant tissue used and preparation for electrophoretic tests

Seeds that were imbibed for 24 hours in the dark, leaf portions and roots from 5- to 7-day-old seedlings were used for extracting enzymes (Table 2.1). 0.5 ml of appropriate extraction buffer was used to grind equal amounts of plant tissue (seed, leaf and root) with pestles in chilled porcelain mortars to squeeze out enzyme in tissue juice. After grinding, the juice was absorbed into equal sized (3 x 8mm) paper wicks and stored in a cool place to preserve enzyme activity.

Buffers used

The following buffer systems were prepared for each tissue type and appropriate dilutions and pH adjustments carried out as summarized in Table 2.2.

Gel preparations

Thirty-three grams of sifted and clump-free hydrolyzed potato starch (Sigma 5-4501) in 250 ml of the appropriate buffer (Table 2.2) was used in preparing the gel. The buffer starch mixture was heated in a 1,000 ml side-arm erlenmeyer flask with occasional vigorous shaking until just boiling to dissolve the starch. The liquid gel was immediately degassed under vacuum to remove air bubbles and quickly poured into the gel mold. Any clumps and air bubbles formed during pouring were removed using pasteur pipettes. The gel thus prepared was then covered with saran wrap and allowed to cool at room temperature overnight.

Table 2.1: Enzyme systems assayed and tissue used to extract enzymes

	Enzyme/Protein	Tissue used		
		Seed	Leaf	Root
1.	Phaseolin (Sdpr)	x		
2.	Malic dehydrogenase	x		
3.	Rubisco		x	
4.	Shikimic dehydrogenase (SKDH)		x	
5.	Malic enzyme			x
6.	Peroxidase-1 (PRX-1)			x
7.	Peroxidase-2 (PRX-2)			x
8.	Diaphorase-1 (DIAP-1)			x
9.	Diaphorase-2 (DIAP-2)			x
10.	Acid phosphatase			x
11.	Esterase-1 (EST-1)			x
12.	Esterase-2 (EST-2)			x

Table 2.2: Buffer systems, tissue and pH of buffer systems

Buffer used	Tissue	*Preparation for electrode (tank) buffer	pH
Lithium borate (Li–Bo) (Weeden I)	Seeds	0.03M lithium hydroxide H_2O (1.2 g/l) 0.19M boric acid (11.9 g/l). pH adjusted with LiOH	8.1
Lithium borate (Li–Do) (Weeden I)	Roots	0.03M lithium hydroxide, H_2O (1.2 g/l) 0.19M boric acid (11.9 g/l) pH adjusted with LiOH	8.1
Citrate–Aminopropyl morpholine (C/M) (Weeden II)	Leaf	0.04M citric acid H_2O (8.2 g/l) pH adjusted to 6.1 with N–(3–aminopropyl) –morpholine	6.1

*One part electrode (tank) buffer: 9 parts tris–citrate buffer used for gel buffer.
**1:10 dilution of electrode (tank) buffer used for gel buffer.

Electrophoresis Run

Gels for electrophoretic determination were run after the method of Weeden (1984) and Weeden and Emmo (n.d.) as modified by Sprecher (1988). To insert enzyme-bearing paper wicks into the gel slab, a horizontal cut (slit) was made using a palette knife about 4 cm from the cathodal end of the gel. The wicks were inserted at about 1 mm intervals between wicks and twenty (20) such wicks were placed in the gel. To monitor the rate of migration, marker dye-bearing wicks were inserted on either side of the gel slab. The gel along with the wicks was then loaded into the tank containing the appropriate buffer. Cellulite sponges touching the tank buffer on one end and spreading near to touching the line of wicks on the other end were used to serve as conductors of electric current. The tank prepared in this manner was placed into a cooling chamber and connected properly to an AC power source.

The first electrophoretic run was done for 20 minutes at 50 amperes and at a voltage of about 200v (< 300 v) during which time enzyme held in the wicks was drawn into the gel via the electric current. The wicks were quickly but carefully removed and the two pairs of gels press together to eliminate space left by the wicks. A plastic straw was used to help the two gels pressed together by placing the straw on the cathodal end of the gel. The tank was then set up as before and left in the cooler for the main electrophoretic run. The main run was continued for four hours at 45 amperes and at a voltage less than 300v. At the end of the four-hour run, the tank was removed from the cooler and the gel prepared for slicing. Five thin slices from the cathodal and anodal portions of the gel were cut by sequentially placing pairs of 1/16 inch plastic strips on either side of the gel slab and drawing (pulling) a monofilament nylon sewing thread through the gel. The gels were then placed into individual trays containing different activity strains to develop specific bands. After optimum development at room temperature in the dark, the gels were fixed in 50 percent ethanol and scored. A total of 12 enzyme systems were assayed on 20 parental and non-parental bean

cultivars including two controls (Montcalm and Sanilac) whose isozyme mobility patterns had been determined in a previous study (Sprecher, 1988). The electrophoretic study was conducted two times to verify original findings.

Isozyme Mobility Score

The alleles for scoring isozyme mobility patterns were designated as fast (F) or slow (S) for convenience. The designation of fast (F) and slow (S) was in relation to the relative position of the fronts of the enzyme migration of the respective isozymes of the 20 cultivars to the mobility of the two control cultivars (Montcalm and Sanilac) whose mobility patterns were known (Sprecher 1988). The mobility scores obtained in this manner were tabulated (Table 2.3) and later converted to an allelic frequency figure to compute the following after Nei (Nei, 1972 and Nei, 1978): 1) Nei's genetic (standard) distance was calculated from the allelic frequencies of 12 (enzyme systems) loci based on the formula of Nei's distance where D = 1n $[J_{xy}/\sqrt{J_x \cdot J_y}]$ and J_x, J_y and J_{xy} are the averages of the Ex_i^2, Ey_i^2, and Ex_iy_i over the r loci (12 loci) examined and where

Ex_i^2 = the sums of squares of the i^{th} allelic frequency in sample or population x

Ey_i^2 = the sums of squares of the i^{th} allelic frequency in sample or population y

Ex_iy_i = the sum of squares of the cross-product of the i^{th} allelic frequencies in population x and population y over the r (12 loci) examined.

Nei's distance (D) measures the accumulated number of gene (allele) differences per locus between two populations (Nei, 1978).

Table 3.1. Isozyme mobility patterns scored as fast (F) and slow (S) of 20 bean cultivars assayed for twelve enzyme systems

Table 2.3: Isozyme mobility patterns scored as fast (F) and slow (S) of 20 bean cultivars assayed for twelve enzyme systems

Cultivar	APS	DIA-1	DIA-2	EST-1	EST-2	MDH	ME	PRX-1	PRX-2	RBCO	SdPr	SKDH
1 CNC-2	S	S	F	S	F	S	F	F	F	F	S	F
2 C-49-242	S	S	F	S	F	S	F	F	F	F	S	F
3 Rico Bajo-1014	S	S	F	S	F	S	F	F	F	F	S	F
4 Cuilapa-72	S	S	F	S	F	S	F	F	F	F	S	F
5 ICA-Pijao	S	S	F	S	F	S	F	F	F	F	S	F
6 B-190	S	S	F	S	F	S	F	F	F	F	S	F
7 LaVega	S	S	S	S	F	S	F	F	F	F	S	F
8 Sanilac	S	S	S	S	F	S	F	F	F	F	S	F
9 Mexico-309	S	S	S	S	F	S	F	F	F	F	S	F
10 UI-111	S	S	S	F	F	S	F	F	F	F	S	F
11 UI-114	S	S	S	S	F	S	F	F	F	F	S	F
12 GN-1140	S	S	S	S	F	S	F	F	F	F	S	F
13 MWHfRnr	S	S	S	S	F	S	F	F	S	F	S	S
14 BAT-1320	S	S	S	S	F	S	F	S	F	F	S	F
15 K.W.-780	S	S	S	S	F	S	F	S	F	F	S	S
16 Nep-2	S	S	F	S	F	S	F	F	S	F	S	F
17 Aurora	S	S	F	S	F	S	F	F	S	F	S	F
18 Montcalm	F	F	S	S	S	F	S	S	F	S	F	S
19 Mexico-235	S	S	S	F	F	S	F	F	F	F	S	F
20 Ecuador-299	S	S	S	S	F	S	F	F	F	F	S	F

2. Nei's identities—this measures the proportion of genes that are common in the two populations being examined. It is computed from the formula: $I = J_{xy}/\sqrt{J_x \cdot J_y}$ where J_x, J_y and J_{xy} are the arithmetic means of the following:

   a) J_x, the probability of identity of two randomly chosen genes in populations or sample x and equal to $\sum x_i^2$ where x_i is the frequency of the i^{th} allele in population or sample X.

   b) J_y, the probability of identity of two randomly chosen genes in population or sample y and equal to $\sum y_i^2$ where y_i is the frequency of the i^{th} allele in population or sample y.

   c) J_{xy}, the probability of identity of a gene for x and a gene for y and equal to $\sum x_i y_i$. The quantity I is unity (= 1.00) when the two populations have the same alleles in identical frequencies, while it is zero (0.00) when they have no alleles in common (Nei, 1972).

3. Draw cluster dendograms based on the value of genetic distance and/or genetic identities computed from allelic frequency data using a computer program, using the unweighted group mean analysis (UWPGMA) developed by Dr. Kermit Ritland of the University of Toronto, Canada, and kindly provided and run by Dr. D. Douches, assistant professor, Michigan State University.

4. Compute similarity index values (SI) from a simple matching coefficient of pairwise comparisons of isozyme mobility patterns of the 12 enzyme systems (assumed to represent 12 loci) for the 20 bean cultivars. The coefficients of similarity were computed from the formula $S=K^1/k$ where cultivars have K^1 similar loci from a total of K loci and K=12 assuming single locus control of the character.

5. Cluster analysis of the 20 bean cultivars based on their isozyme mobility score for 12 enzyme systems. The original 20 cultivar x 12 enzymic data set was converted to a

binary data matrix by assigning numerical values of 1 for F (fast) and 2 for S (slow) to render it suitable for a cluster analysis algorithm appropriate for such data.

RESULTS AND DISCUSSION

Isozyme mobility patterns of 12 enzyme systems for twenty bean cultivars

The results of isozyme mobility score as fast (F) and slow (S) alleles for 12 enzymes of 20 bean cultivars are summarized in Tables 2.3 and 2.4. The isozyme mobility patterns for each cultivar were compared against patterns of the red kidney cultivar Montcalm and the navy bean cultivar Sanilac which were used as checks. For both cultivars isozyme mobility patterns for several enzymes and the storage protein phaseolin have been thoroughly studied and they represented the two major gene pools (Sprecher, 1988), large-seeded and small-seeded gene pools, respectively. The various different cultivars were grouped into seven isozyme mobility pattern groups based on their similar mobility scores for these isozymes (Table 2.5). Five of the tropical small, black commercial class (CNC-2, C-49-242, Cuilapa-72, ICA-Pijao, and B-190) of a total of 8 tropical blacks, along with one small red (Rico-Bajo-1014) had identical scores for all 12 enzyme systems. Two of the small blacks (LaVega and Mexico-309) were grouped with the standard check cultivar, Sanilac, and a small red cultivar, Ecuador-299, in Group 2. Groups 1 and 2 were similar in their allelic score for 11 of the 12 enzymes but differed in their allelic score for the enzyme Diaphorase-2 (DIA-2). Whereas Group 1 had a fast (F) allele score for DIA-2, Group 2 showed a slow (S) allelic score for this enzyme. Group 1 cultivars with predominantly small black cultivars differed by 2, 2, 3, 1 and 10 (Table 2.5) allelic scores (alleles) with cultivars in groups 3, 4, 5, 6 and 7, respectively. The greatest difference of 10 alleles was with the group that contained the one-member cultivar Montcalm (red kidney bean) that represented the large-seeded Andean gene pool.

Table 2.4: Similarities and differences in isozyme mobility patterns for 12 enzyme systems of 20 bean cultivars

Group	Class Designation	Gene Pool Designation	Enzyme System											
			APS	DIA-1	DIA-2	EST-1	EST-2	MDH	ME	PRX-1	PRX-2	RBCD	SdPr	SKDH
1 CNC-2	Small black	Mesoamerican	S	S	F	S	F	S	F	F	F	F	S	F
C-49-242	Small black	Mesoamerican												
Cuilapa-72	Small black	Mesoamerican												
ICA-Pijao	Small black	Mesoamerican												
B-190	Small black	Mesoamerican												
Rico-Bajo-1014	Small red	Mesoamerican												
II														
LaVega	Small black	Mesoamerican	S	S	S	S	F	S	F	F	F	F	S	F
Mexico-309	Med. Sm blk	Mesoamerican												
Sanilac	Pea Bean (Navy)	Mesoamerican												
Ecuador-299	Small red	Mesoamerican												
GN-1140	Great Northern	Mesoamerican												
UI-114	Pinto	Mesoamerican												
III														
UI-111	Pinto	Mesoamerican	S	S	S	F	F	S	F	F	F	F	S	F
Mexico-235	Small red	Mesoamerican												
IV														
BAT-1320	Small black	Mesoamerican	S	S	S	S	F	S	F	F	S	F	S	F
MWHFRnr	Cylindrical Pea Bean	Mesoamerican												
V														
K.W.-780	White Flat	Introgressed Kidney	S	S	S	S	F	S	F	S	F	F	S	S
VI														
Nep-2	Pea Bean	Mesoamerican	S	S	F	S	F	S	F	F	S	F	S	F
Aurora	Small White													
VII														
Montcalm	Lg Red Kidney	Andean	F	F	S	S	S	F	S	S	F	S	F	S

Table 2.5: Isozyme mobility groups, number of allelic score differences (extracted from Tables 2.4 and 2.5) and enzyme differences between cultivars assayed for 12 enzyme systems

Group Pairs	Allele Score Differences	Enzyme Differences
1 vs 2	1	DIA-2
1 vs 3	2	DIA-2, EST-1
1 vs 4	2	DIA-2, PRX-1
1 vs 5	3	DIA-2, PRX-1, SKDH
1 vs 6	1	PRX-2
1 vs 7	10	All except EST-1 & PRX-2
2 vs 3	1	EST-1
2 vs 4	1	PRX-2
2 vs 5	1	PRX-1
2 vs 6	2	DIA-2, PRX-2
2 vs 7	9	All except DIA-2, EST-1 & PRX-2
3 vs 4	2	EST-1, PRX-2
3 vs 5	2	EST-1, PRX-1
3 vs 6	3	DIA-2, EST-1, PRX-2
3 vs 7	10	All except EST-1 & PRX-2
4 vs 5	2	PRX-1, PRX-2
4 vs 6	1	DIA-1
4 vs 7	10	All except DIA-2, EST-1
5 vs 6	4	DIA-2, PRX-1, PRX-2, SKDH
6 vs 7	11	All except EST-1

Single alleles separated Group 2 cultivars (LaVega, Mexico-309, Sanilac, Ecuador-299, UI-114, and GN-1140) from Groups 3, 4 and 5, respectively, while two alleles separated Group 2 from Group 6 whose members included the identically behaving cultivars Nep-2 and Aurora. The maximum separation for Group 2 occurred with Group 7 containing the single member cultivar Montcalm with nine allelic differences.

Similarly, Group 3 was separated by 2, 2, 3 and 10 alleles respectively, from Groups 4, 5, 6 and 7. Group 4 differed from Groups 5, 6 and 7 by 2, 1 and 10 alleles, respectively, whereas Group 5 differed from Groups 6 and 7 by 4 and 7 alleles, respectively. The last two groups with two and one member in each differed at the maximum allelic difference of 11 between them. Member cultivars of each grouping showed the highest difference in allelic numbers with the large-seeded kidney cultivar Montcalm. This may have been due to the predominance of the small-seeded cultivars which resembled in their allelic scores the small-seeded control cultivar, Sanilac, with which they had a one- or two-allele difference.

It is interesting to note that cultivars that were grouped in the same cluster by their disease reaction patterns in an international bean rust nursery (IBRN) (Ghaderi et al., 1984) were also grouped together for isozyme mobility patterns. This is evident from the grouping of the tropical small black and small reds such as cultivars CNC-2 and C-49-242 (Cluster IV), Cuilapa-72 and Rico-Bajo-1014 (Cluster V), Nep-2 and Aurora (Cluster VII), for all 12 enzymes. The clustering by isozyme mobility patterns, however, grouped CNC-2, C-49-242, Cuilapa-72, ICA-Pijao, B-190 and Rico-Bajo-1014 as members of a single large cluster (Figure 2.1). It may therefore be speculative to connect the clustering by disease reaction with similar grouping by isozyme mobility patterns. There appears to be no indication of a direct relation for grouping by isozyme patterns with patterns from reaction for rust isolates. However, there is no denying that clustering by two different sets of variables (isozymes and disease resistance) underscores the existing relationships among these various cultivars.

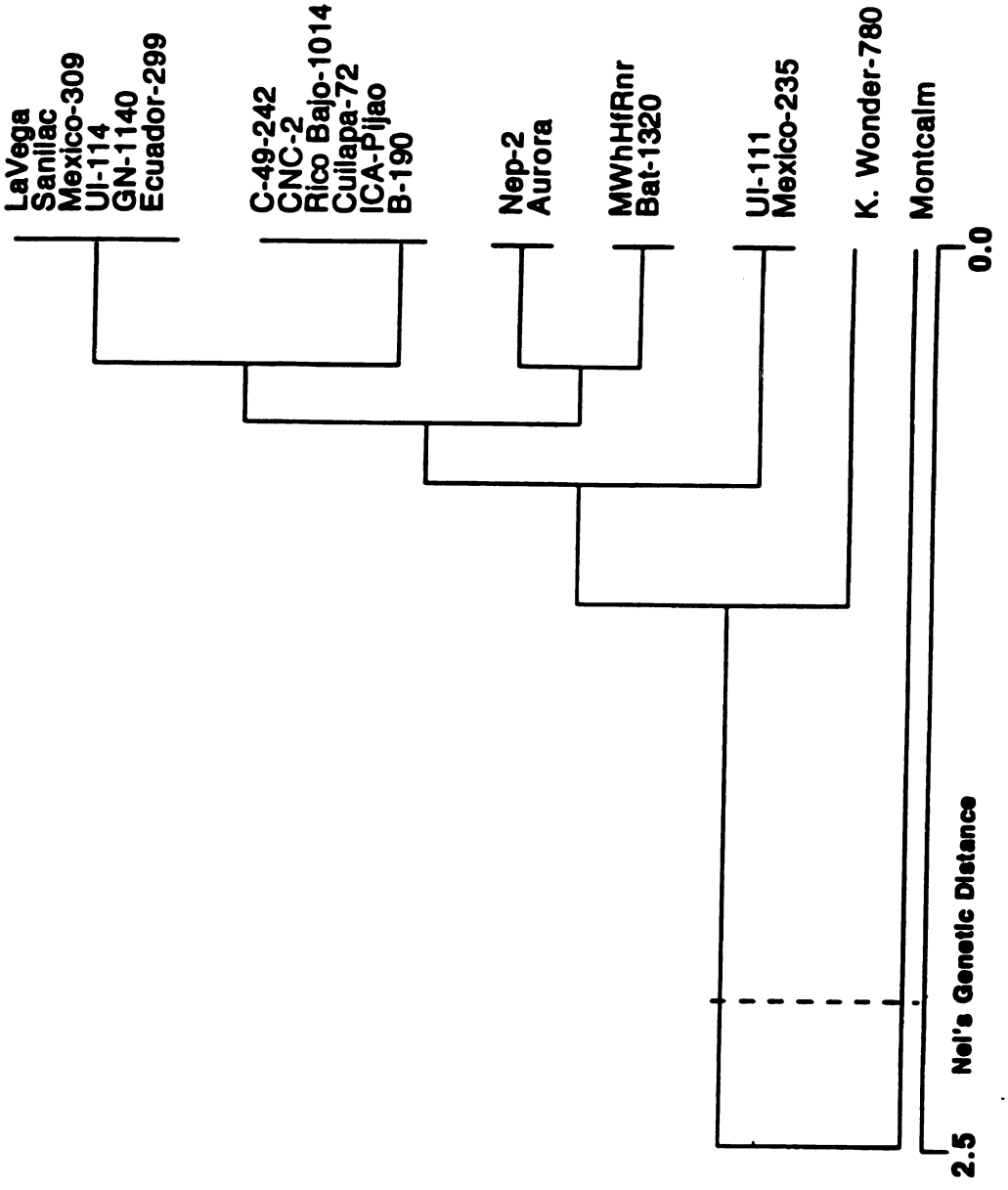


Figure 2.1 Dendogram of genetic distance based on 12 enzyme systems on 20 bean cultivars.

Nei's genetic distance and genetic identities from allele frequencies surveyed for 12 enzyme loci

Nei's genetic distances and genetic identities that were computed from allelic scores of 12 enzyme loci for 20 bean cultivars (Table 2.3) are summarized in Table 2.6. Whereas Nei's genetic distance measures the accumulated number of allelic differences between two populations, the related parameter, Nei's identities, measures the proportion of identical proteins between two related populations. When genetic identities (I) between individuals in a population are high, genetic distance (D) is correspondingly small, and vice versa (Nei, 1972).

Values of genetic identity ranged between 0.000 and 1.000; where I = 1.00 between two populations indicate they have the same alleles in identical frequencies and a value of I = 0.00 indicate no common alleles between the two populations.

All cultivars within each of the seven groupings gave Nei's genetic distance of 0.000 with a corresponding Nei's genetic identity of 1.000 (Table 2.7). This follows from the isozyme mobility pattern for all within-group cultivars which had no allele differences between them. Increasing genetic distance values were observed with corresponding but decreasing values of genetic identities associated with increasing numbers of allelic differences. The maximum genetic distance of 2.485 was between Group 6, which contains the cultivars Nep-2 and Aurora, and Group 7, which consists of only one member, K.W. 780. It also has a corresponding low value of genetic identity at I = 0.083. The maximum allelic difference (11 allelic differences) was also recorded for this pair of groups. In general, the matrix of Nei's coefficients of genetic identities probably depicts the existing natural differences among the cultivar groups on the basis of isozymes mobility patterns. The high genetic identities within groups, particularly for those with several cultivars within groups, reflects underlying similarities among them (Decker, 1985; Adams, 1977).

Table 2.6: Nei's distance (above diagonal) and Nei's identities (below diagonal) based on allele frequency of 12 enzyme loci for 20 parental and non-parental bean cultivars

	CNC-2	C-49 242	RB1014	Cullapa 72	ICA Pijao	B-190	LaVega	Sanilac	M-309	UI-111	UI-114	GN-1140	MWHfr	BAT 1320	KW780	Nep-2	Aurora	Monticalm	M-235	E-299
CNC-2	X	0.000	0.000	0.000	0.000	0.000	0.087	0.087	0.087	0.182	0.087	0.087	0.182	0.182	0.288	0.087	0.087	1.792	0.182	0.087
C-49-242	1.000	X	0.000	0.000	0.000	0.000	0.087	0.087	0.087	0.182	0.087	0.087	0.182	0.182	0.288	0.087	0.087	1.792	0.182	0.087
RB1014	1.000	1.000	X	0.000	0.000	0.000	0.087	0.087	0.087	0.182	0.087	0.087	0.182	0.182	0.288	0.087	0.087	1.792	0.182	0.087
Cullapa 72	1.000	1.000	1.000	X	0.000	0.000	0.087	0.087	0.087	0.182	0.087	0.087	0.182	0.182	0.288	0.087	0.087	1.792	0.182	0.087
ICA Pijao	1.000	1.000	1.000	1.000	X	0.000	0.087	0.087	0.087	0.182	0.087	0.087	0.182	0.182	0.288	0.087	0.087	1.792	0.182	0.087
B-190	1.000	1.000	1.000	1.000	1.000	X	0.087	0.087	0.087	0.182	0.087	0.087	0.182	0.182	0.288	0.087	0.087	1.792	0.182	0.087
LaVega	0.917	0.917	0.917	0.917	0.917	0.917	X	0.000	0.000	0.087	0.000	0.000	0.087	0.087	0.182	0.182	0.182	1.386	0.087	0.087
Sanilac	0.917	0.917	0.917	0.917	0.917	0.917	1.000	X	0.000	0.87	0.000	0.000	0.087	0.087	0.182	0.182	0.182	1.386	0.087	0.000
M-309	0.917	0.917	0.917	0.917	0.917	0.917	1.000	1.000	X	0.087	0.000	0.000	0.087	0.087	0.182	0.182	0.182	1.386	0.087	0.000
UI-111	0.833	0.833	0.833	0.833	0.833	0.833	0.917	0.917	0.917	X	0.087	0.087	0.182	0.182	0.288	0.288	0.288	1.792	0.000	0.087
UI-114	0.917	0.917	0.917	0.917	0.917	0.917	1.000	1.000	1.000	0.917	X	0.000	0.087	0.087	0.182	0.182	0.182	1.386	0.087	0.000
GN-1140	0.917	0.917	0.917	0.917	0.917	0.917	1.000	1.000	1.000	0.917	1.000	X	0.087	0.087	0.182	0.182	0.182	1.386	0.087	0.000
MWHfr	0.833	0.833	0.833	0.833	0.833	0.833	0.917	0.917	0.833	0.917	0.917	0.917	X	0.000	0.288	0.087	0.087	1.792	0.182	0.087
BAT 1320	0.833	0.833	0.833	0.833	0.833	0.833	0.917	0.917	0.917	0.833	0.917	0.917	1.000	X	0.288	0.087	0.087	1.792	0.182	0.087
KW780	0.750	0.750	0.750	0.750	0.750	0.750	0.833	0.833	0.833	0.750	0.833	0.833	0.750	0.750	X	0.405	0.405	0.785	0.288	0.182
Nep-2	0.917	0.917	0.917	0.917	0.917	0.917	0.833	0.833	0.833	0.750	0.833	0.833	0.917	0.917	0.667	X	0.000	2.485	0.288	0.182
Aurora	0.917	0.917	0.917	0.917	0.917	0.917	0.833	0.833	0.833	0.750	0.833	0.833	0.917	0.917	0.667	1.000	X	2.485	0.288	0.182
Monticalm	0.167	0.167	0.167	0.167	0.167	0.167	0.250	0.250	0.250	0.250	0.167	0.250	0.250	0.167	0.417	0.083	0.083	X	1.792	1.386
M-309	0.833	0.833	0.833	0.833	0.833	0.833	0.917	0.917	0.917	0.917	1.000	0.917	0.833	0.833	0.750	0.750	0.750	0.167	X	0.087
E-299	0.917	0.917	0.917	0.917	0.917	0.917	1.000	1.000	1.000	0.917	1.000	1.000	0.917	0.917	0.833	0.833	0.833	0.250	0.917	X

Table 2.7: Summary of ranges of Nei's distance (D) and Nei's identities (I) and allelic differences observed among the various isozyme mobility groups of cultivars

Mobility Group	Nei's (D)	Nei's (I)	Allele Differences	Similarity Index (SI)
All within group cultivars	0.000	1.000	0	1.00
Gp 1 vs Gp 2 Gp 1 vs Gp 6 Gp 2 vs Gp 3 Gp 2 vs Gp 4 Gp 2 vs Gp 5 Gp 2 vs Gp 6	0.087	0.917	1	0.92
Gp 1 vs Gp 3 Gp 1 vs Gp 4 Gp 2 vs Gp 6 Gp 3 vs Gp 4 Gp 3 vs Gp 5 Gp 4 vs Gp 5	0.182	0.833	2	0.83
Gp 1 vs Gp 5 Gp 3 vs Gp 6	0.288	0.750	3	0.75
Gp 5 vs Gp 6	0.405	0.667	4	0.67
Gp 5 vs Gp 7	0.875	0.417	7	0.42
Gp 2 vs Gp 7	1.386	0.250	9	0.25
Gp 1 vs Gp 7 Gp 3 vs Gp 7 Gp 4 vs Gp 7	1.792	0.167	10	0.17
Gp 6 vs Gp 7	2.485	0.083	11	0.08

Similarity Indices for Isozyme Mobility Patterns and Agrophysiological Traits

Similarity indices (SI) computed as single matching coefficient from pairwise matching of isozyme mobility scores for 12 enzymes of 20 bean cultivars, are summarized in Table 2.8 (above the diagonal). SI from isozyme mobility scores were exactly identical to the values of Nei's genetic identities (Table 2.7), ranging from a value of SI = 0.08 for relationship between cultivars Nep–2 and Aurora with Montcalm to SI = 1.00 for several cultivars that indicated the highest degree of relationship. Nei's genetic identities indicate shared alleles for enzyme mobility patterns among these cultivars corroborating the comparison of these same cultivars on the basis of enzyme loci and the corresponding homology of isozyme mobility pattern observed for each cultivar.

Similarity indices based on six agrophysiological traits are summarized in Table 2.8 (below the diagonal). In general, these values, which are mostly based on external characteristics of seed or plant parts of each cultivar, appear to be less discriminative and less able to separate the various cultivars that were easily grouped by isozyme mobility scores. The highest score for similarity index was within the cultivar group that included the small black bean gene pool containing cultivars CNC–2, C–49–242, Cuilapa–72, ICA–Pijao and B–190. The small red cultivar, Rico Bajo–1014, that was included in this group on the basis of isozyme mobility scores, showed a low similarity index value for agrophysiological traits, while a non–member, the small black cultivar LaVega, showed high SI values for its agrophysiological score (Table 2.9) with these cultivars.

The lowest score (S = 0.00) was recorded for cultivars KW–780 and Montcalm with several other cultivars. Among the six traits (Table 2.9) used for comparing cultivars and cluster analysis purposes, seed shape or commercial class trait was the most discriminating among nine classes observed.

Table 2.8: Similarity indices for isozyme mobility patterns (above diagonal) based on 12 enzyme systems and six agrophysiological traits (below diagonal) for 20 parental and non-parental bean cultivars

	CNC-2	C-49 242	RB1014	Cullapa 72	ICA Pijao	B-190	LaVega	Sanilac	M-309	UI-111	UI-114	GN-1140	MWHfr	BAT 1320	KW780	Nep-2	Aurora	Montcalm	M-235	E-299
CNC-2	X	1.00	1.00	1.00	1.00	0.92	0.92	0.92	0.92	0.83	0.92	0.92	0.83	0.83	0.75	0.92	0.92	0.17	0.83	0.92
C-49-242	0.83	X	1.00	1.00	1.00	1.00	0.92	0.92	0.92	0.83	0.92	0.92	0.85	0.83	0.75	0.92	0.92	0.17	0.83	0.92
RB1014	0.83	0.33	X	1.00	1.00	1.00	0.92	0.92	0.92	0.83	0.92	0.92	0.83	0.83	0.75	0.92	0.92	0.17	0.83	0.92
Cullapa 72	0.83	1.00	0.33	X	1.00	1.00	0.92	0.92	0.92	0.83	0.92	0.82	0.83	0.83	0.75	0.92	0.92	0.17	0.83	0.92
ICA Pijao	0.67	0.83	0.50	0.83	X	1.00	0.92	0.92	0.92	0.83	0.92	0.92	0.83	0.83	0.75	0.92	0.92	0.17	0.83	0.92
B-190	1.00	0.83	0.33	0.83	0.67	X	0.92	0.92	0.92	0.92	0.92	0.83	0.83	0.75	0.92	0.92	0 92	0.17	0.83	0.92
LaVega	1.00	1.00	0.33	0.83	0.67	1.00	X	1.00	0.92	1.00	1.00	0.92	0.92	0.83	0.83	0.83	0.25	0.92	0.92	1.00
Sanilac	0.33	0.33	0.50	0.33	0.33	0.33	0.33	X	1.00	0.92	1.00	1.00	0.92	0.92	0.83	0.83	0.83	0.25	0.92	1.00
M-309	0.50	0.50	0.17	0.50	0.33	0.50	0.50	0.17	X	0.92	1.00	1.00	0.92	0.92	0.83	0.83	0.83	0.25	0.92	1.00
UI-111	0.17	0.33	0.33	0.33	0.00	0.17	0.17	0.17	0.33	X	0.92	0.92	0.83	0.83	0.75	0.75	0.75	0.17	1.00	0.92
UI-114	0.17	0.33	0.33	0.33	0.00	0.17	0.17	0.17	0.33	1.00	X	1.00	0.92	0.92	0.83	0.83	0.83	0.25	0.92	1.00
GN-1140	0.33	0.17	0.17	0.17	0.17	0.33	0.33	0.50	0.17	0.17	0.17	X	0.92	0.92	0.83	0.83	0.83	0.25	0.92	1.00
MWHfr	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.67	0.50	0.33	0.33	0.50	X	1.00	0.75	0.92	0.92	0.17	0.83	0.92
BAT 1320	0.67	0.67	0.67	0.67	0.83	0.67	0.67	0.50	0.33	0.33	0.33	0.17	0.17	X	0.75	0.92	0.17	0.83	0.83	0.92
KW780	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.17	0.00	0.00	0.50	0.50	0.00	X	0.67	0.67	0.42	0.75	0.83
Nep-2	0.33	0.50	0.33	0.50	0.33	0.33	0.33	0.83	0.13	0.33	0.33	0.50	0.67	0.33	0.33	X	1.00	0.08	0.75	0.83
Aurora	0.50	0.33	0.33	0.33	0.33	0.50	0.50	0.67	0.17	0.17	0.17	0.67	0.50	0.33	0.33	0.67	X	0.08	0.75	0.83
Montcalm	0.17	0.17	0.33	0.17	0.00	0.17	0.17	0.17	0.17	0.00	0.00	0.00	0.00	0.17	0.17	0.00	0.00	X	0.17	0.25
M-235	0.50	0.33	0.50	0.33	0.50	0.33	0.33	0.33	0.17	0.33	0.33	0.33	0.17	0.50	0.00	0.33	0.50	0.00	X	0.92
E-299	0.33	0.17	0.50	0.33	0.50	0.50	0.33	0.33	0.33	0.33	0.33	0.17	0.33	0.50	0.00	0.33	0.33	0.00	0.83	X

Table 2.9: Agrophysiological characteristics of 22 bean cultivars

Code	Cultivar	Flower Color	Seed Color	Seed Size	Commercial Class Designate	Determi-nancy	Phaseolin Protein Type
1	LaVega	P	BL	SM	SM,BL	IND.3	S
2	Mexico-235	PK	R	SM	SM,R	IND.3	S
3	CNC-3	P	BL	SM	SM,BL	IND.3	S
4	CNC-2	P	BL	SM	SM,BL	IND.3	S
5	C-49-242	P	BL	SM	SM,BL	IND.2	S
6	Cuilapa-72	P	BL	SM	SM,BL	IND.2	S,B
7	Mexico-309	P	BL	M	M,BL	IND.4	S
8	RB-1014	PK	PK	SM	SMPK	DET.1	S
9	Ecuador-299	PK	R	SM	SM,R	IND.4	S
10	NEP-2	W	W	SM	PB	IND.2	S,SB
11	Aurora	W	W	SM	SM,W	IND.3	S,SB
12	ICA-Pajio	P	BL	SM	SM,BL	IND.2	S,B
13	KW-780	W	S	M,L	W,FK	IND.4	T,C
14	UI-111	PK	PO	M	M,PO	IND.2	S,SD
15	GN-1140	W	W	M,L	M,GN	IND.3	S,SD
16	MWHRnr	W	W	M	CY,PB	IND.4	S,C
17	CNC	P	BL	SM	SM,BL	IND.4	S
18	B-190	P	BL	SM	SM,BL	IND.3	S
19	BAT-1320	PK	BL	SM	SM,BL	DET.1	S
20	BAC-87	PK	BL	SM	SM,BL	DET.1	S
21	Sanilac	W	W	SM	PB	DET.1	S
22	Montcalm	P	PK	L	LRK	DET.1	T

P=Purple; PK=Pink; W=White; BL=Black; R=Red; PO=Pinto; SM=Small; M=Medium; M,L=Medium, Large; L=Large

Cluster dendogram based on Nei's genetic identities and isozyme mobility scores

Cluster analysis of 20 bean cultivars using the UWPGMA analysis based on their allelic frequency score for isozyme mobility patterns (Table 2.3) resulted in two major clusters (Figure 2.1) in which the first cluster included 19 members and the second cluster consisted of a single member, Montcalm. However, the same cluster dendogram revealed seven branches with varying numbers of cultivars within each that coincided with the earlier grouping using values of Nei's identities and distances (Table 2.6). The clustering procedure using Ward's method in SAS (Figure 2.1) identified two major clusters that coincided with earlier clustering of Phaseolus spp. accessions into the large-seeded beans of Andean South America with a T or C phaseolin and the small-seeded beans of Mesoamerica with the S phaseolin protein (Gepts et al., 1986; Sprecher, 1988). Given the criteria used for scoring isozyme mobility patterns as fast (F) and slow (S) (Table 2.3), this cluster outcome is not totally unexpected. Adoption of Romesberg's (1984) criteria of cutting the cluster dendogram and relaxing the requirement to a point where the width of the range of the resemblance coefficient is reasonably the largest and therefore least sensitive to error, seven cluster groups were again obtained (Figure 2.2). This grouping coincides with subsequent grouping into seven isozyme mobility pattern groups using isozyme mobility scores.

Six cultivars dominated by four small black beans (Tropical Blacks) formed the first group (Group 1), which had identical scores for all 12 enzymes. None of these cultivars are known to share a common pedigree. Bassiri and Adams (1978b) and Singh et al. (1991) described such homology of banding patterns in the tropical bean classes in their studies of isozymes in the common bean. This group also contains cultivars that were clustered together in another study of reaction to bean rust in the field in international bean rust nurseries in 1976 (Ghaderi et al., 1984); cultivar CNC-2 and C-49-242 in cluster IV and cultivars Cuilapa-72 and Rico-Baja-1014 in cluster V. Group 2 contained six cultivars of diverse

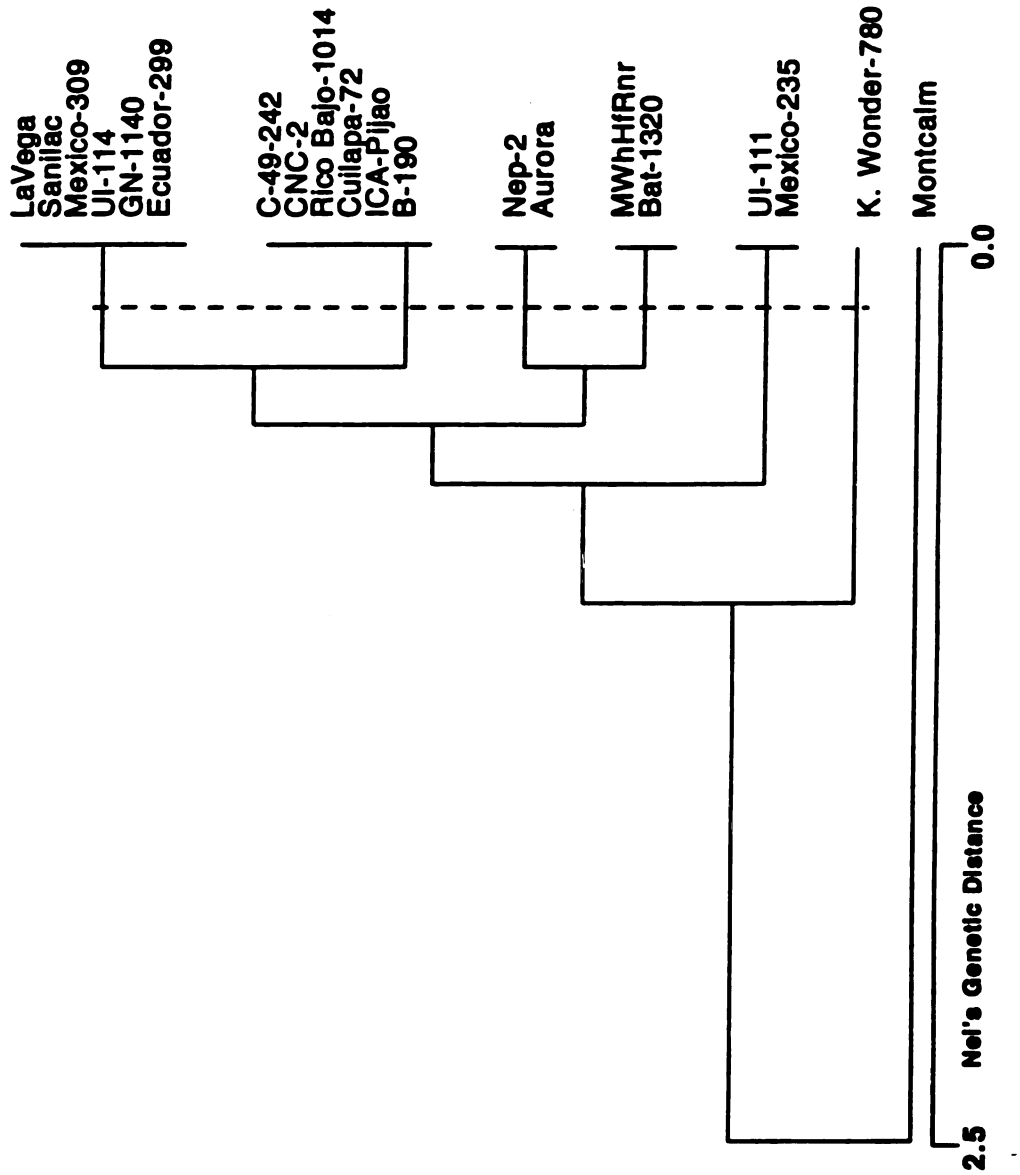


Figure 2.2 New clusters based on genetic distance of 20 bean cultivars on 12 enzyme systems.

background including two tropical blacks (LaVega and Mexico-309), one navy bean (Sanilac), one small red (Ecuador-299), one Great Northern (GN-1140), and one pinto (UI-114). This group was separated from groups 1, 3, 4 and 5 by one allele difference in each group, respectively (Table 2.5). There is no known pedigree relationship among these cultivars from which to predict their similar banding patterns. However, it is also difficult and no reason not to expect such homology in banding patterns on the grounds that these cultivars have no known pedigree relationship.

Singh et al. (1991) identified within the Mesoamerican and Andean cultivated germplasm, clusters of landraces that share a common allozyme that could be traced to a common ancestry. However, cultivars within the same allozyme genotypes were found that have undergone further evolutionary diversification for morphological traits but not for molecular markers. Such diversity were particularly noted for seed type traits such as size, color, shape and color patterns.

Bassiri and Adams (1978b) reported the usefulness of these techniques to provide estimates of genetic relationship but also noted the limitations these isozyme mobility patterns may have as indices of total genetic relationships. A good example in this connection is the relationship between cultivars in Group 2 and cultivars in Group 3. Whereas Group 2 contains the cultivar UI-114, which shares a common pedigree (r=0.56) with UI-111 in Group 3, they are nevertheless separated by one allele difference from total homology of banding patterns that grouped them into two separate mobility groups.

It should also be noted here that these cultivar groupings were based arbitrarily on one or few allelic score differences of isozyme mobility patterns. This classification does not therefore take into consideration existing pedigree relations that are established (Table 3.3 in Chapter 3), for example, between cultivars B-190 in Group 1 and Mexico-309 in Group 2, C-49-242 in Group 1 and Aurora in Group 6, C-49-242 in Group 1 and BAT-1320 in Group 4,

GN-1140 in Group 2 and KW-780 in Group 5, Cuilapa-72 in Group 1 and BAT-1320 in Group 4, and BAT-1320 in Group 4 with Aurora in Group 6. It is interesting to note here that none of the cultivars within each group has any known pedigree relationships but that pedigree relationship has been established among cultivars belonging to Groups 1 and 2 (B-190 and Mexico-309), Groups 1 and 6 (C-49-242 and Aurora), Groups 1 and 4 (C-49-242 and BAT-1320), Groups 2 and 5 (GN-1140 and KW-780), Groups 1 and 4 (Cuilapa-72 and BAT-1320), and Groups 4 and 6 (BAT-1320 and Aurora). This observation appears contrary to accepted expectations of homology of isozyme banding or mobility patterns among cultivars in relation to shared parentage history.

The different cultivar groupings for isozyme mobility patterns were evident whether data were generated from Nei's genetic identities (Table 2.6) based on cultivar allelic frequencies for 12 enzyme loci or when similarity index values (Table 2.8) from isozyme mobility patterns were generated from computations of simple matching coefficients for pairs of cultivars.

Similarity indices computed from six agrophysiological traits were in general higher only for cultivars of the tropical black bean class regardless of whether they were members or non-members of a cultivar group of similar banding patterns. However, most cultivars showed intermediate (S=0.50) to low similarity indices (S=0.17 or S=0.00) for these traits. The generally low similarity indices for agrophysiological traits may have been due to the large number of commercial classes with divergent agronomic traits in addition to the use of only six such traits for computing these indices, which may not be adequate to represent existing variability of these groups of traits.

A comparison in the study by Bassiri and Adams (1978b) of isozyme polymorphic bands using similarity index values from band sharing among the same cultivars was highly correlated to distances as calculated by Adams (1977) using PCA. Smith (1984) and Smith et

al. (1984, 1985), also used PCA on a covariance matrix of allele frequencies of several loci in corn and relatives of corn to assess diversity and to examine the usefulness of isozymes to characterize inbreds and hybrids.

Adams (1977) noted that cultivars that resemble each other very closely for certain obvious plant and seed traits were found to be quite diverse in traits for which no direct selection had been performed. It is also true that phenotypic similarities between two cultivars, based on superficial uniformity resulting from selection of seed traits, may not accurately reflect their overall genetic similarity or dissimilarity (Adams 1977, Murphy et al., 1986).

Bassiri and Adams (1978) observed that while the precision and specificity of isozyme comparison between two cultivars can be very high, the total number of genes involved is such a minor portion of the complete genome that the overall genetic relationship is only approximately predicted. The same authors advised that caution be exercised when isozyme banding is the only basis for assessing cultivar relationships. Cox et al. (1985a, 1985b) suggested using a composite index for estimating genetic relationships that included both coefficients of parentage (r) and indices of similarity (S) computed from other traits such as morphological and biochemical characteristics. These authors noted that both r and s are inadequate estimates of the relationships between two cultivars when used alone, their accuracy being affected by selection, genetic drift, sampling of loci, and unknown relationships among the supposedly unrelated ancestors.

The different bean cultivar groupings following clustering of isozyme mobility patterns (Figure 2.1) provided an added dimension with which to examine and compare the original cluster groups (Table 1.1, General Materials & Methods Ch. 1) based on field reaction to rust in 16 different locations (Ghaderi et al., 1984).

Ward's minimum variance method was used in SAS for clustering the data on field reactions to rust while the unweighted Pair Group Method using arithmetic average (UWPGMA), Ward's method, single linkage (SLINK), complete linkage (CLINK), average linkage and Centroid linkage, were used to cluster cultivars based on enzyme allele frequency of 12 enzyme systems.

The clustering methods by enzyme allele frequency data resulted in the separation of the two major seed classes, small- to medium-seeded bean cultivars in sub-clusters IA-IF and the one cultivar member class Montcalm (Figure 2.2) in the second group, which is a large-seeded bean confirming earlier clustering results (Sprecher, 1988; Gepts et al., 1986). The clustering outcome with enzyme allele frequency data, however, differed significantly from clustering by disease reaction to rusts. Whereas the two major seed classes (small-seeded versus large-seeded) comprised the clusters by enzyme clustering, eight clusters resulted with clustering by rust reaction. Meaningful comparison between the two methods becomes apparent only when sub-clusters for enzyme allele frequency was examined. The consistently behaving cultivars Nep-2 and Aurora remained together as a group in sub-cluster IA without Ecuador-299. Cultivars Cuilapa-72 and Rico-Bajo-1014 (Cluster V) and CNC-2 and C-49-242 (Cluster IV) were grouped together but lumped together with sub-cluster IA cultivars of the isozyme data. Cultivars LaVega, CNC-3 and Mexico-235 (Cluster III) and ICA-Pijao and KW-780 (Cluster VIII) were dispersed by the clustering steps while KW-780 remained by itself in sub-cluster IE of the isozyme data.

SUMMARY AND CONCLUSION

Twelve enzyme systems were surveyed in 20 bean cultivars using seed, leaf and root tissues. Seven isozyme mobility groups of cultivars were observed that were separated as distinct mobility groups on one, two, three, four, seven, nine and eleven allelic differences on the basis of degree of homology of isozyme mobility scores.

The values of Nei's genetic identities computed from allelic frequency for enzyme loci that indicated proportion of identical enzymes between two cultivars was identical to the similarity index values computed as a simple matching coefficient of pairwise mobility patterns. These coefficients indicated high genetic similarities among cultivars within groups.

The cluster dendogram from cluster analysis using the UWPGM method and six other fusion techniques resulted in two major clusters separating the small- to medium-seeded cultivars from the large-seeded cultivar Montcalm primarily. However, all methods produced seven subgroups on the basis of seven isozyme mobility pattern groups whether such scores were based on Nei's genetic identities from allelic frequency of enzyme loci or from isozyme mobility scores.

LIST OF REFERENCES

Adams, M.W. 1977. An estimation of homogeneity in crop plants with special reference to genetic vulnerability in the dry bean, *Phaseolus vulgaris* L. Euphytica 20: 665–679.

Bassiri, A. and Adams, M.W. 1978a. An electrophoretic survey of seedling isozymes in several *Phaseolus* species. Euphytica 27: 447.

Bassiri, A. and Adams, M.W. 1978b. Evaluation of common bean cultivar relationships by means of isozyme electrophoretic patterns. Euphytica 27: 707–720.

Cox, T.S., Y.T. Kiang, M.B. Gorman and D.M. Rogers. 1985. Relationships between coefficients of parentage and genetic similarity indices in soybeans. Crop. Sci. 25: 529–532.

Cox, T.S., G.L. Lookhart, D.E. Walker, L.G. Harrell, L.D. Albers and D.M. Rogers. 1985. Genetic relationships among hard red winter wheat cultivars as evaluated by pedigree analysis and gliadin polyacrylamide gel electrophoretic patterns. Crop Sci. 25(6): 1058–1062.

Decker, D.S. 1985. Numerical analysis of allozyme variation in *Cucurbita pepo*. Econ. Bot. 39(3): 300–309.

Delannay, X., D.M. Rodgers and R.G. Palmer. 1983. Relative genetic contributions among ancestral lines to North American soybean cultivars. Crop. Sci. 23: 944–949.

Gepts, P., T.C. Osborn, K. Rashka and F.A. Bliss. 1986. Phaseolin–protein variability in wild forms and landraces of the common bean (*Phaseolus vulgaris* L.): Evidence for multiple centers of domestication. Econ. Bot 40(4): 451–468.

Ghaderi, A., M.W. Adams and A.W. Saettler. 1984. A quantitative analysis of host–pathogen–environment interaction in International Bean Rust Nurseries (IBRN). Rept. Bean Imp. Coop. vol. 27.

Murphy, J.P., T.S. Cox, D.M. Rodgers. 1986. Cluster analysis of red winter cultivars based upon coefficients of parentage. Crop. Sci. 26(4): 672–676.

Nei, M. 1972. Genetic distance between populations. Amer. Natur. 106(949): 283–292.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583–590.

Romesberg, H.C. 1984. Cluster analysis for researchers. Lifetime Learning Publications, Belmont, CA.

Singh, S.P., R. Nodari and P. Gepts. 1991. Genetic diversity in cultivated common bean: 1 allozymes. Crop Sci. 31: 19-23.

Smith, J.S.C. 1984. Genetic variation within US commercial hybrid maize: Multivariate analysis of allozyme data. Crop Sci. 24: 1041-1046.

Smith, J.S.C., M.M. Goodman and C.W. Stuber. 1985. Genetic variability within US maize germplasm. I. Historically important lines. Crop Sci. 25(3): 550-555.

Smith, J.S.C., M.M. Goodman and C.W. Stuber. 1984. Variation within Teosinte III. Numerical analysis of allozyme data. Econ. Bot. 38(1): 97-113.

Sprecher, S.L. 1988. Allozyme differentiation between gene pools in common bean (*Phaseolus vulgaris* L.), with special reference to Malawian germplasm. Ph.D. dissertation. Michigan State University, E. Lansing.

Weeden, N.F. 1984. Distinguishing among white seeded bean cultivars by means of allozyme genotypes. Euphytica 33: 199-208.

Weeden, N.F. and Emmo, A.C. n.d. Horizontal starch gel electrophoresis laboratory procedures. NYAES. Geneva, New York.

CHAPTER III

EVALUATION OF COMMON BEAN CULTIVAR RELATIONSHIPS BY PEDIGREE ANALYSIS AND GENETIC INDICES OF SIMILARITY

INTRODUCTION

Analysis of patterns of overall genetic variability in crop plants is essential to assess genetic diversity and in planning crosses for pureline or hybrid cultivar development. Estimates of genetic diversity and/or similarity among cultivars, populations or species of plants are usually based on morphological or biochemical genetic markers, quantitative traits or pedigree analysis. Where pedigree information is available the coefficient of parentage (r) provides an estimate of the genetic relationship between two genotypes. The coefficient of parentage or kinship between two genotypes is the probability that a gene chosen at random from one individual is identical by descent with a homologous gene chosen at random from the same or from another individual. Detailed pedigrees of all genotypes are required for computations of coefficients of parentage including the assumptions that the original ancestors of the relevant cultivars are unrelated, or their relationship is unknown.

Coefficients of parentage among 171 pairwise combinations of 19 common bean cultivars representing a subset of the eight clusters (clustered from analysis of the 88 original cultivars in the 1976 IBRN) have been computed. The objective of this study was to compare the overall pattern of relationship of the bean cultivars within and between the groups resulting from clusterings with kinship coefficients calculated from pedigree data.

LITERATURE REVIEW

The coefficient of parentage (r) is a useful measure of the degree of relationship between two genotypes, where r = 0 if the genotypes have no common parentage, and r = 1 if they are identical.

Pedigrees of 158 USA and Canadian soybean cultivars were examined by Delannay et al. (1983) to determine the relative genetic contributions of ancestral lines for both the Northern and Southern USA and Canadian soybean cultivars released in successive time periods and to study trends in germplasm usage to the present day. Relative genetic contribution of the various ancestral lines was determined by analyzing pedigree data. They assumed no relationship among the original introductions and 50 percent of the genes descended from each ancestral parent. Further, they grouped the cultivars by maturity groups and period of release, and for each introduction or ancestral line, the mean of the relative genetic contributions to all cultivars belonging to a group or gene pool formed the mean relative genetic contribution of that introduction or ancestral line for that gene pool. Finally, a cumulative relative genetic contribution was determined. From the above, the authors could trace the North American soybean gene pool to only fifty introductions, and a relative few contributed an increasingly greater proportion of the genetic base. Ten introductions contributed, collectively, more than 80 percent of the northern gene pool, while only seven contributed the same share to the southern gene pool. It was noted that many of the introductions had originated from the same geographic area, confirming previous estimates of the narrowness of the genetic base of present-day soybean cultivars.

Murphy et al. (1986) used cluster analysis and principal coordinates analysis based on coefficients of parentage between pairwise combinations of 110 recently released and historically important soft and hard red winter wheat cultivars in order to observe the overall pattern of relationships between and within the two classes and to obtain genetic clusters within the gene pool of US red winter wheat. Although the two classes contained overlapping germplasm, six clusters among 38 soft red wheats and seven clusters among the 49 hard red winter wheats were formed based on predominant parents within each class. Principal coordinate analysis separated the 13 clusters primarily by class as well as by geographic origin of predominant parents within classes. Cluster analysis was performed on the matrix of coefficient of parentage (r) values using the sequential, agglomerative hierarchical and non-overlapping UPGMA method.

Souza and Sorrels (1989) estimated coefficients of parentage using pedigree data of 205 North American oat cultivars to help them measure 1) relative changes in genetic diversity through time based on diversity of cultivars released for the periods 1951–1960 and 1976–1989; 2) to measure contribution to the germplasm pool of 89 landraces and ancestral introductions; and 3) to identify major grouping of related cultivars by cluster and principal component analysis. Cultivars were clustered based on the coefficient of parentage matrix using the unpaired group mean method of Sneath and Sokal (1973) utilizing the SAS PROC CLUS program. Principal Component Analysis (PCA) of the cultivar–ancestral parent coefficient of parentage matrix was also employed for dimensional reduction of data using SAS PROC PRIN COMP. The authors calculated that the average coefficient of parentage (r_p) among all cultivars released from 1951 to 1960 was 0.09, and for cultivars released from 1975 to 1985 r_p was 0.08. The ten most important ancestral parents in each time period, based on average r_p, declined in their relative contribution to germplasm pool from 79 percent of the parentage of cultivars from 1951 - 1960 to 54 percent for cultivars released from 1976 -

1985. Cluster analysis resulted in seven cultivar groups, six of which corresponded to either the regional germplasm pools or to cultivars with high degrees of relationship to a specific ancestral parent cultivar such as Victoria.

Cox et al. (1985a) compared similarity coefficients (s) based on polyacrylamide gel electrophoresis patterns with coefficients of parentage (r) computed from pedigree analysis for all pairwise combinations of 43 US hard red winter wheat cultivars within the gene pool of US hard red winter wheat. Each index varied from zero for two unrelated cultivars to one for two identical cultivars.

Adams (1977) used PCA to calculate distance metrics using a large number of metrical traits to establish its validity as a measure of genetic homogeneity in the common bean. He argued that by using this distance metric from PCA one can show that related cultivars are separated by smaller distances than are unrelated cultivars. He demonstrated the validity of his arguments by comparison of inter-cultivar distances from PCA with relationship coefficients (r) calculated for particular pairs of cultivars, or sets of cultivars whose pedigrees were known.

Martin et al. (1991) investigated diversity among North American Spring barley cultivars based on coefficients of parentage. A total of 167 spring barley cultivars categorized as two- and six-rowed and by period of release were used for cluster and principal coordinate analysis on the r matrix computed among the cultivars. Principal coordinate analysis of the between cluster r-matrix separated the two-rowed from the six-rowed gene pools while the cluster analysis of the r matrix produced 30 clusters with a limited number of ancestor cultivars contributing largely to the germplasm of the early and recently released barley cultivars. The authors noted that malting barley cultivars were based on a limited sample of germplasm.

The genetic diversity of a large pool of North American dry bean cultivars representing the major market classes was studied by McClean et al. (1993) using the

coefficient of parentage derived from pedigrees of paired cultivars. The r values among 143 dry bean cultivars was used for cluster and principal coordinate analysis. The authors obtained 16 clusters, which were further reorganized into three major clusters corresponding to the small, medium and large kidney seed size groups. Low genetic diversity or variability was indicated from high within cluster estimate of r. The limitation that strict requirement to maintain seed size, color, agronomic and canning characteristics on bean cultivars has been noted by the authors to contribute to the generally low genetic diversity within the various North American dry bean market classes.

MATERIALS AND METHODS

Coefficient of Parentage (r)

Values of coefficient of parentage (r) were determined using the methods of Emik and Terrill (1949) based on pedigree analysis of the individual cultivars and the genetic contributions corresponding to the theoretical proportion of genes coming from an ancestor, if it is assumed that every time a cross is made, 50 percent of the genes come from each parent. The following were also assumed in determining the values:

i) Ancestors are unrelated (r = 0)

ii) All cultivars, ancestors and parental lines are homozygous and homogeneous

iii) A cultivar derived from a bi-parental cross obtains one-half (0.5) of its genes from each parent

iv) The value of r between a cultivar or ancestor and a direct selection from that cultivar or ancestor is assigned an arbitrary value of r = 0.75

v) The value of r between two selections from the same cultivar or ancestor is $(0.75)^2 = 0.56$. The r values so obtained were used to compare genetic similarities from clusters, and other similarity indices. The values of the coefficient of parentage between two genotypes were computed after Emik and Terrill (1949).

$r_{x,y}$ = 0.5 ($r_{xx} + r_{xy}$), i.e., the co-ancestry of individual Y with X is equal to the mean co-ancestry of Y's parents with X.

Parental and non-parental bean cultivars were included (Table 3.1) to determine coefficients of parentage (r) of pairwise comparisons from pedigrees of 46 bean cultivars

Table 3.1: Number, path designation, parental designation, level and names of ancestors in the pedigree of parental and non-parental bean cultivars

#	P1	P2	Level	Name
1	0	0	1	Ecuador-299
2	0	0	1	XA
3	1	2	2	Mexec-1
4	0	0	1	Ecuador-299
5	3	1	3	Mexec-2
6	0	0	1	Ecuador-299
7	5	1	4	Mexec-3
8	0	0	1	Ecuador-299
9	7	1	5	Mexec-4
10	0	0	1	Ecuador-299
11	9	1	6	Mexec-5
12	0	0	1	Ecuador-299
13	11	1	7	Mexico-235
14	0	0	1	CNC
15	0	0	1	Z
16	14	15	1	X1
17	0	0	1	CNC
18	14	16	2	X2
19	0	0	1	CNC
20	14	18	3	CNC-2/CNC-3
21	0	0	1	Black Turtle Soup
22	0	0	1	Cornell 49-242
23	21	22	2	Aurora
24	0	0	1	Porillo Sinth
25	0	0	1	Mexico-11
26	24	25	2	ICA-Pijao
27	0	0	1	GN UI #1
28	0	0	1	Comm. Red. Mex.
29	0	0	1	GNCT 32
30	0	0	1	Common Pinto
31	27	28	2	RM UI-34
32	29	41	2	GN J-378
33	30	31	3	UJ-111
34	32	33	4	UI-114
35	0	0	1	Mexico-309
36	0	0	1	50600
37	35	36	2	B-190
38	0	0	1	Mant. Fosco-11
39	0	0	1	Rico-23
40	38	39	2	Rico B. 1014
41	0	0	1	UI-123
42	0	0	1	K.W. 780
43	0	0	1	Idaho Pinto
44	42	43	2	US #5 Pinto
45	41	44	3	GN 1140

(Table 3.2). The putative parental or ancestral cultivars were assigned permanent numbers andthis identified that particular ancestor. These designated numbers are entered in the parental columns as appropriate depending on the pedigree of the respective cultivars. A number is also assigned to designate the level of relationships. A level of 1 usually identifies a parent or an ancestral parent or landrace or an introduction having no known relationship to any other introduction or landrace; and a level of 2 identifies a derivative or progeny of two level 1's (Table 3.1) or one level 1 and another level 2 and so on. The names are self explanatory and identify a known parent identified by a designated number or is given an assumed name (designation) in the event that parents are unknown.

A computer program written by Dr. Carl Ramm and Dr. Clay Sneller was kindly provided and used to compute the coefficients of parentage.

Table 3.2: Pedigree of parental and non-parental bean cultivars

Cultivar	Pedigree
Ecuador-299	Source unknown; may be the same as Mexico-235 (George Freytag)
Mexico-235	Probably from Hidalgo, Mexico; CIAT ID = G -5732 w/ Hidalgo 41-A-3 entry code
CNC	A composite of Guatemalan black beans by E. Schreiber and M. Gutierrez
CNC-2	Selection from CNC
CNC-3	Selection from CNC
Black Turtle Soup	Very old landrace variety, originally from Venezuela
Nep-2	White-seeded type II bean derived from San Fernando via mutagenesis by Dr. Moh
Cornell 49-242	Perhaps equivalent to PI 326418 from Venezuela (Hubbeling, 1957) and introduced to Cornell by Marcano, source of ARE gene
Aurora	Black Turtle Soup/Cornell 49-242; a white-seeded mutant in the F6 generation
ICA-Pijao	Porillo Sintetico/Mexico-11, a bred line from the National Program in Colombia
UI-111	Common Pinto/UI-34
UI-114	GNJ-378/UI-111
Mexico-309	Probably from Mexico; pedigree unknown
B-190	Mexico-309/50600
Rico Bajo-1014	Manteigo Fosco-11/Rico-23
Kentucky Wonder-780	Pedigree unknown
Great Northern 1140	UI-123/US #5 Pinto
La Vega	A tropical, multiple disease-resistant black bean; pedigree unknown
Cuilapa-72	Released in Guatemala from a line in Costa Rica known as 51051
BAT-1320	BAT-883 (Cuilapa-72X (San Fernando/Cacahuate-72)/BAT-447 (Diacol Nima/Cornell 49-242)
BAC-87	BAT-450 (Cornell 49-242/PI 310797)/(Negro-324/Jules)
GN UI #1	A selection from the landrace common Great Northern
Common Red Mexican	Landrace of Red Mexican
GNCT-32	Curly top resistant GN of unknown parentage
Common Pinto	Landrace of Pinto
Red Mexican UI-34	GN UI #1/Common Red Mexican
GNJ-378	GN UI-123/GN CT-32
50600	A tropical black bean selected by A. Pinchinat, IICA, Turrialba, Costa Rica in 1960
UI-123	A selection from the landrace Common Great Northern
Idaho Pinto	Pedigree unknown; probably a landrace
US #5 Pinto	Kentucky Wonder-780/Idaho Pinto
Rico-23	A black bean selection by C. Vieira, Brazil from the "Rico" line in 1970
Manteigo Fosco-11	Pedigree unknown
San Fernando	A selection from local black bean cultivars in Costa Rica
Diacol Nima	Pedigree unknown
Cacahuate-72	Pedigree unknown
BAT-47	Diacol Nima/Cornell 49-242
BAT-883	This cultivar has Cuilapa-72, S. Fernando and Cacahuate-72 in its pedigree
51051	A line from Costa Rica, possible progenator of Cuilapa-72
Porillo Sintetico	Pedigree unknown
Mexico-11	Pedigree unknown
Common Great Northern	Landrace of Great Northern
BAT-450	It has Cuilapa-72 (PI 310797) and Cornell 49-242 among others in its pedigree
Jules	GN Nebraska #1 sel. 27/GN-1140
GN Nebraska #1 Sel. 27	Selection from GN Nebraska #1
GN Nebraska #1	P. vulgaris cv. Montanas/P. acutifolius var. latifolius cv. tepary

RESULTS AND DISCUSSION

The matrix of values of coefficients of parentage (r) computed from pairwise comparison of pedigree among 19 bean cultivars is summarized in Table 3.3. The majority of these cultivars are unrelated as most of them are either landraces themselves or derived from selections in/of such landraces. This is reflected in the non-integer (zero) value of coefficients of parentage for most cultivar pairs.

Examples of landrace cultivars and their derivatives include Compuesto Negro Chimaltenango (CNC) and its selections CNC-2 and CNC-3. On the assumption that CNC-2 and CNC-3 are direct selections from CNC, r = 0.75 with their common ancestor and r = 0.56 among themselves (Cox et al., 1985a). These cultivars have shown consistently high similarities with respect to their reaction to several races of the bean rust fungus (Table 1.8, Chapter I) and high indices of similarity for morphological traits (Table 2.8, Chapter II). Cultivars Nep-2 and Aurora, which share no known common parentage (r = 0) behave identically for reaction to several rust races and showed complete homology for isozyme banding patterns of 12 enzymes (Table 2.8). Both originated from separate black-seeded landrace parents (Table 3.2). Nep-2 was an EMS-induced white-seeded, mutant from San Fernando (S-182N) and Aurora was a natural white-seeded mutant in the F_5 generation of crosses between Black Turtle Soup and Cornell 49-242 (McClean et al., 1993). From a total of 19 pairwise comparisons on the basis of pedigree, r values were established for two lines from CIAT (BAT 1320 and BAC-87), which are apparently related to many of these cultivars and three other cultivar pairs (C-49-242/Aurora, UI-111/UI-114 of B-190/Mexico-309).

Table 3.3: Coefficients of parentage (r) for all pairwise comparisons of 19 bean cultivars

	C-49 242	CNC	Cuilapa 72	E-299	KW780	M-309	Aurora	B-190	CNC-2	CNC-3	ICA Pijao	Nep-2	RB1014	GN-1140	UI-111	BAT 1320	UI-114	BAC 87	M-235
C-49-242	1.0	0	0	0	0	0	0.50	0	0	0	0	0	0	0	0	0.25	0	0.25	0
CNC		1.0	0	0	0	0	0	0	0.75	0.75	0	0	0	0	0	0	0	0	0
Cuilapa-72			1.0	0	0	0	0	0	0	0	0	0	0	0	0	0.25	0	0	0
Ecuador-299				1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.98
KW-780					1.0	0	0	0	0	0	0	0	0	0	0	0	0	0.03	0
Mexico-309						1.0	0	0.50	0	0	0	0	0	0	0	0	0	0	0
Aurora							1.0	0	0	0	0	0	0	0	0	0.13	0	0.13	0
B-190								1.0	0	0	0	0	0	0	0	0	0	0	0
CNC-2									1.0	0.56	0	0	0	0	0	0	0	0	0
CNC-3										1.0	0	0	0	0	0	0	0	0	0
ICA-Pijao											1.0	0	0	0	0	0	0	0	0
Nep-2												1.0	0	0	0	0	0	0	0
RB-1014													1.0	0	0	0	0	0	0
GN-1140														1.0	0	0	0	0.13	0
UI-111															1.0	0	0.56	0	0
BAT-132																1.0	0	0.06	0
UI-114																	1.0	0.03	0
BAC-87																		1.0	0
Mexico-235																			1.0

Coefficients of parentage values extracted from Table 3.3 provided a non-zero matrix of r for 16 pairs of cultivars (Table 3.4) for comparison with SI values. Coefficients of parentage ranged from values that indicated low relationship (r = 0.0313) for cultivars K.W. 780 and BAC-87 to an r value that indicated a high degree of relatedness (r = 0.9844) for cultivars Mexico-235 and Ecuador-299. The latter two cultivars were almost identical in their reactions to several races of the bean rust fungus and homology in isozyme mobility patterns. Freytag (1989, personal communication) considered these varieties identical, with different names given in separate regions. The high value of coefficient of parentage (r) for these pairs was obtained by the same method for computing r for other cultivars assuming that cultivar Mexico-235 was the progeny in the sixth backcross between the recurrent parent Ecuador-299 and an unknown cultivar named here as XA (Table 3.1). Other cultivar pairs produced coefficients of parentage (r) values in between these.

High similarity indices (SI) were observed for disease reaction response, isozyme mobility patterns and agrophysiological traits in seven cultivar pairs (Table 3.4) that were associated with relatively high values of coefficients of parentage. The only exception to this was the low SI values for the cultivar pair Aurora and C-49-242 for disease reaction response (SI - 0.27) to 26 rust races. However, the SI value for isozyme mobility patterns was higher (SI = 0.92) in contrast. For any value of r, the SI for isozyme mobility patterns was invariably higher perhaps indicating the genetic control of molecular markers than it is for either disease reaction or agrophysiological traits where the environmental component is much greater. This is also evident from the limited data presented in Table 3.4, in which variability of SI for disease reaction ranged from SI = 0.27 to 1.00 and for agrophysiological traits from SI = 0.53 to 1.00.

Coefficient of parentage was used previously to quantify genetic diversity in soybeans (Cox et al, 1985a, 1985b; Delannay et al, 1983), red winter wheat (Murphy et al., 1986),

Table 3.4: Comparison of coefficient of parentage (r) and different indices of similarity (SI) for 16 pairs of bean cultivars

		r	SI[1]	SI[2]	SI[3]
1	C-49-242 vs Aurora	0.5000	0.27	0.92	0.33
2	C-49-242 vs BAT-1320	0.2500	-	0.83	0.67
3	C-49-242 vs BAC-87	0.2500	-	-	-
4	CNC vs CNC-2	0.7500	0.73	-	1.00*
5	CNC vs CNC-3	0.7500	0.73	-	1.00*
6	Cuilapa-72 vs BAT 1320	0.2500	-	0.83	0.67
7	Ecuador 299 vs Mexico-235	0.9844	0.85	0.92	0.83
8	K.W.-780 vs BAC-87	0.0313	-	-	-
9	K.W.-780 vs GN 1140	0.2500	-	0.83	0.50
10	Mexico-309 vs B-190	0.5000	1.00	0.92	0.50
11	Aurora vs BAT-1320	0.1250	-	0.92	0.33
12	Aurora vs BAC-87	0.1250	-	-	-
13	CNC-2 vs CNC-3	0.5625	0.73	-	1.00*
14	GN-1140 vs BAC-87	0.1250	-	-	-
15	UI-111 vs UI-114	0.5625	1.00	0.92	1.00
16	BAT-1320 vs BAC-87	0.0625	-	-	-

[1]SI = Similarity index for disease data (Table 3)
[2]SI = Similarity index for isozyme mobility patterns (above diagonal) Table 4
[3]SI = Similarity index for agrophysiological traits (below diagonal) Table 4

*Not shown in the respective SI tables

spring barley (Martin et al., 1991), oats (Souza and Sorrels, 1989) and beans (Adams, 1977, Singh et al., 1991, and McClean et al., 1993). Cox et al. (1985a, 1985b) suggested the use of similarity indices based upon several loci revealed by electrophoretic data to supplement coefficients of parentage data. They found that by including a similarity index (S) with coefficients of parentage values (r), improved evaluation of genetic similarities could be achieved among soybean cultivars. High indices of similarity were associated with high values of coefficients of parentage (r) for winter wheat cultivars (Cox et al., 1985b) and the authors proposed the possibility of using s as a means of identifying closely related cultivar pairs when pedigrees are not known.

Although r is not necessarily reliable as an indicator of the proportion of shared germplasm between two relatives (Adams, 1977), it is a valid genetic measure to establish relatedness. A substantial genetic implication was suggested by Adams (1977) from high correlations between r and "distance" based on PC scores of cultivars for 18 chemical-agronomic characteristics. Singh et al. (1991) identified within the Mesoamerican or Andean cultivated germplasm, clusters of landraces that share a common allozyme that are also traceable to a common ancestor. However, they observed that cultivars with the same allozyme genotype exhibiting similarities for certain morphological traits could diverge considerably for other morphological or agronomic traits.

Cultivated germplasm may be identical-in-state or may share genes in common without evidence of traceable ancestry or resemble each other very closely for the selected plant and seed traits important in agronomy and in commerce and yet be quite diverse in genes for which no direct selection has been practiced (Adams, 1977; Singh et al., 1991; McClean et al., 1993).

Various approaches have been suggested to compute r for assessing relatedness either as a stand-alone parameter (Wright, S., 1917, Malecot, G., 1948, Emik and Terrill, 1949;

Delannay et al., 1983; Souza and Sorrels, 1989) or in combination with other measures of similarity (Adams, 1977, Cox et al., 1985a, 1985b). There is agreement, however, among researches to use r along with other indices of similarity such as molecular markers and/or agronomic traits to substantiate assessments of genetic diversity. The use of similarity indices, S (Cox et al., 1985a); distance metric measures based on PCA (Adams, 1977) and disease reaction response patterns employing several isolates of disease pathogens (Ghaderi et al., 1984) have shown the usefulness of the various indices in helping in the assessment of genetic relatedness.

SUMMARY AND CONCLUSION

1. Coefficients of parentage values (r) among 171 pairwise combinations of 20 bean cultivars representing a subset of the eight cluster groups have been computed.
2. The majority of these cultivars were unrelated landrace cultivars whose coefficient of parentage values were zero.
3. The highest value of r was between cultivars Ecuador–299 and Mexico–235 (both small reds and with broad resistance for several rust races) at r = 0.9844. The r value was determined by using the second cultivar as a sixth generation backcross progeny of a cross involving the first cultivar as recurrent parent and an assumed donor.
4. For those cultivars with non–zero values of coefficient of parentage (r), it appears that high values of similarity indices (SI) from isozyme mobility patterns and disease reaction response patterns are related to r. However, high values of similarity indices (SI) for isozyme patterns and disease reaction response patterns for the majority of cultivars with non–integer (zero) values of coefficient of parentage (r) cannot be explained by the same reasoning, i.e. by shared pedigree. In other words, possessing high values of indices of similarity for both isozyme mobility patterns and disease reaction response patterns without basis of a common pedigree can only be attributed to the ubiquitous genes in common, i.e., belonging to the same gene pool.

LIST OF REFERENCES

Adams, M.W. 1977. An estimation of homogeneity in crop plant with special reference to genetic vulnerability in the dry bean, *Phaseolus vulgaris L.* Euphytica 20: 665–679.

Cox, T.S., Y.t. Kiang, M.B. Gorman and D.M. Rogers. 1985a. Relationships between coefficients of parentage and genetic similarity indices in soybean. Crop Sci. 25: 529–532.

Cox, T.S., G.L. Lookhart, D.E. Walker, L.G. Harrell, L.D. Albers and D.M. Rogers. 1985b. Genetic relationships among hard red winter wheat cultivars as evaluated by pedigree analysis and gliadin polyacrylamide gel electrophoretic patterns. Crop Sci. 25(6): 1058–1062.

Delannay, X., D.M. Rodgers and R.G. Palmer. 1983. Relative genetic contributions among ancestral lines to North American Soybean cultivars. Crop Sci. 23: 944–949.

Emik, L.O. and C.C. Terrill. 1949. Systematic procedures for calculating inbreeding coefficients. J. of Heredity 40: 51–55.

Ghaderi, A., M.W. Adams and A.W. Saettler. 1984. A quantitative analysis of host–pathogen–environment interaction in International Bean Rust Nurseries (IBRN). Rept. Bean Imp. Coop. vol. 27.

Malecot, G. 1948. Les Mathematiques de l'heredite. Masson Cie, Paris.

Martin, J.M., T.K. Blake and E.A. Hockett. 1991. Diversity among North American spring barley cultivars based on coefficient of parentage. Crop. Sci. 31: 1131–1137.

McClean, P.E., J.R. Myers, J.J. Hammond. 1993. Coefficient of parentage and cluster analysis of North American dry bean cultivars. Crop Sci. 33: 190–197.

Murphy, J.P., T.S. Cox and D.M. Rodgers. 1986. Cluster analysis of red winter wheat cultivars based upon coefficients of parentage. Crop Sci. 26(4): 672–676.

Singh, Shree P., R. Nodari and P. Grepts. 1991. Genetic diversity in cultivared common bean: 1. Allozymes. Crop Sci. 31: 1923.

Sneath, P.H.A. and R.R. Sokal. 1973. Numerical taxonomy. W.H. Freeman and Co. San Francisco, CA 573p.

Souza, E. and M.E. Sorrels. 1989. Pedigree analysis of North American oat cultivars released from 1951 to 1985. Crop Sci. 29(3): 545-601.

Wright, S. 1917. Coefficient of inbreeding and relationship. Am. Nat. 51: 545-559.

CHAPTER IV

GENETIC RELATIONSHIPS AND RESISTANCE IN BEANS (*PHASEOLUS VULGARIS* L.) TO THE BEAN RUST (*UROMYCES APPENDICULATUS*) (PERS.) UNGER VAR. APPENDICULATUS

INTRODUCTION

Rust caused by *Uromyces appendiculatus* is an important disease of beans, contributing to yield reduction in many parts of the world. Basic to the implementation of yield stabilization through the avoidance of risk of rust attack on bean crops entails understanding the various facets of a disease triangle involving pathogenic variability, host resistance reactions, and the influence of environment on this interplay for development of disease epidemics.

The existence of considerable variability in the rust pathogen with unnecessary virulence genes unrelated to host resistance challenges has been noted (Stavely, 1984a; Groth and Urs, 1985). Host susceptibility to a wide range of rust races has been implicated for the prevalence of unnecessary virulence genes in the rust fungus, in addition to its autoecious, macrocyclic life cycle that enhances recombination and appearance of new races (Stavely, 1984a, 1984b).

The availability of a wide range of pathogenic variability, although a challenge, can be utilized to facilitate the identification of host resistance mechanisms and resistance genes that are distinct from already recognized resistance genes. Stavely (1984a) noted the existence of

natural pathogenic diversity in the form of pathogenic races in the bean rust fungus the diversity of which has been equally matched by the presence of several kinds of resistance genes in the host (Stavely, 1984a, 1984b). Host resistance and pathogenic virulence data would allow analysis and prediction of host resistance and pathogenic virulence interactions on the basis of the gene-for-gene system for a long-term breeding program.

Analysis of long-term disease reaction data or multi-locationally tested disease reaction data using appropriate cluster analysis and other multi-variate statistical techniques permits the partitioning of the cultivars and/or the pathogens into groups with similar reaction and/or virulence patterns. These are helpful in furnishing tentative information on the nature of cultivar and/or pathogen relationships (similarities with regard to resistance and/or virulence genes). Using cluster analysis, 88 bean cultivars that were tested in the 1976 International Bean Nursery (IBRN) were grouped into eight cluster groups with similar reaction response patterns (Ghaderi et al., 1984). Based on this, the authors postulated that genotypes within clusters are more similar or possibly identical for genes or genetic complexes conditioning reaction to rust, than randomly selected cultivars, or cultivars between clusters.

The understanding of the relationships between the various resistance genes from the different germplasm sources is also of fundamental importance to: 1) understanding the genetics of resistance to pathogenic races; 2) the understanding of linkage and pleiotropic relationships of the various resistance genes; and 3) devising appropriate breeding methods to provide stable disease resistance.

The main objective of this study was to determine the genetics of rust resistance using four distinct rust isolates simultaneously inoculated to a plant on several parental bean cultivars that were previously included in IBRNs, and secondly, to utilize the information on the number of gene differences for resistance and susceptibility to support or refute the main

hypothesis that cultivars within clusters are genetically more similar than cultivars between clusters.

LITERATURE REVIEW

Fromme and Wingard (1921), seventy years ago, recognized a reduced intensity of uredinia per unit of leaf area and decreased spore production as potentially useful forms of resistance to bean rust.

Reduced uredinial intensity (low receptivity) for all races has been tested on such cultivars as Royal Red Kidney (Groth and Urs, 1982) and Jamaica Red (Shaik, 1985a). A polygenic mechanism of resistance was deemed important (Simons, 1972). Polygenic inheritance was suggested by the analysis of relationship of stomatal and hair density to uredinium density on bean cultivars to several races (Shaik, 1985a). Stomatal density and uredinial intensity were positively correlated (Shaik, 1985a), whereas uredinium intensity was negatively correlated with mean hair density on both leaf surfaces (Shaik, 1985a).

A longer latent period (LP) from infection to sporulation (slow rusting) not associated with the reduced intensity type of resistance was reported by Shaik (1985a). The presence of substantial "horizontal" resistance equally effective against all races was suggested by Vieira (1972) in Brazilian material. Eight bean lines varied in incubation period, latent period, infection frequency, infection type and infection intensity against different rust isolates. Menten and Filho (1981) analyzed the variability found in horizontal resistance components of nine rust isolates and reported a significant differential interaction between rust isolates and bean lines, according to the classical theory of Van der Plank. They believed that vertical resistance genes do play at least some role in expression of these races.

The possibility of reducing pathogen variability was investigated to see if virulence in basiodispores and uredeospores is under independent genetic control in the bean fungal pathogen (Groth and Roelfs, 1982b). However, it appears that the pathogen genes for virulence and avirulence in both basidiospores and uredeospores are the same (Kolmer et al., 1984). If such was the case, basidiospore resistance could be used to decrease chances for pathogen variability.

Aust et al. (1984) reported resistance expressed by sporulation on three bean cultivars (one susceptible and two with horizontal resistance). The total number of spores produced/per pustule in the susceptible cultivar Rosinha G-2/C-21 was two times more than that produced by either of the cultivars with theoretically horizontal resistance. One-third less spores were produced by either of the theoretically horizontal resistant cultivars Carioca/C-224 and Roxo/C-740.

Potentially useful non-biological resistance mechanisms include variation in length of dew or drying period that enhance resistance with plant development (Ballantyne, 1974; Berger, 1977).

Rodriguez et al. (1977) noted tolerance in the cultivar Mexico-309, which was susceptible to race CR-29 but able to yield as well as cultivars resistant to CR-29. Unnecessary virulence was noted during monitoring of virulence changes in a polymorphic rust population over five asexual generations by Alexander et al. (1985), which revealed that changes in virulence may be independent of pathogen exposure to host resistance.

Genetic studies of resistance indicate that reaction grade is controlled by single dominant genes and that there are many such genes in beans. Wingard (1933) was the first to study the inheritance of resistance in beans to *U. appendiculatus*. His studies in 1933, conducted before the discovery of large numbers of physiological races of the organism,

showed resistance to be dependent on a single dominant factor, suggesting that he worked only with one race.

Zaumeyer and Harter (1941) reported on the inheritance of resistance to five physiologic races of bean rust. They found that single dominant factors commonly conditioned resistance to most of the races of *U. appendiculatus* that were included in their studies. They dealt primarily with the hypersensitive type of resistance (HR). In their results, resistance to races 1 and 2 in the hybrids was governed by a single dominant factor, but more than one dominant factor was involved in the resistance to races 6 and 12 and incompletely dominant factors were involved in conditioning reaction to races 11 and 17.

Augustine et al. (1972), in the studies with the Brazilian race B11, found that in crosses between resistant Great Northern 1140 and four susceptible lines, a major dominant gene controlled disease resistance.

Ballantyne (1974) reported field reactions of 158 bean lines to natural infection by rust resulted in only slight effects on bush snap and red kidney cultivars, suggesting a non-race-specific type of resistance. On the other hand, pole and most dry beans showed either a high level of specific resistance or were severely rusted with no apparent non-specific resistance. This supports the gene pool theory in which tolerance reaction to US races is exhibited by Andean germplasm and resistance/susceptibility is exhibited by the Meso-American beans (Kelly, 1989, personal communication; Stavely, 1982a).

Ballantyne and McIntosh (1975) examined the variation in *U. appendiculatus* virulence in eastern Australia and the genetic basis of resistance in the host under greenhouse and field conditions. Twenty races were identified from a total of 163 collections. Application of the gene-for-gene hypothesis allowed them to predict the presence of at least nine distinctive genes for resistance in the eight host genotypes used to distinguish the races. Genetic studies involving nine genotypes revealed either dominant or incompletely dominant resistance.

Ballantyne (1978), using Australian races, studied the genetics of several kinds of rust resistance. She indicated that resistance in beans to single races of *U. appendiculatus* is controlled by one dominant gene regardless of whether the resistance is expressed as hypersensitive reaction (HR) or as small pustule resistance. Individual resistance genes could be effective against more than one race, suggesting that she may have been working with a single linkage block for multiple race resistance such as Stavely found in B-190 (Stavely, 1982b).

Carvalho et al. (1978) has also shown that the immune reaction of the cultivar 1458 to five Brazilian races of rust was under monogenic dominant control.

Meiners (1979; 1981) concluded, as did Ballantyne (1978), that all genetic data on rust resistance in beans obtained to date have indicated an oligogenic mode of inheritance, but it has been postulated that considerable horizontal resistance may be available in already identified germplasm.

Christ and Groth (1982a; 1982b), investigating the interaction of virulence and resistance genes in the rust fungus and the host bean plant, showed a gene-for-gene relationship between virulence in *U. appendiculatus* and resistance in beans. In the same study, the authors found single gene resistance to rust isolate P10-1 in the snap bean cultivar Early Gallatin, but that resistance to isolate S1-5 was controlled by complementary dominant factors.

Monogenic dominant control of a minute uredinium reaction was reported for the differential cultivar Kentucky Wonder 814 by Kolmer and Groth (1984). In the same study, it was established that the genes for resistance in KW-814 and US #3 against isolate S1-5 were independent and dominant. The gene in KW-814 was epistatic to the gene conditioning necrotic fleck in US#3. Similarly, F_2 segregation in the cross KW-814 x Early Gallatin

indicated that the gene conditioning resistance to rust isolate P10-1 and the gene in KW-814 are independent of each other.

Stavely (1984b) investigated the genetics of rust resistance in a breeding line B-190 that possesses resistance to most races of rust in the continental U.S. In one test, the cultivar B-190 was crossed to the moderately susceptible cultivar Green Giant 447 and to a pinto cultivar Olathe. Tests on F_1, F_2 and F_3 progenies with the first cross inoculated to eight races simultaneously indicated that resistance was controlled by a single dominant gene. B-190 expressed resistance as a limitation of uredinium size to less than 0.3 mm in diameter for the first seven races and as a small, necrotic spot without sporulation (HR) for the eighth race. The study indicated that resistance to two of the eight races was controlled by the same resistance gene and that it and the remaining six R genes and the HR gene were closely linked to one another. The cross B-190 x Olathe indicated that the R genes in B-190 were independent of the dominant single genes that condition R or HR in Olathe. The genes in Olathe conferring HR to three races were closely linked to one another and epistatic to the genes in B-190 that condition resistance against the same races. The resistance in B-190 was expressed as very small uredinia against 15 races and small necrotic spots against races 38 and 39, and appeared to be conditioned by 17 dominant genes (linked series of monogenic dominant factors), one per race, that are linked in coupling. He suggested the strategy of gene pyramiding in the development of new rust resistant cultivars, particularly when resistance genes are closely linked.

Stavely (1984c) reported on the genetic relationship of resistance in two broadly rust-resistant bean cultivars, Compuesto Negro Chimaltenango (CNC) and B-190. Whereas CNC has the small pustule type of resistance or immunity (I) to 20 recently described races of *U. appendiculatus*, B-190 has similar resistance genes to many (15) races but it is susceptible to three races to which CNC has resistance. In the cross between CNC x B-190, and its

reciprocal, Stavely (1984) found that none of the 468 F_2 plants had a susceptible reaction to races to which CNC had I or R and B-190 had R or HR. He concluded that both parents had the same or different alleles for resistance at a single locus for reaction to each of the races to which both have resistance (R), or CNC had I and B-190 has R or HR. The resistance (R) gene in CNC to the races to which B-190 is susceptible is regulated by additional linked single dominant resistance gene regulated on a gene-for-gene basis.

Qualitative inheritance of resistance to two races of the bean rust fungus (races 44 and 52) for three dry bean cultivars was reported by Grafton et al. (1985). Resistance in the F_2 plants of crosses between Aurora x UI-114 and Olathe x UI-114 and T-39 x UI-114 indicated that resistance to each race was controlled by a single dominant gene. On the other hand, F_2 segregation ratios of the cross Olathe x T-39 inoculated only with race 44 indicated that complementary dominant genes controlled resistance to race 44. F_2 segregation ratios in the cross Aurora x Olathe for resistance to both races 44 and 52 indicated independent assortment without epistasis that suggests that each cultivar has dominant alleles at one of the two loci expressing complementary gene action.

Stavely and Steinke (1985) reported on four white-seeded, green-podded bush snap bean germplasm lines released as bulks in the F_5 (BARC RR-2 and -3) from single, homozygous rust-resistant F_3 plant and/or F_8 bulks (BARC RR-4 and -5) from a single, homozygous rust-resistant F_4 plant. The germplasm lines were developed specifically for resistance to 20 races of the bean rust fungus and are the first snap beans homozygous for resistance to all available U.S. races of the pathogen (28 races). Resistance to 15 races is expressed as restricted uredinia, while resistance to races 49, 50 and 51 was conditioned by monogenic, dominant factors obtained from the backcross parents and expressed as necrotic spots less than 1.0 mm in diameter.

In a review of research accomplishments (Biennial Review, ARS), Stavely (1985) noted the existence of a high level of pathogenic specialization in *U. appendiculatus*, pointing out that perhaps it was the most variable pathogen currently in existence. Stable control of this disease through resistance poses difficulty because of the potential for development of races capable of overcoming resistance. The author noted that in one of the broadly resistant cultivar where resistance is controlled by one dominant gene per race with many such genes linked in coupling its occurrence has influenced strategies about rust control through host resistance. It has been learned that a second line resistant to 29 races has not only the same set of resistance genes and linked group as that of the first line (B–190), but it also has a second independent linkage group over that of the first line. A third line has two such linkage groups that are independent of those in the first two lines. Two additional lines have at least three more independent linkage groups of resistance (R) genes.

Stavely and Grafton (1985) described the genetics of resistance to eight races of *U. appendiculatus* in a *P. vulgaris* cultivar, Mexico–235. Mexico–235, as does the cultivar B–190, has small uredinium resistance (R) or necrotic hypersensitive resistance (HR) to most races of *U. appendiculatus*. F_2 segregation from a susceptible Fiesta x Mexico–235 cross indicated that a single, dominant pleiotropic gene or group of tightly linked genes control HR of Mexico–235 to races 40, 52, 53 and 54. These genes are epistatic to the R genes for the races in B–190. Mexico–235 also contained a second, independent group of apparently linked single dominant genes for resistance to races 40, 45 and 48 and for high resistance (HR) to races 49 and 50. The genes for HR to races 49 and 50 in Mexico–235 were apparently influenced by modifier genes, environment or both so that their expression varied from HR to R in the F_2. The F_2 of B–190 x Mexico–235 also indicated allelism of the R genes for races 40 and 48, but one plant in 64 was susceptible to race 45, indicating triplicate factor dominant epistasis.

Kardin and Groth (1985) investigated the inheritance of resistance in two white-seeded dry bean cultivars against seven bean rust isolates. Simultaneous inoculations of the F_1 and F_2 generations from crosses between Aurora x UI-111, Fleetwood x UI-111, and Aurora x Fleetwood with seven rust isolates indicated that the resistance of Aurora and Fleetwood to each isolate was controlled by a single dominant gene. The authors reported that Aurora possessed at least two resistant genes. They also hypothesized that the same resistance allele and locus in cultivar Aurora conditions incompatibility to all six isolates. The small fleck gene in Aurora was epistatic to that in Fleetwood that produces a minute uredinium. The gene in Aurora for resistance to the seventh isolate segregated independently from that which conditioned resistance to the other six isolates, and was independent of and epistatic to a third gene, in cultivar UI-111, that gave an intermediate reaction to this isolate.

Finke et al. (1985) studied the inheritance and association of resistance to bean rust and common blight on parental bean cultivars and their F_2 progenies. They found no interaction between the two pathogens that permitted separate analysis of inheritance in both. The F_2 segregation of resistance and susceptible plants to three races of rust showed a good fit to 13:3 resistant-susceptible plants, respectively, which suggested that two major genes determined the reaction, with a dominant gene for resistance exhibiting epistasis. Rust susceptibility was expressed only in the presence of the dominant allele for susceptibility and homozygous recessive alleles at the other locus.

Webster and Ainsworth (1988) reported on the inheritance and stability of the more moderate form of resistance in which uredinia are reduced to 0.5 to 0.7 mm in diameter. Uredinia of this size are categorized as a moderately susceptible reaction in the commonly used rating scale (Stavely and Pastor-Corales, 1989). The authors found from data on parentals, F_1, backcrosses and F_2 populations that this kind of resistance to race 38 was conditioned by a single dominant allele. The same allele was present in both parents

exhibiting small pustule resistance. While the test for stability of this resistance was inconclusive, tests using near-isogeneic lines indicated its stability or consistency to be due to factors other than the small pustule resistant gene in the different genetic backgrounds.

In a cross between resistant cultivar PC-50 and a susceptible snap bean cultivar E-Z, Zaiter et al. (1989) reported that resistance was determined by a monogenic recessive allele.

MATERIALS AND METHODS

Hybridization and F_2 seed production

Crosses were planted between cultivars within and among the clusters (Table 4.1). Hybridization to obtain F_1 progenies between the various entries in a cross-combination was accomplished by transferring pollen from a pollen-laden stigma of an already open flower of a male parent into a flower bud of a female parent whose stamens were removed by emasculation. After rubbing the pollen-laden stigma from the male parent, it was left in contact within the stigma of the emasculated flower bud. The flower bud was then covered with thin plastic adhesive tape to ensure contact and prevent desiccation. All emasculated buds were labelled by identifying the male and female parents, initialed and dated. Later, F_1 seeds from the different cross-combinations were harvested individually and stored in labelled envelopes until required for testing and/or producing F_2 seeds.

F_2 seed was produced by allowing each F_1 hybrid to self-pollinate. For F_3 production, each F_2 plant of known reaction to the different rust isolates used in this study was identified and allowed to self-pollinate and produce seed. F_3 seeds from each F_2 plant were identified as a family and stored in labelled envelopes for verification of homozygosity or heterozygosity. No F_3s were tested to verify genotypes of F_2 in this study.

Inoculation and disease-reaction grading

Inoculation, incubation and disease reaction grading for F_1, F_2 and their parental checks were carried out similarly as for other test plants that were mentioned in the general materials

Table 4.1: Reactions to four *U. appendiculatus* isolates (41, 46, 49 and 53) of 13 parental bean cultivars

Parental Cultivar	Predominant Reaction to Race			
	41	46	49	53
LaVega	R	R*a	S	R*b
Mexico-235	HR	R	R	HR
CNC-3	R*c	R*d	R	R
CNC-2	HR*e	R*f	S	HR*g
C-49-242	R*h	S*i	S*j	R*k
Mexico-309	R*l	R	S	R*m
Rico-Bajo-1014	R*n	R	R	R*o
Cuilapa-72	HR	R*p	S*q	HR
Ecuador-299	HR	R	R	HR
Nep-2	HR	S	S	HR
Aurora	HR	S	S	HR
KW-780	S	S	HR	S
ICA-Pijao	S	R*r	S*s	S

*a = LaVega produced few susceptible plants to race 46
*b = LaVega produced few susceptible plants to race 53
*c = CNC-3 produced few hypersensitive resistant (HR) plants to race 41
*d = CNC-3 produced few susceptible (S) plants to race 46
*e = CNC-2 produced few resistant (R) plants to race 41
*f = CNC-2 produced few susceptible (S) plants to race 46
*g = CNC-2 produced few resistant (R) plants to race 53
*h = C-49-242 produced few susceptible (S) plants to race 41
*i = C-49-242 produced few resistant (R) plants to race 46
*j = C-49-242 produced few resistant (R) plants to race 49
*k = C-49-242 produced few susceptible (S) plants to race 53
*l = Mexico-309 produced few hypersensitive resistant (HR) plants to race 41
*m = Mexico-309 produced few hypersensitive resistant (HR) plants to race 53
*n = Rico-Bajo-1014 produced few hypersensitive resistant (HR) plants to race 41
*o = Rico-Bajo-1014 produced few hypersensitive resistant (HR) plants to race 53
*p = Cuilapa-72 produced few susceptible (S) plants to race 46
*q = Cuilapa-72 produced few resistant (R) plants to race 49
*r = ICA-Pijao produced few susceptible (S) plants to race 46
*s = ICA-Pijao produced few resistant (R) plants to race 49

and methods section. However, reaction grades that were converted from the conventional scale of Davison and Vaughn (1963), as modified by an international bean rust workshop in Puerto Rico in 1983 (Stavely et al., 1983), were further categorized into hypersensitive resistance (highly resistant = HR), resistant (R), and susceptible (S) for purposes of mendelian genetic analysis of their F_2 (Tables 1 and 2 in Chapter 1). Segregation in the F_2 for resistance (R) and susceptibility (S) to each race for all four races (41, 46, 49 and 53) was examined by utilizing fixed-ratio Chi-square tests. Later, joint segregation ratios were examined for pairwise F_2 data to assess linkage/pleiotropic relationships or establish independent assortment of reaction phenotypes in each cross-combination. F_2 populations from a total of 68 cross-combinations were tested for individual and joint segregations. Contingency chi-square analysis was performed on all F_2 data for all 68 cross-combinations to establish homogeneity of crosses before submitting it to a joint fixed ratio chi-square test utilizing appropriate monohybrid segregation ratios obtained from individual fixed-ratio chi-square tests of F_2 for each race. The expected values corresponding to the observed values for each reaction category to each race was computed on the fixed ratio (hypothesis) assumed. The deviations from the assumed ratio were tested by chi-square using the formula: $X^2 = \sum_{i=1}^{k}(O_i-E_i)^2/E_i$ with k-1 degrees of freedom, where:

$\sum$ = summation over all classes (categories)

O = observed

E = expected, and

n = number of classes (categories)

Deviations were considered significant when the calculated chi-square value exceeded the tabular value at the 0.05 probability level with 1 df in the individual analysis and 3 df for joint segregation tests. The most appropriate ratio was assumed to be the most probable with the smallest computed chi-square value for the degrees of freedom in question after testing several

other likely fixed-ratios for individual segregation tests. For joint segregation tests, the genes controlling the resistance and susceptible reactions were considered independent when the calculated chi-square value was less than the tabular value at the 0.05 probability level with 3 degrees of freedom. If the chi-square value was more than the tabular value, different hypotheses (linkage and/or pleiotropy) were postulated.

RESULTS AND DISCUSSION

Parental reactions to four rust isolates

The reactions of 13 parental bean cultivars, belonging to eight cluster groups of a previous cluster analysis study that were tested as inoculated control plants along with their F_1 and F_2 progenies to four races of the bean rust fungus, are summarized in Table 4.1. For Mendelian genetic analysis of F_2 data, the conventional scale of Davison & Vaughn (1963) as adopted and modified in the 1983 Bean Rust Workshop was employed to categorize plant reactions as hypersensitive resistant (HR), resistant (R) and susceptible (S).

A. Crosses made within clusters

Of the fourteen possible within-cluster crosses, seven were attempted and were successful. The data on F_2 segregation for reaction to simultaneous inoculation to four races, chi-square values and associated probability (P) are summarized in Table 4.2.

1. Cluster III x Cluster III

F_1 and F_2 from LaVega x Compuesto Negro Chimaltenango-3 cross: All 6 F_1 plants that were produced from LaVega (R) and CNC-3 (R) were all hypersensitive resistant (HR) to races 41 and 53. Five and four F_1 plants of the same cross were resistant (R) to races 46 and 49 respectively. Occasionally plants with hypersensitivity resistance reaction are produced by the cultivar CNC-3. Although only 27 F_2 plants were produced, the F_2 were all resistant. This absence of segregation indicated that genes for resistance to race 41 in LaVega and CNC-3 may be allelic. Similarly, all F_1 plants of the cross LaVega x CNC-3 were

Table 4.2: Segregation for reaction to *Uromyces appendiculatus* races 41, 46, 49 and 53 in cross combinations between parental bean cultivars within cluster groups

Percentage of F_2 population	41					46					49					53				
	HR/R	MS/S	Exp. Ratio	X^2	P	HR/R	MS/S	Exp. Ratio	X^2	P	HR/R	MS/S	Exp. Ratio	X^2	P	HR/R	MS/S	Exp. Ratio	X^2	P
LaVega (iii) x CNC-3 (iii)	27	0	NS		-	27	0	NS		-	27	0	NS	-	-	27	0	NS	-	-
C-49-242 (iv) x CNC-2 (iv)	121	0	NS		-	31	0	NS		-	2	119	1:63	0.0065	0.75>P >0.75	120	1	63:1	0.4258	0.75>P> 0.50
Rico Bajo-1014 (v) x Mexico-309 (v)	77	0	NS	-	-	21	0	NS	-	-	22	61	1:3	0.1000	0.90>P>	77	0	NS	-	-
Mexico-309 (v) x Cuilapa-72 (v)	79	14	13:3	0.8351	0.50>P >0.25	90	3	15:1	1.4497	0.25> P>0.10	11	81	3:13	2.7488	0.10>P> 0.05	76	12	13:3	1.5105	0.25>P> 0.10
Ecuador-249 (vii) x Aurora (vii)	109	0	NS	-	-	21	6	3:1	0.1111	0.75 > P >0.50	84	25	3:1	0.2480	0.75>P> 0.50	110	0	NS	-	-
Nep-2 (vii) x Aurora (vii)	100	0	NS	-	-	0	53	NS	-	-	0	101	NS	-	-	100	0	NS	-	-
ICA-Pijao (viii) x KW-780 (viii)	0	101	NS	-	-	38	25	9:7	0.4227	0.75 >P> 0.50	74	27	3:1	0.1610	0.75P>4 0.50	93	4	1:15	0.7470	0.50>P> 0.25

NS = Non-segregating

HR = Hypersensitivity/Resistant

MS/S = Moderately Susceptible/Susceptible

resistant to races 46, 49 and 53 and 27 plants of the same cross tested simultaneously to these races did not segregate. Absence of segregation in these F_2 plants for races 41, 46, 49 and 53, indicated these genes for resistance to these races may be allelic.

2. Cluster IV x Cluster IV

F_1 and F_2 from the cross C–49–242 x Compuesto Negro Chimaltenango–2 (CNC–2): The 12 F_1 plants produced from the cross of the resistant cultivar C–49–242, which occasionally produces a few susceptible plants to race 41 x CNC–2 (HR), which occasionally produces resistant plants to race 41, were all resistant. All F_2 plants that were produced from this cross did not segregate for reaction to race 41 indicating that genes for resistance to race 41 in the cross C–49–242 x CNC–2 were identical (allelic).

Nine F_1 plants from the race 49 susceptible cultivar C–49–242 (which also produces occasional resistant plants to race 49) x CNC–2 (also S to 49) displayed susceptible reactions. F_2 progenies segregated into 1R (reaction grade 4, 43): 63S ratio indicating a three–dominant factor control of susceptibility in the cultivar CNC–2 to race 49 and triple, homozygote recessive, genes for resistance in the cultivar C–49–242. The reaction of CNC–2 in this study was expressed as 3,4,5; 3,4,5,6; 4,5,6; 5,6; and 6, all reactions that are categorized as moderately susceptible (MS) to susceptible (S). The cultivar C–49–242 produces predominantly susceptible reactions to race 49 as does cultivar CNC–2 but with occasional 4,3 and 4 reaction grades that are classified as moderately resistant (MR). In this instance the parent plant of the cultivar C–49–242 used had reaction grades of 4 or 4,3. The F_1 and F_2 genotypes from a cross CNC–2 x C–49–242 would therefore be expected to depend on the genotype of the plants in C–49–242 that are used in making the initial cross to CNC–2. It appears from the outcomes of the F_2 data with a segregation ratio of 1R:63S that a plant that was resistance (4 or 4,3 grade) to race 49 was used in the initial cross to CNC–2, which has uniform susceptibility to race 49.

The cross CNC-2 x C-49-242 was also simultaneously tested to race 53. CNC-2 has a hypersensitive reaction to race 53 with occasional resistant reactions of the minute uredinia < 0.3mm diameter. The second parent, C-49-242, is resistant to race 53, predominantly producing small uredinia less than 0.3mm in diameter (reaction grades 3 or 3,4) and an occasional 3,4,5 and 4,5 grades that are categorized as moderately susceptible. All F_1 plants that were produced from this cross were resistant and the F_2 segregated in a 63R:1S ratio suggesting a three dominant epistatic gene control of resistance in the cross C-49-242 x CNC-2 to race 53.

3. Cluster V x V

F_1 and F_2 from the cross Rico-Bajo-1014 x Mexico-309: Cultivars Rico Bajo-1014 and Mexico-309 are both resistant to race 41 with reactions in which uredinia 0.3mm - 0.5mm in diameter are predominant and occasional non-sporulating necrotic spots less than 0.3mm in diameter (HR) are produced. Their F_1 plants were all resistant and 77 F_2 plants were all resistant. The absence of segregation in the F_2 suggests that the genes for resistance to race 41 in Mexico-309 and Rico-Bajo-1014 are similar (allelic).

Both cultivars Rico Bajo-1014 and Mexico-309 are resistant (R) to race 46, with reactions producing predominantly uredinia 0.3 mm in diameter and an occasional lesion 0.3 - 0.5 mm in diameter in Rico Bajo-1014. The F_1 plants were all resistant (R) and all 21 F_2 progenies were resistant (R) as were their parents. Although the number of F_2 tested in the cross is very low, the absence of segregation for R and S to race 46 suggested identical or similar genes for reaction to race 46 in the cultivars Rico-Bajo-1014 and Mexico-309.

The reactions to race 49 of the cultivars Rico-Bajo-1014 and Mexico-309 are quite contrasting. While Rico-Bajo-1014 was moderately resistant producing minute uredinia 0.3mm - 0.5mm in diameter, Mexico-309 was susceptible to race 49. The only surviving F_1

plant available for testing was susceptible and 83 F_2 plants segregated into a 1R:3S ratio that indicated resistance in Rico-Bajo-1014 to race 49 was controlled by a single recessive gene.

Mexico-309 and Rico-Bajo-1014 behaved identically to race 53 by producing uredinia < 0.3mm in diameter predominantly categorized as resistant (and uredinia 0.3mm - 0.5mm in diameter for Rico-Bajo-1014) with occasional non-sporulating necrotic spots (HR) less than 0.3mm in diameter. The F_1 plants from this cross were all resistant and similar in reaction to either parent. The lack of segregation in the F_2 of this cross also suggested that genes for resistance to race 53 in both cultivars are identical (allelic).

4a. Cluster VII x Cluster VII

F_1 and F_2 from the cross Ecuador-299 x Aurora: Both parental cultivars Ecuador-299 and Aurora, reacted identically to race 41 by producing non-sporulating necrotic spores < 0.1mm - 0.3mm in diameter (HR). Seven F_1 plants produced from this cross were highly resistant (HR) and identical in reaction to their parents. The 109 F_2 plants that were produced were also predominantly HR with a few R plants. The absence of segregation for susceptibility in the F_2 progenies indicated resistance genes for reaction to race 41 in both Ecuador-299 and Aurora were allelic.

Cultivar Ecuador-299 reacted to race 46 by producing small uredinia predominantly 0.3mm - 0.5mm in diameter (R) whereas Aurora was susceptible (S). The seven F_1 plants were all resistant and the 27 F_2 plants segregated in a manner that satisfactorily fit a 3R:1S ratio. This indicated that resistance to race 46 in Ecuador-299 was controlled by a single dominant gene. Similarly, the cultivar Ecuador-299 reacted to race 49 as resistant (R) and Aurora susceptible (S). All 7 F_1 plants were resistant and the 109 F_2 plants also segregated in a manner that satisfactorily fit a 3R:1S ratio suggesting that resistance in Ecuador-299 was controlled by a single dominant gene.

Both cultivars react identically to race 53 by producing non-sporulating necrotic spores less than 0.3mm in diameter (HR). All 7 F_1 plants from the cross Ecuador-299 x Aurora were resistant (R) and 110 F_2 plants were all resistant. The lack of segregation in the F_2 indicated that the genes for reaction to race 53 in both cultivars were probably identical (allelic).

4b. Cluster VII x Cluster VII

F_1 and F_2 from the cross Nep-2 x Aurora: Nep-2 and Aurora reacted identically to all four races (41, 46, 49 and 53). In response to both races 41 and 53, they predominantly produce non-sporulating necrotic spots (2) less than 0.3mm in diameter (HR) with occasional necrotic spots of 0.3 - 1.0 mm in diameter (2+) encountered. Both cultivars were susceptible (S) to races 46 and 49. All F_1 plants from the cross Nep-2 x Aurora were identical in reaction to either parent when inoculated to race 41, producing hypersensitive type reactions (HR). The 100 F_2 progenies were hypersensitive resistant (HR), like their parents, and non-segregating. The absence of segregation in the F_2 to race 41 and the identical reaction of the F_1, F_2 and parents suggested that resistance genes to race 41 in both parental cultivars Nep-2 and Aurora were identical. Similarly for race 53, all five F_1 plants from the same cross were resistant (HR) and 100 F_2 plants were non-segregating and identical in reaction to race 53 just as were their parents. Lack of segregation in the F_2 of the same cross for reaction to race 53 also suggested similar genes for resistance to race 53 in Nep-2 and Aurora. It appears from the identical reactions of both parental cultivars, their F_1 and F_2 for races 41 and 53 and lack of segregation for R and S to these races that both cultivars have identical genes for reaction to both races (41 and 53). Similarly, 53 F_2 plants tested against race 46 did not segregate suggesting that genes for reaction to race 46 and 49 respectively, were similar in both cultivars.

5. Cluster VIII x Cluster VIII

F_1 and F_2 from the cross ICA-Pijao x KW-780: Twenty-seven F_1 plants were produced from the cross between cultivars ICA-Pijao x KW-780. Both cultivars reacted identically to race 41, being susceptible (S). All 27 F_1 plants were susceptible to race 41 and the 101 F_2 progenies were all susceptible to race 41 indicating that the genes for susceptibility in both parental cultivars are identical.

The reaction of KW-780 and ICA-Pijao to race 46 were not identical. KW-780 was susceptible (S) to race 46 whereas ICA-Pijao was predominantly resistant (R) with occasional production of sporulating uredinia of size greater than 0.3mm in diameter (3,4,5; 4,3,5; 4,5). This behavior in ICA-Pijao may indicate that it was heterogeneous. It is therefore important to note what genotypes of the parental plants that were used for producing F_1 and F_2 progenies. The interpretation of Mendelian segregation data will be dealt with in this light.

The 23 F_1 progenies from the cross KW-780 x ICA-Pijao tested for reaction against race 46 were all resistant (R) like the resistant parent ICA-Pijao. The 63 F_2 progenies segregated in a manner that satisfactorily fit a theoretical 9R:7S ratio. This indicates that two complementary dominant genes controlled resistance to race 46.

The reactions of KW-780 and ICA-Pijao to race 49 were not the same. KW-780 was hypersensitive resistant (HR) producing non-sporulating necrotic spots of size 1.00-3.00mm in diameter and greater than 3.00 mm in diameter (2+, 2++) whereas ICA-Pijao was predominantly susceptible to race 49 with occasional resistant plants (3,4 pustules). All 27 F_1 plants from the cross KW-780 x ICA-Pijao were hypersensitive resistant and the 101 F_2 progenies segregated in a manner that satisfactorily fit a theoretical 3R:1S ratio ($X^2 = 0.16$) suggesting that resistance in KW-780 for race 49 was controlled by a dominant monogenic factor. The same F_2 segregation data were tested for a theoretical ratio of 9 HR:3R:4S and was as probable as the 3R:1S ratio but this ratio had a higher X^2 value (0.3206). It is

noteworthy though that the combined 9 HR + 3R ratio = 12 R with a 3S would produce the same 3R: 1S segregation ratio.

KW–780 and ICA–Pijao have nearly identical reactions to race 53, both being ranked susceptible (S). The pustules in KW–780 were larger (0.5 – 0.8mm; in some greater than 0.8mm in diameter) whereas ICA–Pijao produced uredinia that were no larger than 0.8mm in diameter [grades 3,4,5; 4,5; 5) which would lead to a moderately susceptible grade. The 27 F_1 plants from the cross KW–780 x ICA–Pijao were all susceptible to race 53, similar to both parents, and the 97 F_2 progenies segregated in a manner that satisfactorily fit a theoretical ratio of 1R:15S, indicating that duplicate dominant genes (the action of either of two dominant loci required to produce the susceptibility (S) reaction) controlled the susceptibility reaction to race 53. Single gene recessive control of resistance was also indicated as a corollary.

B. Crosses made between clusters

The reactions of the 13 parental cultivars, including those cultivars among which between–cluster crosses were made, are given in Table 4.1. Of the 81 possible half–diallel, between–cluster cross combinations, 22 were attempted; 19, 9, 15 and 18 between–cluster cross–combinations were analyzed for reaction to races 41, 46, 49 and 53, respectively (Tables 4.3, 4.4, 4.5 and 4.6). The interpretation of F_2 segregation data for each cross combination has been replaced with a summary table (Tables 4.3–4.6) for brevity.

Segregation patterns and numbers of genes proposed for reaction to Race 41: F_2 from a total of 19 between–cluster combinations were examined for segregation of R and S to race 41 (Table 4.3), simultaneously inoculated with three other races (46, 49 and 53). Segregation ratios of 3R:1S (in two cross combinations that indicated a monogenic, dominant factor control of R to race 41); 9R:7S (in two cross–combinations that indicated two complementary dominant gene control of R or at least one dominant gene or both genes

Table 4.3: Segregation for reaction to *Uromyces appendiculatus* race 41 in cross-combinations among parental bean cultivars between cluster groups

Cross Combination	R	S	Ratio	X^2	P	Mendelian Genetic Interpretations
LaVega x C-49-242	48	1	63:1	0.0698	0.90<P<0.75	All F_1 and R, F_2 segregated in a ratio of 63R:1S; 3 factor dominant control of resistance
CNC-3 x Rico-Bajo-1014	83	0	NS	-	-	All F_1 were R and identical to either parent; no segregation in F_2 genes for R in CNC-3 and R. Bajo-Rico-1014 for reactions to race 41 identical (allelic).
LaVega x Cuilapa-72	119	5	15:1	1.0405	0.50<P<0.25	F_1 was R and F_2 segregated 15R:15; duplicate dominant genes control resistance in the cross LaVega x Cuilapa-72 to race 41
LaVega x Aurora	81	0	NS	-	-	All F_1 were R and F_2 did not segregate for R & S to race 41. Genes for resistance in LaVega & Aurora to race 41 may be identical. Aurora (HR); LaVega (R, HR)
LaVega x ICA-Pijao	88	17	13:3	0.4523	0.75>P>0.50	All F_1 are R; 105 F_2 segregated 13R:3S ratio; dominant and recessive epistasis indicated for reaction to race 41
C-49-242 x Mexico-309	130	0	NS	-	-	All F_1 were R. No segregation in 130 F_2 for R and S to race 41. Gene for R in C-49-242 and Mexico-309 for race 41 identical (allelic)
C-49-242 x Cuilapa-72	105	0	NS	-	-	All F_1 were R. No segregation for R and S in 105 F_2 tested. Genes for R reaction to race 41 in C-49-242 and Cuilapa-72 probably the same?
C-49-242 x Rico Bajo-1014	91	0	NS	-	-	All 12 F_1 were R; no segregation in F_2 for R and S to race 41. Gene for R reaction to race 41 in C-49-242 and Rico Bajo-1014 identical
C-49-242 x Nep-2	89	2	63:1	0.2407	0.75<P<0.50	All 5 F_1 were R; 93 F_2 segregated into 63R:1S ratio. Indicated 3 factor dominant control of resistance.

(continued)

Table 4.3: (continued)

Cross Combination	R	S	Ratio	X^2	P	Mendelian Genetic Interpretations
C-49-242 x KW-780	63	30	3:1	2.6129	0.25<P<0.10	All F_1 were R; 93 F_2 segregated into 3R:1S ratio. Dominant monogenic factor control of resistance in C-49-242 to race 41.
CNC-2 x ICA-Pijao	113	11	15:1	1.4538	0.25<P<0.10	All F_1 were R; 124 F_2 segregated into 15R:1S ratio. Duplicate dominant gene control of resistance to race 41 indicated in the cross CNC-2 x ICA-Pijao
Mexico-309 x Aurora	104	3	63:1	1.0760	0.50<P<0.25	All F_1 were R; 107 F_2 segregated 63R:1S ratio; 3 factors dominant control of resistance to race 41 in the cross.
Rico-Bajo-1014 x Nep-2	91	0	NS	-	-	All F_1 were R. No segregation in the 113 F_2 for R and S to race 41. Identical gene for R to race 41 to test Nep-2 and Cuilapa-72
Cuilapa-72 x Nep-2	113	0	NS	-	-	All F_1 were R. No segregation in a 3R:1S ratio indicating dominant monogenic factor control of resistance in Rico-Bajo-1014 for race 41
Rico-Bajo-1014 x ICA-Pijao	81	27	3:1	0.00	P = 1	All 16 F_1 were R; 108 F_2 segregated in a 3R:1S ratio indicating dominant monogenic factor control of resistance in Rico-Bajo-1014 for race 41.
Aurora x ICA-Pijao	39	38	9:7	0.9803	0.50<P<0.25	All 5 F_1 were R; 77 F_2 segregated in a 9R:7S ratio. Two complementary dominant gene control (at least one dominant gene on both loci needed) of R for race 41
Nep-2 x ICA-Pijao	92	11	15:1	3.4441	0.10<P<0.05	All F_1 were R; 103 F_2 segregated 15R:1S. Duplicate dominant gene control of R in the cross Nep-2 x ICA-Pijao for race 41
Aurora x KW-780	39	25	9:7	0.5710	0.50<P<0.25	All 14 F_1 were R; 64 F_2 segregated 9R:7S ratio. Indicated two complimentary dominant gene control of R in the cross Aurora x KW-780 for race 41.

Table 4.4: Segregation for reaction to *Uromyces appendiculatus* race 46 in cross-combination among parental bean cultivars between cluster groups

Cross Combination	R	F_2	Theor. Ratio	X^2	P	Mendelian genetic interpretations
CNC-3 x Rico Bajo-1014	45	0	NS	-	-	All F_1 were R; 45 F_2 did not segregate. Identical reaction of parentals F_1 and F_2. Indicated similar genes for reaction to race 46
LaVega x Cuilapa-72	87	0	NS	-	-	One F_1 was R; 45 F_2 did not segregate. Identical reaction of parentals F_1 and F_2. Indicated similar genes for reaction to race 46
LaVega x Aurora	12	29	1:3	0.3984	0.75<P<0.50	F_1 destroyed; 41 F_2 segregated in a 1R:3S ratio. Monogenic recessive factor controlled resistance to race 46 in LaVega.
C-49-242 x Cuilapa-72	36	10	3:1	0.2609	0.75<P<0.50	One F_1 was R; 46 F_2 plants segregated into a 3R:1S ratio. Dominant monogenic factor control of R to race 46 in Cuilapa-72
C-49-242 x Aurora	0	61	NS	-	-	All F_1 were S and 61 F_2 did not segregate. Parents F_1 and F_2 reaction to race 46 identical. Similar gene for reaction to race 46 proposed
C-49-242 x Nep-2	0	63	NS	-	-	All F_1 were S and 63 F_2 did not segregate. Parents F_1 and F_2 reaction to race 46 identical. Similar gene for reaction to race 46 proposed
CNC-2 x ICA-Pijao	37	0	NS	-	-	All F_1 were R; 37 F_2 did not segregate. Parents F_1 and F_2 reaction to race 46 identical. Similar genes for R to race 46 indicated
Rico-Bajo-1014 x Nep-2	34	7	3:1	0.2331	0.75<P<0.50	All F_1 were R; 44 F_2 segregated 3R:1S, indicating monogenic dominant factor control of R in Rico-Bajo-1014 to race 46
Aurora x ICA-Pijao	20	20	9:7	0.6349	0.50<P<0.25	All F_1 were R; 40 F_2 segregated into a 9R:7S ratio indicating two complementary dominant gene control of R in the cross Aurora x ICA-Pijao to race 46

Table 4.5: Segregation for reaction to *Uromyces appendiculatus* race 49 in cross-combinations among parental bean cultivars between cluster groups

Cross combination	R^{F_2}	S	Theor. Ratio	X^2	P	Mendelian genetic interpretations
LaVega x C-49-242	0	48	NS	-	-	All F_1 were S; 48 F_2 did not segregate. Identical reaction of parents F_1 and F_2 to race 49 identical genes for reaction to race 49 proposed.
CNC-3 x RB-1014	82	0	NS	-	-	All F_1 were R; no segregation in 82 F_2 plants. Identical reaction of parents F_1 and F_2 to race 49. Identical genes for reaction to race 49 proposed
LaVega x Cuilapa-72	0	114	NS	-	-	No F_1 tested; 114 F_2 did not segregate. Identical reaction of P_1, P_2 and F_2. Probably identical genes for reaction to race 49
LaVega x Aurora	0	79	NS	-	-	All F_1 were S; 79 F_2 did not segregate. Identical reaction of P_1, P_2, F_1 and F_2. Identical genes for reaction to race 49 proposed
C-49-242 x Mex-309	0	129	NS	-	-	All F_1 were S; 129 F_2 did not segregate. Identical reaction to race 49 by P_1, P_2, F_1 and F_2. Similar gene for reaction to race 49 proposed
C-49-242 x RB-1014	52	35	9:7	0.4373	0.75<P<0.50	All F_1 were R; 87 F_2 segregated 9R:7S indicating two complementary dominant genes control of R to race 49
C-49-242 x Aurora	0	117	NS	-	-	All F_1 were S; 117 F_2 did not segregate. Identical reaction of P_1, P_2, F_1 and F_2 to race 49. Similar genes for reaction to race 49 proposed
C-49-242 x Nep-2	5	87	1:15	0.1043	0.75<P<0.50	All F_1 were S; 92 F_2 segregated 1R:15S ratio. Duplicate dominant gene control of S parentage. Double recessive factor for R to race 49

(continued)

Table 4.5: (continued)

Cross combination	R^{F_2}	S	Theor. Ratio	X^2	P	Mendelian genetic interpretations
C-49-242 x ICA-Pijao	11	38	1:3	0.1701	0.75<P<0.50	All F_1 were S; 49 F_2 segregated 1R:3S ratio. Monogenic recessive factor controlled R for race 49
C-49-242 x KW-780	74	21	3:1	0.4245	0.75<P<0.50	All F_1 were R; 95 F_2 segregated 1R:63S. Three factor dominant controlled of S in this cross. Monogenic dominant factor control of R in KW-780
Mex-309 x Aurora	3	100	1:63	1.0760	0.50<P<0.25	All F_1 were S and 103 F_2 segregated 1R:63S. Three factor dominant controlled of S in this cross. Monogenic dominant factor control for R to race 49
Cuilapa-72 x Nep-2	23	91	3:13	0.1511	0.75<P<0.50	All F_1 were S. The 114 F_2 segregated 3R:13S indicating recessive and dominant epistasis for reaction to race 49
RB-1014 x ICA-Pijao	72	28	3:1	0.4800	0.50<P<0.25	All F_1 were R; 100 F_2 segregated 3R:1S ratio. Monogenic dominant factor control of R reaction in RB-1014
Nep-2 x ICA-Pijao	10	91	1:15	2.3017	0.25<P<0.10	All F_1 were S and 101 F_2 were segregated in 1R:15S ratio indicating duplicate dominant gene control for S in race 49. Recessive resistance in ICA-Pijao
Aurora x KW-780	50	15	3:1	0.1283	0.75<P<0.50	All F_1 were R and 75 F_2 segregated in 3R:1S ratio. Monogenic dominant factor control of R in KW-780

Table 4.6: Segregation for reaction to *Uromyces appendiculatus* race 53 in cross combination among parental bean cultivars between cluster groups

Cross combination	R^{F_2}	S	Theor. Ratio	X^2	P	Mendelian genetic interpretations
LaVega x C-49-242	47	1	63:1	0.0846	0.90<P<0.75	All F_1 were R; 48 F_2 segregated 63R:1S indicating a three factor dominant control of R to race 53
CNC-3 x RB-1014	82	0	NS	-	-	All F_1 were R; 82 F_2 did not segregate. P_1, P_2, F_1 and F_2 were all R to 53. Similar genes for R reaction to 53 proposed.
LaVega x Cuilapa-72	118	5	15:1	1.0038	0.50<P<0.25	F_1 were R, 123 F_2 segregated 15R:1S. Duplicate dominant factor control of R to 53 indicated
LaVega x Aurora	77	0	NS	-	-	All F_1 were R; 77 F_2 did not segregate; similar genes for R reaction to race 53 indicated.
LaVega x ICA-Pijao	79	16	13:3	0.2263	0.75<P<0.50	All F_1 were R; 75 F_2 segregated in a ratio of 13R:3S. Two factors with dominant and recessive epistasis for R phenotype repression indicated
C-49-242 x Mexico-309	129	0	NS	-	-	All F_1 were R; 129 F_2 did not segregate. P_1, P_2, F_1 and F_2 were all R to race 53. Similar gene from R reaction to race 53 indicated
C-49-242 x Cuilapa-72	100	0	NS	-	-	All F_1 were R; 100 F_2 did not segregate; P_1, P_2, F_1 and F_2 were all R to race 53. Similar genes for R reaction to race 53 indicated.
C-49-242 x RB-1014	78	0	NS	-	-	All F_1 were R; 78 F_2 did not segregate; P_1, P_2, F_1 and F_2 were all R to race 53. Similar gene for R to race 53 indicated
C-49-242 x Aurora	112	4	15:1	1.5570	0.25<P<0.10	All F_1 were R; 116 F_2 segregated 15R:1S. Duplicate dominant factor control of R to race 53 prepared

(continued)

Table 4.6: (continued)

Cross combination	R^{F_2}	S	Theor. Ratio	X^2	P	Mendelian genetic interpretations
C-49-242 x Nep-2	92	0	NS	-	-	All F_1 were R; 92 F_2 did not segregate. P_1, P_2, F_1 and F_2 were all R to race 53. Similar gene for R to race 53 indicated.
CNC-2 x ICA-Pijao	108	12	15:1	2.8800	0.10<P<0.05	All F_1 were R; 120 F_2 segregated 15R:1S. Duplicate dominant factor control of R to race 53 indicated.
Mexico-309 x Aurora	102	2	63:1	1.194	0.50<P<0.25	All F_1 were R; 104 F_2 segregated 63R:1S. Three factor dominant control of R to race 53 indicated
RB-1014 x Nep-2	82	0	NS	-	-	All F_1 were R; 82 F_2 did not segregate. P_1, P_2, F_1 and F_2 were all R to race 53. Similar gene for R to race 53 indicated.
Cuilapa-72 x Nep-2	111	0	NS	-	-	All F_1 were R; 111 F_2 did not segregate. P_1, P_2, F_1 and F_2 were all R to race 53. Similar gene for R to race 53 indicated
RB-1014 x ICA-Pijao	67	31	3:1	2.2993	0.25<P<0.10	All F_1 were R; 98 F_2 segregated 3R:1S indicating monogenic dominant control of R to race 53 in RB-1014
Aurora x ICA-Pijao	39	35	9:7	0.3769	0.75<P<0.50	All F_1 were R; 74 F_2 plants segregated 9R:7S ratio. Two complementary dominant gene control of R to 53 in this cross
Nep-2 x ICA-Pijao	92	6	15:1	0.0025	0.95<P<0.95	All F_1 were R; 98 F_2 segregated 9R:7S. Two complementary dominant factor control of R to race 53 in this cross

needed for R to race 41); 13R:3S dominant and recessive epistasis situation in one cross-combination, LaVega x ICA-Pijao, in which the dominant gene and its allelic form in one locus are epistatic to the second gene and its allelic form in the other locus; 15R:1S (in four cross-combinations, which indicated duplicate dominant gene control of R to race 41); 63R:1S (in three cross-combinations that indicated 3 factors, dominant control of R to race 41) and lack of segregation in seven cross-combinations that indicated genes for R to race 41 in the respective cultivars were allelic or identical.

Segregation patterns and numbers of genes proposed for reaction to race 46: F_2 plants from a total of 9 cross-combinations were examined for segregation of R and S to race 46 (Table 4.4). The full array of segregation ratios were not observed for race 46 since the number of cross-combinations were smaller owing to the difficulties of inoculum viability in race 46 that was encountered in several test schedules. Whether this problem of viability was due to sensitivity of race 46 to the 0.1 percent tween-20 added as a wetting agent, the practice of spore increase and storage followed, or, as indicated by Augustine, Coyne and Schuster (1972), the effect of the Freon-113 propellant agents, was not determined here.

Absences of segregation in the F_2 for R and S to race 46 was predominant. F_2 population in three cross-combinations had plants that were all resistant (R), as were the F_1 and parental cultivars. This lack of segregation indicated that genes for R to race 46 in the respective genotypes are allelic (identical). Similarly, all F_2 plants in two cross-combinations (C-49-242 x Aurora and C-49-242 x Nep-2) did not segregate. All F_2 plants in the two crosses were susceptible (S), as were their F_1 and parental cultivars. This absence of segregation for R and S in these cross-combinations indicated similar or identical (allelic) genes for susceptibility (S) to race 46. The F_2 of the cross LaVega x Aurora segregated in a 1R:3S ratio indicating a single recessive gene control of R to race 46 in LaVega. F_2 populations in two cross combinations, C-49-242 x Cuilapa-72 and Rico Bajo-1014 x Nep-

2, segregated in a 3R:1S ratio, which indicated monogenic, dominant factor control of R to race 46 in Cuilapa–72 and Rico Bajo–1014, respectively. The F_2 of the cross Aurora x ICA–Pijao segregated in a ratio of 9R:7S that indicated two complementary dominant genes for control of R in this cross.

Segregation pattern and number of genes proposed for reaction to race 49: F_2 data from a total of 15 different cross–combinations were examined for reactions to race 49 (Table 4.5). A total of six cross–combinations showed lack of segregation in the F_2. Eighty–two F_2 plants of the CNC–3 x Rico–Bajo–1014 did not segregate and all were resistant (R) to race 49 as were their F_1 and parental cultivars. This absence of segregation in the F_1 suggests identical (allelic) genes for R reaction to race 49 in CNC–3 and Rico–Bajo–1014. F_2 plants from the other five cross–combinations were all susceptible (S) like their F_1 progenies and the parental cultivars. This indicated similar (allelic) genes for susceptibility (S) to race 49 in the respective cultivars. The F_2 of the cross C–49–242 x Rico–Bajo–1014 segregated 9R:7S, indicating control of two complementary dominant genes for resistance (R) to race 49. The F_2 of the cross C–49–242 x Nep–2 segregated in a 15R:1S ratio that suggested duplicate dominant gene control of the reaction to race 49. Double recessive genes were also indicated for resistance to race 49 in the same cross. The F_2 for the cross between the predominantly susceptible (S) with occasional R (4,43 grade) C–49–242 x ICA–Pijao also predominantly susceptible (S) with occasional R (4,43 grade) segregated in a 1R:3S ratio, suggesting monogenic recessive factor control of R to race 49. The F_2 from three cross–combinations, C–49–242 x KW–780, Rico–Bajo–1014 x ICA–Pijao, and Aurora x KW–780, segregated in a 3R:1S ratio, suggesting a single, dominant factor control of R to race 49 in KW–780, Rico–Bajo–1014 and KW–780, respectively.

The F_2 of the cross Mexico–309 x Aurora segregated in a 1R:63S ratio that indicated a 3–factor dominant gene control of the susceptibility (S) reaction to race 49. The F_2 of the

cross Cuilapa–72 x Nep–2 segregated in a 3R:13S ratio that indicated respectively, recessive and dominant epistasis of the two loci concerned for the R and S reaction to race 49.

Segregation patterns and numbers of genes proposed for reaction to race 53: F_2 data from 18 cross–combinations were examined for segregation to race 53 (Table 4.6). Segregation ratios of 3R:1S that indicated single dominant factor control of R to race 53 (Rico Bajo–1014 x ICA–Pijao); 9R:7S that indicated two complementary dominant factor control of R to race 53 (Aurora x ICA–Pijao and Aurora x KW–780); 13R:3S that indicated two factor control with dominant and recessive epistasis, respectively, for R reaction to race 53 (LaVega x ICA–Pijao); 15R:1S that suggested duplicate, dominant factor control of R to race 53 (LaVega x Cuilapa–72, C–49–242 x Aurora, CNC–2 x ICA–Pijao and Nep–2 x ICA–Pijao), were observed. The F_2 in two cross–combinations (LaVega x C–49–242 and Mexico–309 x Aurora) segregated 63R:1S that suggested three factors, dominant control of R to race 53 in these two crosses, respectively.

Gene differences for R and S reactions to the four races (41, 46, 49 and 53) that were simultaneously applied to each F_2 plant in the various cross–combinations are summarized in Table 4.7.

C. Gene differences for resistance and susceptibility for within–cluster crosses

Gene differences (number of genes) for reaction to race 41 (Table 4.7) for five within–cluster crosses was zero (0) that suggested identical alleles for R to race 41. One cross (Mexico–309 x Cuilapa–72) showed a two–gene difference, based upon the 13R:3S F_2 segregation.

Two cross–combinations showed lack of segregation for race 46 in the F_2, which indicated the same gene for reaction to race 46 in the respective crosses. A one–gene difference was observed in one cross for R to race 46 (Ecuador–299 x Aurora), whereas

Table 4.7: Gene differences for resistance and susceptibility in crosses between pairs of bean cultivars tested against four rust isolates in the greenhouse

Cluster Code	Cross-Combination	Genetic Segregation Ratios for Rust Isolates															
		41				46**				49				53			
		R	S	Segre. Ratio	Gene Diff.	R	S	Ratio	Diff.	R	S	Ratio	Diff.	R	S	Ratio	Diff.
III x III	LaVega x CNC-3	27	0	NS	0	27	0	NS	0	27	0	NS	0	27	0	NS	0
IV x IV	CNC-2 x C-49-242	121	0	NS	0	-	-	-	-	2	119	1:63*	3	120	1	63:1	3
V x V	RB-1014 x Mexico-309	77	0	NS	0	-	-	-	-	22	61	1;3*	1	77	0	NS	0
V x V	Mexico-309 x Cuilapa-72	79	14	13:3	2	90	3	15:1	2	11	81	3:13	2	76	12	13:3	2
VII x VII	Ecuador-299 x Aurora	109	0	NS	0	21	6	3:1	1	84	25	3:1	1	110	0	NS	0
VIII x VIII	ICA-Pijao x KW-780	0	101	NS	0	38	25	9:7	2	74	27	3:1	1	4	93	1:15*	2
III x IV	LaVega x C-49-242	48	1	63:1	3	-	-	-	-	0	48	NS	0	47	1	63:1	3
III x V	CNC-3 x RB-1014	83	0	NS	0	45	0	NS	0	82	0	NS	0	82	0	NS	0
III x V	LaVega x Cuilapa-72	119	5	15:1	2	87	0	NS	0	0	114	NS	0	118	5	15:1	2
IIX x VII	LaVega x Aurora	81	0	NS	0	12	29	1:3*	1	0	79	NS	0	77	0	NS	0
III x VIII	LaVega x ICA-Pijao	88	17	13:3	2	-	-	-	-	-	-	-	-	79	16	13:3	2
IV x V	C-49-242 x Mexico-309	130	0	NS	0	-	-	-	-	0	129	NS	0	129	0	NS	0
IV x V	C-49-242 x Cuilapa-72	105	0	NS	0	36	10	3:1	1	-	-	-	-	100	0	NS	0
IV x V	C-49-242 x RB-1014	91	0	NS	0	-	-	-	-	52	35	9:7	2	78	0	NS	0
IV x VII	C-49-242 x Aurora	113	4	15:1	2	0	61	NS	0	0	117	NS	0	112	4	15:1	2
IV x VII	C-49-242 x Nep-2	89	2	63:1	3	0	63	NS	0	5	87	1:15*	2	92	0	NS	0
IV x VIII	C-49-242 x ICA-Pijao	-	-	-	-	-	-	-	-	11	38	1:3*	1	-	-	-	-
IV x VIII	C-49-242 x KW-780	63	30	3:1	1	-	-	-	-	74	21	3:1	1	-	-	-	-
IV x VII	CNC-2 x ICA-Pijao	113	11	15:1	2	37	0	NS	0	-	-	-	-	108	12	15:1	2
V x VII	Mexico-309 x Aurora	104	3	63:1	3	-	-	-	-	3	100	1:63*	3	102	2	63:1	3
V x VII	RB-1014 x Nep-2	91	0	NS	0	34	7	3:1	1	-	-	-	-	108	12	15:1	2
V x VII	Cuilapa-72 x Nep-2	113	0	NS	0	-	-	-	-	23	91	3:13	2	111	0	NS	0
V x VIII	RB-1014 x ICA-Pijao	81	27	3:1	1	-	-	-	-	72	28	3:1	1	67	31	3:1	1
VII x VIII	Aurora x ICA-Pijao	39	38	9:7	2	20	20	9:7	2	-	-	-	-	39	35	9:7	2
VII x VIII	Nep-2 x ICA-Pijao	92	11	15:1	2	-	-	-	-	10	91	1:15	2	92	6	15:1	2
VII x VIII	Aurora x KW-780	39	25	9:7	2	-	-	-	-	50	15	3:1	1	38	26	9:7	2

* Recessive resistant; ** for race 46, reaction scores recorded from relatively fewer plants in each cross combination due to poor viability of inoculant; NS = not segregating

two-gene differences were obtained for F_2 of two other crosses (Mexico-309 x Cuilapa-72 and ICA-Pijao x KW-780) tested against race 46.

In the test for R and S reactions to race 49, segregation in the F_2 showed no segregation in two crosses, one-gene difference in three crosses, three-gene differences in the cross Mexico-309 x Cuilapa-72, and two-gene differences in the cross CNC-2 x C-49-242. Similarly for R and S tests in the F_2 against race 53 (Table 4.7), four crosses showed no segregation; two-gene differences for two crosses and three-gene differences for the cross CNC-2 x C-49-242.

D. Gene differences for resistance and susceptibility for between-cluster crosses

Seven cross combinations showed absence of segregation in the F_2 tested against race 41, indicating similar genes for reaction to race 41 while gene differences of one (two cross-combinations), two gene differences (in seven cross-combinations), and three gene differences (in three cross-combinations) were recorded. In F_2 tested against race 46, no genetic difference in five cross-combinations, one gene difference for three cross combinations, and two gene differences for the cross Aurora x ICA-Pijao were recorded (Table 4.7).

F_2 segregation tests for reaction to race 49 also showed six cross combinations with no genetic differences implying similar gene or genes, for reaction to race 49 and one gene difference in four cross combinations, two gene differences in four cross combinations and three gene differences for S reaction in the cross Mexico-309 x Aurora (Table 4.7).

F_2 tests for reaction to race 53 similarly showed similar genes for reaction to race 53 in eight cross combinations, one gene difference in the cross Rico-Bajo 1014 x ICA-Pijao, two gene differences in seven cross combinations and three gene differences in two cross combinations (Table 4.7).

Independence and linkage/pleiotropic relationships of genes from pairwise segregation analysis

Pairwise segregation ratios (Tables 4.8 and 4.9) were examined for 17 crosses exhibiting joint segregation reactions to six paired race combinations (41/46, 41/49, 41/53, 46/49, 46/53 and 49/53) to establish whether segregations observed in the F_2 were independent or whether linkage/pleiotropy might be involved. Linkage is the tendency of genes located on the same chromosomes to be associated in inheritance whereas pleiotropy is the condition in which a single gene affects two or more distinct and seemingly unrelated traits. Of the total examined in this manner, a little over 50 percent (25 out of the 49) exhibited independence of gene assortment for reaction phenotypes observed while the remainder of the crosses showed segregation patterns that differed significantly from that of independence and suggested that linkage/pleiotropy might be operative in these crosses. On the basis of linkage/pleiotropic analysis results (Table 4.9) and depending on the paired segregation ratios for reaction to these races, 24 linkage/pleiotropic patterns were obtained for genes that confer resistance to the respective races.

Discussion

Reactions to the four races were graded using the conventional scale of Davison and Vaughn (1983) later converted to a 1 to 7 scale for computational purposes. In general these reactions fell into three discrete categories: hypersensitive resistance (HR), resistance (R) and susceptible (S), which were convenient for categorization into two classes (resistant, which included HR and R, and susceptible (S) classes) for Mendelian genetic analysis. None of these reactions that were determined using the above-mentioned grading scale bear any relationship to other categories of resistance that conferred equal protection across cultivars and otherwise known generally as horizontal resistance (Van der Plank, 1968; Vieira and Wilkinson, 1972) mechanisms that are manifested in reduced uredinial intensity (Fromme & Wingard, 1921;

Table 4.8: Pairwise (joint) segregation ratios, chi-square (X^2) values and probability levels (P) on 17 basic within- and between-cluster groups crosses examined for independent assortment and linkage/pleiotropy

Cross	Joint segregation reaction to races	Joint segregation ratios	X^2	P	Category	
					Independent	Linkage/ pleiotropy
Mexico-309 x Cialapa-72	41/46	13:3/15:1	2.5485	0.50<P<0.25	X	
LaVega x Cuilapa-72	41/46	15:1/63:1	3.9558	0.25<P<0.10	X	
C-49-242 x Nep-2	41/46	63:1/1:63	0.9761	0.90<P<0.75	X	
Nep-2 x ICA-Pijao	41/46	15:1/9:7	4.9278	0.25<P<0.10	X	
Aurora x ICA-Pijao	41/46	9:7/9:7	0.5067	0.95<P<0.50	X	
Mexico-309 x Cuilapa-72	41/49	13:3/3:13	3.8429	0.50<P<0.25	X	
LaVega x ICA-Pijao	41/49	13:3/1:63	1.0315	0.90<P<0.75	X	
C-49-242 x Nep-2	41/49	63:1/1:15	0.4746	0.95<P<0.90	X	
C-49-242 x KW-780	41/49	3:1/1:3	1.8566	0.75<P<0.50	X	
CNC-2 x ICA-Pijao	41/49	15:1/13:3	7.0379	0.10<P<0.05	X	
Mexico-309 x Aurora	41/49	63:1/1:63	2.6615	0.50<P<0.25	X	
RB-1014 x ICA-Pijao	41/49	3:1/1:3	79.9034	P < 0.01		X
Nep-2 x ICA-Pijao	41/49	15:1/1:15	5.5716	0.25<P<0.10	X	
Aurora x ICA-Pijao	41/49	9:7/1:63	1.0978	0.90<P<0.75	X	
Aurora x KW-780	41/49	9:7/3:1	2.7249	0.50<P<0.25	X	
Mexico-309 x Cuilapa-72	41/53	13:3/3:13	10.5975	0.05<P<0.01		X
LaVega x Cuilapa-72	41/53	15:1/15:1	37.3751	P < 0.01		X
LaVega x ICA-Pijao	41/53	13:3/13:3	17.6985	P < 0.01		X
C-49-242 x Aurora	41/53	15:1/15:1	42.9870	P < 0.01		X
CNC-2 x ICA-Pijao	41/53	3:1/9:7	22.1790	P < 0.01		X
Mexico-309 x Aurora	41/53	63:1/63:1	297.1904	P < 0.01		X
RB-1014 x ICA-Pijao	41/53	3:1/3:1	97.8890	P < 0.01		X
Nep-2 x ICA-Pijao	41/53	15:1/15:1	126.0791	P < 0.01		X
Aurora x ICA-Pijao	41/53	9:7/9:7	56.1213	P < 0.01		X
Aurora x KW-780	41/53	9:7/9:7	46.6694	P < 0.01		X
Mexico-309 x Cuilapa-72	46/49	15:1/3:13	6.3372	0.10<P<0.05		X
Ecuador-299 x Aurora	46/49	3:1/3:13	23.3740	P < 0.01		X
ICA-Pijao x KW-780	46/49	9:7/3:1	1.7410	0.75<P<0.50	X	
C-49-242 x Nep-2	46/49	1:63/1:15	15.6677	P < 0.01		X
RB-1014 x Nep-2	46/49	3:1/15:1	84.7750	P < 0.01		X
Cuilapa-72 x Nep-2	46/49	3:1/3:13	12.3018	P < 0.01		X
Cuilapa-72 x KW-780	46/49	63:1/1:15	0.4726	0.95<P<0.90	X	
Nep-2 x ICA-Pijao	46/49	9:7/1:15	20.0912	P < 0.01		X
Aurora x ICA-Pijao	46/49	9:7/1:63	8/2917	0.05<P<0.01		X
Mexico-309 x Cuilapa-72	46/53	15:1/13:3	6.8097	0.01<P<0.05		X
ICA-Pijao x KW-780	46/53	9:7/1:15	1.9998	0.75<P<0.50	X	
LaVega x Cuilapa-72	46/53	63:1/15:1	51.3014	P < 0.01		X
Nep-2 x ICA-Pijao	46/53	9:7/15:1	1.6702	0.75<P<0.50	X	
Aurora x ICA-Pijao	46/53	9:7/9:7	0.4216	0.95<P<0.90	X	
Mexico-309 x Cuilapa-72	49/53	13:3/13:3	5.5191	0.25<P<0.10	X	
ICA-Pijao x KW-780	49/53	3:1/1:15	1.7802	0.75<P<0.50	X	
C-49-242 x KW-780	49/53	9:7/3:1	4.2932	0.25<P<0.10	X	
CNC-2 x ICA-Pijao	49/53	13:3/15:1	7.0379	0.10<P<0.05	X	
Mexico-309 x Aurora	49/53	1:63/63:1	2.5567	0.50<P<0.25	X	
RB-1014 x ICA-Pijao	49/53	3:1/3:1	99.7795	P < 0.01		X
Nep-2 x ICA-Pijao	49/53	15:1/1:15	2.8404	0.50<P<0.25	X	
Aurora x ICA-Pijao	49/53	1:63/9:7	0.7056	0.90<P<0.75	X	
Aurora x KW-780	49/53	3:1/9:7	1.5561	0.75<P<0.50	X	

Table 4.9: Conditions for gene transmission for various cross combinations in the F_2 suggested from analysis of joint segregations ratios for reactions to pairs of rust races

Cross	Joint segregation reaction to races	ratios	Segregation Condition of gene transmission (independent or linkage)
Mexico-309 x Cuilapa-72	41/46	13:3/15:1	Two dom. genes (with rec. & dom. epist.) for R to 41 indep. of 2 dup. dom. gene for R to 46
Mexico-309 x Cuilapa-72	41/49	13:3/3:13	Two dom. genes (with rec. & domin. epist.) for R to 41 indep. of rec. epist. gene to R to 49
Mexico-309 x Cuilapa-72	41/53	13:3/13:3	Two dom. gene (with rec. & domin. epist.) for R to 41 and for R to 53 linked
Mexico-309 x Cuilapa-72	46/49	15:1/3:13	Two dup. dom. genes for R to 46 linked to 2 dom. epist. genes for R to 49
Mexico-309 x Cuilapa-72	46/53	15:1/13:3	Two dup. dom. genes for R to 46 linked to 2 dom. epist. genes for R to 49
Mexico-309 x Cuilapa-72	49/53	13:3/13:3	Two dom. genes (w/ rec. & dom. epist.) for R to 49 and R to 53 indep.
LaVega x Cuilapa-72	41/46	15:1/63:1	Two dup. domin. gene for R to 41 indep. of 3 dom. epist. factor for R to 46
LaVega x Cuilapa-72	41/53	15:1/15:1	Two dup. domin. genes for R to 41 and for R to 53 are linked
LaVega x Cuilapa-72	46/53	63:1/15:1	Three dom. epist. factor for R to 46 linked to 2 dup. dom. gene for R to 53
C-49-242 x Nep-2	41/46	63:1/1:63	Three dom. epist. factor for R to 41 indep. of 1 triple homoz. rec. gene for R to 46
C-49-242 x Nep-2	41/49	63:1/1:15	Three dom. epist. factor for R to 41 indep. of 1 double rec. for R to 49
C-49-242 x Nep-2	46/49	63:1/1:15	One triple homoz. rec. gene for R to 46 linked to one dbl rec. for R to 49
Nep-2 x ICA-Pijao	41/46	15:1/9:7	Two dup. dom. gene for R to 41 indep. of 2 compl. dom. gene for R to 46
Nep-2 x ICA-Pijao	41/49	15:1/1:15	Two dup. dom. gene for R to 41 indep. of 1 dbl. rec. gene for R to 49
Nep-2 x ICA-Pijao	41/53	15:1/15:1	Two dup. dom. genes for R to 41 and for R to 53 linked
Nep-2 x ICA-Pijao	46/49	9:7/1:15	Two compl. dom. genes for R to 46 and 1 dbl. rec. gene for R to 49 linked
Nep-2 x ICA-Pijao	46/53	9:7/15:1	Two compl. dom. genes for R to 46 and 2 dup. dom. gene for R to 53 indep.
Nep-2 x ICA-Pijao	49/53	15:1/1:15	Two dup. dom. gene for R to 49 indep. of 1 dbl. rec. gene for R to 53
Aurora x ICA-Pijao	41/46	9:7/9:7	Two compl. dom. gene for R to 41 and for R to 46 are independent
Aurora x ICA-Pijao	41/49	9:7/1:63	Two compl. dom. gene for R to 41 indep. of 1 triple rec. gene for R to 49
Aurora x ICA-Pijao	41/53	9:7/9:7	Two compl. dom. gene for R to 41 and for R to 53 linked
Aurora x ICA-Pijao	46/49	9:7/1:63	Two compl. dom. gene for R to 46 and 1 triple rec. gene for R to 49 linked
Aurora x ICA-Pijao	46/53	9:7/9:7	Two compl. dom. gene for R to 46 and for R to 53 are independent
Aurora x ICA-Pijao	49/53	1:63/9:7	One triple homoz. rec. gene for R to 49 indep. of two comp. dom. gene for R to 53
LaVega x ICA-Pijao	41/49	13:3/1:63	Two dom. genes (w/ dom. and rec. epist.) for R to 41 indep. of 1 triple rec. for R to 49
LaVega x ICA-Pijao	41/53	13:3/13:3	Two dom. genes (w/ dom. and rec. epist.) for R to 41 and for R to 53 linked

(continued)

Table 4.9: (continued)

Cross	Joint segregation reaction to races	ratios	Segregation Condition of gene transmission (independent or linkage)
C-49-242 x KW-780	41/49	3:1/3:1	One dom. gene for R to 41 indep. of one rec. gene for R to 49
C-49-242 x KW-780	41/53	3:1/9:7	One dom. gene for R to 41 linked to 2 compl. dom. genes for R to 53
C-49-242 x KW-780	49/53	9:7/3:1	Two compl. dom. genes for R to 49 indep. of one dom. gene for R to 53
C-49-242 x Aurora	41/53	13:1/15:1	Two dup. dom. genes for R to 41 and for R to 53 linked
CNC-2 x ICA-Pijao	41/59	15:1/13:3	Two dup. dom. genes for R to 41 and two dom. genes (w/ dom. & rec. epist.) for R to 49 linked
CNC-2 x ICA-Pijao	41/53	15:1/15:1	Two dup. dom. genes for R to 41 and for R to 53 linked
CNC-2 x ICA-Pijao	49/53	13:3/15:1	Two dom. genes (w/ rec. & dom. epist.) for R to 49 linked to 2 dup. dom. genes for R to 53
Mexico-309 x Aurora	41/49	63:1/1:63	Three dom. epist. genes for R to 41 indep. of 1 triple rec. gene for R to 49
Mexico-309 x Aurora	41/53	63:1/63:1	Three dom. epist. genes for R to 41 and for R to 53 linked
Mexico-309 x Aurora	49/53	1:63/63:1	One triple homoz. rec. gene for R to 49 indep. of 3 dom. epist. genes for R to 53
RB-1014 x ICA-Pijao	41/49	3:1/2:1	One dom. gene for R to 41 and for R to 49 linked
RB-1014 x ICA-Pijao	41/53	3:1/3:1	One dom. gene for R to 41 and for R to 53 linked
RB-1014 x ICA-Pijao	49/53	3:1/3:1	One dom. gene for R to 49 and for R to 53 linked
Aurora x KW-780	41/49	9:7/3:1	Two compl. dom. genes for R to 41 indep. of one dom. gene for R to 49
Aurora x KW-780	41/53	9:7/9:7	Two compl. dom. genes for R to 41 and for R to 53 linked
Aurora x KW-780	49/53	3:1/9:7	One dom. gene for R to 49 indep. of two compl. dom. genes for R to 53
Ecuador-299 x Aurora	46/49	3:1/3:1	One dom. gene for R to 46 and for R to 49 linked
ICA-Pijao x KW-780	46/49	9:7/3:1	Two compl. dom. genes for R to 46 and one dom. gene for R to 49 indep.
ICA-Pijao x KW-780	46/53	9:7/1:15	Two compl. dom genes for R to 46 and one dlb. rec. gene for R to 53 indep.
ICA-Pijao x KW-780	49/53	3:1/1:15	One dom. gene for R to 49 indep. of one dbl. rec. gene for R to 53
RB-1014 x Nep-2	46/49	3:1/15:1	One dom. gene for R to 46 and 2 dup. dom. genes for R to 49 linked
Cuilapa-72 x Nep-2	46/49	3:1/3:13	One dom. gene for R to 46 and rec. epist. gene for R to 49 linked
Cuilapa-72 x KW-780	46/49	63:1/1:15	Three dom. epist. genes for R to 46 indep. of 1 dbl rec. gene for R to 49

Shaik, 1985a; Groth and Urs, 1982) reduced spore production (Aust, 1981) longer latent period (Shaik, 1985a) or tolerance (Rodriguez, 1977) reactions.

In none of the crosses was there any segregation patterns that indicated segregation that could arise from either incompletely dominant factors as proposed by Zaumeyer and Harter (1941) and Ballantyne and McIntosh (1975) or multifactor control (polygenic) of inheritance or non-specific resistance (Ballantyne, 1974). All of the segregation patterns observed indicated oligogenic control of resistance or susceptibility (Table 4.7) depending on the dominance relationship of the interacting factors (Meiners, 1979, 1981; Ballantyne, 1978; Carrijo et al., 1980; Harter and Zaumeyer, 1941; Stavely, 1984b; Stavely, 1984c).

Single-gene differences for resistance were indicated in 14 cross-combinations (Table 4.7) with single dominant factor control in 10 cases and single recessive factor control in four of the cases studied. Wingard (1937) was probably the first to suggest single-dominant factor control of resistance in rust. The findings in this study agree with reports from several investigations (Zaumeyer and Harter, 1941; Augustine et al., 1972; Ballantyne, 1974; Ballantyne & McIntosh, 1975; Ballantyne, 1978; Christ and Groth, 1982; Kolmer and Groth, 1982; Stavely, 1984a, 1984b; Grafton et al., 1985; Stavely and Steinke, 1985; Stavely & Grafton, 1985; Kardin & Groth, 1985), which suggested that resistance in beans to single races of *U. appendiculatus* is controlled predominantly by monogenic, dominant factors. Zaiter et al. (1989) reported monogenic, recessive factor control of resistance in a cross between a resistant cultivar PC 50 and a susceptible snap bean cultivar E-Z.

Two gene differences for resistance were indicated in this study in 25 cross-combinations in which resistance was controlled in 20 out of 25 cases (Table 4.7) by dominant factor epistasis involving two loci. Of these, seven crosses exhibited complementary dominant factor control (9R:7S) of resistance, four crosses showed a combination of dominant and recessive epistasis (13R:3S) and nine crosses showed duplicate dominant factor control

(15R:1S) of resistance in the crosses to the races they were tested against. These findings agree with reports of a complementary dominant factor control of resistance as proposed by Christ and Groth (1982) for one of the races (race S1-5) and Grafton et al. (1985) who reported a similar segregation ratio in the F_2 of the cross Olathe X T-39 when inoculated with race 44. Similar findings were also reported by Finke et al (1985) on F_2 segregation to three races that suggested control by two major genes in a ratio of 13R:3S plants in which rust susceptibility was expressed in the presence of dominant allele for susceptibility and homozygous recessive alleles at the other locus. In these same 25 cross-combinations, segregation ratios of 7R:9S, 3R:13S and 1R:15S were obtained in five cross-combinations (Table 4.7).

F_2 segregations that indicated three gene differences were observed in eight cross-combinations, six of which showed segregations that suggested a three-factor, dominant epistasis (63R:1S) in agreement with the suggestion by Stavely and Steinke (1985) and Stavely (1984b) and Stavely and Grafton (1985). In two cases a segregation ratio of 1R:63S was indicated. This suggested a triple recessive factor for resistance in these crosses. In contrast segregations that indicated two, three gene differences for reaction and single, double and triple recessive gene control of reaction for R and S suggest that resistance in beans to single races of *U. appendiculatus* is not the exclusive function of monogenic, dominant factors.

Examination of the F_2 segregation data in the various cross-combinations of the within- and between-cluster groups revealed a preponderance of absence of segregation for resistance and susceptibility. A key observation here is that non-segregation, i.e. genic identity, was encountered in both the within-cluster and between-cluster crosses and more often in the within-cluster crosses (Table 4.10) than in the between-cluster crosses (Table 4.11). This was evident particularly for race 41 in 6 out of 7 crosses and in 4 out of 7 crosses for races 46 and 53. Occasional lack of segregation was also observed for race 49

Table 4.10: Number of segregating (S) and non-segregating (NS) F_2s encountered for within-cluster crosses, ratios and percentages of nonsegregation for each race and total

Cluster combinations	41	46	49	53	Total
III x III	NS	NS	NS	NS	4
IV x IV	NS	NS	S	S	2
V x V	NS	NS	S	NS	3
V x V	S	S	S	S	0
VII x VII	NS	S	S	NS	2
VII x VII	NS	NS	NS	NS	4
VIII x VIII	NS	S	S	S	1
Total non-segregating	6	4	2	4	16
Ratio of non-segregating to total NS of	6/7	4/7	2/7	4/7	16/28
Percent non-segregating	85.7	57.1	28.6	57.1	57.1

NS = no non-segregating F_2s
S = segregation observed

Table 4.11: Number of segregating (S) and non-segregating (NS) F_2s encountered for between-cluster crosses, ratio and percentages of non-segregation for each race and total

Cluster combinations	41	46	49	53	Total
III x IV	S	--	NS	S	1
III x V	NS	NS	NS	NS	4
III x V	S	NS	NS	S	2
III x VII	NS	S	NS	NS	3
III x VIII	S	--	--	S	0
IV x V	NS	S	NS	NS	3
IV x V	NS	--	--	NS	2
IV x V	NS	--	--	NS	2
IV x VII	S	NS	NS	S	2
IV x VII	S	NS	S	NS	2
IV x VIII	S	NS	S	--	1
IV x VIII	S	--	S	S	0
V x VII	S	S	S	S	0
V x VII	NS	--	--	NS	2
V x VII	NS	--	S	NS	2
V x VIII	S	--	S	S	0
VII x VIII	S	S	--	S	0
VII x VIII	S	--	S	S	0
VII x VIII	S	--	S	S	0
Total non-segregating	7	5	6	8	26
Ratio of ns: total number of crosses	7/19	(5/9)	6/15	8/18	21/52
Percent non-segregating	36.8	(55.6)	40.0	44.4	40.0

NS = number of non-segregating F_2s
S = segregation observed

(Nep–2 x Aurora and Lavega x CNC–3). This indicates similar genes for resistance in the parental cultivars included in each cross for the respective rust races used to test for such similarity.

Comparisons of non–segregation in the F_2s were made among seven basic crosses in the within–clusters combinations (Table 4.10) and nineteen crosses among the between–cluster combinations (Table 4.11). Of the total seven within–cluster crosses tested, six (85.5 percent), four (57.1 percent) two (28.6 percent) and four (37.1 percent) non–segregating F_2s were observed that were tested against races 41, 46, 49 and 53 respectively. This is in large part much higher when contrasted to the F_2s among the between–cluster crosses that indicated non–segregation when tested to the same four rust races. On the average, percent non–segregating F_2s in the within–cluster crosses was much higher (57.1 percent) than in the between–cluster crosses (40.0 percent).

The position was taken in this study that cultivars within clusters would be genetically more similar than cultivars between clusters. The finding that more crosses in the within–cluster crosses resulted in F_2s with non–segregation than in the between–cluster crosses provides support to this position. The presence of substantial number of non–segregating F_2s among the between–cluster crosses and segregation in the F_2 of within–cluster crosses, although not totally unexpected, may have been due to a number of reasons. Cultivars could be incorrectly scored as resistant, for example, in those environments where the disease was not present or in instances where the cultivar may be afforded with an escape mechanism unlike true disease resistance in the sense of a genetic host–pathogen interaction. Under these circumstances, cultivars may appear as having the same reaction grade, i.e., false resistance and therefore similar. Even when rust incidences occur, the presence of non–differentiating or poorly differentiating rust races could give the impression of cultivar similarity. In this study

(Chapter One), the differentiating capacity of race 53 which was much better than for race 41 is a case in point.

Situations also exist in the IBRNs in unusual years where disease epidemics may have been heavy or light and cultivars may have been given high or low disease scores that did not reflect optimum situations. This view is particularly paralleled by the same observation in greenhouse tests conditions in which inoculum concentration has an important relationship with symptom expression. Finally, discrepancies such as the presence of 43 percent segregation in the within-cluster crosses and the same amount (43 percent) of non-segregation in the F_2 of the between-cluster crosses can be accounted for if we assume that the four races used for the genetic study (41, 46, 49 and 53) may not be representative of the rust races encountered in the field by the 88 bean lines screened in 16 locations in the 1976 IBRN. Possibly, the bean lines selected to cross in the within-cluster and between-cluster crosses may have been too few to fairly represent the genetic situation.

The role that linkage/pleiotropy plays in genetic similarities among cultivars has been alluded to by Anderson (1949). Anderson argued that some amount of linkage is the normal condition in crop plants when large numbers of genes are involved in character expression and transmission. According to Anderson, the general effect of linkage is to cause a complex multiple gene system to simulate a single gene system in its breeding behavior and to increase greatly the proportion of F_2 individuals that resemble one or the other parent. The end result is strong correlation in the direction of the parental character combinations.

The occurrence of linkage and/or pleiotropic relationships in 50 percent of the cross combination in this study (Tables 4.8 and 4.9) suggested the control of resistance or susceptibility reactions to rust races by sets of several linked genes that agree well with views expressed above on genetic similarities. These findings are also in agreement with several similar reports by Stavely (1983, 1984a, 1984b and 1984c; and Stavely et al., 1989). A case

in point is the linkage relationship that is observed for the cross Rico-Bajo-1014 x ICA-Pijao. In this cross, monogenic dominant factors control resistance, one for each of the races 41, 49 and 53. These single, dominant genes, linked in a series, confer resistance to races41, 49 and 53. In a cross between two broadly resistant cultivars (CNC x B-190), Stavely (1984c) reported lack of segregation in 468 F_2 plants to which both parents were resistant and proposed similar genes for resistance (same or different allele for R) at a single locus for reaction to each race. Additional linked single dominant resistant genes operating on a gene-for-gene basis were also reported in this same cross (CNC x B-190) for races to which CNC was resistant and to which B-190 was susceptible. Stavely (1983) believes that such linked groups of resistance genes may occur in several of the bean cultivars that are resistant to multiple races. The possibility now exists that such linked groups of resistance genes are not truly allelic but "pseudo-allelic," that is, they are very closely linked genes giving the same reaction phenotypes to the disease in question (Anderson 1949; Adams, personal communication, 1990). The testing of very large F_2 population is required to distinguish true from "pseudo" alleles.

The findings in this study, although tentative, inasmuch as the segregating genotypes in the F_2 were not verified by F_3 segregation data, seem to support such hypotheses when examined in the light of a substantial number of crosses exhibiting linked genes for resistance to several races. Ghaderi et al. (1984) proposed a simple but logically sound model to elaborate the fundamental genetic causes for cultivar similarities of reaction phenotypes to several pathogenic races. The model was projected to explain the clustering of several (88) bean cultivars that may or may not have common pedigree relationships that clustered into eight separate groups based on their similar reaction patterns when submitted to a cluster analysis algorithm. The model proposed examines the genetic factors of both host and pathogen that may give rise to differential rust reactions for the similarly behaving members of

a cluster across environments in a given sampling year. In the model, two genotypes, G_1 and G_2, representing two members of the same cluster along with two rust environments, E_1 and E_2, are assumed for purposes of drawing views on their similarity. Further assumptions were that both members show susceptibility and resistance reactions in both E_1 and E_2, respectively. The authors argued that susceptibility of G_1 and G_2 in E_1 can be attributed to the virulent action of either the same or of different races of a pathogen. On the other hand, if G_1 and G_2 are resistant in E_2, it has to be logically attributed to similar resistance genes in both genotypes (G_1 and G_2) in accordance with the gene-for-gene concept. The model may be limited to the extent that it can only explain ideal situations in which R and S reactions are assumed in both environments E_1 and E_2. However, in reality, resistance could be ascribed to a genotype in locations that don't have the appropriate pathogenic race to which the cultivar is susceptible, or in which an escape mechanism is afforded by the cultivar. This does not, however, negate the whole model, it only warns on such situations that may give rise to genetically unfounded similarities. It would only suffice to look at the reaction summary in Table 4.9 to make one's point about genetic similarities through clustering of cultivars by their reaction response patterns. Given the few though perhaps significant shortcomings of chance seed mixtures encountered in these studies, it nevertheless provides sound reason to interpret the data in terms of genetic similarities since environmental effects have been controlled and tests were conducted on described rust races on pure line cultivars. If we were to use the four races to cluster the 13 parental cultivars (Table 4.9) we would have ended up with two clusters each time we use one variable (one race in this case) to run the cluster analysis. Since the clustering is usually done using more than one variable (race vs test locations), it is not as simple as depicting cultivar response patterns in a model and rationalizing where its cluster membership would fall.

After a thorough series of systematic studies on pathogenic specialization in *U. appendiculatus* and rust resistance in beans, Stavely (1983, 1984a; Stavely et al., 1989; Stavely, Steadman and McMillan, 1989) sums it up that the pathogenic variability described altogether was sufficient to indicate genetic similarities and differences in rust resistance for the different cultivars and germplasms used in these tests. Stavely's (1982) views on cultivar genetic similarities agree well with the model proposed above and states that the occurrence of two different kinds of resistance reactions to a single race on any two cultivars suggests that the resistant reactions of these cultivars may be conditioned by different genes. Likewise, the occurrence of similar resistant reactions to a single race on any two cultivars may indicate that the same genes control the reaction on both cultivars.

The similarities for reaction expressed among some of the parental bean cultivars to simultaneous inoculation to four races (41, 46, 49 and 53) and the number of instances of possession of similar genes for resistance or susceptibility to these same races and the complex linkage/pleiotropic relationships indicated in these results point to existing fundamental genetic interrelationships that also agree very well with several findings in similar studies (Zaumeyer & Harter, 1941; Augustine et al., 1972; Ballantyne, 1974, 1978; Ballantyne & McIntosh, 1975; Carvalho et al., 1978; Chris & Groth, 1982a, 1982b; Kolmer & Groth, 1984; Kardin & Groth, 1985; Stavely, 1983, 1984b and 1984c).

SUMMARY AND CONCLUSION

The reaction of 13 parental bean cultivars tested against four races (41, 46, 49 and 53) provided the basis for a Mendelian genetic analysis of the F_2 for several within- and between-cluster crosses.

Although many of the cultivars appeared to have their own unique interaction pattern to each rust race affording a unique reaction response pattern at times, there were several instances in which certain cultivars exhibited similarities in their response patterns to the four rust races they were tested against:

1. Cultivars LaVega, CNC-2 (in clusters IV of Ghaderi et al., 1984) and Mexico-309 and Cuilapa-72, both in cluster V showed similar reaction patterns to the four described races with occasional R or HR responses to express resistance.

2. Cultivars Mexico-235 and CNC-3 (both in cluster III), Rico-Bajo-1014 (Cluster V) and Ecuador-299 (Cluster VII) were resistant (R or HR) to all four races producing the same reaction response patterns to the four races.

3. Nep-2 and Aurora (cluster VII) were identical in their reactions to all four races, being HR to races 41 and 53 and S to races 46 and 49. The landrace cultivar C-49-242 (Cluster IV), which also is one of the parents in the pedigree of Aurora, has the same pattern for reaction response to the four races as Aurora but with a slight difference in degree of resistance.

4. Kentucky Wonder-780 and ICA-Pijao (Cluster VIII) were similar in reaction for two races (41 and 53) but displayed contrasting reactions to races 46 and 49.

5. When race 41 was considered, 11 cultivars out of 13 were resistant (R or HR) and only two cultivars were identically susceptible (KW-780 and ICA-Pijao). Nine cultivars of the 13 tested against race 46 were resistant and four cultivars (Nep-2, Aurora, C-49-242 and KW-780) were susceptible. Conversely, eight cultivars out of the 13 tested were susceptible to race 49 and five cultivars had resistance reactions (R or HR). Perhaps race 49, being the race to which most cultivars proved susceptible, may be the most virulent of the four. It is the most widely virulent, but not necessarily the most fit in natural environments.

6. The reaction of the 13 parental cultivars to race 53 was similar to that observed for race 41, with most of the cultivars, except two (KW-780 and ICA-Pijao), being resistant.

7. Similar reaction responses by cultivars to the four races suggested similar genes for resistance to the races.

8. Mendelian genetic analysis of F_2 data from within- and between-crosses revealed the following:

a) Lack of F_2 segregation in five, two, two and four within-cluster group crosses for reaction to races 41, 46, 49 and 53, respectively. This lack of segregation indicated genes for resistance to the respective races were probably identical.

b) Segregation in the F_2 that indicated a one, two and three gene difference were observed in the different within-cluster group crosses, with epistatic interactions.

c) Similarly, absence of segregation in the F_2 was observed from seven, five, six and eight between-cluster crosses for races 41, 46, 49 and 53, respectively. The absence of segregation similarly leads to the conclusion that genes for R and S in the respective parental cultivars for reaction to the four races individually were probably identical.

d) Segregation in the F_2 suggested a one, two and three gene difference for R and S was also observed for the various between-cluster group crosses, with epistatic interactions.

e) Linkage/pleiotropy relationships between genes for R and S reactions to the four races were detected. This finding also indicated linked dominant monogenic, digenic and trigenic factors for R and S to the races tested.

LIST OF REFERENCES

Alexander, H.M., J.V. Groth and A.P. Roelfs. 1985. Virulence changes in *Uromyces appendiculatus* after five asexual generations on a partially resistant cultivar of *Phaseolus vulgaris*.Phytopathology. 75:449–453.

Anderson, E. 1949. Introgressive hybridization. John Wiley, New York.

Augustine, E., D.P. Coyne and M.L. Schuster. 1972. Inheritance of resistance in *Phaseolus vulgaris* to *Uromyces phaseoli typica*; Brazilian rust race B11 and of plant habit. J. Am. Soc. Hort. Sci. 97: 526–529.

Ballantyne, B. 1978. The genetic basis of resistance to rust, caused by *Uromyces appendiculatus* in bean (*Phaseolus vulgaris*). Ph.D. thesis, University of Sydney, Australia. 262 pp.

Ballantyne, B. 1974. Resistance to rust (*Uromyces appendiculatus*) in bean (*Phaseolus vulgaris*). Proc. Linn. Soc. of N.S.W. 98:107–121.

Ballantyne, B. and R.A. McIntosh. 1975. The genetic basis of rust resistance in bean. Mimeograph of Plant Breeding Institute, Castle Hill, N.S.W.

Berger, R.D. 1977. Application of epideiological principles to achieve plant disease control. Ann. Rev. Phytopath. 15: 165–183.

Carvalho, L.P. De, G.M. Chavez and A.A. Pereira. 1978. Inheritance of resistance to five physiological races of *Uromyces phaseoli* var. *typica* Arth. in Phaseoli vulgaris L. Fitopat. Bras. 3:181–185.

Christ, B.J. and J.V. Groth. 1982a. Inheritance of resistance in three cultivars of beans to the bean rust pathogen and the interaction of virulence and resistance genes. Phytopathology 72: 771–773.

Christ, B.J. and J.V. Groth. 1982b. Inheritance of virulence to three bean cultivars in three isolates of the bean rust pathogen. Phytopathology 72:767–770.

Davison, A.D. and E.K. Vaughan. A simplified method for identification of races of *Uromyces phaseoli* var. *phaseoli*. Phytopathology 53: 456–459.

Finke, M.L., D.P. Coyne, J.R. Steadman and A.K. Vidaver. 1985. The inheritance and association of resistance to bean rust (*U. appendiculatus*) and common blight (*X. campestris* Pv *phaseoli*) in dry beans (*P. vulgaris*). Ann. Rep. Bean Improv. Coop. 28: 55–56.

Fromme, F.D. and S.A. Wingard. 1921. Varietal susceptibility of beans to rust. J. Agr. Res. 21: 385–404.

Ghaderi, A., M.W. Adams, A.W. Saettler. 1984. A quantitative analysis of host–pathogen–environment in International Bean Rust Nurseries (IBRN). Mimeograph. Crops and Soil Sci. Dept., MSU, East Lansing, Michigan, 48824.

Grafton, K.F., G.C. Weiser, L.J. Littlefield and J.R. Stavely. 1985. Inheritance of resistance to two races of *Uromyces appendiculatus* (Pers. ex. pers.) Unger var. appendiculatus) in dry edible beans. Crop. Sci. 25: 527–539.

Groth, J.V. and A.P. Roelfs. 1982. Genetic diversity for virulence in bean rust collections. Phytopathology 72: 982–983.

Groth, J.V. and R.D. Shrum. 1977. Virulence in Minnesota and Wisconsin bean rust collections. Plant Dis. Reptr. 61: 982–983.

Groth, J.V. and N.V. Rama Raja Urs. 1982. Differences among bean cultivars in receptivity to *Uromyces phaseoli* var. *typica*. Phytopathology 72: 374–378.

Kardin, M.K. and J.V. Groth. 1985. The inheritance of resistance in two white–seeded dry bean cultivars to seven bean rust isolates. Phytopathology 75: 1310 (Abstr.).

Kolmer, J.A., B.J. Christ and J.V. Groth. 1984. Comparative virulence of monkaryotic and dikaryotic stages of five isolates of *Uromyces*.

Kolmer, J.A. and J.V. Groth. 1984. Inheritance of a minute uredinium infection type of bean rust in bean breeding line 814. Phytopathology 74(2): 205–207.

Meiners, J.P. 1981. Genetics of disease resistance in edible legumes. Ann. Rev. Phytopathology 19: 189–209.

Meiners, J.P. 1979. Sources of resistance to U.S. bean rust—update. Ann. Rep. Bean Improv. Coop. 22:62–63.

Menten, J.O.M. and A.B. Filho. 1981. Monocyclic components of beans (*Phaseolus vulgaris* L.) resistance to *Uromyces appendiculatus* (Pers.) Ung.a nd their relationship with the epidemiological parameters X_0 and r. Ann Rept. Bean Improv. Coop. 24:6.

Rodriguez, C., E. Vargas and E. Portella. 1977. Resistance of cultivars of common beans to rust (*Uromyces appendiculatus*) (Urper) Fr. and comparison of the methods of evaluation with visual ratios (S). In Reunion Annual del PCCMCA 23a, Panama.

Simons, M.D. 1972. Polygene resistance to plant disease and its use in ressitant cultivars. J. Env. Quality 1:232–40.

Stavely, J.R., Steadman, J.R. and McMillan, R.T., J.R. 1989. New pathogenic variability in *Uromyces appendiculatus* in North America. Plant Disease 73:428–432.

Stavely, J.R. and M.A. Pastor–Corrales. Rust. Chap. 7. In: Bean production problems in the tropics. H.F. Schwartz and M.A. Pastor–Corrales, eds. CIAT, Cali, Columbia (In press).

Stavely, J.R. and Grafton, K.F. 1985. Genetics of resistance to eight races of *Uromyces appendiculatus* in *Phaseolus vulgaris* cultivar Mexico–235. Phytopathology 75: (Abstr.).

Stavely, J.R. and J. Steinke. 1985. BARC–rust resistance –2, –3, –4 and –5 dry bean germplasm. Hort. Sci. 20(4): 779–780.

Stavely, J.R. 1985. The modified Cobb Scale for estimating bean rust intensity. Ann. Rept. Bean Improv. Coop. 28: 31–32.

Stavely, J.R. 1984a. Pathogenic specialization in *Uromyces phaseoli* in the U. States and rust resistance in beans. Plant Disease 68: 95–99.

Stavely, J.R. 1984b. Genetics of resistance to *Uromyces phaseoli* in a *Phaseolus vulgaris* line resistant to most races of the pathogen. Phytopathology 74(3): 339–344.

Stavely, J.R. 1984c. Genetic relationship of resistance in two broadly rust resistant beans (Abstr.). Phytopathology 74: 834.

Stavely, J.R. 1983. Pathogenic specialization in *U. phaseoli* and rust resistance in bean germplasm. Paper presented at the BIC–NDBRC meeting, St. Paul, MN. Mimeograph.

Stavely, J.R. 1982a. The potential for controlling bean rust by host resistance. Pages 28–30. In: Report of Bean Improvement Cooperative and National Dry Bean Research Conference, University of Florida, Gainsville.

Stavely, J.R. 1982b. Genetics of resistance to *Uromyces phaseoli* in *Phaseolus vulgaris* breeding line B–190 (Abstr.), Phytopathology 72: 1004.

Stavely, J.R., Freytag, G.F., Steadman, J.R. and Schwarz, H.F. 1983. The 1983 bean rust workshop. Ann. Rep. Bean Improv. Coop. 26: iv–vi.

Van der Plank, J.E. 1968. Disease resistance in plants. Acad. Press, New York, NY. 206 p.

Vieira, C. and R.E. Wilkinson. 1972. The importance of field resistance and genetic diversity in bean breeding programs in south–central Brazil. Ann. Rep. Bean Improv. Coop. 15: 94–97.

Webster, D.M. and P.M. Ainsworth. 1988. Inheritance and stability of a small pustule reaction of snap beans to *Uromyces appendiculatus* (Pers.) (Ung.). J. Amer. Soc. Hort. Sci. 113(6):938–40.

Wingard, S.A. 1933. The development of rust resistant beans by hybridization. J.Agric. Exp. Stn. Tech. Bull. S1:40 pp.

Zaiter, H.Z., D.P. Coyne and J.R. Steadman. 1989. Inheritance of resistance to a rust isolate in beans. Ann. Rep. Bean Improv. Coop. 32: 126–127.

Zaumeyer, W.J. and L.L. Harter. 1941. Inheritance of resistance to six physiologic races of bean rust. J. Agr. Res. 65:599–622.

Zaumeyer, W.J. and J.P. Meiners. 1975. Disease resistance in beans. Ann. Rev. Phytopath. 13: 320–322.

CHAPTER V

GENETIC RELATIONSHIPS AMONG BEAN CULTIVARS AS EVALUATED BY CLUSTER AND OTHER MULTIVARIATE ANALYSES OF DISEASE REACTIONS, ISOZYME MOBILITY PATTERNS AND AGROPHYSIOLOGICAL CHARACTERISTICS

INTRODUCTION

Breeders routinely assess genetic relationships among genetic stocks with which they work. Resort to visual evaluations of relationships or the use of simple statistics of correlation are employed for quick assessment of relationships. More recently, the need has given rise to the development and use of powerful multivariate statistical techniques as tools for cursory examination of variability in biological data. With these methods, the opportunity exists to better examine and assess genetic interrelationships between and within biological units and the possibility of quantifying potential genetic variability in breeding materials.

Different multivariate statistical techniques have been used for different purposes but all with the main purpose of condensing information generated in the course of the investigations. The ability of most of these techniques in data reduction has helped investigators to focus attention on a few major component variables that are important in understanding existing interrelationships in the material of interest.

A judicious choice of biological traits that represents the diversity of inherent characters and best describes the biological entity in question together with a good understanding of the purposes and limitations of the different multivariate statistical techniques

will help toward a better understanding of existing interrelationships. Such variables as taxonomic metric traits, morphological, agronomic, yield and fitness data, biochemical, physiological and disease reaction data have been used for examining relationships. Almost all of the above variables have been subjected to one or another of the several kinds of cluster analysis algorithms, either singly or in combination with one or the other of the following multivariate statistical techniques: principal component analysis (PCA), canonical variate analysis (CVA) and Mahalanobis distance statistic (D^2), all with the objective of examining patterns and assessing interrelationships.

Data have been compiled or obtained in this thesis on the following:

1) Field reaction of several bean lines to bean rusts in an internationally coordinated bean rust nursery (IBRN) for three years (1975, 1976 and 1977).

2) Disease reaction to four, nine and twenty-six described rust isolates in East Lansing, Michigan, and Beltsville, Maryland, in the greenhouse, on a subset of the entries from the 1976 IBRN maintained as purelines.

3) Isozyme mobility pattern, agrophysiological and pedigree data on several bean cultivars.

The objectives of this study were:

1) To compare the clustering pattern of the original entries in the 1976 IBRN using Ward's minimum variance technique on CLUSTAN (Ghaderi et al, 1984) with the repeat clustering pattern of the same data using Ward's minimum variance method on SAS (SAS Inst., 1982) and SPSS-X (Release 2.2, 1988) and the clustering pattern of the subset entries that were maintained as purelines, and tested in controlled environments in the greenhouses using described rust isolates.

2) To compare the clustering pattern of the above original clustering with the clustering of the subset entries on the basis of their isozyme mobility, agrophysiological and disease reaction patterns to multiple races singly or as combined attributes.

3) To substantiate various clustering patterns with coefficients of parentage and Mendelian genetic analyses results.

LITERATURE REVIEW

A. Multivariate statistical procedures

1. Cluster analysis

Cluster analysis is concerned with the partitioning of a multivariate multi-observational set of data into homogeneous groups. Its basic premise is that objects should be placed in the same group if measurements of variables associated with these objects are highly similar such that subsets of the original data that have high internal consistency and maximum separability from other subsets or groups are evident. Sokal (1974) stated that the main purpose of cluster analysis is to describe the structure and relationship of the constituent objects to each other and to similar objects, and to simplify these relationships in such a way that general statements can be made about classes of objects.

However, cluster analysis is highly empirical with different methods leading to very different grouping both in number and content (Afifi and Clark, 1984). Furthermore, since the groups are not known *a priori* (or can be found from the data only), it may be difficult to judge whether the results make sense in the context of the problem being studied (Afifi and Clark, 1984).

Romesburg (1984) chooses to define a "cluster" as follows: "It is a set of one or more objects that one is willing to call similar to each other. To call two or more objects similar, one must be willing to neglect some of the details that makes them non-identical; that is, one must be tolerant of some of their differences." Owing to the problem of subjectivity of

selecting the best grouping, the user is advised to exercise reasonable judgment in the choice of cluster algorithms, resemblance coefficients and the number of groups.

Among several methods of clustering, one of the most basic approaches to clustering consists of maximizing hierarchical clustering procedures (Johnson, 1967). In this procedure the objects are treated as separate groups composed initially of one member each. The next step is to create n−1 groups by combining the two one-member groups where the aggregation or clustering will cause the least impairment to the character of either, i.e., the smallest value of Euclidian distance or Mahalanobis distance (D^2) or any other resemblance or measure of relationship. At this point, the distance from the newly formed, two-member clusters (X_1, X_2 for example) to any other one-member cluster (X_i where i = 1, 2, n) may be defined as exactly the diameter of the new set (X_1, X_2) U (X_i) where U = union. This is the simplest means of visualizing the clustering process--the maximizing method that minimizes the diameter of the clusters on each iteration. This clustering procedure is believed to yield the most homogeneous (smallest diameter) groupings if the variables selected are representative of the character of the objects to be clustered (Johnson, 1967). The clustering process continues until all n objects are included in one cluster. Some help regarding the appropriate number of groups in the cluster that is implicit from the clustering method is the sharp increase in the value of the measure of relationship (distance measure) selected as the number of cluster approaches 1 (when all n objects are considered). Plotting the changes of the distance measure (D^2 or Euclidian distance) at each iteration of the clustering procedure would reveal abrupt increase or drops of the distance measure that indicates that the last cluster formed is less homogeneous than the previously formed clusters (Johnson, 1967). It is believed that plotting the changes in the selected measures of relationship reveals the changes in the internal homogeneity. Romesburg (1984) suggested a strategy for cutting the tree (dendogram) in cluster analysis for a general purpose classification. The suggestion was to cut the tree at

some point within a wide range of the resemblance coefficient for which the number of clusters remain constant, because a wide range indicates that the clusters are well separated in the attribute space. He argued that the decision as to where to cut the tree (dendogram) is least sensitive to error when the width of the range is largest.

2. Principal component analysis (PCA)

The major intended purpose of principal component analysis (PCA) was to help reduce the complexity of multivariate data (reduce dimensionality) to a more manageable set of compound variables. Essentially, it is a multivariate technique that consists of standardization and orthogonal angular rotation of the original axes (variables) into a new set of axes that are uncorrelated variables known as principal components (PCs).

Each principal component in reality is a linear combination of the original variables (for example, varietal score on the original variables) whose variance (latent roots or eigenvalues) is one measure of the amount of information conveyed by each PC (Afifi & Clark, 1984). The PCs were arranged in order of decreasing variance with the first PC being the most informative, the second PC, the next best informative and so on until the last PC, which is the least informative. Usually, interest is focused on the first few PCs, those that account for the majority of the total variation. In addition, orthogonality of the PCs to each other indicates independent genetic contribution to variance. In matrix notation, the equation has the following form:

$[R_c - \lambda I]b = 0$ where λ = the diagonal matrix of the latent roots (eigenvalues or variance; b = matrix of latent vectors (eigenvectors) that comprise the orthogonal transformation matrix; I = the identity matrix and R_c = the matrix of correlation coefficients between pairs of variables or it could be the variance-covariance matrix depending on the objective of the user. A majority of researchers prefer to use the correlation matrix that compensates for the differential units of measurement in the different variables. The above

matrix represents a set of m homogeneous equations in m unknowns the solution of which depends on the requirement that the determinant $|R_c - I| = 0$ if the original data matrix contains n cultivars x m variables. An m^{th} degree polynomial in λ (lambda) is generated and solved for λ to produce m latent roots (eigenvalues). Reinsertion of the λ values into the original set of homogeneous equations produces the vector value b.

Once the number of the PC is selected, the investigator should examine the coefficient defining each of them in order to assign an interpretation to the components. A high coefficient in a PC on a given variable is an indication of high correlation between the variable and the PC. These PCs are interpreted in the context of the variables with high coefficients (Afifi and Clark, 1984).

In PCA it is suggested that characteristics be selected that are representative of the fundamental structure of the biological system with sufficient diversity to represent the most important dimensions of the system. In PCA there are no objective statistical testing procedures to allow measurement or evaluation of the significance of the results generated by PCA. Therefore, sound biological judgments based on the researcher's insight is very important.

3. Mahalanobis distance

One commonly used measure of distance between populations (groups) is known as the Mahalanobis distance statistic, D^2, named after its originator, an Indian statistician. Unlike simple Euclidean distance that suffers from the disadvantage that two objects may be viewed as different because their values on one variable differ markedly, D^2 takes into account 100 percent of the variance and compensates for the correlation between variables (Afifi and Clark, 1984).

The formula for calculating D^2 is as follows: $D^2 = d's^{-1}d$ where s^{-1} is the inverse of pooled within group variance-covariance matrix and d is the vector of mean differences. The

first step in the calculation of D^2 is to obtain the vector of the means for the two groups being compared followed by the calculation of s (the pooled within-group, variance-covariance matrix) and its inverse s^{-1}. Finally, the statistic D^2 is calculated from the above formula and the distance (d) between the two groups determined. Whether the distance between the two populations is significant is tested by calculating Hotelling's T^2 and then using an F-test as follows:

$$T^2 = N_1N_2/N_1 + N_2(D^2)$$

where

N_1 = size of group 1

and N_2 = size of group 2

An F statistic can be determined from the following relationship to test significance:

$$F = N_1 + N_2 - P-1/(N_1 + N_2-2-P)T^2$$

where

P = the number of variables used in the study.

B. Multivariate treatment of morphological, agronomic, taxonomic, yield and fitness traits

The methods of numerical taxonomy and other related cluster analysis methods using extensive sets of observations of metric traits have been applied to help in the interpretation of intra- and inter-specific classifications based on classical taxonomic methods (Sneath and Sokal, 1962; Sokal and Sneath, 1963; Sneath and Sokal, 1973; Sokal, 1974).

Systematic investigation of variation within *Oryza perennis* was made by Morishima (1969) using data for 24 characters, including F_1 sterility relationships of 65 strains by methods of numerical taxonomy from both phenetic and phylogenetic standpoints. Correlation coefficients and taxonomic distances were computed in a cluster analysis with the unweighted pair group method (UPGMA) algorithm and with arithmetic averages used as the clustering

method. The methods of cluster analysis and principal component analysis (PCA) gave consistent results in this study by showing that the phenetic variation patterns in *O. perennis* can be largely represented by the differentiation of strains into several geographic groups and into the perennial and annual types.

A feature of the traditional systematic classification has been to utilize a few characters and weigh those characters unequally and subjectively and where the phylogenetic relationships ultimately constructed are based on the judgment of the investigator.

In an exploratory study to test the reliability of numerical taxonomic classification techniques as applied to very closely related genotypes of barley, Molina-Cano (1976) scored 41 characters on 38 very closely related barley cultivars and subjected the standardized data matrix to two cluster analysis methods: Weighted Pair Group Method using Arithmetic Averages (WPGMC) and the Unweighted Pair-Group Method using Arithmetic Averages (UPGMC). The study was augmented by PCA to substantiate findings from cluster analysis. The results with the centroid fusion technique (UPGMC) showed two clearly separable clusters (two-rowed and six-rowed barley cultivars). The same general pattern for cultivar grouping was obtained with the arithmetic average linkage (WPGMA) without reversals. The author preferred the WPGMA over the UPGMA method. In the same study it was noted that although cluster analysis shows phenetic similarity, there were examples where a common genetic origin did not mean close phenetic similarity. This could be explained from the standpoint of the breeder's actions in which two divergent selection trends may have been followed starting from the same cultivars used as parents; or these two selection trends could be directed towards phenotypes very different from the cultivars used as parents in the cross. The author also used principal component analysis (PCA) on the data, which substantiated the same general patterns of groupings.

With the view to minimize subjectivity and classify the species in accordance with their probable phylogeny, Liang and Cassady (1966) employed the method proposed by Michener and Sokal (1957) on 22 morphological characters in 21 species of sorghum to examine the pattern of interspecific variation. The correlation matrix of the 21 sorghum species with the 22 traits was used as the basis for a quantitative index of affinity (similarity) between any two species. The analysis resulted in subdividing the species into three series comprising 14, 6 and 1 species, respectively.

Akinola and Whiteman (1972) emphasized the importance of applications of numerical analysis to agronomic and morphological variabilities in classification of crops. Ninety-five pigeon pea (*Cajanus cajan*) accessions from 11 countries were subjected to hierarchical clustering on 31 original characters of both numerical (metric) and discrete multivariate data that were weighted and standardized attributes using Euclidean distance as the similarity criterion. The analysis resulted in 15 major groups (clusters).

Numerical taxonomic techniques have been valuable for the study of variation within germplasm collections. Broich and Palmer (1980) used a cluster analysis technique to examine phenotypic variation within the USDA Soybean germplasm collection and in particular to establish more accurately the position of one gracilis-like phenotype in the subgenus Soja. Forty-nine traits were measured on 30 genotypes (OTUs) comprising three subgroups of the soybean primary gene pool. Clusters were generated by clustering the OTUs by traits (Q-analysis) and then clustering the traits by OTUs (R-analysis). The correlation coefficient was used to calculate similarity between pairs of OTUs and the resulting similarity matrices clustered by the unweighted pair group mean (UWPGM) method. The clustering showed two morphologically distinct entities (*Glycine max* and *Glycine soja*) and a third one (*Glycine gracilis*) as conspecific with *G. max* because of the weedy features of *G. gracilis*.

Investigations to estimate the extent of genetic divergence among groups based on multiple characters have been submitted to various measures of statistical distance including Mahalanobis's D^2 statistic. These and other multivariate methods, such as CVA, PCA and factor analysis have been used to augment the customary clusters analysis techniques by revealing preliminary groupings and important variable characters that influence the final clustering.

Vairavan et al. (1973) employed quality and agronomic characters of 194 rice genotypes to estimate genetic divergence. Principal component analysis (PCA) and canonical variate analyses (CVA) were employed for a preliminary grouping of genotypes owing to the large number of genotypes included. The resultant 42 groups were further classified using Mahalanobis's D^2 statistic. Nine divergent clusters were obtained in the final step of grouping. Three *indica* standards were clustered in three different clusters whereas the *japonica* formed a separate cluster, thus indicating the wide availability of variability among them. The authors noted characters that figured high for either primary or secondary differentiation. Geographical origin was found not to be related to genetic divergence.

Lee and Kaltsikes (1973) applied Mahalanobis's D^2 statistic to agronomic traits of ten durum wheat cultivars to examine genetic divergence and whether or not genetic diversity could be attributed to their geographic and/or ecological background. The authors found no association between genetic divergence and geographic origin but they succeeded in differentiating between those cultivars of tropical origin adapted to short day length and those of temperate origin requiring longer days. They also noted a better grouping of the cultivars by exclusion of two traits which were anomalous in their distributions.

A general method for quantitatively assessing genetic similarity among a set of cultivars of a given crop was proposed by Adams (1977) who also illustrated its application to dry beans in the U.S. The method is based on principal component analysis (PCA) which

computes a "distance" metric between any two cultivars in the set, the distance of which was highly inversely correlated with genetic relationships estimated from a knowledge of breeding ancestry or pedigree. On the basis of calculated distances among cultivars within given production regions (states) and a knowledge of the acreage of each cultivar grown in the region, an average weighted distance metric appropriate for each region was computed that served as an index of "genetic homogeneity" for the crop in that region. With respect to the bean crop, he pointed out that the high degree of within-class homogeneity based on biochemical and morphological trait similarities found in the various commercial classes made common beans particularly vulnerable to genotype-specific problems.

The usefulness of various measures of statistical distance between races of maize, relative to their F_2 generations, was investigated by Martinez et al. (1983). Five morphological characters of the ear and six statistical distance procedures (Euclidean, Mahalanobis Generalized distance, Modified generalized distance, approximate Dempster's distance, and Dempster's distance) were used to obtain estimates of genetic divergence between pairs of races involved in a cross (30 F_2 populations from crosses of 47 major races) and to learn the interrelationships, and facility of computation among the various distance measures. The authors concluded that Euclidean distance and Dempster's distance would be useful in studying pair-wise relationships.

Eight quantitative characters related to yield and fitness were used by Narayan and Macefield (1976) to assess the nature of genetic divergence in a world germplasm collection of chickpeas (5477 cultivars from 17 countries). They used Mahalanobis distance statistic (D^2), canonical variate and factor analysis. With the D^2 statistic, 6 clusters with substantial genetic divergences between them were identified. Further independent analysis using canonical variate analysis confirmed the results obtained from D^2 analysis. It was noted that despite an overall parallelism between genetic diversity and geographic distance, stringent natural and

human selection or geographic barriers preventing gene flow were important in the genetic divergence of the material studied.

A model which gives information on genotypic similarity in terms of mean differences, relative stability and comparative stability measures was suggested by Johnson (1977). He used cluster analysis with weighted Euclidean distance as the measure of similarity to obtain information on similarity of 49 maize hybrids grown in 18 locations. The clustering scheme arranged the hybrids into similarity groups that were differentiatable in terms of means and regression coefficients (stability index). Differences among means was the greatest source of variation among clusters.

Information on the diversity of the components of yield in parental cultivars was investigated by Ghaderi et al. (1979) in 16 genotypes of mung beans that were subjected to 18 treatment combinations (environments). Cluster analysis was used to provide an index of similarity of the genotypes in their response across environments. A hierarchical, agglomerative and polythetic algorithm (SAS from N.C. State) was used with the unstandardized Euclidean matrix in the calculation of distances among genotypes. Genetic similarity of genotypes was reflected in the phenetic similarity of the five clusters formed in an 18 dimensional space corresponding to 18 environments.

The use of cluster analysis as an adjunct to other ways of evaluating genotypic behavior was suggested by Ghaderi et al. (1980). While investigating the contribution of testing sites in Michigan to GXE interactions for test weight of wheat cultivars, they also used the same test weight data and stability parameters (mean, coefficient of regression and deviation from regression) to classify 41 genotypes of winter wheat and 16 environments (2 year x 8 locations). A hierarchical, agglomerative and polythetic clustering scheme as described by Johnson (1967) and the complete linkage method as a fusion strategy was used. Whereas cultivars were grouped in 10 clusters with regard to their test weight similarity across

16 environments, the clustering of locations into four groups was achieved by the deletion of one of the locations in the analysis of variance (AOV) which resulted in a non-significant within group G x L interaction. Cluster analysis of genotypes using stability parameters also effectively grouped genotypes according to their stability responses.

Using yield data for 39 entries common to seven of the test locations out of 98 cultivars and breeding lines of different bean types planted, Ghaderi et al. (1982) showed that cluster analysis classified the cultivars into subsets of clusters almost identically coinciding with their commercial class designations; this finding was also corroborated by the canonical variate analysis. The data were subjected to a hierarchical, agglomerative and polythetic clustering technique with the complete linkage procedure used as a fusion option on the simple correlation matrix of genotypes over environments. The authors selected the truncation level of the number of clusters to be 9 corresponding to the number of commercial bean classes known to date. The authors found that 1) two clusters could possess almost identical cluster mean yields and yet deviate in opposite directions over the same range of environments; 2) the behavior of the other members of the class across a similar range of environments can be predicted from the behavior of a given cultivar belonging to the same cluster; 3) the cluster x environment variance was substantial over the total genotype x environment variation. Adams (1977) observed that a narrow genetic base within the common bean germplasm could account for a major portion of within-cluster similarities.

Brown et al. (1983) proposed a methodology to improve the efficiency of cultivar testing first by clustering nursery environments based on selected environmental variables and secondly by identifying optimum selection environments within clusters by linear regression of the performance of genotypes within an environment on mean genotypic performance over all environments. Initially, the most predictive subset of variables was identified by regressing the site mean response for the trait on environmental variables at each site. The selected predictor

variables were converted to standard units and then weighted by the sum of squares from the multiple regression. The weighted predictor variables were finally used in a cluster analysis in order to group sites. The authors proposed a genotypic index regression method to identify sites that consistently discriminated genotypes. The authors provided a worked example of the method and asserted that an optimum selection environment that discriminates genotypes and predicts performance of genotypes should have high values for regression coefficient (b) and coefficient of determination (r^2). The authors presented a numerical illustration of the method on data from the International Rice Cold Tolerance Nursery (IRCTN) by the International Rice Research Institute (IRRI). Cluster analysis was performed by SAS PROC STEPWISE. Clustering the sites using mean heading data resulted in four clusters, whereas sites clustered by the criterion of sterility score resulted in three clusters. The analysis of data for different years gave similar results.

Carver et al. (1987) characterized responses of 70 hard red winter wheat genotypes (semi-dwarf purelines, tall purelines and F_1 hybrids) to environmental variations using linear regression and cluster analysis methods. The cluster analysis was used to classify genotypes into groups of homogeneous environmental responses. Average linkages and Ward's minimum variance method where two clusters resulting in the smallest increase in the sum of squares index were used as the clustering strategy. A cluster hierarchy was produced for each year using the CLUSTER and TREE procedure of SAS. Their results indicated high similarity between environmental responses of hybrids and semi-dwarf purelines. Responses of hybrids and tall purelines, however, were dissimilar.

Janoria et al. (1976) used 50 metric traits to classify 18 dwarf rice cultivars, which included genotypes that derived their dwarfing genes from a common grandparent, and which were grown into two different environments (high and low level of fertility). The UPGMA clustering method was used to obtain clusters from the correlation coefficient matrix. They

showed that the grouping of cultivars into seven major clusters satisfactorily matched the grouping based on pedigrees and that environment (fertility) had only a minor effect on clustering patterns. The authors observed that since cultivars were selected from populations sharing genes from a common grandparent, and possibly other germplasm as well, it would be expected that selection pressure for traits of agronomic value could well lead to an accumulation of common genes resulting in high degrees of overall similarity among various cultivars.

Acquaah (1987) employed multivariate analysis procedures (Multiple regression, PCA, PFA and Discriminant Analysis) and genetic analysis methods to elucidate the underlying interrelationships within and between two germplasm pools and to evaluate populations in a phenotypic recurrent selection scheme of a dry bean ideotype breeding program. The extent of recombination between the small-seeded architectural germplasm and the large-seeded pinto germplasm pool was revealed by PCA, while both PFA and PCA revealed optimum bean plant architectural traits defined principally by height, hypocotyl diameter, branch angle and the number of pods on the main stem. Independent loading of the architectural traits and the seed-pod traits in a principal factor analysis suggested the two sets of traits may be under separate genetic systems control.

Singh et al. (1991a) analyzed patterns of diversity at nine polymorphic loci in 227 cultivated landraces of the common bean and confirmed previous findings of the existence of two major groups (Meso-american and Andean) on the basis of variation of phaseolin seed protein at a single locus. The authors noted within each group, clusters of landraces that share a common allozyme that can also be traced to a common ancestor. Landraces representing hybrids (introgressions) between the Mesoamerican and Andean groups were also noted that indicated occasional gene flow through a mechanism of outcrossing. The same study suggested that cultivars within the same allozyme genotype, following their origination from a

common ancestor had undergone further diversification for morphological traits but not for molecular markers.

Singh et al. (1991b) in a companion paper examined diversity for morphological and agronomic traits in 306 landraces of the cultivated bean from Latin America and its relationships to phaseolin seed protein and allozyme patterns. PCA showed that Mesoamerican and Andean groups had distinct morphology confirming prior phaseolin and allozyme data. The study revealed the existence of subgroups within each of the major Andean and Mesoamerican groups with distinct morphology, adaptation and disease resistance. Hybrids of landraces with Mesoamerican phaseolin and Andean morphology and vice versa were discovered. Fifth inter-node length, number of nodes to first flower, leaflet size, and seed weight were major traits distinguishing Mesoamerican from Andean with the latter generally being larger than the former.

C. Rust disease

Disease monitoring using appropriate differential bean cultivars representing resistance sources is customary for tracking disease incidence and to learn about the extent of race composition. Over a period of time, such nurseries will yield data that reveal information on virulence relationships among the existing pathotypes and genetic similarities of the cultivars used in the test when such data are subjected to appropriate multivariate statistical techniques. In addition, changing patterns in virulence relationships of certain pathotypes and presence or lack of resistance genes in the cultivar against such virulent pathotypes can be learned when nursery data are subjected to cluster analysis and other multivariate statistical methods to look at patterns by location, year, rust pathotype or cultivar.

In two-cluster analysis studies performed separately on bean rust isolates collected from two different regions (Region 1 = Nebraska and Colorado, 1979-1986, and Region 2 =

the Dominican Republic, 1982–1985), Miles and Steadman (1989) clustered 78 rust isolates for region 1 (58 isolates collected from Nebraska and Colorado, plus 20 previously described races by Stavely, 1984a) and 91 rust isolates from region 2 (71 isolates collected from the Dominican Republic and the same 20 previously described races mentioned above). The authors used the most common (predominant) primary leaf reaction for their analysis which resulted in three cluster groups for region 1 and seven cluster groups for region 2. The study revealed the virulence relationship that existed among the *U. appendiculatus* isolates. For both regions, isolates that had similar reaction patterns were clustered together with isolates that were of the same race, having the smallest distance between them. Clusters contained isolates from different locations or years for region 1, indicating the presence of virulence patterns that may be reappearing, while in region 2 isolates clustered by field collections, year or geographic region. In both cases, unnecessary virulence was observed within the local pathogen population.

Using a large sample of bean germplasm subjected to a wide array of rust races from diverse geographic areas in an International Bean Rust Nursery (IBRN), Ghaderi et al. (1984) partitioned the cultivars into groups with similar response patterns (clusters) using quantitative statistical procedures and cluster analysis techniques. The authors used a hierarchical, agglomerative clustering scheme merging cultivars based on Ward's method that yields the least increase in the error sum of squares. They selected the number of clusters to be 8 since this gave the greatest contrast of within–cluster to between–cluster sum of squares. Similarly, the 16 geographic locations were subjected to the same clustering scheme that grouped them into 6 clusters on the basis of eliciting similar response from the 88 genotypes used in the initial clustering scheme. In the same study, they found support for their hypothesis of race specificity among sites and race–specific host response. The study gave rise to the suggestion

that genotypes within clusters would be similar or possibly identical for the genes or genic complexes conditioning reactions to rust.

The various multivariate analysis techniques applied to biological data singly or in combination have resulted in patterns that reveal inherent relationships within and among the various interacting units. These patterns are translated by the investigators in terms of genetic identity or similarity of genotypes, or virulence relationships among several pathotypes if disease data were used.

MATERIALS AND METHODS

The following data were subjected to different cluster analysis algorithms to compare cluster memberships with the original clustering results of the bean cultivars included in the 1976 IBRN (Ghaderi et al., 1984).

Cluster analysis of bean cultivars

A. Disease reaction grades in the greenhouse

1. Disease reactions grades of 19 bean cultivars tested against 26 rust races in Beltsville, MD.
2. Disease reaction grades of 23 bean cultivars tested against four and nine rust races in Beltsville, MD.

In the cluster analysis of 19 x 26, 23 x 4 and 23 x 9 cultivar by rust race data, respectively, each cultivar was represented by a vector whose elements correspond to the rust scores when inoculated by each of four, nine and twenty-six races, respectively. Measures of similarity were based on Euclidean distance among cultivars calculated on the basis of a geometrical model of four, nine and twenty-six dimensions, respectively (Table 5.1).

B. International bean rust nurseries (IBRN) 1975, 1976 and 1977

International Bean Rust Nurseries (IBRN) coordinated by the Centro Internacional de Agricultura Tropical (CIAT) were conducted in 1975, 1976 and 1977 with 15, 17 and 17

Table 5.1 Dimensions of matrices for various different experiments for cluster and other multivariate analyses

Experiment	Data Summary	Geometric Matrix of Dimensions	Matrix of Distances
Greenhouse tests 19 cultivars vs 26 races, Beltsville	19 cultivars, 26 races	26	19 x 19
Greenhouse tests 23 cultivars vs 9 races (Beltsville)	23 cultivars, 9 races	9	23 x 23
Greenhouse tests of 23 cultivars vs 4 races (E. Lansing)	23 cultivars 4 races	4	23 x 23
IBRN 1976, 88 cultivars and 16 locations	88 cultivars 16 locations	16	88 x 88
IBRN 1975, 46 cultivars and 6 locations	46 cultivars, 6 locations	6	46 x 46
IBRN 1977, 52 cultivars and 14 locations	52 cultivars 14 locations	14	52 x 52
Greenhouse tests 26 races x 19 cultivars in Beltsville, MD	26 races 19 cultivars	19	26 x 26
Greenhouse tests 33 races x 19 cultivars in Beltsville, MD	33 races, 19 cultivars	19	33 x 33
16 cultivars x 27 traits (combined)	16 cultivars 27 traits	27	16 x 16
38 cultivars x 22 locations IBRN 1975 and 1976 comb.	38 cultivars, 22 locations	22	38 x 38
20 cultivars x 12 enzymes	20 cultivars, 12 enzymes	12	20 x 20
22 cultivars x 6 agrophys.	22 cultivars 6 agron.	6	22 x 22

cooperating locations, respectively. One hundred thirty-two, 132 and 118 cultivars were included in these same nurseries in 1975, 1976 and 1977, respectively. For clustering purposes, only 6, 16 and 14 locations were selected along with their respective cultivars of 46, 88 and 52 that were uniformly tested in 1975, 1976 and 1977, respectively. Each cultivar was represented by a vector whose elements correspond to the rust scores in each of 6, 16 and 14 locations for 1975, 1976 and 1977, respectively. Euclidean distances among cultivars for each year were calculated separately to serve as a measure of similarity on the basis of a geometrical model of 6, 16 and 14 dimensions for 1975, 1976 and 1977, respectively.

The resulting matrix from the greenhouse tests in both East Lansing (MI) and Beltsville (MD) and the IBRN data for 1975, 1976 and 1977 seasons gives the following dimensions of matrices, listed in Table 5.1.

C. Combined and transposed data

1. Transposed data

The disease reaction response data of 19 cultivars x 26 races were transposed to give a 26 race x 19 cultivar matrix. This matrix was subjected to a cluster analysis algorithm to investigate the cluster grouping pattern of the rust races on the basis of eliciting similar responses on the 19 cultivars. The resulting matrix of distances (26 x 26) based on a geometrical model of 19 dimensions is shown in Table 5.1.

2. Rust collections in the U.S.

Thirty-three bean rust collections in continental U.S. made by Stavely et al. (1989) and disease reaction data on 19 different bean cultivars in Beltsville, MD (Stavely et al., 1989) were used for cluster analysis purposes. The 33 races x 19 cultivars matrix was subjected to various hierarchical clustering algorithms in order to see the cluster grouping patterns on the basis of eliciting similar responses on 19 differential cultivars. The resulting matrix of

distances (33 x 33) on the basis of a geometrical model of 19 dimensions is shown in Table 5.1.

3. Combined agronomic, disease and isozyme mobility data

The data on six agrophysiological traits, disease reaction grades to nine bean rust races in the greenhouse, and isozyme mobility pattern for 12 enzyme systems were combined for 16 cultivars that were uniformly scored for these traits. The matrix of 16 cultivars x 27 traits was subjected to several cluster analysis algorithms to investigate the cluster grouping of the bean cultivars based on their scores on the combined parameters. The resulting matrix of distances (16 x 16) on the basis of a geometrical model of 27 dimensions is shown in Table 5.1.

4. Combined IBRNs

The data for 1975 and 1976 IBRN was combined for 38 cultivars and 22 locations, giving a raw data matrix of 38 x 22. This was subjected to various cluster analysis algorithms to study the cluster grouping patterns of the cultivars common to both years on the basis of their reaction responses to the rust races prevalent during these years. The resulting matrix of distances (38 x 38) on the basis of a geometrical model of 22 dimensions is shown in Table 5.1.

The matrices of distances in Table 5.1 were then subjected to a hierarchical, agglomerative clustering scheme in separate runs following initial cluster search employing principal component analysis (PCA) and a non-hierarchical clustering procedure on SAS (FASTCLUS). Merging or fusion of cultivars was done using single linkage (SLINK), complete linkage (CLINK), CENTROID, AVERAGE and WARDS method (Romesburg, 1984) for the respective data using either the SAS (1985) or SPSS-X programs for running the clusters. The number of clusters in each data set was determined by cutting the tree or dendogram from cluster analysis of each data set at a point or value with a wide range of the resemblance coefficient for which the number of cluster remains constant, i.e., at the widest

range of the resemblance coefficient where the clusters are well separated in attribute space (Romesburg, 1984).

D. Mahalanobis distance

The Mahalanobis distance (D^2) between pairs of clusters was calculated from the relationship $D = (d's^{-1}d)^{1/2}$ for each cluster analysis data set where d is the vector of differences and S^{-1} is the inverse of the pooled within-group variance-covariance matrix. The SAS (1985) MAH option was specified in the CANDISC procedure to obtain the generalized distances among pairs of clusters.

RESULTS AND DISCUSSION

A. Field reactions to endemic races in international bean rust nurseries (1975–1977)

1. Cluster analysis results of field reactions of 88 bean cultivars to bean rust in the 1976 IBRN

Field reaction scores for 88 bean cultivars to endemic (prevalent) rust races in the 1976 IBRN are shown in Table 5.2.

Following the lead from an initial cluster search using PCA and the non-hierarchical clustering scheme of FASCLUS in SAS, the final decision for clustering was based on Romesburg's (1984) criterium that states: to achieve best results, it is desirable to cut the cluster dendogram (tree) at some point in the hierarchical clustering where the width of the ranges in the resemblance coefficient is the largest and therefore least sensitive to error. Using this criterium, six cluster groups were obtained in SAS program when Ward's minimum variance method was used as fusion option (Figure 5.1, Table 5.3). Mahalanobis's distance (D) calculated for the clusters by Ward's method that are reflections of contrasting response patterns among the clusters, ranged from 4.24, the distance between clusters I and II to 10.58, the distance between clusters II and IV (Table 5.4). Figure 5.2 displays the relationship or differences in response patterns of cluster members along the first, second and thirdprincipal axes and accounting for 54.6 percent of the total variation of a principal component analysis of the reaction responses in sixteen environments in the 1976 IBRN. Ghaderi et al. (1984) using Ward's minimum variance method on CLUSTAN and the criteria of the greatest contrast of

Table 5.2: The reaction of 88 bean lines uniformly tested in 16 locations internationally in the 1976 International Bean Rust Nursery (IBRN)

Variety Name	Locations															
	A	C1	C2	E1	E2	G	M	P1	P2	P3	U1	U2	B1	Bz	D	E3
1 4691-54-1	3	4	4	3	1	2	3	1	4	3	2	3	3	4	2	3
2 Redlands Pion	3	1	2	3	3	3	2	3	1	2	4	2	3	2	4	2
3 11411	1	3	4	3	2	4	3	1	3	4	2	2	3	3	1	2
6 Diacol Calima	3	2	2	3	2	2	4	1	2	2	3	2	3	4	4	2
7 Comp. Chimal-3	1	1	2	2	2	3	4	1	2	1	2	2	2	3	1	2
8 SH-30CI-PM-PI	1	2	3	4	2	3	4	1	3	3	2	1	2	2	3	2
9 Cullapa-72	1	2	4	3	1	4	1	1	1	1	2	1	1	3	3	2
10 PR 12	3	2	2	4	3	2	4	4	2	2	4	2	3	2	3	2
11 PR 19	1	1	3	3	4	4	1	2	3	2	2	2	2	3	1	2
13 Mexico-309	1	1	4	3	4	3	1	1	1	1	2	2	1	2	1	3
14 Turrialba 1	1	1	3	3	3	2	4	2	2	3	2	1	2	3	3	2
15 ICA-Guali	3	1	2	4	2	2	3	4	2	2	3	2	3	3	3	2
16 Villa Guerrero	1	4	3	4	3	3	4	1	2	4	2	1	4	4	4	3
18 San Pedro Pinula	1	2	3	2	3	3	4	1	3	4	2	1	1	2	3	2
19 Turrialba 4	1	1	4	4	3	2	4	1	1	1	2	1	1	3	3	2
20 Westralia	1	3	4	4	4	4	4	1	4	4	4	2	3	4	4	3
21 PI 319649	1	2	4	3	4	4	4	1	2	4	2	2	1	2	4	2
22 Porrillo 1	1	4	4	3	3	3	4	1	3	4	3	2	3	4	4	2
23 Rico Pardo 896	1	2	3	3	4	4	4	1	3	3	1	1	2	2	1	2
25 Linea 37	1	2	3	2	4	4	4	1	2	3	2	3	1	4	1	2
26 Ecuador-299	1	4	3	2	3	3	4	1	2	2	2	1	2	3	3	2
27 Porrilla 70	3	4	4	3	3	2	4	1	2	4	3	2	3	3	4	2
28 142-ML-PM-PI	1	4	3	3	3	3	4	1	3	4	2	1	2	4	4	2
29 ICA-Tui	1	4	4	3	3	4	4	1	2	4	3	1	3	4	4	2
32 Venezuela-54	1	4	4	3	2	3	4	2	3	4	2	1	4	4	4	2
33 PR-5	1	1	4	2	3	3	4	1	3	2	2	1	2	3	3	2
34 CNC-3	1	1	2	4	4	4	4	1	1	3	2	1	1	1	4	2
35 Porrillo Sint.	3	4	4	3	3	2	4	1	3	4	3	2	3	3	4	2
36 173-ML-PM-PI	1	3	4	4	4	4	4	1	2	4	3	2	3	3	4	3
37 PI-31377	3	1	3	4	3	2	4	4	2	3	3	2	3	4	4	2
38 PR-4	1	4	4	4	3	3	4	1	3	4	1	1	3	4	4	2
39 PR-9	1	4	4	4	3	3	4	1	4	4	2	2	4	4	4	2
40 PR-3	1	3	4	3	3	3	4	1	4	4	2	2	3	4	4	2
41 Linea 34	1	2	4	3	4	3	4	1	4	4	3	4	3	4	4	2
42 PR-1	1	2	3	3	3	2	4	1	2	4	2	2	3	2	1	2
44 Cornell-49-242	1	2	3	3	3	3	4	1	3	3	2	2	3	3	3	2
45 Negro SR #5	3	1	4	4	3	4	4	1	4	2	3	2	3	3	1	4
46 PR-21	1	4	4	4	3	2	4	1	3	3	3	3	3	4	4	2
48 PI 163372	1	4	4	4	3	4	1	1	3	4	2	1	4	4	2	2
49 Nep-2	1	4	4	3	3	4	3	1	3	4	2	1	3	3	4	2
50 Diacol Nima	3	2	2	4	3	2	3	4	2	2	3	2	3	3	1	2
52 ICA-Pijao	3	4	2	3	2	2	1	1	3	3	3	3	2	1	3	2
55 Rico Bajo 1014	1	3	2	2	3	4	1	1	2	3	4	1	1	3	3	4
56 PI 313664	3	3	1	4	3	2	1	4	2	3	4	1	3	2	3	3
57 PI 165426	1	4	4	4	3	4	4	1	4	4	2	1	3	3	4	4

Table 5.2: (continued)

Variety Name	Locations															
	A	C1	C2	E1	E2	G	M	P1	P2	P3	U1	U2	B1	Bz	D	E3
58 Jamapa	1	4	4	3	3	3	4	1	3	3	2	2	4	4	4	2
60 PI 226883	3	3	3	4	3	3	3	4	2	4	4	1	3	3	4	4
61 PI 152326	1	4	4	4	3	4	4	1	4	4	1	1	3	4	4	3
62 PI 307824	3	4	4	4	3	4	4	1	4	4	1	3	4	4	4	3
64 Actop x San 37	1	4	4	2	3	4	4	1	4	4	2	2	2	2	4	2
65 Actop x San 39	1	4	4	3	3	3	3	1	3	4	2	2	2	2	3	3
66 Actop. x San 51	1	3	4	2	3	4	3	1	4	4	2	1	1	2	1	3
68 Aurora	1	4	4	4	4	4	4	1	3	4	2	2	3	3	4	2
73 Cacahuate 72	3	2	3	4	2	2	3	4	2	3	3	2	2	3	4	2
75 Comp. Cotaxtla	3	4	4	4	3	4	4	1	4	4	3	2	3	4	4	2
76 Costa Rica 1031	1	4	3	3	2	3	3	1	4	3	1	1	2	2	4	3
77 Guatemala 416	1	4	4	4	4	4	4	1	4	4	3	2	3	4	4	3
78 Honduras 46	3	4	4	4	4	4	4	1	4	4	3	3	3	4	4	2
79 LaVega	1	2	4	3	4	4	4	1	4	3	2	1	2	2	3	3
80 Manteigao PR 20	3	2	2	4	3	2	4	4	2	3	4	2	3	3	3	2
82 Mexico-235	1	3	2	2	3	4	4	1	1	2	1	1	2	3	1	3
83 Miss Kelly	3	4	4	4	3	4	4	1	3	3	2	1	3	4	4	3
84 Mogul	3	4	4	3	3	4	4	1	2	3	3	2	3	4	4	3
85 M/WhfRnr	3	4	3	4	3	2	4	2	4	3	4	3	3	3	4	3
88 Panamito Corr.	1	3	4	3	3	3	4	1	3	4	3	1	3	3	4	2
90 PI 165435	1	4	4	3	4	4	4	1	3	4	4	1	3	3	4	3
91 PI 207262	1	4	4	4	3	4	4	4	4	4	4	2	3	3	4	3
92 PI 310814	3	4	4	4	4	4	4	1	3	4	4	2	2	4	4	3
94 PI 310878	1	4	4	4	3	4	4	1	3	4	2	2	3	4	4	3
95 PI 313524	1	4	3	3	3	3	4	1	1	3	2	1	3	2	4	3
97 Portland Red	3	4	4	4	4	4	1	4	4	4	3	3	3	4	4	3
99 Preto 897	1	4	4	3	3	3	4	1	3	4	1	1	3	3	4	2
101 PR-6	1	1	4	4	4	4	4	1	4	3	2	2	2	2	2	3
103 PR-15	3	1	2	4	2	2	4	4	2	4	3	2	2	3	4	3
104 PR-1	3	2	2	4	3	2	4	3	4	3	3	2	3	3	4	2
105 Redlands AC	3	2	2	4	2	2	3	4	2	2	3	2	2	3	3	2
106 Redlands GL-C	3	3	2	3	2	2	4	2	3	3	2	1	3	2	4	2
108 Redlands GL-B	3	3	2	3	2	2	4	1	3	3	2	2	2	2	4	2
109 Bountiful-181	3	3	2	4	2	2	4	4	3	3	4	2	3	3	4	2
110 Brown Beauty	3	3	3	4	3	2	4	4	3	3	4	2	3	3	4	3
111 Canario-101	4	2	2	4	2	2	3	4	2	3	4	2	3	2	2	3
112 CA Sm. Wht. 643	3	4	2	3	3	2	4	1	2	2	3	1	3	2	4	2
113 CCGB-44	3	4	3	3	3	3	4	1	4	4	1	1	4	4	4	2
115 Epicure	4	4	4	4	4	4	4	1	4	4	4	3	3	3	2	3
118 KW-780	4	4	3	4	3	3	1	2	4	4	4	3	3	3	4	3
119 KW-814	1	4	4	3	4	2	4	1	4	3	3	3	3	3	4	3
123 Veracruz 1A6	4	3	4	3	3	3	4	1	4	4	3	3	3	3	4	3

A = Australia C1 = CIAT C2 = Costa Rica U1 = USA, Beltsville G = Guatemala P1 = Peru P2 = Puerto Rico Is.
P3 = Puerto Rico Lim. E2 = El Salvador B1 = Brazil D = Dominican Republic E3 = El Salvador E1 = Ecuador U2 = USA, Michigan
M = Mexico Bz = Brazil, 77

Table 5.3: Ward's minimum variance clustering in SAS of field reactions of 88 bean cultivars in the 1976 IBRN

I	II	III	IV	V	VI
4691-54-1	Villa Guerero	Redland Pioneer	PR-12	1141	Cuilapa-72*
Porillo-70	142-ML-PM-PI	Diacol Calima	Manteigo Preto-20	PR-1	Mexico-309*
Porillo Sintetico	PR-4	Redlands GLF-B	Diacol Nima	Rico Pardo 896	Rico-Bajo-1014*
M/WhfRnr	Preto-897	Redlands GLF-C	Canario-101	LaVega*	
Veracruz 1A6	Nep-2*	Ca Sm White 643	ICA Guali	PR-6	
PI 307824	Venezuela-34	ICA-Pijao*	Redlands Autumn Cr	Actopan x San 51	
CCGB-44	PR-9	KW-780*	Cacahuate-72	Negro San Ram #5	
Miss Kelly	Jamapa		PI 31377	CNC-3*	
Mogul	PR-3		PR-15	Mexico-235*	
Comp Cotaxtala	PI 165426		PR-1	PR-19	
Honduras 46	PI 152326		Bountiful-181	Linea-37	
PI 310814	Aurora*		Brown Beauty	SH-30CI-PM-PI	
Portland Red	PI 310878		PI 313664	C-49-242*	
Epicure	PI 163372		PI 226883	Turrialba-1	
	Ecuador-299*		PR-5	PR-5	
	PI 313524		Turrialba-4		
	Actopan x San. 37		San Pedro Pinula		
	Actopan x San. 39		PI 319649		
	Costa Rica 1031		CNC-2*		
	Westralia				
	Guatemala-416				
	173-ML-PM-PI				
	PI 165435				
	Porillo-1				
	Panamito Corr.				
	ICA TU1				
	PR-21				
	PI 310739				
	PI 207762				
	Linea-34				
	KW-814				

* Subset of cultivars that clustered together in the 1976 IBRN by Ghaderi et al. (1984).

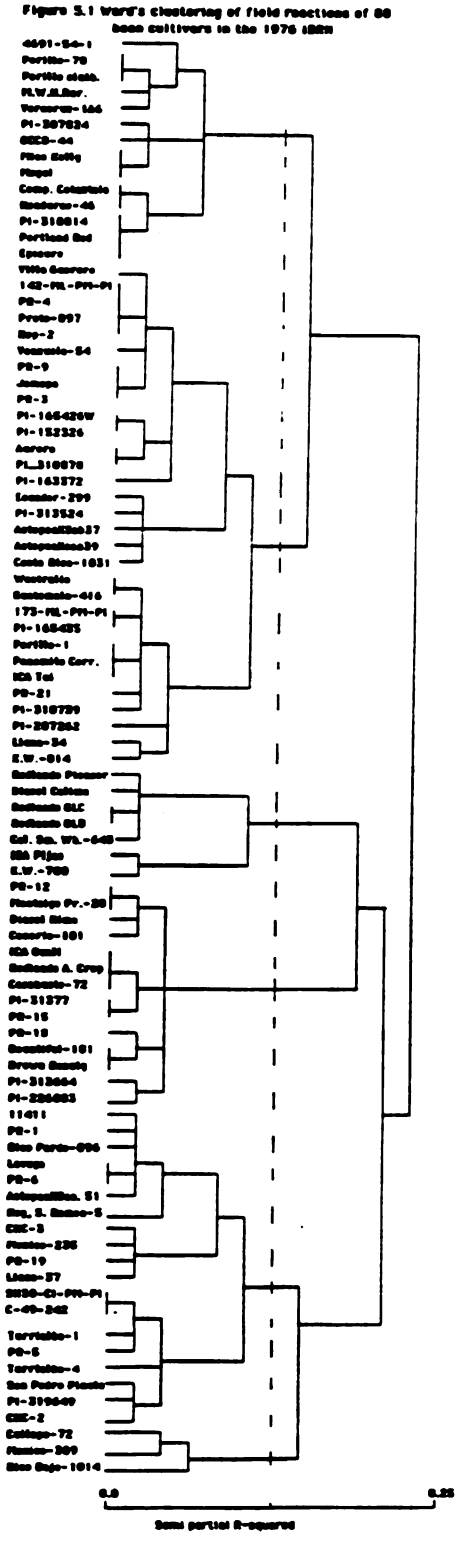


Figure 5.1 Ward's clustering of field reactions of 88 bean cultivars to the 1976 SBCM

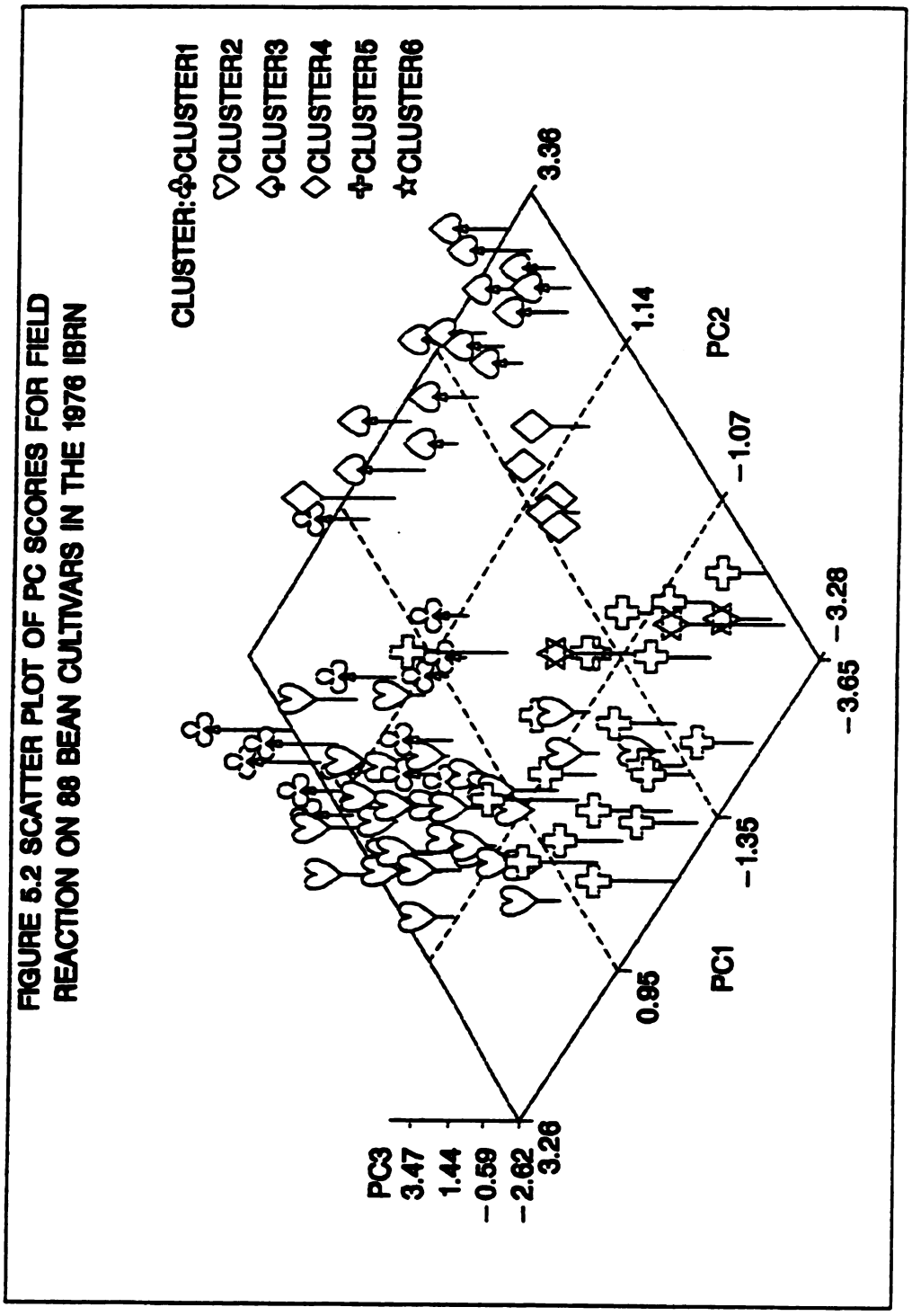


FIGURE 5.2 SCATTER PLOT OF PC SCORES FOR FIELD REACTION ON 88 BEAN CULTIVARS IN THE 1976 IBRN
CLUSTER:ΦCLUSTER1
▽CLUSTER2
◇CLUSTER3
◇CLUSTER4
✣CLUSTER5
☆CLUSTER6
PC3
3.47
1.44
−0.59
−2.62
3.26
PC1
0.95
−1.35
PC2
3.36
1.14
−1.07
−3.28
−3.65

within cluster to between cluster sum of squares in the analysis of variance found eight cluster groups to be optimal (Table 5.5).

Table 5.4. Mahalanobis' distance (D^2) among 6 clusters with different rust reaction patterns in the 1976 IBRN

Clusters	I	II	III	IV	V	VI
I	0.00					
II	5.20	0.00				
III	4.24	5.89	0.00			
IV	4.98	8.41	5.63	0.00		
V	5.62	7.75	4.93	5.28	0.00	
VI	9.84	10.58	7.99	9.88	7.44	0.00

For purposes of comparison, field reaction data of the same 88 bean cultivars in the 1976 IBRN was subjected to complete and average linkages in SAS and by complete linkage and Ward's method using SPSS-X Release 2.2 (SPSS Inc., 1988). The cluster outcomes, whether average linkage, complete linkage and Ward's methods in SAS, or Ward's method in CLUSTAN, or complete linkage and Ward's method in SPSS-X Release 2.2 was the consistent cluster grouping by all methods of the subset of cultivars: CNC-2, LaVega and Mexico-235 (III in Ghaderi et al., 1984), CNC-2 and C-49-242 (IV in Ghaderi et al, 1984), Cuilapa-72, Mexico-309 and Rico-Bajo-1014 (V in Ghaderi et al., 1984), Nep-2, Aurora and Ecuador-299 (VII in Ghaderi et al., 1984) and ICA-Pijao and KW-780 (VIII in Ghaderi et al., 1984) into the same pattern of clustering as in the original clustering by Ghaderi et al., 1984. One exception was the separation of LaVega from the group with complete linkage. However, in all cases, the two distinct clusters in the original clustering comprising CNC-3, LaVega and Mexico-235 (Cluster III) and cultivars CNC-2 and C-49-242 (Cluster VI) were lumped together as members of one large cluster. The pattern of clustering was remarkably similar regardless of system program or cluster algorithm used in the remainder of cultivars not

Table 5.5: Composition of original eight clusters of bean cultivars with varying response patterns across 16 locations

CLUSTER I
1. 4961-54-1
2. Porillo 70
3. Porillo Sintetico
4. PI-307824
5. Compuesto Cotaxtala
6. Honduras-46
7. Miss Kelly
8. Mogul
9. Mountaineer Whitehalf Runner
10. PI-310814
11. Portland Red
12. CCGB-44
13. Epicure
14. Veracruz 1A6

CLUSTER II
15. Redlands Pioneer
16. Diacol Calima
17. Redlands Greenleaf C
18. Redlands Greenleaf B
19. California Small White 643

CLUSTER III
20. 1141
21. Compuesto Negro Chimatenango-3
22. PR-19
23. Rico Pardo 896
24. Linea 37
25. PR-1
26. Negro San Ramon No. 5
27. Actopan x Sanilac-51
28. LaVega
29. Mexico-235
30. PR-6

CLUSTER IV
31. SB-30-CI-PM-PI
32. Turrialba 1
33. San Pedro Pinula
34. Turrialba-4
35. PI-319649
36. PR-5
37. Compuesto Chimaltenango-2
38. C-49-242

CLUSTER V
39. Cuilapa-72
40. Mexico-309 (B-190)
41. Rico-Bajo-1014

CLUSTER VI
42. PR-12
43. ICA-Guali
44. PI-313667
45. Diacol Nima
46. PI 313664
47. PI 226883
48. Cacahuate 72
49. Manteigao Preto 20
50. PR-15
51. PR-18
52. Redlands Autumn Crop
53. Bountiful 181
54. Brown Beauty
55. Canario 101

CLUSTER VII
56. Villa Guerrero
57. Westralia
58. Porrillo-1

CLUSTER VII (continued)
59. Ecuador-299
60. 142-ML-PM-PI
61. ICA-TUI
62. Venezuela-54
63. 173-ML-PM-PI
64. PR-4
65. PR-9
66. PR-3
67. Linea-34
68. PR-21
69. PI 163372
70. Nep-2
71. PI 165426 (white)
72. Jamapa
73. PI 152326
74. Actopan x Sanilac-37
75. Actopan x Sanilac-39
76. Aurora
77. Costa Rico-1031
78. Guatemala-416
79. Panamito Corriente
80. PI 165435
81. PI 207262
82. PI 310739
83. PI 310878
84. PI 313524
85. Petro 897
86. Kentucky Wonder 814

CLUSTER VIII
87. ICA-Pijao
88. Kentucky Wonder 780

included in the subset of selected clusters mentioned above and comprising the majority of the lines. Similarly, the 16 locations were subjected to two cluster analysis methods (complete linkage and Ward's method) on the basis of their eliciting similar responses on the 88 bean lines. The composition of clusters of these tests sites appear in Figure 5.3 and Table 5.6. The clustering in SPSS-X by the complete linkage method produced four clusters which were at variance with the grouping of the 16 locations into 6 clusters on the basis of their eliciting similar responses from the 88 genotypes by Ward's method on both SPSS-X and CLUSTAN programs. The test sites of the same clusters producing similar types of reaction on the bean cultivars reflects the presence of the same or similar pathogenic races in these sites.

2. Cluster analysis results of field reactions of 52 bean cultivars in the 1975 IBRN

Rust reaction scores of 52 bean cultivars to population races in six locations in the 1975 IBRN is presented in Table 5.7. Five, five and four cluster groups were obtained for complete linkage, average linkage and Ward's minimum variance method (Figure 5.4, Table 5.8) when Romesburg's (1984) criteria for classification was applied on the cluster dendogram (tree). Figure 5.5 shows the relationships and differences in reaction response patterns of cluster members along the first, second and third principal axes (accounting for 75.1 percent of total variation) of a principal component analysis of the reaction responses in 6 locations in the 1975 IBRN. Mahalanobis's distance (D) calculated for the four clusters by Ward's method ranged from 3.21, the distance between Clusters I and II to 5.55, the distance between clusters II and IV (Table 5.9).

Of the 52 bean cultivars in the 1975 IBRN and 88 bean cultivars in the 1976 IBRN, there were 38 bean cultivars that were common to both IBRNs. These 38 cultivars common to both years of clustering in both years were used for comparing the various features of the patterns. The two most important factors that give distinctions between the 1975 IBRN and

Table 5.6: Cluster analysis of sixteen rust environments (using complete linkage and Ward's method on SPSS-X) in the 1976 IBRN for eliciting similar rust reaction patterns on 88 bean cultivars

Complete Linkage

C1	C2	C3	C4
El Salvador	USA, Beltsville, MD	Mexico	Australia
Guatemala	El Salvador 77	Dominican Republic	USA, Michigan
Costa Rica			Peru
Puerto Rico Limani			
Puerto Rico - Isabella			
Centro Int. Agri. Tropic.			
BRAV			
Brazil 77			
Ecuador			

Ward's Method

C1	C2	C3	C4	C5	C6
El Salvador	BRAV	Mexico	USA, Beltsville, MD	Australia	Peru
Guatemala	Brazil 77	Dominican Republic	El Salvador 77	USA, Michigan	
Costa Rica	Ecuador				
	Puerto Rico - Isabella				
	Puerto Rico Limani				
	Centro Int. Agri. Tropic.				

Table 5.7: The reaction of 52 bean cultivars uniformly tested in 6 locations in the 1975 IBRN coordinated by CIAT

Variety Code	Name	Location					
		Brazil, Vicosa	CIAT, Palmira (Feb.)	CIAT, Palmira (Apr.)	CIAT, Palmira (Oct.)	Costa Rica, Alajuela	USA, Beltsville, MD
1	4691-54-1	3	2	3	4	2	2
3	11411	3	3	1	3	3	2
5	27-R	2	1	2	3	3	4
6	Diacol Calima	3	3	2	2	1	2
7	Comp. Chimal.-3	1	1	1	4	3	2
9	Cuilapa-72	2	1	1	2	3	2
10	PR-12	3	3	3	1	3	4
13	Mexico-309	2	1	2	3	3	2
14	Turrialba-1	1	2	1	3	3	2
15	ICA-Guali	3	1	2	3	1	1
17	Negro Jalpatagua	2	2	2	2	3	1
18	San Pedro Pinula	1	2	3	2	3	1
19	Turrialba 4	1	1	1	4	3	2
21	PI-319649	1	2	1	1	3	3
22	Porrillo-1	2	3	3	3	2	1
23	Rico Pardo-896	2	1	1	2	1	2
25	Linea-37	1	2	1	2	3	2
26	Ecuador-299	2	2	2	3	1	2
27	Porrillo-70	3	3	3	4	3	3
29	ICA-Tui	3	4	3	4	3	1
30	Canario Divex-8120	3	2	2	3	1	3
33	PR-5	3	2	1	2	3	2
34	Comp. Chimal.-2	1	1	1	1	1	2
35	Porrillo Sintetico	3	3	4	3	1	3
38	PR-4	4	3	4	4	3	2
40	PR-3	3	3	4	4	3	1
41	Linea-34	3	3	1	2	1	2
42	PR-1	1	3	3	3	2	2
44	Cornell-49-242	3	2	2	4	3	2
45	Negro San Ramon-5	3	3	1	1	1	2
47	PR-17	4	3	3	4	1	2
48	PI-163372	3	3	2	4	2	1
49	Nep-2	3	2	2	3	2	1
51	PI-165426	4	3	2	4	3	2
52	ICA-Pijao	1	3	3	4	2	3
53	Rico-23	4	2	3	4	3	2
54	PI-199044	3	2	1	3	1	4
56	PI-313664	2	3	2	3	1	1
57	PI-165426 (white)	3	3	3	4	3	2
59	PI-203958	3	2	3	3	3	4
60	PI-226883	3	2	2	3	1	4
61	PI-152326	4	3	3	4	3	1
62	PI-307824	3	2	4	4	3	3
63	PI-226895	2	2	1	3	1	2
107	Cuva 168-N	2	2	2	3	1	2
109	Bountiful-181	3	3	2	3	2	4
116	Golden Gate Wax	3	3	3	4	3	2
117	KW-765	2	2	1	4	1	2
118	KW-780	4	3	4	4	3	4
119	KW-814	2	3	2	4	3	3
121	Pinto No. 650	4	4	4	4	4	4
122	US No. 3	3	4	4	4	4	4

Table 5.8: Number of clusters and cultivars within clusters of cluster analysis of field reactions of 52 bean cultivars to rust (*U. appendiculatus*) in six different locations in the 1975 IBRN

Cluster Methods				
CI	CII	CIII	CIV	CV
		Complete Linkage		
4691-54-1	11411	CNC-3*	27-R	Porillo-70
C-49-242	PR-5	Turr.-4	PR-12	PI-165426B
PI-165426W	Discol Calima	Cuilapa-72*	PI-203958	Gold. Gt. Wax
Rico-23	Linea 34	Mexico-309*	Bount.-181	KW-814
PI-115326	NSR #5	Turr.-1	Porillo-1	PI-307824
ICA-TUI	ICA Guali	Linea-37	PI-313664	PR-1
PR-4	CD-8120	Neg. Jal.	PI-163372	ICA-Pijao*
PR-3	PI-199044	San PP	Nep-2*	
KW-814	PI-226883	PI-319649	Porillo S.	
Pinto-650	Ecuador-299*	Rico P-896	PR-17	
US No. 3	Cuva 168-N	CNC-2*		
	PI-226895			
	KW-765			
		Average Linkage		
4691-54-1	27-R	KW-780*	11411	Diacol Calima
C-49-242*	PI-203953	Pinto-650	PR-5	Linea-34
Porillo-70	Bount.-181	US No. 3	Cuilapa-72	NSR-5
PI-165426B	PR-12		Mexico-309*	ICA-Guali
GG Wax			Turr.-1	Can. Div. 8120
PI-165426W			Linea-37	PI-199044
Rico-23			PI-319649	PI-226883
PI-1152326			Neg Jal	Ecuador-299*
ICA-TUI			San PP	Cuva 168-N
PR-4			CNC-3*	PI-226895
PR-3			Turr.-4	KW-765
PI-307824				Rico-Pardo 896
Porillo-1				CNC-2*
PI-313664				
PI-163372				
Nep-2*				
Porillo Sintetico				
PR-17				
PR-1				
ICA-Pijao*				
KW-814				
		Ward's Method		
4691-54-1	27-R	11411	Diacol Colima	
C-49-242*	PI-203958	PR-5	Linea-34	
Porillo-70	Bount.-181	Cuilapa-72*	Negro San Ramon #5	
PI-165426W	PR-12	Mexico-309*	Rico-Pardo-896	
GG Wax	KW-780*	Turrialba-1	CNC-2*	
KW-814	Pinto-650	Linea-37	ICA-Guali	
PI-307824	US No. 3	PI-319649	Canario Divex 8120	
JCA Tui		Nego Jal	PI-199044	
PR-4		San PP	PI-226883	
PR-3		CNC-3*	Ecuador-299*	
PI-165426B		Turrialba-4	Cuva 168-N	
Rico-23			PI-226895	
PI-152326			KW-765	
Porillo-1				
PI-313664				
PI-163372				
Nep-2*				
PR-1				
ICA-Pijao				
Porillo Sintetico				
PR-17				

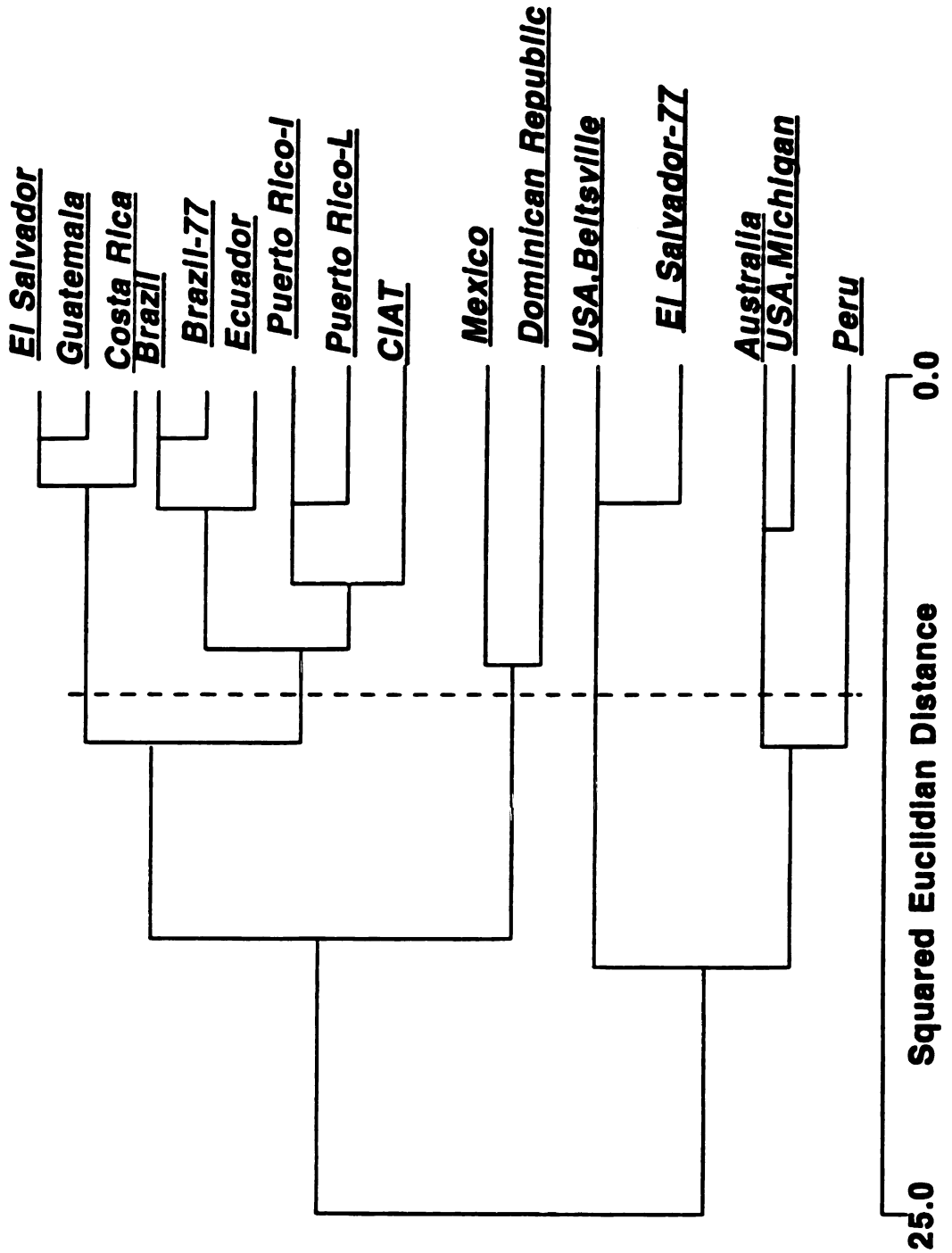
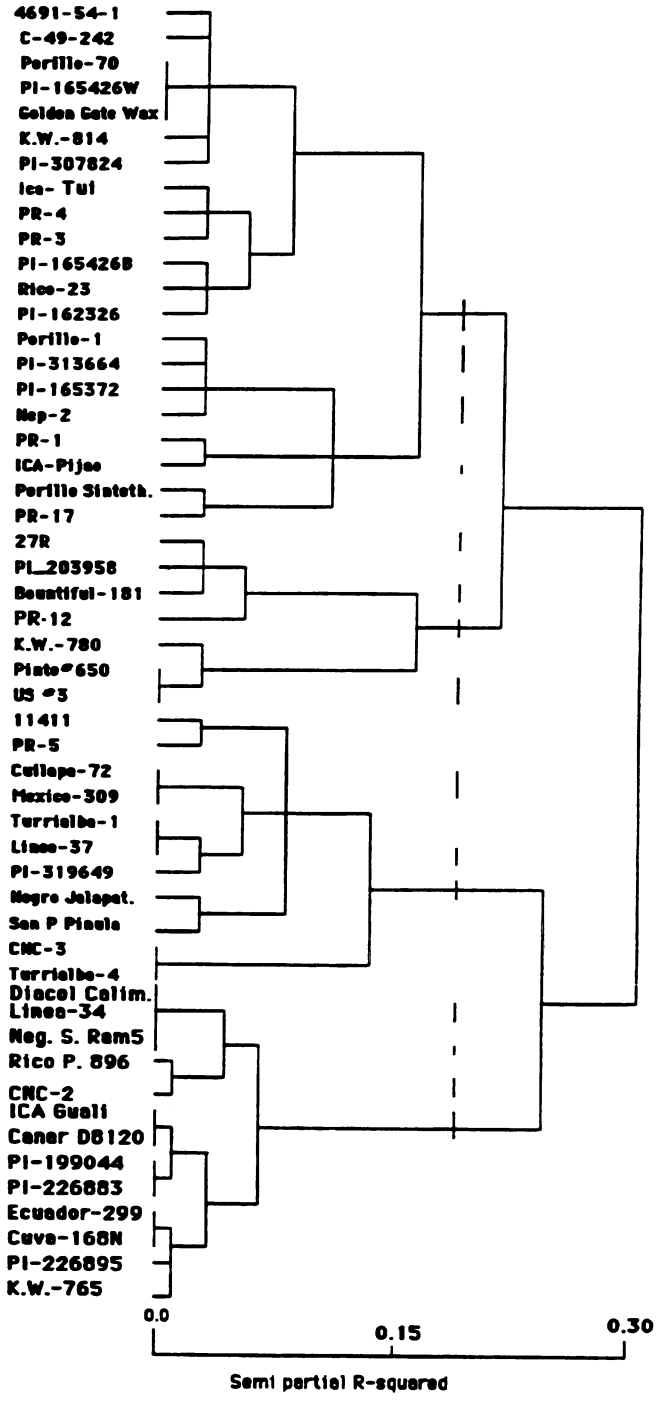


Figure 5.3 Ward's clustering of 16 sites in the 1976 IBRN.

Figure 5.4 Ward's clustering of field reactions of 52 bean cultivars in the 1975 IBRN



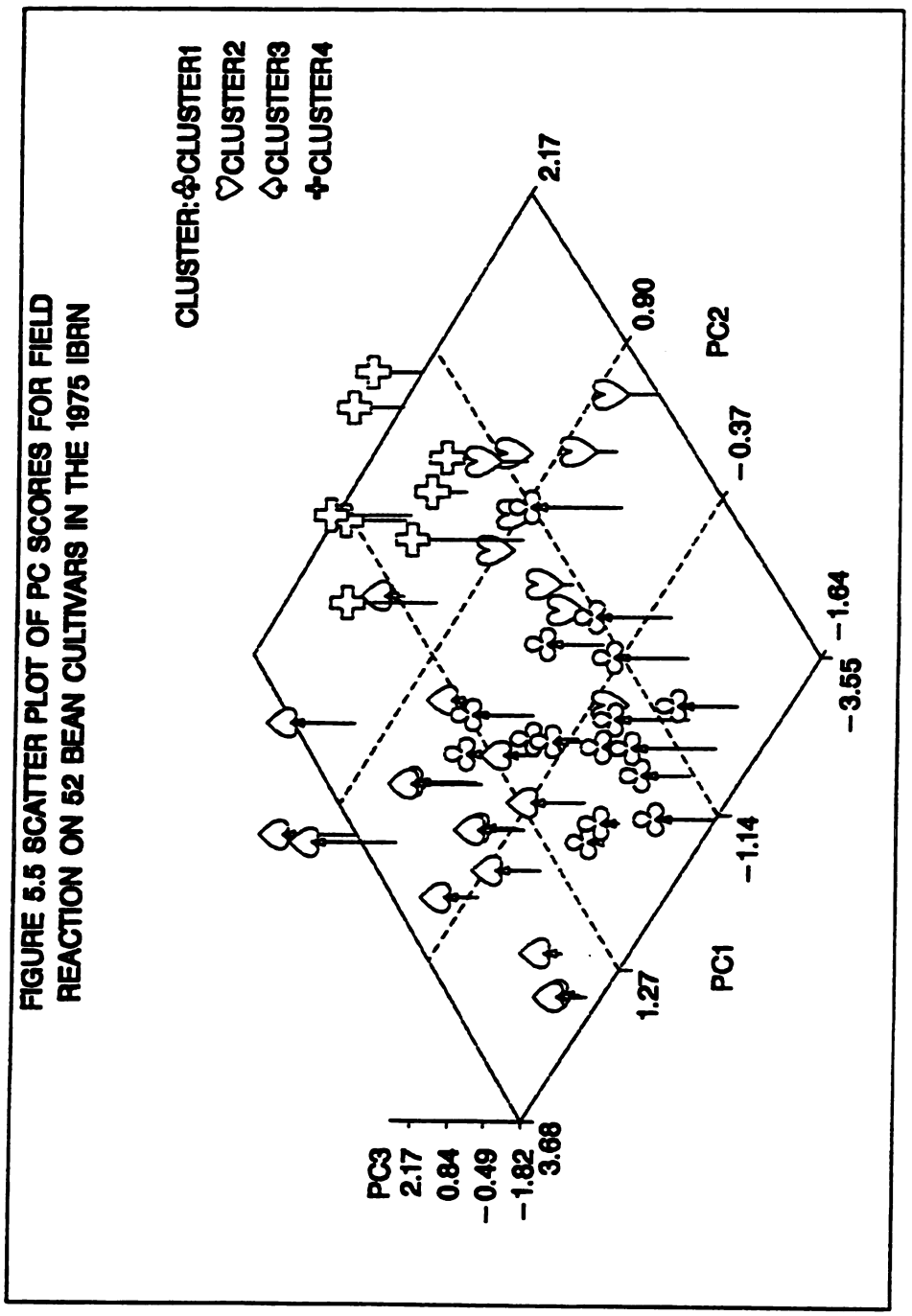


FIGURE 5.5 SCATTER PLOT OF PC SCORES FOR FIELD REACTION ON 52 BEAN CULTIVARS IN THE 1975 IBRN

Table 5.9: Mahalanobis's distance (D^2) among four clusters with different rust reaction patterns in the 1975 IBRN

Cluster	I	II	III	IV
I	0.00			
II	3.21	0.00		
III	3.23	4.51	0.00	
IV	4.39	5.55	4.77	0.00

the 1976 IBRN and, for that matter, the 1977 IBRN, are testing season and test locations. These variables can influence cultivar behavior particularly reactions to diseases that will have a bearing on how one perceives and makes conclusions on fundamental genetic interrelationships (Vander Plank, 1968). Cluster membership by bean cultivars to one or another cluster will have to be looked and compared in light of this possibility. At this juncture, it would be appropriate to point out the obvious differences, in particular, between the 1975 IBRN and the 1976 IBRN:

1) The number of bean cultivars used in the test analysis in each year, i.e., 88 cultivars in the 1976 IBRN vs 52 cultivars in the 1975 IBRN.

2) Two different test seasons with attendant differences in test conditions.

3) Different test locations, i.e., six locations in the 1975 IBRN vs 16 locations in the 1976 IBRN. Of these, four locations were common to both years.

4) After cluster analysis of the 1976 IBRN data using the CLUSTAN program and Ward's minimum variance method, eight clusters were accepted for the 1976 IBRN. The clustering for the 1975 IBRN was done using SAS programs with three cluster analysis algorithms, i.e., complete linkage, average linkage and Ward's minimum variance method. Ward's method was used for final comparison purposes.

Other than their slight differences in the number of cluster groups formed, there is an almost one-to-one agreement in the ordering of the cultivars in average linkage and Ward's method (Table 5.8). The slight difference is in the ordering of the cultivars in the hierarchical clustering scheme that is resident in the respective cluster analysis algorithms.

Complete linkage clustering resulted in the same number of clusters as average linkage clustering but as expected with an entirely different ordering and content of cultivars in the cluster groups.

A look at the cultivar membership in the clustering results of the 1975 IBRN for the 38 cultivars common to the 1975 IBRN and the 1976 IBRN reveals that several cultivars have retained their membership as in the old cluster grouping of the 1976 IBRN. The majority have been assigned to entirely new cluster groupings composed of cultivars other than their former group mates. This is particularly evident in the break-up of the cluster grouping of CNC-2 and C-49-242 (IV in Ghaderi et al., 1984), Nep-2 and Ecuador-299 (VII in Ghaderi et al., 1984), KW-780 and ICA-Pijao (VIII in Ghaderi et al., 1984) and forming new group alignments with other cultivars. Examples of this are cultivars 4901-54-1, Porillo-70, Porillo-Sintetico, and PI-207824, which clustered together in Cluster I of both 1975 and 1976 IBRN along with other cultivars from other groups. The only exceptions were Mexico-309 and Cuilapa-72, which were consistently clustered together regardless of the cluster method used or even the expected difference between testing seasons that may have some bearing on their cluster outcome. This indicates the existence of such broadly resistant cultivars with presumably several genes for resistance to multiple races that enable them to behave (cluster together) similarly from season to season. Both cultivars have been reported to possess broad resistance genes to many rust races (Stavely, 1989).

Although the break-up of the old cluster groupings and the assignment of these cultivars to new clusters is not totally unexpected given the use of different procedures (Afifi

and Clark, 1984) and more importantly, to presumably different testing conditions (Van der Plank, 1968), it is, nevertheless, important to look beyond these cluster formations, since these groupings and the assignment of cultivars to new clusters reveal a new pattern that may have explanations in fundamental biological rationale. The fact that these same cultivars were tested in two different growing seasons and in probably different locations has a great deal to do with their assignments into new clusters with cultivars other than their former cluster mates. Since these cultivars were grouped on the basis of field reactions to endemic population races of rust in the field, it is also logical to expect these cultivars to produce disease reaction grades in response to races that may be different from previous seasons or different from the other test locations consistent with the host pathogen interaction system (Van der Plank, 1968), and therefore, to cluster into groups with similar response patterns based on these new reactions. These new reactions, if they occur, are manifestations of the basic interactions of the host genotype with the prevalent dominant pathogenic races during that season at that location and the interplay of these factors with the environment to produce the reaction response in question. If indeed these cultivars were clustered into these new groupings as shown in Table 5.8, because of the above biological reasons, it brings into focus the principle of the gene–for–gene system (Person, 1959). This is a biological switch in which cultivars whose disease resistance gene(s) expressed in one test condition was (were) activated for a prevalent field race, which may or may not be the same as the previous one, producing one set of reaction, may in turn produce another set of reactions in a different test condition for a different prevalent field race in accordance with the gene–for–gene system (Person, 1959; Flor, 1955). If this theory holds, the clustering into the same group of the cultivars in the analysis by Ghaderi et al. (1984) may have been due to the prevalence during that test period of dominant rust races that could elicit similar reactions, i.e., switching of the same reaction phenotype contingent on the presence of a corresponding host genotype.

Similarly, the appearance of new reaction phenotypes in different test conditions (testing season and test locations) of the same cultivars resulting in new cluster groups with new cluster alignments indicates the presence of a new dominant race eliciting similar reactions on cultivars possessing corresponding genes for reaction to these races. The presence of diverse pathogenic potential as indicated by location specifics and differences in racial composition by time of planting was reported for these sites during the 1975, 1976 and 1977 IBRN tests (CIAT, 1979), which substantiates the above assertion.

3. Cluster analysis of field reaction of 38 bean cultivars common to both 1975 and 1976 IBRN

The field reactions of 38 bean cultivars tested in 22 locations (6 locations in the 1975 IBRN and 16 locations in the 1976 IBRN) appears in Table 5.10. Four test locations of the total six locations in the 1975 IBRN were the same in the 1976 IBRN.

Cluster analysis results of the 38 bean cultivars using three cluster analysis methods are shown in Figure 5.6 and Table 5.11. Three, five and four groups were obtained for complete linkage, average linkage and Ward's method respectively. Figure 5.7 displays the relationships and differences in reaction response patterns of cluster members along the first, second and third principal axes (accounting for 51.8 percent of total variation) of a principal component analysis of the reaction responses in the 22 locations of the combined 1975–1976 IBRN. Mahalanobis's distances (D^2) that are reflections of the contrasting response patterns among the clusters are shown in Table 5.12. Values ranged from 5.94, the distance between clusters I and IV to 15.43, the distance between clusters III and IV. The number of groups formed for each clustering method may have been influenced by the relatively large number of attributes (variables), i.e., 6 and 16 variables (test locations) for the 1975 and 1976 IBRN vs 22 variables (6 + 16) for the combined 1975 and 1976 IBRN and the relatively few observations (38) in the combined 1975/1976 IBRN vs 88 in the 1976 IBRN. It is recognized

Table 5.10: The reaction of 38 bean varieties uniformly tested in 22 environments in the 1975 and 1976 IBRN*

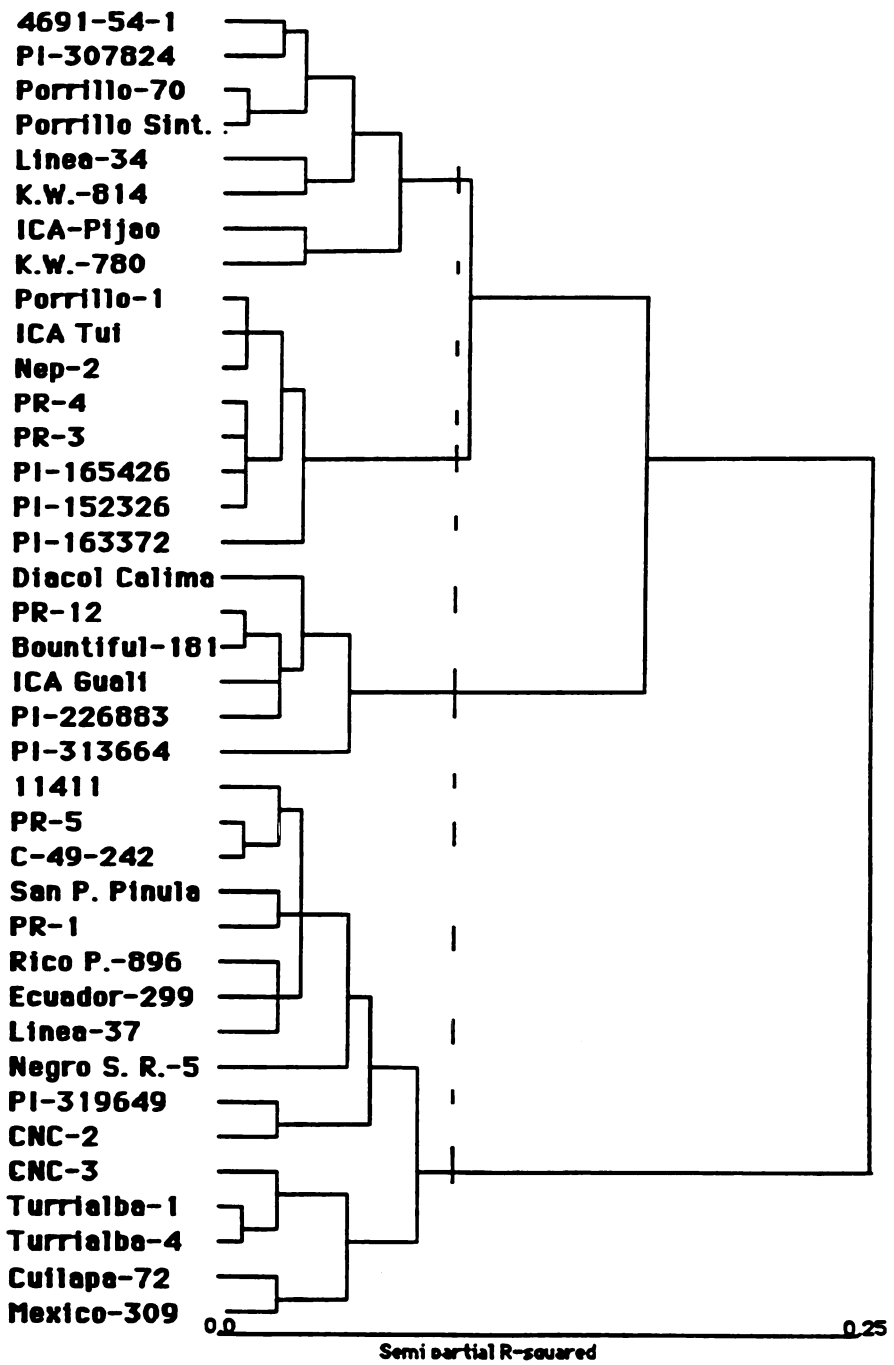
4691-54-1	3	2	3	4	2	2	3	4	4	3	1	2	3	1	4	3	2	1	3	3	1	3
11411	3	3	1	3	3	2	1	2	4	3	2	4	3	1	3	4	2	2	3	3	1	3
Diacol Calima	3	3	2	2	1	2	3	2	2	3	2	2	4	1	2	2	3	2	3	4	4	2
CNC-3	1	1	1	4	3	2	1	1	2	2	2	3	4	1	2	1	2	2	2	3	1	2
Cuilapa-72	2	1	1	2	3	2	1	2	4	3	1	4	1	1	1	1	2	1	1	3	3	2
PR-12	3	3	3	1	3	4	3	2	2	4	3	2	4	4	2	2	4	2	3	2	3	2
Mexico-309	2	1	1	2	3	1	1	1	4	3	4	3	1	1	1	1	2	2	1	2	1	3
Turrialba-1	1	2	1	4	4	2	1	1	3	3	3	2	4	2	2	3	2	1	2	3	3	2
ICA-Guali	3	1	2	3	1	3	3	1	2	4	2	2	3	4	2	2	3	2	3	3	3	2
San PP	1	2	3	2	3	1	1	2	3	2	3	3	4	1	3	4	2	1	1	2	3	2
Turrialba-4	1	1	1	4	3	2	1	1	4	4	3	2	4	1	1	1	2	1	1	3	3	2
PI-319649	1	2	1	1	3	3	1	2	4	3	4	4	4	1	2	4	2	2	1	2	4	2
Porrillo-1	2	3	3	3	2	1	1	4	4	3	3	3	4	1	3	4	3	2	3	4	4	2
Rico P. 896	2	1	1	2	1	2	1	2	3	3	4	4	4	1	3	3	1	1	2	2	1	2
Linea-37	1	2	1	2	3	2	1	2	3	2	4	4	4	1	2	3	2	3	1	4	1	2
Ecuador-299	2	2	2	3	1	2	1	4	3	2	3	3	4	1	2	2	2	1	2	3	3	2
Porrillo-70	3	3	3	4	3	3	3	4	4	3	3	2	4	1	2	4	3	2	3	3	4	2
ICA-Tui	3	4	3	4	3	1	1	4	4	3	3	4	4	1	2	4	3	1	3	4	4	2
PR-5	3	2	1	2	3	2	1	1	4	2	3	3	4	1	3	2	2	1	2	3	3	2
CNC-2	1	1	1	1	1	2	1	1	2	4	4	4	4	1	1	3	2	1	1	1	4	2
Porrillo Sintetico	3	3	4	3	1	3	3	4	4	3	3	2	4	1	3	4	3	2	3	3	4	2
PR-4	4	3	4	4	3	2	1	4	4	4	3	3	4	1	3	4	1	1	3	4	4	2
PR-3	3	3	4	4	3	1	1	3	4	3	3	3	4	1	4	4	2	2	3	4	4	2
Linea-34	3	3	1	2	1	2	1	2	4	3	4	3	4	1	4	4	3	4	3	4	4	2
PR-1	1	3	3	3	2	2	1	2	3	3	3	2	4	1	2	4	2	2	3	2	1	2
C-49-242	3	2	2	4	3	2	1	2	3	3	3	3	4	1	3	3	2	2	3	3	3	2
Negro SR #5	3	3	1	1	1	2	3	1	4	4	3	4	4	1	4	2	3	2	3	3	1	4
PI-163372	3	3	2	4	2	1	1	4	4	4	3	4	1	1	3	4	2	1	4	4	2	2
Nep-2	3	2	2	3	2	1	1	4	4	3	3	4	3	1	3	4	2	1	3	3	4	2
ICA-Pijao	1	3	3	4	2	3	3	4	2	3	2	2	1	1	3	3	3	3	2	1	3	2
PI-313664	2	3	2	3	1	1	3	3	1	4	3	2	1	4	2	3	4	1	3	2	3	3
PI-165426	3	3	3	4	3	2	1	4	4	4	3	4	4	1	4	4	2	1	3	3	4	4
PI-226883	3	2	2	3	1	4	3	3	3	4	3	3	3	4	2	4	4	1	3	3	4	4
PI-152326	4	3	3	4	3	1	1	4	4	4	3	4	4	1	4	4	1	1	3	4	4	3
PI-307824	3	2	4	4	3	3	3	4	4	4	3	4	4	1	4	4	1	3	4	4	4	3
Bountiful-181	3	3	2	3	2	4	3	3	2	4	2	2	4	4	3	3	4	2	3	3	4	2
KW-780	4	3	4	4	3	4	4	4	3	4	3	3	1	2	4	4	4	3	3	3	2	3
KW-814	2	3	2	4	3	3	1	4	4	3	4	2	4	1	4	3	3	3	3	3	4	4

*First six columns are the same locations as in the 1975 IBRN and the next 16 columns are the same locations as in the 1976 IBRN.

Table 5.11: Clusters analysis of combined field reaction score in the 1975 and 1976 International Bean Rust Nursery on 38 bean cultivars using three clustering methods

Cluster Methods				
CI	CII	CIII	CIV	CV
		Complete Linkage		
4691-34-1	Diacol Calima	11411		
Porillo-70	PR-12	PR-5		
Porr. Sin.	Bountiful-181	C-49-242*		
PI-207824	ICA-Guali	Linea-37		
Porillo-1	PI-313654	San Pedro Pinulo		
ICA-Tui	PI-226883	PR-1		
Nep-2	ICA-Pijao*	Rico Pardo-896		
PR-4	KW-780*	Ecuador-299*		
PR-3		Neg. San. Ram. 5		
		CNC-3*		
		Turrialba-1		
		Turrialba-4		
		Cuilapa-72		
		Mexico-309*		
		PI-319649		
		CNC-2*		
		Average Linkage		
4691-34-1	11411	Neg. San.	Diacol Calima	ICA-Pijao*
Porillo-1	PR-5		PR-12	KW-780*
ICA-Tui	C-49-242*		Bountiful-181	
Nep-2*	Rico Pardo 896		ICA-Guali	
PR-4	Ecuador-299*		PI-226883	
PR-3	San P. Pinula		PI-313664	
PI-165426	PR-1			
PI-152326	Linea-37			
Porillo-70	CNC-3*			
Porillo-S	Turrialba-1			
PI-307824	Turrialba-4			
KW-814	PI-319649			
PI-163372	CNC-2*			
Linea-34	Cuilapa-72*			
	Mexico-309*			
		Ward's Method		
4691-34-1	Porillo-1	Diacol Calima	11411	
PI307824	ICA-Tui	PR-12	PR-5	
Porillo-70	Nep-2*	Bountiful-181	C-49-242*	
Porr. Sinth.	PR-4	ICA-Guali	San PP	
Linea-34	PR-3	PI-226883	PR-1	
KW-814	PI-165426	PI-312664	Rico P.-896	
ICA-Pijao*	PI-152326		Ecuador-299*	
KW-780*	PI-163392		Linea-37	
			Neg. 5 R-5	
			PI-319649	
			CNC-2*	
			CNC-3*	
			Turrialba-1	
			Turrialba-4	
			Cuilapa-72*	
			Mexico-309	

Figure 5.6 Ward's clustering of field reactions of 38 bean cultivars in the 1975 and 1976 IBRN



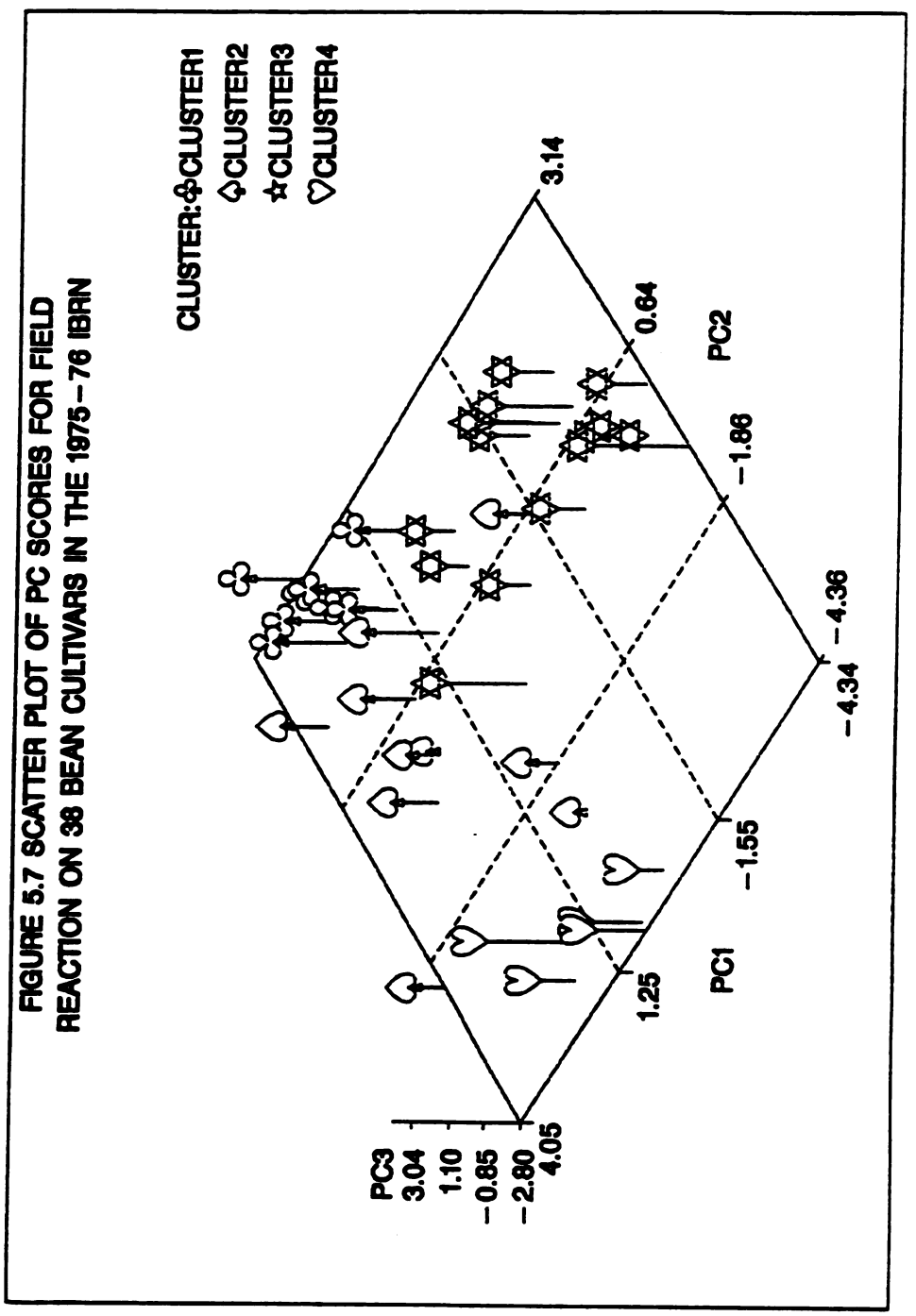


FIGURE 5.7 SCATTER PLOT OF PC SCORES FOR FIELD REACTION ON 38 BEAN CULTIVARS IN THE 1975–76 IBRN
CLUSTER:♣CLUSTER1
◇CLUSTER2
☆CLUSTER3
♡CLUSTER4
PC3
3.04
1.10
−0.85
−2.80
4.05
PC1
1.25
−1.55
PC2
3.14
0.64
−1.86
−4.34
−4.36

that some clusters depend on the discriminating power of a key or a few key variables (Anderberg, 1973). In particular, the 16 variables (16 test locations in the 1976 IBRN) appear to have a greater bearing and have to a large extent forced the cluster outcome. This latter observation appears to be in the right direction because earlier cluster patterns on the basis of the 16 varieties have reappeared as shown in the clustering together of ICA-Pijao with KW-780 (old Cluster VIII), Mexico-309 and Cuilapa-72 (old Cluster IV), and CNC-2 and C-49-242 (old Cluster V). Although the purpose of the cluster analysis of the combined data was made with the objective of assessing cultivar interrelationships on a greater number of attributes (variables), the cluster outcome has resulted in no gain of information. It may simply be inappropriate to lump together biological data from two different growing season for classification purposes. It would perhaps be more appropriate, therefore, to extract field reaction data of the 38 cultivars from the four common test sites (Brazil--Vicosa, USA, Beltsville, Maryland, Costa Rica and CIAT) for both years for definitive comparison purposes provided that the racial composition for both test conditions in these locations are identical. However, the likelihood of such a situation may be remote.

Table 5.12: Mahalanobis' distance (D^2) among four clusters with different rust reaction patterns for the combined 1975 and 1976 IBRN data

Cluster	I	II	III	IV
I	0.00			
II	7.43	0.00		
III	14.17	13.99	0.00	
IV	5.94	6.54	15.43	0.00

4. Cluster analysis of field reactions of 46 bean cultivars in the 1977 IBRN

The reaction to rust in the field of 46 bean cultivars tested in 14 locations is shown in Table 5.13.

Table 5.13: The reaction of 46 bean varieties uniformly tested in 14 locations in the 1977 IBRN coordinated by CIAT

Variety Name	B	C1	C2	C3	C4	D	E1	E2	G	J1	J2	U1	U2	U3
4 Cocacho	3	3	3	2	1	3	2	2	2	3	3	1	2	2
7 Caballero	4	3	2	1	1	3	1	4	2	2	4	2	1	4
11 Ormiston	2	2	3	1	1	1	4	2	2	3	4	3	2	2
13 PR-2	4	4	4	4	1	4	4	2	4	4	2	4	4	2
16 Comp. Chi. 3 (G5712)	2	2	2	2	3	3	1	4	3	2	2	2	2	1
17 Mexico-309 (G5652)	2	2	1	2	1	1	1	4	3	2	2	2	2	2
18 Turrialba 1 (G4485)	2	3	4	2	1	4	1	2	3	4	2	1	2	1
19 Ecuador-299 (G5653)	3	2	2	2	1	3	1	4	4	3	2	2	2	3
20 Mexico-235	3	2	2	1	1	4	1	0	4	2	2	1	2	1
23 Cacahuate 72 (G5481)	2	2	3	1	2	3	4	2	2	3	4	1	2	1
24 27-R (G4458)	4	1	3	3	3	3	4	2	3	3	4	4	4	2
25 ICA-Pijao (G4525)	2	4	4	3	1	4	2	2	2	3	2	4	4	3
26 Cuilapa-72 (G4489)	3	4	4	1	1	3	1	2	3	2	2	1	2	1
27 Turrialba-4 (G4465)	3	4	4	1	1	3	1	2	3	2	2	1	2	1
28 Redlands Pioneer	2	2	2	1	1	3	1	2	2	2	2	1	3	1
29 4691-54-1	3	4	4	4	4	4	2	4	3	3	3	2	2	2
31 Porrillo 70 (G4142)	2	4	4	3	3	4	1	3	4	3	2	3	3	3
33 PR-3	4	4	4	4	3	4	3	3	4	3	2	4	3	3
34 Cornell-49-242	3	3	4	3	1	4	3	3	4	3	2	1	2	2
35 Nep-2 (G4459)	3	4	4	3	1	4	2	2	2	4	2	2	2	2
36 Rico-23 (G3827)	4	4	4	3	1	4	3	4	3	3	2	2	2	2
37 Rico-Bajo-1014	3	1	2	1	3	3	1	4	3	2	2	1	3	1
38 Jamapa	3	3	3	3	3	3	2	3	3	2	2	4	2	3
39 PI-226883	3	2	3	2	3	4	4	3	2	3	4	2	2	2
40 PI-226895 (G1423)	4	2	3	1	3	4	2	3	3	2	3	4	4	3
41 Miss Kelly	4	4	4	3	1	3	2	2	2	4	2	4	3	3
42 Mountain White HR	4	4	4	3	2	4	2	3	4	3	2	1	2	1
43 Redlands Autumn CR	3	1	3	3	2	3	4	2	2	3	4	4	4	3
44 Redlands Gr. Lf. B	2	1	2	2	1	3	2	2	2	1	2	4	4	2
45 Cuva 168-N	2	2	3	4	1	4	1	3	4	3	2	2	3	2
46 Redlands Gr. Lf. C	2	1	3	2	1	3	1	2	2	2	2	1	2	1
48 Brown Beauty	3	2	3	3	3	4	4	4	2	3	4	3	3	2
50 Ca Sm White No. 643	3	4	2	3	1	4	1	2	2	3	2	4	4	2
51 CCGB-44 (G3607)	3	4	4	4	3	4	1	4	3	3	1	4	2	3
53 Epicure	4	4	4	4	2	4	3	4	4	4	2	4	2	3
54 Golden Gate Wax	4	4	2	2	1	4	2	4	2	2	3	2	2	1
55 Kentucky Wonder 765	3	3	2	3	4	4	2	3	2	2	2	4	4	4
57 Kentucky Wonder 814	3	4	4	3	1	4	3	2	4	3	2	3	3	2
58 Mulatinho	3	4	4	4	3	4	3	4	3	3	2	4	3	3
61 Veracruz 1-A-6	4	4	4	2	3	4	2	4	3	2	2	4	3	3
62 Aguascalientes 13	4	4	4	4	4	4	3	4	4	3	3	2	2	1
63 Guerrero 6	3	4	4	3	1	4	2	3	3	3	2	3	3	4
64 Guerrero 9	4	3	3	2	1	4	1	4	2	3	2	4	4	4
66 Jalisco 33	2	3	2	3	1	4	1	4	2	2	2	3	2	1
67 Mexico 6	3	2	2	3	1	4	1	3	3	2	2	4	3	2
68 Mexico 12	4	2	3	4	2	3	2	3	3	2	2	4	3	3

B = Brazil, Goiania
C1 = Columbia, Palmira (A)
C2 = Columbia, Palimira (S)
C3 = Columbia, Popayan
C4 = Columbia, Rio Negro
D = Dominican Republic
E1 = Ecuador
E2 = El Salvador
G = Guatemala
J1 = Jamaica, Top Mount
U1 = USA, Beltsville 1
U2 = USA, Beltsville 2
U3 = USA, Michigan

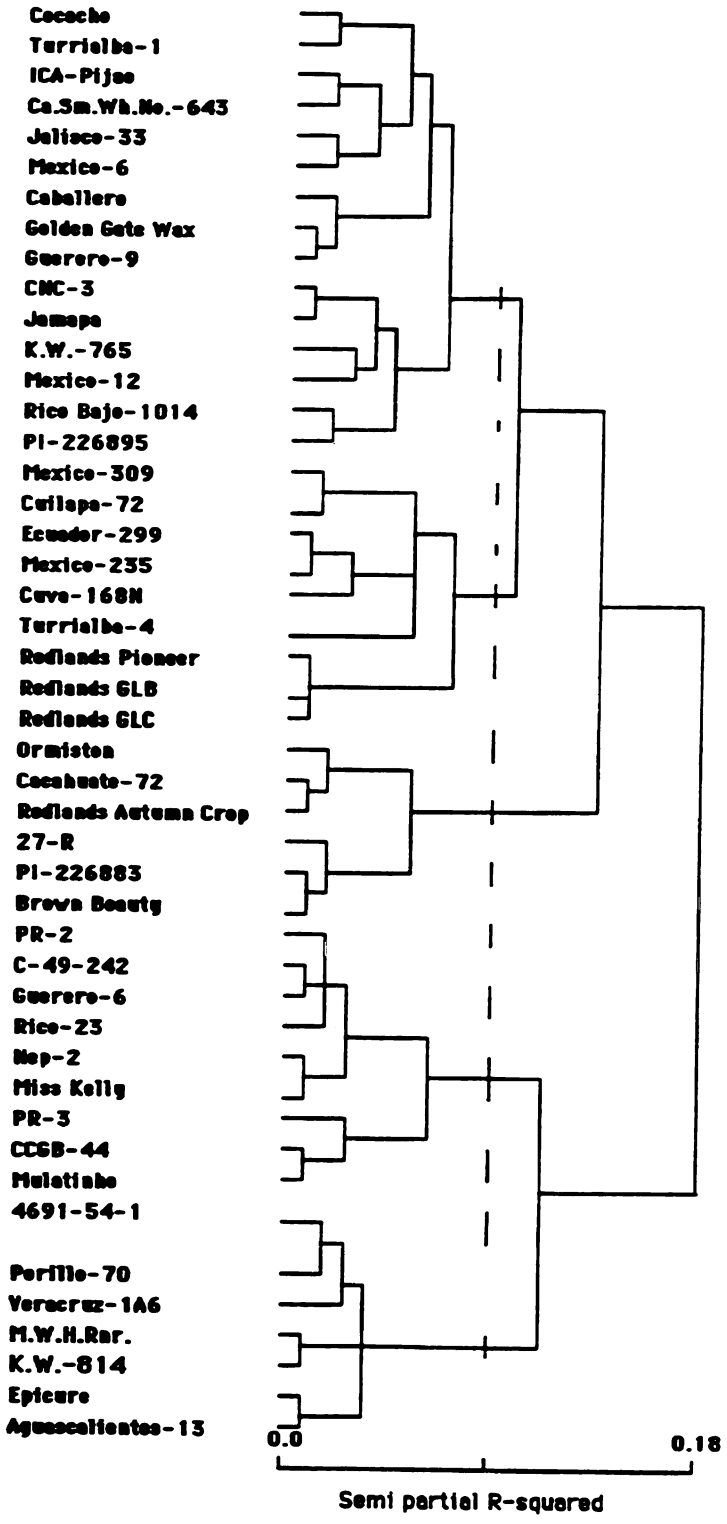
Five clusters each were obtained for average linkage and Ward's method, respectively (Figure 5.8, Table 5.14) and seven clusters for complete linkage (Appendix A6). There was almost a complete agreement in the cluster memberships for average linkage and Ward's minimum variance method of clustering with similar cultivar ordering in the hierarchies. Complete linkage differed as expected for a different algorithm (Afifi and Clark, 1984). from not only to the greater number of clusters formed, which has a tendency to break up cluster groups, but also in casting some cultivars in separate clusters that were clustered together in the same group with average linkage and Ward's method (Table 5.14). However, it agrees with the clustering of the broadly resistant cultivar groups (Mexico–309, Ecuador–299, Mexico–235 and Cuilapa–72), which were clustered together and the cultivars C–49–242 and Nep–2 into the same but distinct group with other cultivars.

Comparison of the clustering patterns of the 46 bean cultivars in the 1977 IBRN by Ward's minimum variance method with the pattern in the 1976 IBRN reveals interesting features of cultivars behavior (Table 5.14). The clustering pattern for the cultivars in the 1977 IBRN was remarkably similar to the clustering pattern observed in the 1975 and 1976 IBRNs. For example, five cultivars from a total of seven that were clustered together in the 1976 IBRN in Cluster I were also clustered together in the 1977 IBRN in Cluster V. These included cultivars 4691–54–1, Porillo–70, M.Wh.Hf.Rnr., Epicure and Veracruz–1A6. Similarly, of the four cultivars that were common to both 1976 and 1977 IBRN and which were clustered together in Cluster II of the 1976 IBRN, three (Redlands Pioneer, Redlands GLB and Redlands GLC) were also clustered together in Cluster II of the 1977 IBRN. Cultivars PI–226883, Cacahuate–72, Redlands Autumn Crop and Brown Beauty also common to both 1976 and 1977 IBRNs clustered together in Cluster III of the 1977 IBRN. In general, the same tendencies in clustering behavior that were apparent in the clustering pattern of the cultivars common to 1975 and 1976 IBRNs were also observed in the clustering of the

Table 5.14: Cluster analysis using average and Ward's clustering methods of field reaction of 46 bean cultivars in the 1977 International Bean Rust Nursery

Cluster Methods				
CI	CII	CIII	CIV	CV
		Average Linkage		
Cocacho	Mexico-309*	PR-2	4691-54-1	Ormiston
Turrialba-1	Ecuador-299*	C-49-242	Porillo-70	Cacahuato-72
ICA-Pijao*	Mexico-239*	Guerero-6	Veracruz-1A6	Redlands A.Cr.
Ca Sm White-643	Cuilapa-72*	Rico-23	M/WhfRnr	27-R
Caballero	Cuva-168-N	Nep-2*	KW-814	PI-226883
Golden Gate Wax	Turrialba-4	Miss Kelly	Epicure	Brown Beauty
Guerero-9		PR-3	Aguascal.-13	
CNC-3*		CCGB-44		
Jamapa		Mulatinho		
Jalisco-33				
Mexico-6				
Mexico-12				
Redlands Pioneer				
Redlands GLF-B				
Redlands GLF-C				
Rico-Bajo-1014*				
PI-226895				
KW-765				
Ormiston				
		Ward's Method		
Turrialba-1	Cuilapa-72	Cacahuate-72	C-49-242*	Porillo-70
ICA-Pijao*	Ecuador-299*	Redlands A.Cr.	Guerero-6	Veracruz
Ca Sm White 643	Mexico-239*	27-R	Rico-23	M/WhfRnr
Jalisco-33	Cuva-168-N	PI-226883	Nep-2*	KW-814
Mexico-6	Turrialba-4	Brown Beauty	Miss Kelly	Epicure
Calballero	Redlands Pioneer		PR-3	Aguascal.-13
GG Wax	Redlands GLF-B		CCGB-44	
Guerero-9	Redlands GLF-C		Mulatinho	
CNC-3*				
Jamapa				
KW-765				
Mexico-12				
Rico-Bajo-1014				
PI-226895				

Figure 5.8 Ward's clustering of field reactions of 46 bean cultivars in the 1977 IBRN



cultivars common to both 1976 and 1977 IBRNs. Besides, an apparent tendency by cultivars with known pedigree relationships to cluster together was noted in the 1977 IBRN.

Ward's clustering of the 46 bean cultivars into five clusters and the distribution of the relationships and differences in reaction response patterns of cluster members along the first, second and third principal axes (accounting for 61.0 percent of total variation) of a principal components analysis of reaction responses in 14 locations is shown in Figure 5.9. Mahalanobis's distance (D^2) that are reflections of contrasting response patterns among the clusters ranged from 5.90, the distance between clusters I and V, to 11.94, the distance between Clusters III and IV (Table 5.15). Of these groups, only cluster II with cultivars Mexico–309 and Cuilapa–72 together with other cultivars, including Ecuador–299 and Mexico–235, constituted the old cluster V in the 1976 IBRN (compromising Mexico–309, Cuilapa–72 and Rico Bajo–1014), which clustered together regardless of clustering methods or testing condition differences. Cultivars Cuilapa–72, Mexico–309, Ecuador–299, Mexico–235, Nep–2, Aurora, CNC and its derivatives CNC–2 and CNC–3, are known to have broad resistance to several rust races (Stavely et al., 1989).

Table 5.15: Mahalanobis's distance (D^2) among five clusters with different rust reaction patterns in the 1977 IBRN

Cluster	I	II	III	IV	V
I	0.00				
II	8.02	0.00			
III	8.52	10.46	0.00		
IV	8.74	6.27	11.94	0.00	
V	5.90	9.99	10.28	7.64	0.00

Probably, the consistent clustering of these cultivars together is the result of their possession of a broad genetic base for resistance to several races of the rust fungus. Other cultivars have not demonstrated such consistency for clustering together from year to year

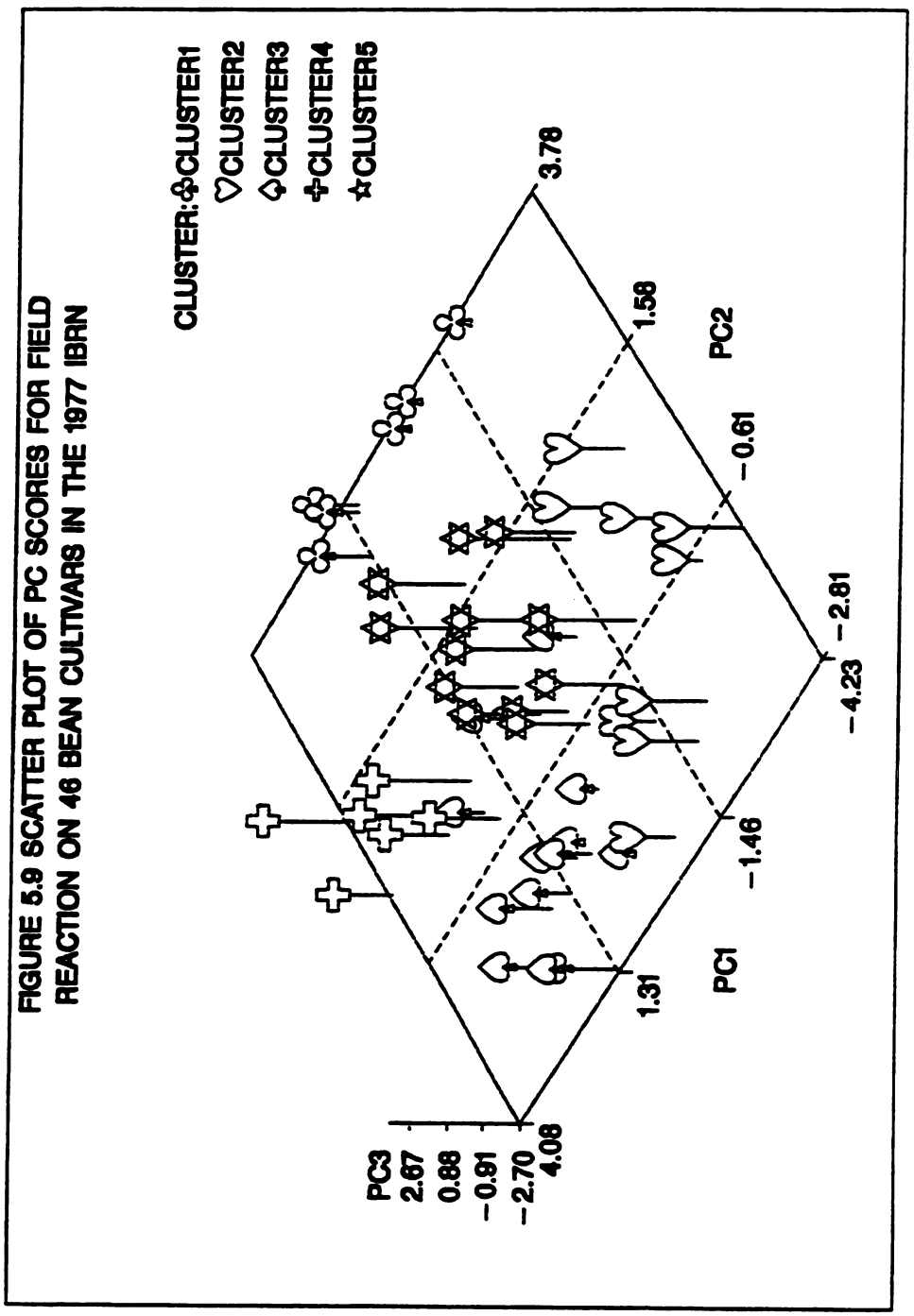


FIGURE 5.9 SCATTER PLOT OF PC SCORES FOR FIELD REACTION ON 46 BEAN CULTIVARS IN THE 1977 IBRN
CLUSTER: CLUSTER1
CLUSTER2
CLUSTER3
CLUSTER4
CLUSTER5
PC3
2.67
0.88
−0.91
−2.70
4.08
PC1
1.31
−1.46
−4.23
PC2
3.78
1.58
−0.61
−2.81

(1975–1977) due presumably to possession of single genes for reaction to each race and exposure to a changing pathogenic pressure as would be expected from the differences in testing conditions. This view is supported by assertion that differences in racial composition within and between sites quantitatively as well as qualitatively existed in the 1977 IBRN. The rust population also varied between planting seasons at one location in CIAT (CIAT, 1979).

In summary, separate cluster analysis results of the IBRNs (1975, 1976 and 1977) and the combined data analysis of the 1975 and 1976 IBRNs, the following were observed:

1. Cluster analysis of the 88 bean cultivars in the 1976 IBRN using three different computer programs, CLUSTAN (with Ward's minimum variance method), SAS (with several fusion techniques to merge observation) and SPSS–X Release 2.2 (with complete linkage and Ward's method) have all resulted in the same cluster grouping of the cultivars as in the cluster grouping of the 1976 IBRN (Ghaderi et al., 1984). The main difference was the number of cluster groups formed by the SAS and SPSS–X route. Eight clusters were formed in the CLUSTAN program by Ghaderi et al. (1984). This introduced a slight alignment of the cultivars that were clustered in separate but otherwise adjacent clusters in the hierarchical scheme, i.e., cultivars CNC–3, Mexico–235 and Lavega in Cluster III (Ghaderi et al., 1984), CNC–2 and C–49–242 in Cluster IV (Ghaderi et al., 1984) were lumped together as a result of the few numbers of clusters formed by the SAS and SPSS–X programs.
2. New cluster groups were observed in the cluster analysis of the 1975 IBRN and the 1977 IBRN test data indicative of the influence of a different test year (season) and test locations which probably has a great deal of bearing on the racial composition and eventually on the cluster outcome.

3. An exception to the formation of new cluster groups in different test years and test locations was the clustering of the broadly rust resistant cultivars Mexico-309 and Cuilapa-72 regardless of clustering methods used, and at times CNC-2, CNC-3 and Mexico-235 in the same cluster group. These cultivars have been noted by Stavely et al. (1989) CIAT (1979) to have broad resistance to several races in the U.S.

4. There is also an apparent tendency for cultivars with known pedigree relationships (C-49-242 and Aurora) or with presumed shared pedigree (Redlands Green Leaf B and Redlands Green Leaf C), or Mexico-235 and Ecuador-299 to cluster together.

It seems appropriate to point out here that further characterization of a reduced number of cultivars maintained as purelines by testing their disease reaction patterns to described races of rust in controlled environments; isozyme assay studies, agronomic characteristics, Mendelian genetic studies and pedigree analysis will shed more light on their interrelationships.

B. Disease reaction in uniform test conditions

1. Cluster analysis of disease reaction of 23 pureline bean cultivars to four described rust isolates (41, 46, 49 and 53) in the greenhouse

The reaction to four rust races (41, 46, 49 and 53) of 23 pureline bean cultivars on a 1-7 scale is presented in Table 5.16. Four cluster analysis methods were used in SAS to group the 23 observations. Four cluster groups each were formed for the complete, average (Table 5.17) and centroid fusion techniques (Table 5.18), respectively, with similar ordering and grouping of cultivars. Three clusters were formed by Ward's method (Table 5.18, Figure 5.10) in which cultivar Olathe and GN-1140, which were in separate clusters of their own in the complete, average and centroid methods were lumped together in Cluster I of Ward's

Table 5.16: Disease reaction of 23 bean cultivars to four races of the bean rust fungus

Cultivars	R41	R46	R49	R53
LaVega	2	5	7	2
Mexico-235	4	4	2	2
CNC-3	4	7	4	4
CNC-2	4	4	2	4
C-49-242	6	6	6	6
Mexico-309	4	4	7	3
RB-1014	4	4	4	4
Cuilapa-72	2	4	6	2
Ecuador-299	2	4	4	2
Nep-2	2	7	7	2
Aurora	2	7	7	2
KW-780	7	7	3	7
ICA-Pijao	7	7	4	7
CNC	4	4	4	4
B-190	4	4	7	4
UI-111	7	7	7	7
M/WhfRnr	7	7	3	7
GN-1140	4	4	4	7
Olathe	2	4	4	7
Pindak	7	6	6	7
Seafarer	7	7	7	7
C-20	4	7	7	2
51051	2	4	7	2

Table 5.17: Cluster analysis of 23 bean cultivars for reaction to four rust isolates in the greenhouse using complete and average linkage method

Cluster Methods			
CI	CII	CIII	CIV
	Complete Linkage		
LaVega	Olathe	Mexico-309	C-49-242
Ecuador-299	GN-1140	B-190	Pindak
Mexico-235		Cuilapa-72	Pinto-111
CNC-2		51051	Seafarer
Rico-Bajo-1014		Nep-2	KW-780
CNC		Aurora	M/WhfRnr
CNC-3		C-20	ICA-Pijao
	Average Linkage		
LaVega	Olathe	Mexico-309	C-49-242
Ecuador-299	GN-1140	B-190	Pindak
Mexico-235		Cuilapa-72	Pinto-111
CNC-2		Nep-2	Seafarer
CNC		Aurora	KW-780
CNC-3		C-20	ICA-Pijao

Table 5.18: Cluster analysis of 23 bean cultivars for reaction to four rust isolates in the greenhouse using centroid and Ward's minimum variance methods

Cluster Methods			
CI	CII	CIII	CIV
	Centroid Linkage		
LaVega	Mexico-309	Olathe	C-49-242
Ecuador-299	B-190	GN-1140	Pindak
Mexico-235	Cuilapa-72		Pinto-111
CNC-2	51051		Seafarer
RB-1041	Nep-2		KW-780
CNC	Aurora		M/WhfRnr
CNC-3	C-20		ICA-Pijao
	Ward's Method		
LaVega	Mexico-309	C-49-242	
Ecuador-299	B-190	Pindak	
Mexico-235	Cuilapa-72	Pinto-111	
CNC-2	51051	Seafarer	
RB-1014	Nep-2	KW-780	
CNC	Aurora	M/WhfRnr	
CNC-3	C-20	ICA-Pijao	
	Olathe		
	GN-1140		

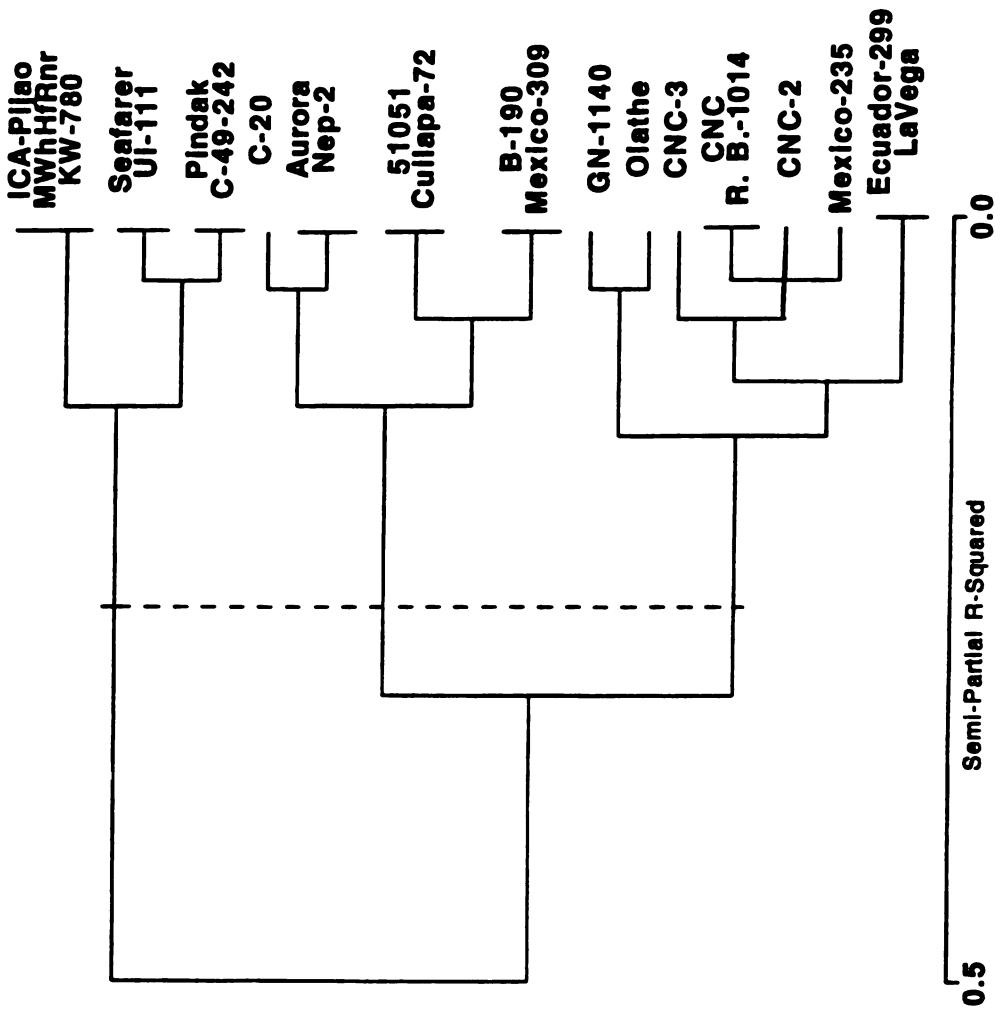


Figure 5.10 Ward's clustering of reactions of 23 bean cultivars to four rust races.

method (3 clusters). All clustering methods had the same cultivars in the hierarchies and similar cultivars membership in most cluster groups. It is apparent that all the clustering steps have grouped the cultivars into three or four reaction phenotype categories, depending on the method used. Complete, average and centroid linkage methods produced four groups each that virtually translated into four reaction phenotype categories of 1) the small pustule resistance reaction category R (Cluster I); 2) the predominant hypersensitive resistance reaction category, HR (Cluster II); 3) the moderately resistant category, MR (Cluster III), comprising Olathe and GN-1140; and 4) the moderately to highly susceptible reaction category (Cluster IV). On the other hand, Ward's method produced three groups that separated the cultivars into three major reaction phenotype categories: (1) cultivars with predominantly small pustule resistance less than 0.3 mm in diameter (Cluster I); (2) cultivars with predominantly hypersensitive resistance category (non-sporulating pustules less than 0.3 mm to 0.5 mm in diameter (Cluster II); and (3) cultivars with moderately susceptible to highly susceptible reaction categories (Cluster III). All clustering methods also agree in clustering together the subset cultivars belonging to clusters III, IV, V, VII and VIII of the 1976 IBRN by Ghaderi et al. (1984). In particular, Ward's method clustered cultivars LaVega, Mexico-235 and CNC-3 (Cluster I), cultivars Mexico-309 and Cuilapa-72 (Cluster II), Nep-2 and Aurora (Cluster II), and cultivars KW-780 and ICA-Pijao (Cluster III) together as in the 1976 IBRN by Ghaderi et al. (1984). The new grouping, however, separated Rico-Bajo-1014 from the original grouping with Mexico-309 and Cuilapa-72. Similarly, Ecuador-299 was separated from the clusters with Nep-2 and Aurora. The cluster that included cultivars C-49-242 and CNC-2 was also broken up because of their divergent reaction responses to the races. There is an apparent tendency for cultivars that share a common pedigree to cluster together. Examples of these include cultivars CNC, CNC-2 and CNC-3 in Cluster I; Ecuador-299 and Mexico-235 (Cluster I);

Mexico-309 and its progeny B-190 (Cluster II); Cuilapa-72 and 51051 and probably Pindak and Pinto III (Cluster III).

The efficiency of the clustering outcome has become more apparent by the appropriate use of pureline cultivars, described rust isolates, and testing in uniform test environments (greenhouse test). This was particularly obvious, with some exceptions, by the separation of the cultivars into groups that express correct classification into precise reaction phenotypes that reflect similar genes for reaction to the rust isolates they were tested against. Differences in reaction response patterns of cluster members along the first, second and third principal axes (accounting for 95.4 percent of total variation) of a principal component analysis of reaction responses to four described races is shown in Figure 5.11. Mahalanobis's distance (D^2) among the clusters ranged from 3.72, the distance between clusters I and II, to 7.60, the distance between clusters I and III (Table 5.19).

Table 5.19: Mahalanobis's distance (D^2) among three clusters with different rust reaction patterns to 4 rust isolates in the greenhouse

	I	II	III
I	0.00		
II	3.72	0.00	
III	7.60	5.02	0.00

2. Cluster analysis of disease reaction of 23 pureline bean cultivars to nine described rust isolates in the greenhouse

The reaction of 23 bean cultivars to 9 rust races on a 1-7 scale is presented in Table 5 (Chapter I). Three cluster analysis methods in SAS were used to cluster the 23 observations. Three, two and three cluster groups were obtained for complete linkage, average linkage and Ward's minimum variance method, respectively (Figure 5.12, Table 5.20). The differences in response patterns of cluster members along the first, second and third principal axes

Table 5.20: Cluster analysis of 23 bean cultivars based on their reactions response patterns to nine bean rust races

Cluster Methods		
CI	CII	CIII
	Complete Linkage	
LaVega	Mexico-309	C-49-242
CNC-3	B-190	KW-780
Mexico-235	Nep-2	M/WhfRnr
Ecuador-299	Cuilapa-72	ICA-Pijao
CNC-2	51051	Pinto-111
Rico-Bajo-1014	Aurora	Seafarer
CNC	C-20	Pindak
Olathe		GN-1140
	Average Linkage	
LaVega	C-49-242	
CNC-3	KW-780	
Mexico-235	M/WhfRnr	
Ecuador-299	ICA-Pijao	
CNC-2	Pinto-111	
Rico-Bajo-1014	Seafarer	
CNC	Pindak	
Mexico-309	GN-1140	
B-190		
Cuilapa-72		
51051		
Aurora		
C-20		
Nep-2		
Olathe		
	Ward's Method	
LaVega	Mexico-309	C-49-242
CNC-3	B-190	ICA-Pijao
Mexico-235	Nep-2	Pinto-111
Ecuador-299	Cuilapa-72	Seafarer
CNC-2	51051	Pindak
Rico-Bajo-1014	Aurora	GN-1140
CNC	C-20	KW-780
Olathe		M/WhfRnr

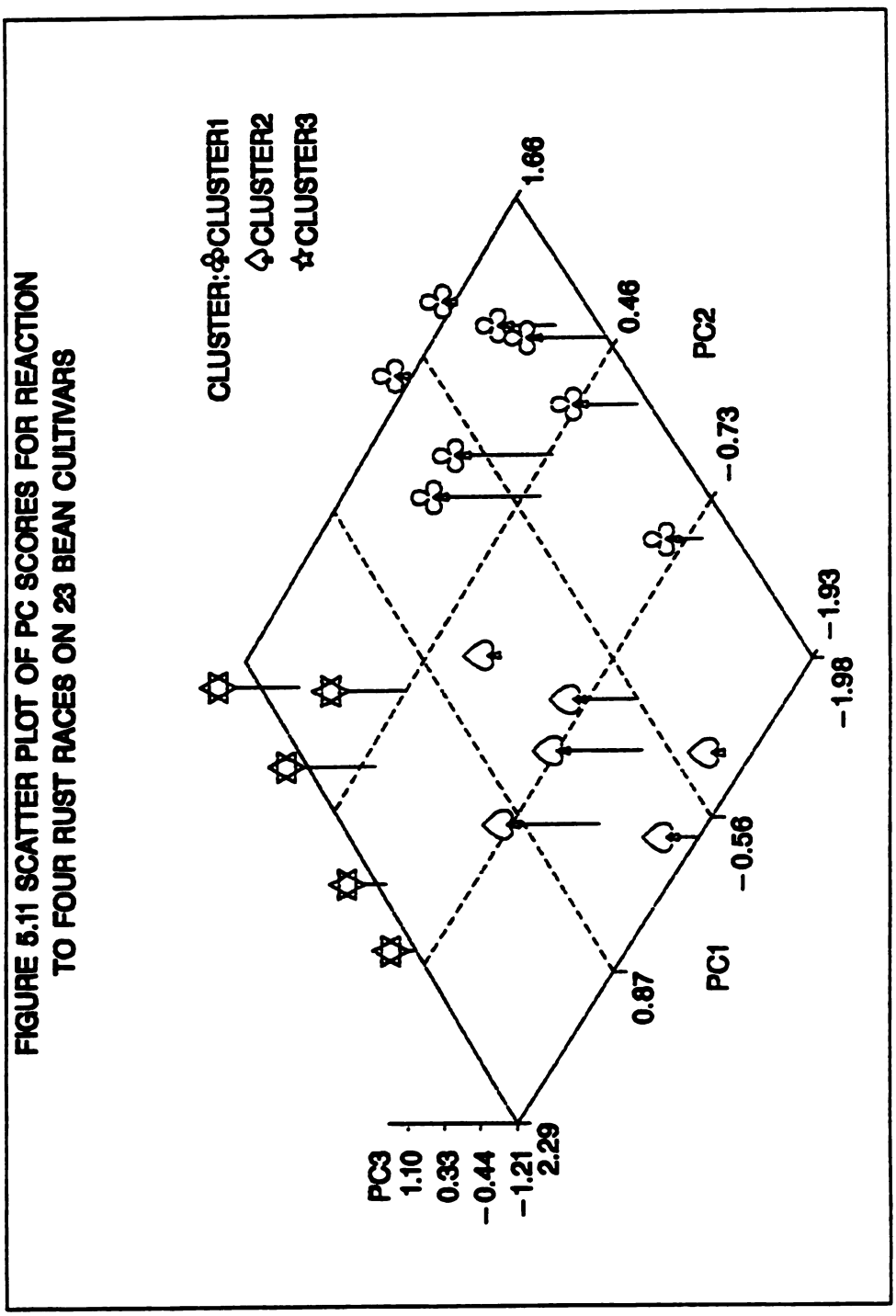


FIGURE 5.11 SCATTER PLOT OF PC SCORES FOR REACTION TO FOUR RUST RACES ON 23 BEAN CULTIVARS

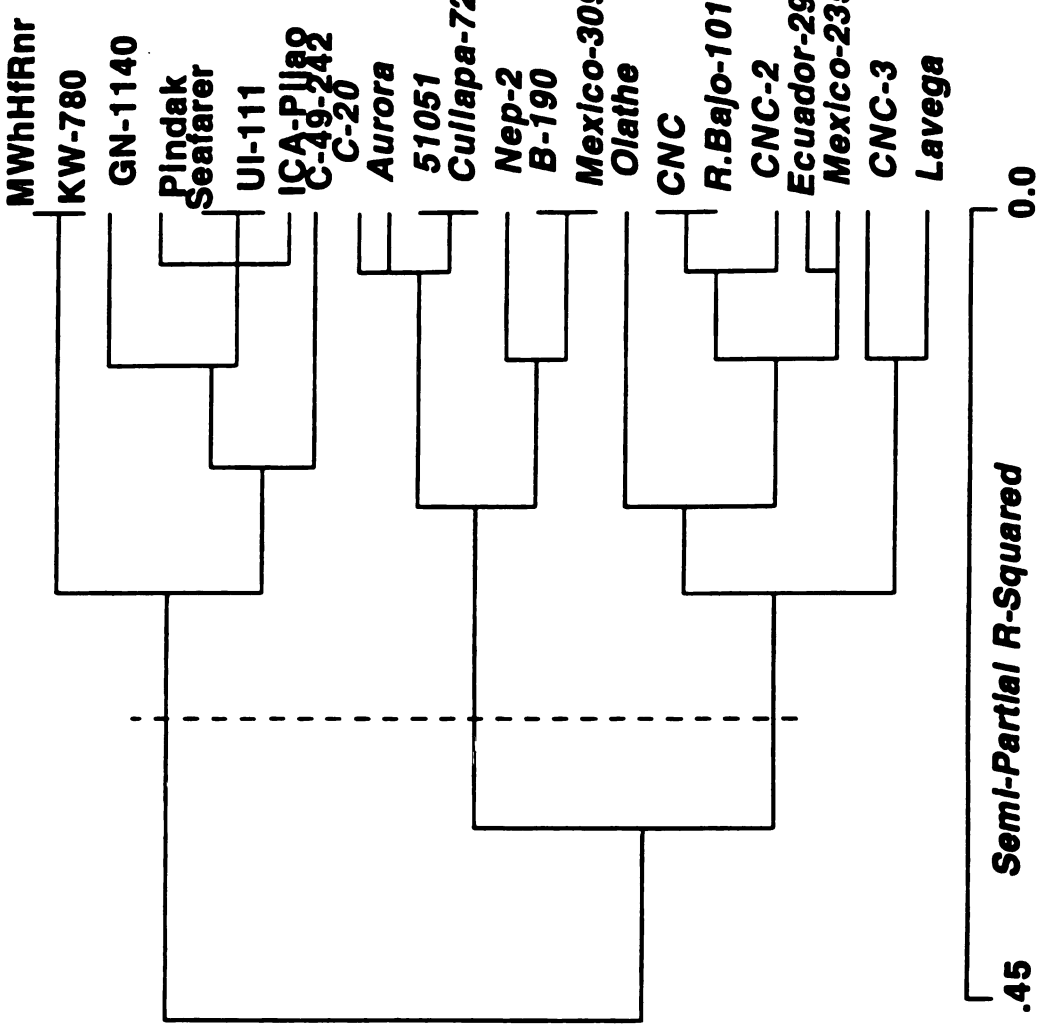


Figure 5.12 Ward's clustering of disease reaction of 23 bean cultivars to nine bean rust races.

(accounting for 77.0 percent of total variation) of a principal component analysis of 23 cultivars for reaction to nine rust races is shown in Figure 5.13. Mahalanobis's distance (D^2) among the clusters ranged from 5.02, the distance between clusters II and III, to 10.73, the distance between clusters I and II (Table 5.21).

Table 5.21: Mahalanobis' distance (D^2) among three clusters with different rust reaction patterns for nine described rust races in the greenhouse

Clusters	I	II	III
I	0.00		
II	10.73	0.00	
III	7.32	5.02	0.00

The cultivar members in Cluster II of complete linkage and Cluster II of both average linkage and Ward's method were the same except the ordering of the cultivars in the hierarchy (Figure 5.12, Table 5.20). The clustering procedure of the complete linkage algorithm and Ward's minimum variance method with three cluster groups in each produced certain interesting features that are similar to the original grouping by Ghaderi et al. (1984). The procedures clustered cultivars LaVega, CNC-3 and Mexico-235 in Cluster I along with other cultivars with both broad resistance genes (Ecuador-299, CNC-2 and CNC), Nep-2 and Aurora in Cluster II along with Mexico-309 and Cuilapa-72 in the same cluster including other similarly broadly resistant cultivars such as B-190 (which is the progeny of Mexico-309), and cultivars 51051 and C-20. In the third group, KW-780 and ICA-Pijao were clustered with other cultivars of moderate resistance and with cultivars such as Seafarer, Pinto-111 and C-49-242 with susceptibility to several races.

In the case of average linkage method, two clusters were produced separating the resistant cultivars (Cluster I) from the susceptible cultivars (Cluster II). Owing to the small number of cluster groups (two clusters) formed in the average linkage method, 15 cultivars

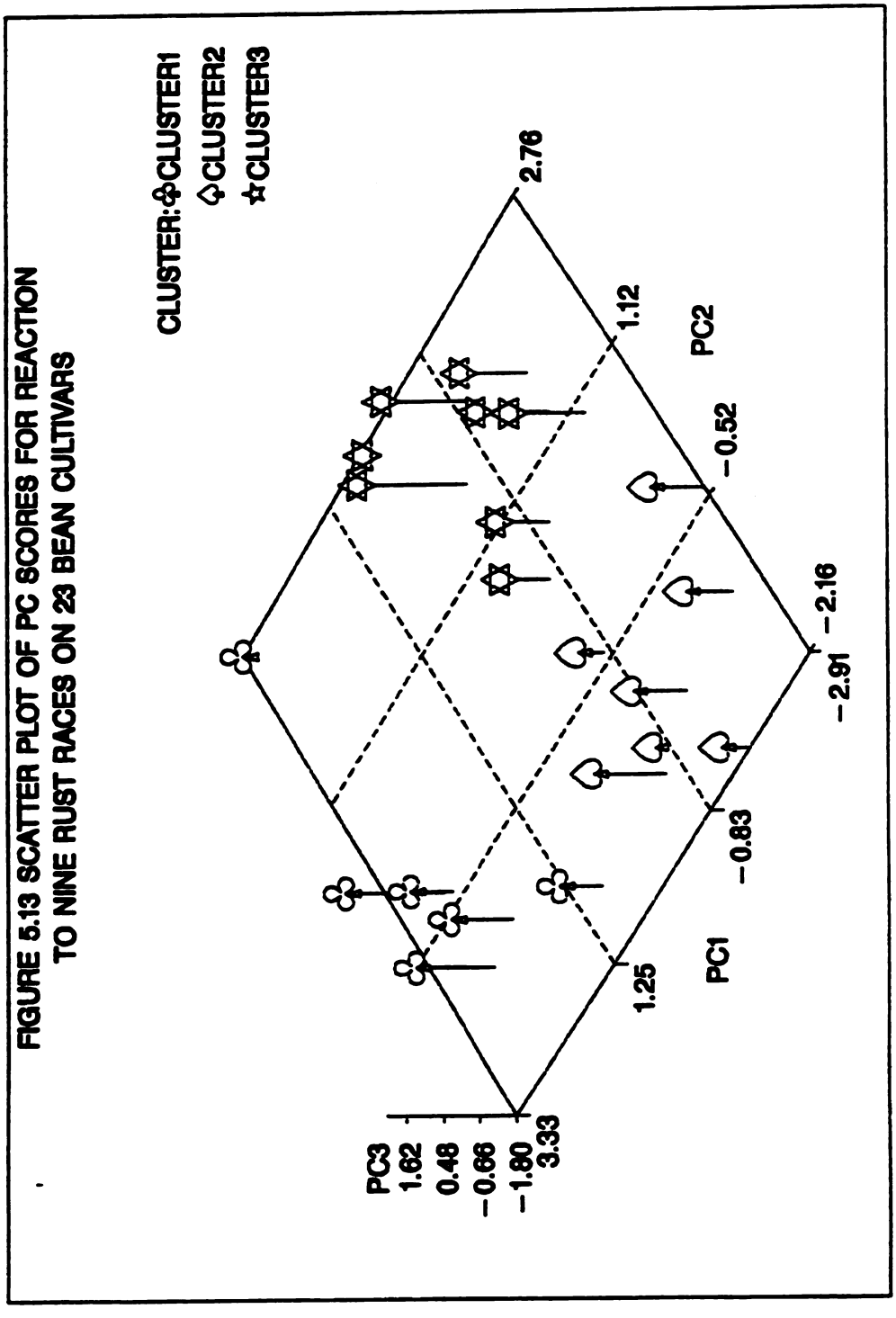


FIGURE 5.13 SCATTER PLOT OF PC SCORES FOR REACTION TO NINE RUST RACES ON 23 BEAN CULTIVARS

that were grouped in two separate clusters in complete linkage (Table 5.20) were grouped together in one cluster (Cluster I, Table 5.20). Although all 15 cultivars have been lumped together in one cluster for the average method, the original clustering patterns of the 1976 IBRN have still been recreated, i.e., cultivars LaVega, CNC-3 and Mexico-235 (III of Ghaderi et al., 1984), Cuilapa-72, Mexico-309 and Rico Bajo 1014 (V of Ghaderi et al., 1984), Nep-2, Aurora and Ecuador-299 (VII of Ghaderi et al., 1984). The only exception has been the breakup of C-49-242 and CNC-2 (IV of Ghaderi et al., 1984). Improved efficiency in clustering of cultivars was also apparent in this case where cultivars were separated into groups that express correct classification into reaction phenotypes that reflect similar genes for reaction to the races they were tested against. Although not identified in the original clustering by Ghaderi et al. (1984), the clustering together of the cultivars CNC with its progenices CNC-2 and CNC-3 and Cuilapa-72 with 51051 and Ecuador-299 with Mexico-235, which are known to share common pedigrees and are also broadly resistant to many rust races, underscores the importance of testing conditions (pureline cultivars described races and controlled environments, etc.) for characterization of cultivar relationships.

3. Cluster analysis of disease reactions of 19 pureline bean cultivars to 26 described rust isolates in the greenhouse

The reactions to 26 described races of 19 bean cultivars on a 1 to 7 scale are presented in Table 7 (Chapter 1).

Cluster analysis of the data using three cluster analysis methods resulted in five, five and four cluster groups for complete linkage, average linkage and Ward's minimum variance method respectively (Figure 5.14, Table 5.22). Figure 5.15 displays the differences in reaction response patterns of cluster members along the first, second and third principal axes (accounting for 75 percent of total variance) of a principal components analysis of 19 cultivars for reaction to 26 rust races. Mahalanobis's distance (D^2) was impossible to calculate between

Table 5.22: Cluster analysis of 19 bean cultivars based on their reaction response patterns to 26 bean rust isolates

Cluster Methods				
CI	CII	CIII	CIV	CV
		Complete Linkage		
LaVega	KW-780	Olathe	Mexico-235	Mexico-309
C-49-242	M/WhfRnr		Ecuador-299	B-190
Pindak			CNC-2	Cuilapa-72
UI-111			CNC	51051
Seafarer				Aurora
				C-20
		Average Linkage		
LaVega	Mexico-235	Olathe	KW-780	Pindak
C-49-242	Ecuador-299		M/WhfRnr	UI-111
Mexico-309	CNC-2			Seafarer
B-190	CNC			
51051	Cuilapa-72			
Nep-2				
Aurora				
C-20				
		Ward's Method		
LaVega	Mexico-235	KW-780	Pindak	
C-49-242	Ecuador-299	M/WhfRnr	UI-111	
Nep-2	CNC-2	Seafarer		
Aurora	CNC			
C-2	Olathe			
Mexico-309				
B-190				
Cuilapa-72				
51051				

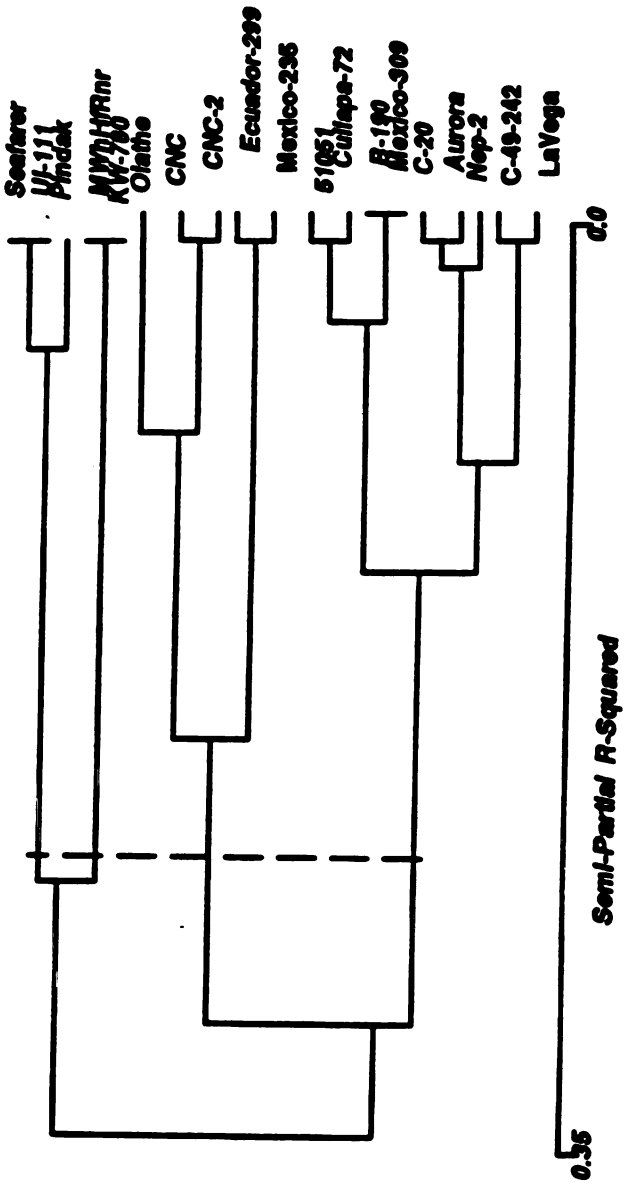


Figure 5.14 Ward's clustering of disease reaction of 19 bean cultivars to 26 bean rust races.

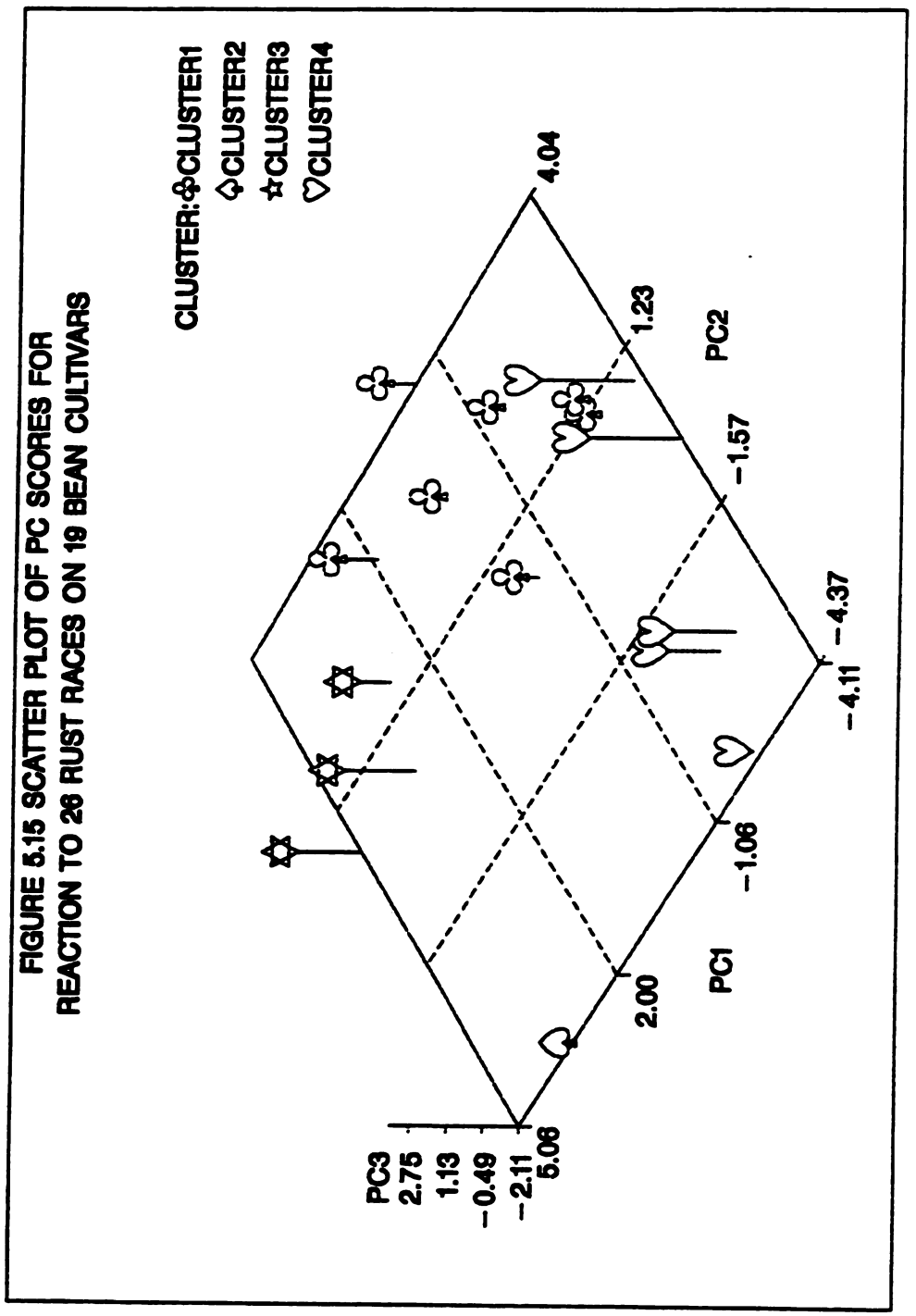


FIGURE 5.15 SCATTER PLOT OF PC SCORES FOR
REACTION TO 26 RUST RACES ON 19 BEAN CULTIVARS
CLUSTER:♣CLUSTER1
◇CLUSTER2
☆CLUSTER3
▽CLUSTER4
PC3
2.75
1.13
-0.49
-2.11
5.06
PC1
2.00
-1.06
-4.11
PC2
4.04
1.23
-1.57
-4.37

clusters due to problems of singularity. This is a situation in which any row or column of a matrix in A, for example, is equal to a linear combination of the other rows or columns (Ramm, 1989; personal communication). This situation leads to a pooled within correlation matrix with values of zero, making it impossible to compute D^2. The clustering of the cultivars into groups, although a characteristic of the clustering algorithm, seems to be affected by the criterion (Afifi and Clark, 1984; Romesburg, 1984) used to specify the numbers of cluster groups on the cluster dendogram (tree). The grouping of the cultivars based on their reaction response patterns to 26 rust races appears to be affected by just that criterion. With the complete linkage procedure using the same criterion, five cluster groups were obtained as in the average linkage procedure. The clusters in this procedure appear more realistic in that the cultivars are cast into their correct reaction phenotype categories. Cultivars in clusters II, III and IV of the complete linkage procedure were the same cultivars in clusters IV, III and II (in reverse order) of the average linkage method. The two methods were similar in separating the most susceptible group from the most resistant group with one cultivar (Olathe) being midway between these groups. One very obvious difference was in the grouping of the variable behaving cultivars LaVega and C-49-242. In both cases, cultivars that have known pedigree relationships such as CNC and CNC-2 (Cluster IV in complete linkage and Cluster II in average linkage), Mexico-239 and B-190, Cuilapa-72 and 51051 (Cluster V in complete linkage and Cluster I in average linkage) or those with presumed pedigree relationships such as Ecuador-299 and Mexico-235 (Cluster IV in complete linkage and Cluster II in average linkage) clustered together. The same trend is also apparent in Ward's minimum variance method, which produced only four clusters.

Considering the cluster outcome from Ward's method, four cluster groups were formed comprising cultivars LaVega, C-49-242, Nep-2, Aurora, C-20, Mexico-309, B-190, Cuilapa-72 and 51051 in Cluster I; Mexico-235, Ecuador-299, CNC-2, CNC and Olathe in

Cluster II; KW-780 and M/WhfRnr in Cluster III; and Pindak, UI-111 and Seafarer in Cluster IV. In this study cultivar pairs Nep-2 and Aurora (cluster VII of Ghaderi et al., 1984) and Cuilapa-72 and Mexico-309 (Cluster V of Ghaderi et al., 1984), which were included in Cluster I along with other cultivars did cluster together as in the 1976 IBRN. It also appears in this study that the clustering step has separated the most susceptible cultivars (Clusters III and IV) from the most resistant cultivars (Clusters I and II, Table 5.22). However, close examination of these cultivars by reaction response patterns to certain races or groups of races exhibiting similar reactions on these cultivars, reveals the clustering outcome may have been influenced by a certain dominant variable (Anderberg, 1973). This can be seen in the unlikely alignment of cultivars C-49-242 and LaVega, with variable response patterns to individual races (landrace behavior), along with cultivars Nep-2, Aurora, C-20, Cuilapa-72, Mexico-309, B-190 and 51051, cultivars that reportedly possess broad resistance genes to several races. Considering only the four described rust races (41, 46, 49 and 53), the only time the cluster outcome observed in this study can occur is if race 49 was the dominant variable to cluster the 19 bean cultivars. If race 49 was the dominant variable responsible for the cluster outcome as proposed here, the cultivars in Cluster I (with susceptible reaction to race 49) would have clustered together as susceptible groups; the cultivars in Cluster II (with resistant reaction to race 49) would have clustered together as resistant groups; the cultivars in Cluster III (with highly resistant reaction to race 49) would have clustered together as a highly resistant group; and the cultivars in cluster IV (with recognized susceptible reaction to race 49 and other races (universal susceptibles) would have clustered together as the more susceptible group thus confirming the above cluster outcome.

In all these methods, the earlier cluster alignment as in the 1976 IBRN reappeared with the clustering together of the cultivar Mexico-309 and Cuilapa-72 and cultivars Nep-2 and

Aurora. The stability in clustering together of the cultivars Mexico-309 and Cuilapa-72 in both field and controlled environment tests is particularly to be noted.

Observation of the cluster outcomes in general, from using four, nine or 26 described rust races on 23, 23 and 19 pureline cultivars in controlled test conditions resulted in three or four cluster groups that separated the cultivars into three or four reaction phenotype categories of homogeneous groups that more or less expresses correct classification of reaction phenotypes that in turn reflect similarity of genes or genetic complexes for reaction to the races in question.

Overall, it may be worthwhile noting the following:

i) that the use of pureline cultivars along with described rust isolates in controlled environments has allowed cultivar separation on the basis of correct reaction phenotypes that can be interpreted in terms of the gene-for-gene host-parasite system.

ii) although the clustering procedure selected for comparing (Ward's minimum variance method) cultivar relationship on variable attributes is reported as producing "compact" clusters with few cluster numbers, the procedure appears to have been particularly constrained by the inadvertent use of few number of observations in the controlled test conditions.

iii) the selection of the subset of cultivars from clusters III, IV, V, VII and VIII of Ghaderi et al. (1984) for further studies, although random, may have been biased toward selection of predominantly resistant entries (nine bean lines were reportedly highly resistant to several rust races as compared to four bean lines with variable reaction to several of the races). This bias is evident in the consistent clustering of these same cultivars together in many instances regardless of test conditions or clustering method used.

C. Rust isolate characterization

1. Cluster analysis of reactions elicited by 26 rust isolates on 19 bean cultivars

The reaction of 19 bean cultivars elicited by 26 rust races is presented in Table 1.9 (Chapter I). These reaction grades were submitted to several cluster analysis algorithms to identify the relationships of the various rust races that were used in clustering the cultivars. The results from three cluster analysis procedures on SAS are presented in Table 5.23. Four clusters in each were obtained for complete linkage, average linkage and Ward's minimum variance methods, respectively (Figure 5.16, Table 5.23). Races 38, 39, 63 and 64 were all grouped into Cluster I of the three grouping methods. The racial grouping for all methods was also identical in Clusters II, III and IV of complete linkage, average linkage and Ward's minimum variance method. The ordering of the races in the hierarchy in Cluster II of average linkage was different. Principal components analysis of the reactions elicited by the 26 races on the 19 bean cultivars along three axes (PC1, PC2 and PC3) accounting for 73.2 percent of total variation shows the relationships and differences in response patterns elicited by the 26 isolates (Figure 5.17).

2. Cluster analysis of reactions elicited by 33 rust isolates on 19 different bean cultivars

Reactions elicited by 33 rust isolates from collections in continental U.S., Puerto Rico and the Dominican Republic (Stavely et al., 1989) on 19 bean differentials is presented in Table 5.24. This includes the 26 isolates in Table 5.22 and races 44, 54, 55 and 62 described by Stavely (1984) and two new races (69 and 70).

Four cluster analysis methods were used to cluster the rust isolates and produced six, four, three and four cluster groups for complete, average, centroid and Ward's methods, respectively (Figure 5.18, Table 5.25). Figure 5.19 displays the differences in reaction patterns elicited on cluster members along the first, second and third principal axes accounting for 66

Table 5.23: Cluster analysis of the reaction elicted by 26 rust isolates on 19 bean cultivars in the greenhouse

Cluster Methods			
CI	CII	CIII	CIV
	Complete Linkage		
R38	R40	R65	R43
R39	R41	R66	R45
R63	R42	R67	R46
R64	R60		R47
	R59		R58
	R61		R48
	R52		R49
	R53		R50
	R57		R51
	R56		
	Average Linkage		
R38	R40	R65	R43
R39	R41	R66	R45
R63	R60	R67	R46
	R42		R47
	R52		R58
	R53		R48
	R57		R49
	R56		R50
	R59		R51
	R61		
	Ward's Method		
R38	R40	R65	R43
R39	R41	R66	R45
R63	R42	R67	R46
R64	R60		R47
	R59		R58
	R61		R48
	R52		R49
	R53		R50
	R57		R51
	R56		

Table 5.24: Rust isolates collected from different states in the U.S., Puerto Rico and the Dominican Republic in different years and the reaction* elicited on 19 different bean cultivars

Table 5.24: Rust isolates collected from different states in the U.S., Puerto Rico and the Dominican Republic in different years and the reaction* elicited on 19 different bean cultivars

Pathogenic Race	Collection and Isolate #	Origin State, states or country where originally obtained	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	s
38	73-16	NDm NJ, NY, VA, TN, MI, FL--on snap beans--J.P. Meinas	4.0	2.0	4.0	2.0	3.0	2.0	2.0	7.0	2.0	1.0	1.0	2.0	7.0	2.0	2.0	2.0	2.0	2.0	1.0
39	73-16	MD, VA, FL	4.0	2.0	4.0	2.0	3.0	2.0	7.0	7.0	2.0	1.0	1.0	2.0	7.0	2.0	2.0	2.0	2.0	2.0	1.0
40	73-23	MD, NJ, MI, ND, NE	7.0	6.0	7.0	6.0	3.0	7.0	2.0	2.0	2.0	2.0	2.0	4.0	6.0	2.0	2.0	2.0	2.0	2.0	4.0
41	73-32	MD, NJ, VA, NY, SC	7.0	4.0	7.0	4.0	7.0	6.0	3.0	6.0	5.0	2.0	2.0	4.0	5.0	2.0	2.0	2.0	2.0	2.0	4.0
42	79-4	FL (Belle Glade)	7.0	4.0	7.0	6.0	7.0	6.0	3.0	5.0	4.0	2.0	2.0	4.0	5.0	4.0	2.0	2.0	2.0	2.0	4.0
43	75-22	Arenac County, MI; NJ	7.0	4.0	7.0	2.0	7.0	4.0	3.0	6.0	4.0	4.0	4.0	4.0	5.0	2.0	2.0	2.0	6.0	7.0	4.0
44	79-15F	ND--Single collection in 1979 on Pinto UI-111	7.0	4.0	7.0	4.0	3.0	6.0	2.0	2.0	2.0	6.0	7.0	4.0	2.0	2.0	4.0	4.0	6.0	6.0	4.0
45	79-6-1	Belle Glade, FL--single collection in 1979 on Aurora	6.0	4.0	7.0	3.0	7.0	4.0	1.0	6.0	5.0	4.0	4.0	4.0	5.0	2.0	2.0	6.0	6.0	4.0	4.0
46	76-6A-1	Belle Glade, FL--single collection in 1979 on Aurora	7.0	4.0	7.0	4.0	7.0	4.0	6.0	6.0	5.0	4.0	4.0	4.0	6.0	4.0	6.0	7.0	6.0	4.0	2.0
47	79-6C-1	Belle Glade, FL--single collection in 1979 on Aurora	6.0	4.0	7.0	4.0	7.0	4.0	2.0	5.0	6.0	4.0	4.0	4.0	6.0	2.0	4.0	6.0	6.0	4.0	1.0
48	82-7-1-1	North Platte, NE--single collection in 1982	7.0	4.0	7.0	5.0	3.0	6.0	6.0	2.0	6.0	4.0	4.0	4.0	6.0	4.0	6.0	6.0	6.0	4.0	1.0
49	82-7-1-2	North Platte, NE--single collection in 1982	7.0	4.0	7.0	4.0	3.0	6.0	6.0	2.0	6.0	4.0	2.0	7.0	6.0	4.0	2.0	6.0	6.0	7.0	4.0
50	79-15B-1	ND--single collection in 1979 on Pinto UI-111	7.0	4.0	7.0	4.0	3.0	6.0	7.0	2.0	5.0	4.0	2.0	6.0	2.0	4.0	2.0	6.0	7.0	6.0	4.0
51	79-15G-1	Hatton, ND--1982 collection as race #44 on Pinto UI-111	3.0	7.0	7.0	6.0	3.0	4.0	2.0	2.0	2.0	6.0	7.0	6.0	2.0	2.0	2.0	4.0	6.0	6.0	4.0
52	79-15A-1	ND (Forest River-1981); MI (Sanilac, 1975)--collection on Pinto UI-111	7.0	4.0	7.0	6.0	7.0	6.0	6.0	5.0	4.0	2.0	2.0	4.0	5.0	7.0	4.0	2.0	2.0	2.0	4.0
53	79-4B-1	Belle Glade--1979, Florida	7.0	7.0	7.0	6.0	7.0	7.0	6.0	5.0	5.0	2.0	2.0	4.0	5.0	7.0	4.0	2.0	2.0	2.0	4.0
54	81-10A-1	ND, Valentine (Tacas-1981)--same collection as race #44 on Pinto UI-111	7.0	4.0	7.0	6.0	3.0	7.0	6.0	2.0	4.0	2.0	2.0	4.0	2.0	7.0	4.0	2.0	2.0	2.0	4.0
55	79-15A-4	North Dakota	7.0	5.0	7.0	7.0	7.0	6.0	6.0	5.0	4.0	2.0	2.0	4.0	5.0	7.0	4.0	2.0	2.0	2.0	4.0
56	82-10A-1	Crossville, Texas--1982 collection on white-seeded Blue Lake Snap Bean	7.0	4.0	7.0	6.0	7.0	6.0	6.0	5.0	4.0	2.0	2.0	2.0	5.0	7.0	2.0	2.0	2.0	4.0	4.0
57	81-10A-3-1	Valentine, Texas--1981 collection from Cu. San Juan Silver & Pinto UI-111	7.0	6.0	7.0	6.0	7.0	6.0	6.0	6.0	6.0	2.0	2.0	4.0	5.0	7.0	2.0	2.0	2.0	2.0	4.0
58	84-2B	Higuey, Dominican Republic through J.R. Steadman	7.0	6.0	7.0	6.0	7.0	4.0	6.0	5.0	5.0	4.0	4.0	6.0	6.0	6.0	4.0	6.0	7.0	4.0	2.0
59	84-3A	Scotts Bluff, NE, USA through J.R. Steadman	3.0	4.0	7.0	4.0	7.0	6.0	2.0	6.0	4.0	2.0	2.0	4.0	6.0	2.0	4.0	2.0	2.0	2.0	4.0
60	84-3-2	Scotts Bluff, NE, USA through J.R. Steadman	7.0	4.0	7.0	4.0	7.0	6.0	2.0	5.0	6.0	2.0	2.0	4.0	6.0	2.0	4.0	2.0	2.0	2.0	4.0
61	84-3-3	Scotts Bluff, NE, USA through J.R. Steadman	7.0	2.0	7.0	4.0	7.0	4.0	2.0	6.0	4.0	2.0	2.0	4.0	6.0	2.0	4.0	2.0	2.0	2.0	4.0
62	84-2-2	Hiquez, Dominican Republic through J.R. Steadman	7.0	4.0	7.0	4.0	3.0	6.0	6.0	2.0	5.0	4.0	4.0	4.0	4.0	4.0	6.0	7.0	7.0	4.0	2.0
63	84-3-5	Scotts Bluff, NE, USA through J.R. Steadman	6.0	7.0	7.0	6.0	3.0	4.0	2.0	2.0	2.0	7.0	7.0	4.0	2.0	2.0	2.0	4.0	7.0	1.0	4.0
64	84-3-7	Scotts Bluff, NE, USA through J.R. Steadman	3.0	7.0	7.0	6.0	3.0	4.0	2.0	2.0	5.0	7.0	4.0	4.0	2.0	2.0	2.0	4.0	7.0	4.0	4.0
65	84-5	Isabella, Puerto Rico from B-190 through G.F. Freytag	7.0	4.0	7.0	4.0	3.0	7.0	7.0	2.0	7.0	4.0	4.0	7.0	2.0	4.0	7.0	7.0	7.0	6.0	4.0
66	84-4E	Homestead, FL--Rogers Bros. Seed Co.--Claude Dean	6.0	7.0	7.0	6.0	3.0	4.0	2.0	2.0	6.0	7.0	7.0	4.0	2.0	2.0	4.0	4.0	6.0	4.0	4.0
67	85-2B	Homestead, FL--Robert McMillan	7.0	4.0	7.0	4.0	7.0	6.0	6.0	5.0	5.0	4.0	2.0	6.0	5.0	4.0	6.0	6.0	6.0	6.0	5.0
68	85-4D	Homestead, FL--Robert McMillan	7.0	4.0	7.0	6.0	7.0	6.0	5.0	6.0	4.0	2.0	2.0	4.0	5.0	6.0	4.0	2.0	2.0	2.0	4.0
69	87-5D	Homestead, FL--Pinto UI-111--Robert McMillan	7.0	4.0	7.0	6.0	3.0	6.0	6.0	2.0	6.0	2.0	2.0	4.0	4.0	6.0	4.0	2.0	2.0	2.0	4.0
70	87-3A	Homestead, FL--McCoslan Pole--Robert McMillan	7.0	7.0	7.0	6.0	3.0	6.0	6.0	2.0	5.0	2.0	2.0	4.0	2.0	7.0	4.0	2.0	2.0	2.0	4.0

*reaction converted to 1-7 scale

**previously reported US races 38-57 by Stavely, J.R., 1984, Plant Dis. 68: 95-99

a = US 3
b = CSW 643
c = Pinto 650
d = KW-765
e = KW-780
f = KW-814
g = Golden Gate Wax
h = Early Gallatin
i = Redlands Pioneer
j = Ecuador 299
k = Mexico 235
l = Mexico 309
m = Brown Beauty
n = Olate
o = Ax537
p = Nep-2
q = Aurora
r = 51051
s = CNC

Table 5.25: Cluster analysis of 33 bean rust races (*U. Appendiculatus*) based on their ability to elicit similar reaction responses to 19 different bean cultivars

Cluster Methods					
CI	CII	CIII	CIV	CV	CVI
		Complete Linkage			
R38	R40	R52	R43	R48	R44
R39	R41	R55	R45	R62	R51
	R60	R68	R47	R65	R63
	R61	R53	R46	R49	R64
	R42	R57	R58	R50	R66
	R59	R56	R67		
	R54				
	R69				
	R70				
		Average Linkage			
R38	R40	R43	R44		
R39	R41	R45	R51		
	R60	R47	R63		
	R61	R46	R64		
	R42	R58	R66		
	R59	R67			
	R52	R48			
	R55	R62			
	R68	R65			
	R53	R49			
	R57	R50			
		R56			
		R54			
		R69			
		R70			
		Centroid Method			
R38	R40	R44			
R39	R41	R51			
	R60	R63			
	R61	R64			
	R42	R66			
	R59				
	R52				
	R55				
	R68				
	R53				
	R57				
	R56				
	R54				
	R69				
	R70				
	R43				
	R45				
	R47				
	R46				
	R58				
	R67				
	R48				
	R62				
	R65				
	R49				
	R50				
		Ward's Method			
R38	R52	R43	R44		
R39	R55	R45	R51		
R40	R68	R47	R63		
R41	R56	R46	R64		
R60	R53	R58	R66		
R61	R57	R67			
R42	R54	R48			
R59	R69	R62			
	R70	R65			
		R49			
		R50			

Figure 5.16 Ward's clustering of reactions elicited

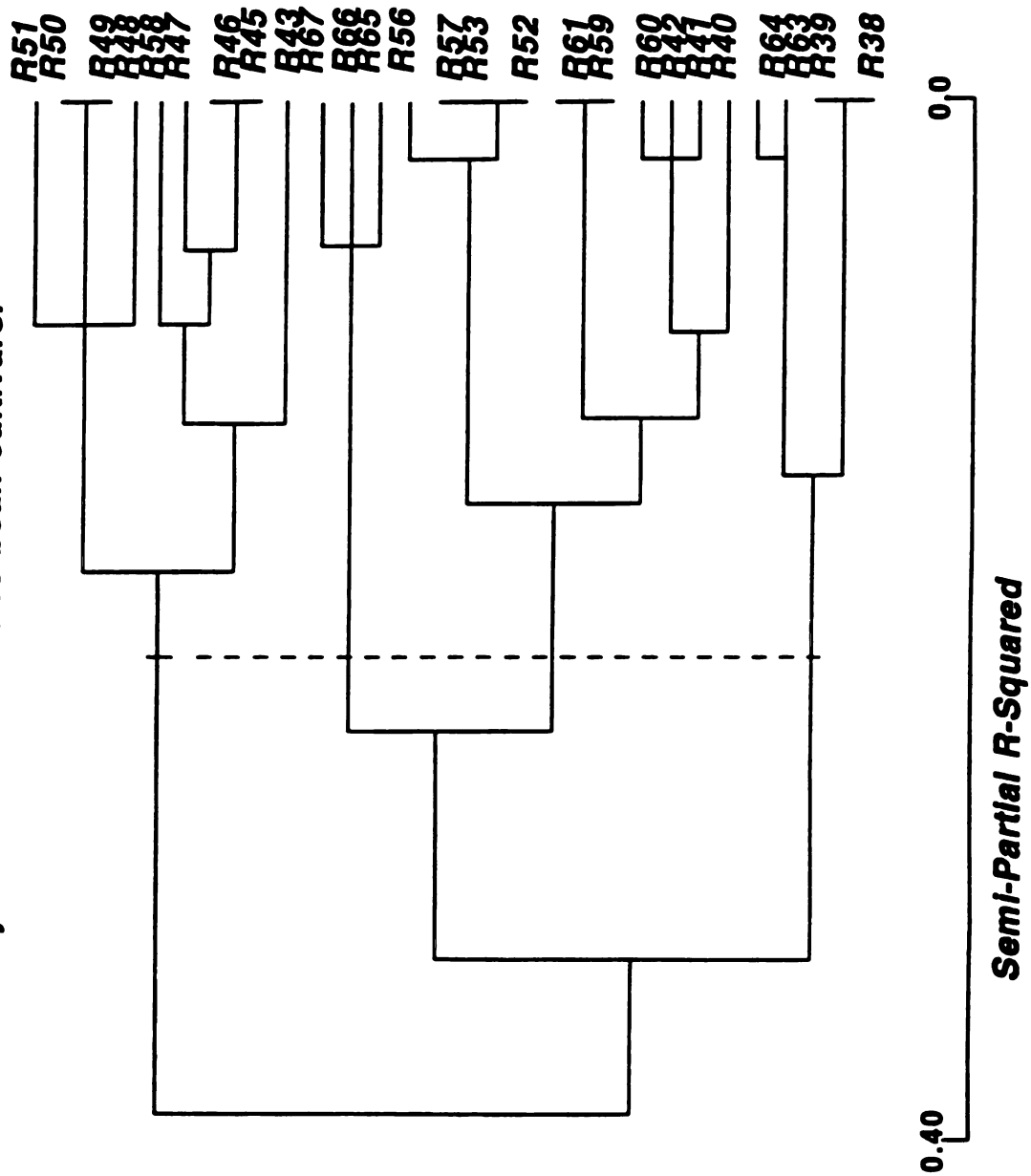


Figure 5.16 Ward's clustering of reactions elicited by 26 rust races on 19 bean cultivars.

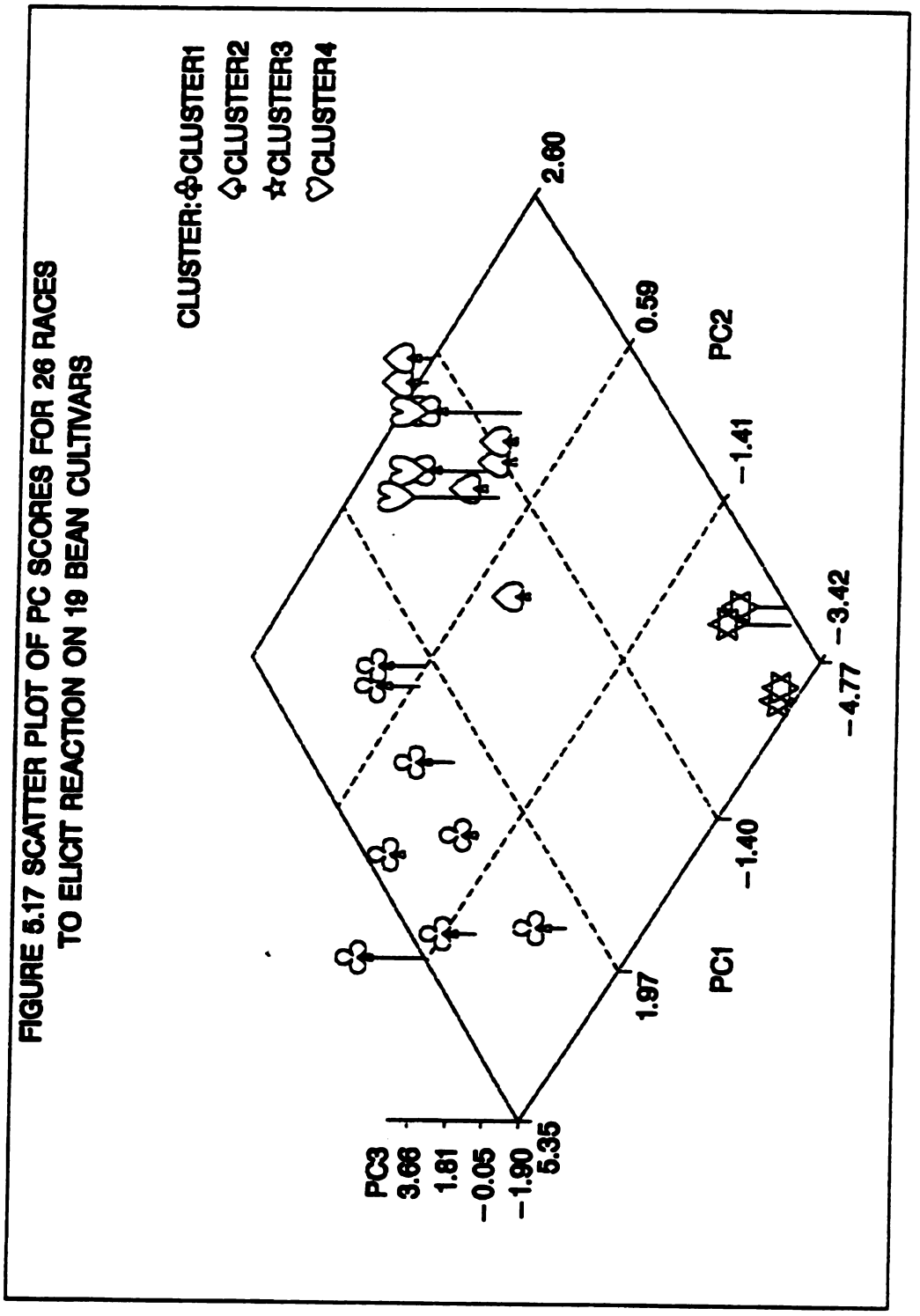
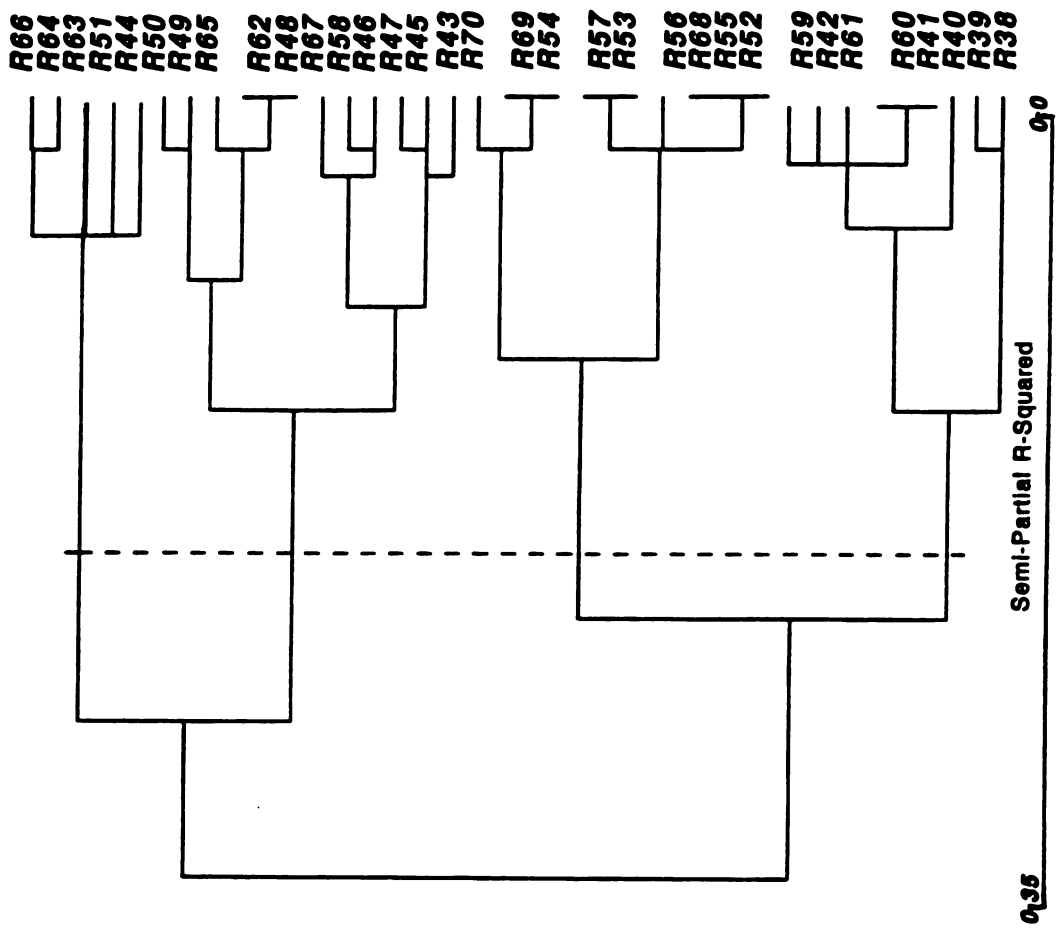


FIGURE 5.17 SCATTER PLOT OF PC SCORES FOR 26 RACES TO ELICIT REACTION ON 19 BEAN CULTIVARS

Figure 5.18 Ward's clustering of reactions elicited by 33 rust races on 19 bean cultivars.



Fgure 5.18 Ward's clustering of reactions ellcited by 33 rust races on 19 bean cultivars.

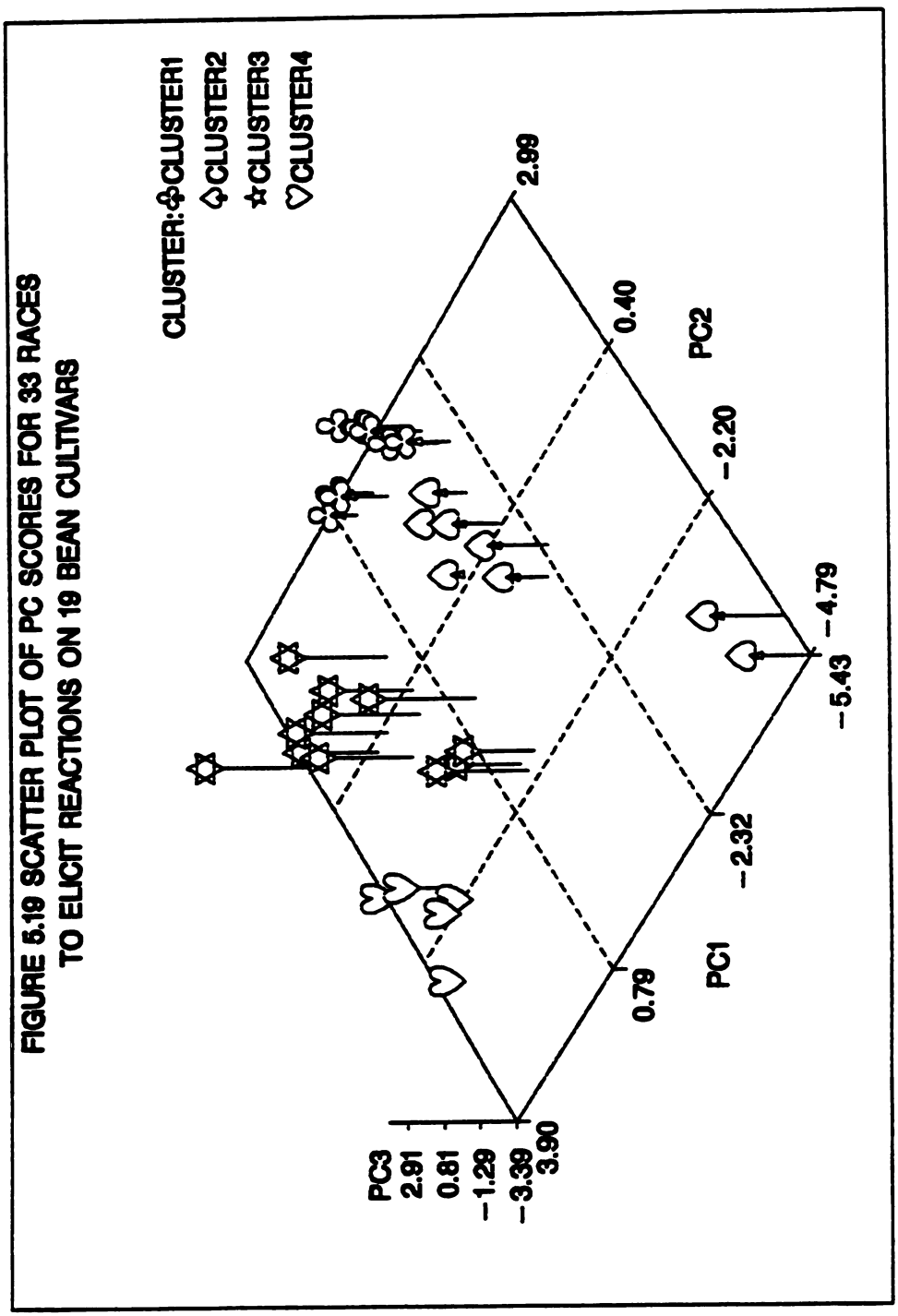


FIGURE 5.19 SCATTER PLOT OF PC SCORES FOR 33 RACES TO ELICIT REACTIONS ON 19 BEAN CULTIVARS

percent of total variation of a principal component analysis of 33 races for eliciting similar reactions on 19 differential bean cultivars. Mahalanobis's distance (D^2) ranged from 7.07, the distance between clusters II and III, to 63.48, the distance between clusters III and IV (Table 5.26). All four clustering methods had similar clustering for races 44, 51, 63, 64 and 66, which were clustered together in clusters VI, IV, III and IV for complete, average, centroid and Ward's methods, respectively. Races 38 and 39, which are the most common races on snapbeans (Stavely 1984a), were grouped together separately in Cluster I of complete, average and centroid methods, but combined with other races (40, 41, 60, 61, 42 and 59) with Ward's method. Notwithstanding the equal number of clusters formed in each of average and Ward's methods, they all agreed in the races that were associated in Cluster III by both methods. As indicated earlier, their difference was in the cluster grouping of races 40 through 70. The most variant of the methods appeared to be centroid clustering. Although it agreed with most other methods in clustering races 38 and 39 in Cluster 1 and races 44 through 66 in Cluster III, its Cluster II contained all of the races clustered into four groups with complete linkage, two clusters in average linkage and almost three clusters by Ward's method. Essentially similar patterns of clustering were observed between the two data sets with slight differences that had valid biological explanations.

Table 5.26: Mahalanobis's distance (D^2) among four clusters with different rust reaction eliciting patterns for 33 rust isolates in the greenhouse

Cluster	I	II	III	IV
I	0.00			
II	34.18	0.00		
III	36.96	7.07	0.00	
IV	31.32	60.97	63.48	0.00

The addition of seven more rust isolates (races 44, 54, 55, 62, 68, 69 and 70) over the 26 rust isolates in Table 9 (Chapter I) for cluster analysis of reactions elicited on 19 differentials did not change essentially the recurring similarities in the cluster patterns of the methods used in this study. Despite the consistent performance of the clustering methods used in producing very similar clustering patterns within each data set, the main difference appears to have come from the composition of the cultivars in each data set. This difference has a biological basis. As similar cultivars are used to identify pathogenic races, similar pathogenic races are also required to identify the cultivars. The use of different sets of cultivars in each data coupled with differences in number and kinds of isolates was thus the main reason for the clustering patterns observed between the two data sets.

Rust isolates that were clustered by their ability to elicit similar reaction responses on 19 bean cultivars in two separate cluster steps were employed to learn their relationships on one another. The first set consisted of the 26 described rust isolates that were applied on 19 bean cultivars while the second set consisted of 33 described rust isolates that were tested on 19 standard bean differentials. Of these, nine bean cultivars and 26 rust isolates were common to both test sets. The second test set with 33 isolates contained seven more isolates (44, 54, 55, 62, 68, 69 and 70) that were not included in the first test set.

Four racial cluster groups were produced for each test set when Ward's minimum variance method was used on each data set separately. The pattern of clustering and assignment of rust isolates within cluster for each test set was basically similar. Several isolates that were clustered together in the test set with 19 cultivars x 26 isolates, were also clustered together in the test set consisting of 19 cultivars x 33 isolates. The clustering pattern, however, displayed groups far from complete agreement. In spite of having 26 isolates in common, obvious differences in the clustering patterns were noted, this difference emanating from their differences in the composition of the differential cultivars within each

test set (nine common differentials from a total of 19) and the differences in the number of isolates in each test set (26 vs 33 isolates). Nevertheless, the clustering of the rust isolates identified four groups and, by extension of the same theory from cultivar clusters, each group belongs to the same virulence/pathogenicity group possessing similar genes or genic complexes for eliciting similar pathogenic reaction phenotypes on the sets of host differentials.

The clustering of rust isolates into homogeneous groups of similarly behaving entities reveal groups of variables that are similar in behavior in a number of basic characteristics. It appears that as much as it is important to learn the presence of basic similarities/identities in the host system, it is equally important to assess the same in the pathotypes for a coherent understanding of the interacting entities in a host-parasite system.

D. Evaluation of cultivar relationships by biochemical and agrophysiological traits

1. Cluster analysis of 22 bean cultivars on the basis of scores for six agrophysiological traits

Scores on varying scales of six agrophysiological characters on 22 bean cultivars are presented in Table 2.9 (Chapter II). The results of cluster analysis using three procedures of clustering produced four, four and six clusters for complete linkage, average linkage and Ward's method, respectively (Figure 5.20, Table 5.27).

The dominant pattern for the first two clustering methods (complete linkage and average linkage) is the clustering of the tropical small blacks along with two small reds and a small pink into one group (Cluster I). The influence of a particular variable (attribute) may be responsible (Anderberg, 1973) for this outcome. Complete and average linkage methods with the same number of groups had an almost identical clustering outcome with the exception of the ordering of cultivars within clusters in each method.Cultivars in Cluster I of complete and average linkage were split and formed clusters I and II of Ward's method. One cultivar

Table 5.27: Cluster analysis of 22 bean cultivars based on their scores for six agrophysiological traits using three clustering methods

Cluster Methods					
CI	CII	CIII	CIV	CV	CVI
		Complete Linkage			
LaVega	Nep-2	Sanilac	Montcalm		
CNC-3	Aurora				
CNC-2	Pinto-111				
B-190	KW-780				
Mexico-309	GN-1140				
CNC	M/WhfRnr				
Mexico-235					
Ecuador-299					
C-49-242					
Cuilapa-72					
ICA-Pijao					
Bat-1320					
Bac-87					
R-B-1014					
		Average Linkage			
LaVega	Sanilac	Montcalm	LaVega		
Nep-2	Aurora				
CNC-3	KW-780				
CNC-2	GN-1140				
B-190	M/WhfRnr				
Mexico-309	Pinto-111				
CNC	Mexico-235				
Mexico-235	Ecuador-299				
Ecuador-299					
C-49-242					
Cuilapa-72					
ICA-Pijao					
Bat-1320					
Bac-87					
RB-1014					
		Ward's Method			
LaVega	C-49-242	RB-1014	KW-780	Sanilac	Montcalm
CNC-3	Cuilapa-72	Nep-2	GN-1140		
CNC-2	ICA-Pijao	Aurora	M/WhfRnr		
B-190	Bat-1320	Pinto-111			
Mexico-309	Bac-87				
CNC					
Mexico-235					
Ecuador-299					

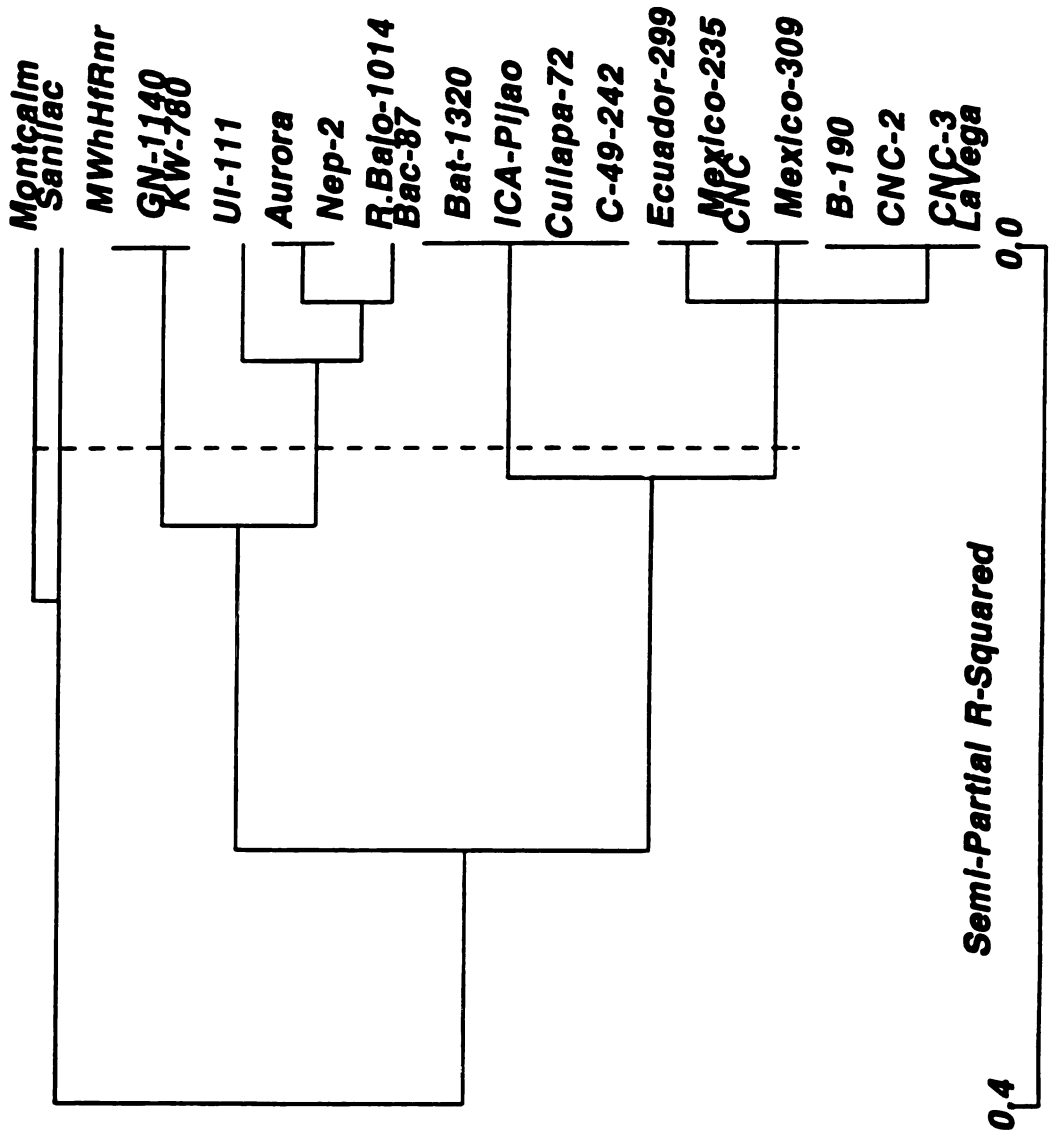


Figure 5.20 Ward's clustering of 22 bean cultivars on the basis of six agrophysiological traits.

member each constituted cluster III (Sanilac, a pea bean) and IV (Montcalm, a large red kidney bean) of both complete and average linkage methods and clusters V and VI in Ward's method (Figure 5.20, Table 5.27).

In the clustering of the 22 bean cultivars based on the six agrophysiological traits, cluster patterns that were obtained by Ghaderi et al. (1984) on the 1976 IBRN were also evident here. For complete and average clustering, cultivar LaVega and CNC-3, CNC-2 and C-49-242, Mexico-309, Cuilapa-72 and Rico-Bajo-1014 and Nep-2 and Aurora were in the same large cluster. These were included in Clusters III, IV, V and VII, respectively, of Ghaderi et al. (1984). The one exception was the breakup of cluster VIII of Ghaderi et al. (1984) composed of a white, flat, kidney bean (KW-780) and ICA-Pijao (a tropical small bean). These cultivars had earlier clustered together on the basis of field reactions in the 1976 IBRN and for disease reaction to described races in controlled environments. For Ward's method, only the clusters containing cultivars Nep-2 and Aurora (Cluster III) and LaVega and CNC-3 (Cluster I) had similar groupings as in the 1976 IBRN. The other cluster groups appeared to have clustered by certain variables that influenced (Anderberg, 1973) the cluster outcome.

The clustering of the cultivars into six groups by Ward's method caused a slight difference in cultivar cluster membership in clusters I, II, III and IV. Scattering of PC scores on the first, second and third principal axes accounting for 83.9 percent of total variation of a PCA on six agrophysiological traits of 22 bean cultivars is shown in Figure 5.21. Mahalanobis's distance (D^2) among the clusters ranged from 3.39, the distance between clusters III and IV (Table 5.28) to 20.95 between clusters II and III.

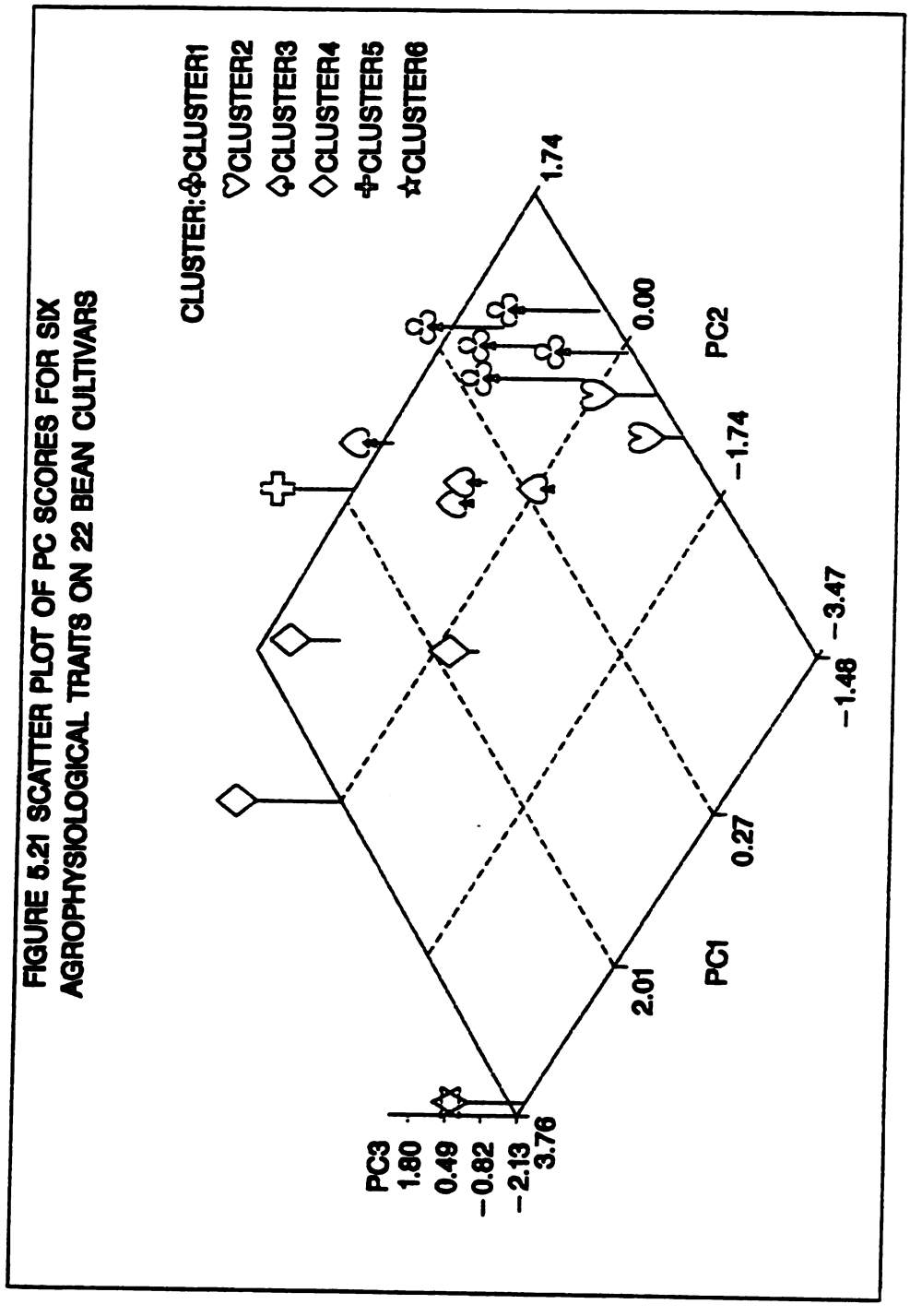


FIGURE 5.21 SCATTER PLOT OF PC SCORES FOR SIX AGROPHYSIOLOGICAL TRAITS ON 22 BEAN CULTIVARS
CLUSTER:♣CLUSTER1
♡CLUSTER2
♤CLUSTER3
◇CLUSTER4
✚CLUSTER5
☆CLUSTER6
PC3
1.80
0.49
−0.82
−2.13
3.76
2.01
PC1
0.27
−1.48
−3.47
PC2
1.74
0.00
−1.74

Table 5.28 Mahalanobis's distance (D^2) among six clusters with different patterns for agrophysiological scores of six seed traits

Cluster	I	II	III	IV	V	VI
I	0.00					
II	9.35	0.00				
III	13.23	20.95	0.00			
IV	10.55	18.59	3.39	0.00		
V	4.23	11.36	10.75	8.77	0.00	
VI	10.62	7.54	19.17	11.65	9.18	0.00

2) Cluster analysis of 20 bean cultivars on the basis of Isozyme mobility scores as fast(1) and slow(2) for 12 enzyme systems

Isozyme mobility scores, as fast (1) and slow (2), for 12 enzyme systems assayed on 20 bean cultivars are shown in Table 2.3 (Chapter II). Cluster analysis results from five cluster analysis methods (single linkage, complete linkage, average linkage, centroid method, and Ward's method) produced two cluster groups for each method (Figure 5.22, Table 5.29) when Romesburg's (1984) criterion was applied.

The dominant feature of the cluster analysis's results with the above five methods is the grouping of cultivars into two groups with the small to medium seeded cultivars clustering together in cluster I and the large-seeded cultivar, Montcalm, clustering as a single cultivar cluster in Cluster II. This result was in agreement with the result of the clustering into two cluster groups of the isozyme mobility score using the UWPGMA method on the basis of allelic frequencies of enzyme loci. These clustering results also concur with the identification of two major clusters that coincide with the earlier clustering of Phaseolus spp. into the large-seeded beans of the Andean South American with a T or C phaseolin protein type and the small-seeded bean of Meso-American with the S phaseolin protein types (Gepts et al., 1986; Sprecher, 1988). However, this outcome although not unexpected has limited relevance in this study as far as its utility for assessing cultivar interrelationships is concerned. The formation

Table 5.29: Cluster analysis of 20 bean cultivars based on isozyme mobility scores of 12 enzyme systems.

Single Linkage		Complete Linkage		Average Linkage		Centroid Method		Ward's Method	
CI	CII	CI	CII	CI	CII	CI	CII	CI	CII
CNC-2	Montcalm	*	Montcalm	*	Montcalm	*	Montcalm	*	Montcalm
C-49-242									
RB-1014									
Cuilapa-72									
ICA-Pijao									
B-190									
LaVega									
Sanilac									
Mexico-309									
Pinto-114									
GN-1140									
Ecuador-299									
M/WhfRnr									
Bat-1320									
Nep-2									
Aurora									
Pinto-111									
Mexico-235									
KW-780									

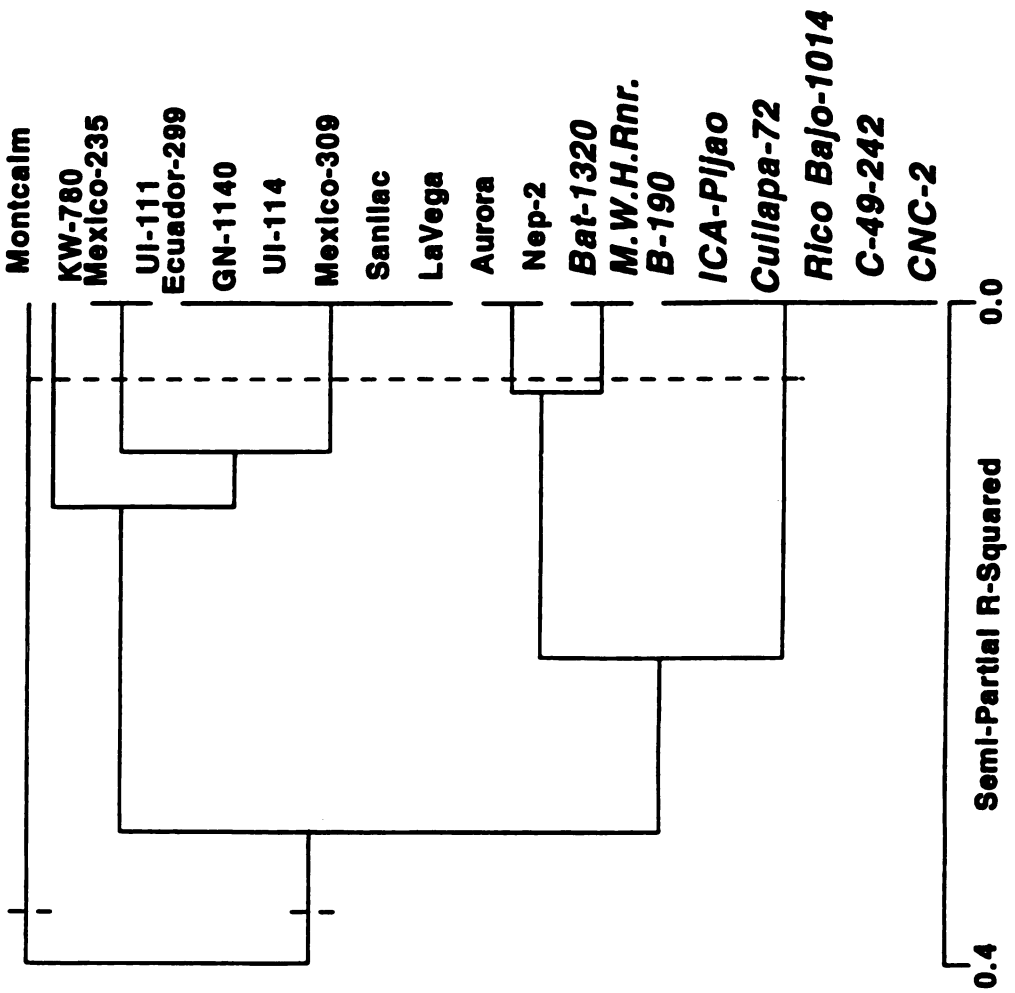


Figure 5.22 Ward's clustering of 20 bean cultivars on the basis of isozyme mobility scores

of seven clusters appears appropriate since it also coincides with the clustering outcome (seven allelic groups) based on Nei's genetic identities (Chapter 3). This is achieved by relaxing the requirement of the criterion for cutting the cluster dendogram. Figure 5.23 displays the differences among clusters for isozyme mobility patterns on the first, second and third PC axes of a PCA accounting for 87.7 percent of total variation. Overall comparison of cluster formation for purposes of assessing cultivar relationships by either agrophysiological traits or isozyme mobility patterns appears to be limited. This limitation possibly was the outcome of using too few traits and limited biochemical variability in beans.

Six seed traits (Table 2.9, Chapter II) were scored on variable scales as agrophysiological traits for 22 bean cultivars. Similarly, 12 enzyme systems were studied to monitor isozyme mobility patterns for 20 bean cultivars. Clustering of the 22 observations produced six cluster groups, the clustering pattern of which was strongly influenced (Anderberg, 1973) by certain variables. The clustering pattern gave the impression of a "gene-pool" or "race" type of cluster. This is shown by the clustering of tropical small blacks together as a group (Cluster I, Table 5.27) and in general the tendency for cultivars in the same commercial class designations to cluster together. The clustering by agrophysiological traits in which certain seed classes pooled together revealed that most cultivars within these classes were resistant to several races of the rust fungus. A good example is cultivar groups in Cluster I (Table 5.27), which included LaVega, CNC-2, CNC-3, CNC, B-190, Mexico-309 (all tropical small blacks), Ecuador-299 and Mexico-235 (both small reds). The clustering by agrophysiological traits also produced two groups that clustered cultivars LaVega, Mexico-235 and CNC-3 (Cluster III of Ghaderi et al., 1984) together and Nep-2 and Aurora (Cluster VII of Ghaderi et al., 1984) together as in the 1976 IBRN. It is also interesting to note that three cultivars with white seed coat color (KW-780, GN-1140 and Mountain White Half-Runner) and seed size of medium to large seeds that clustered together in Cluster VI (Table 5.27,

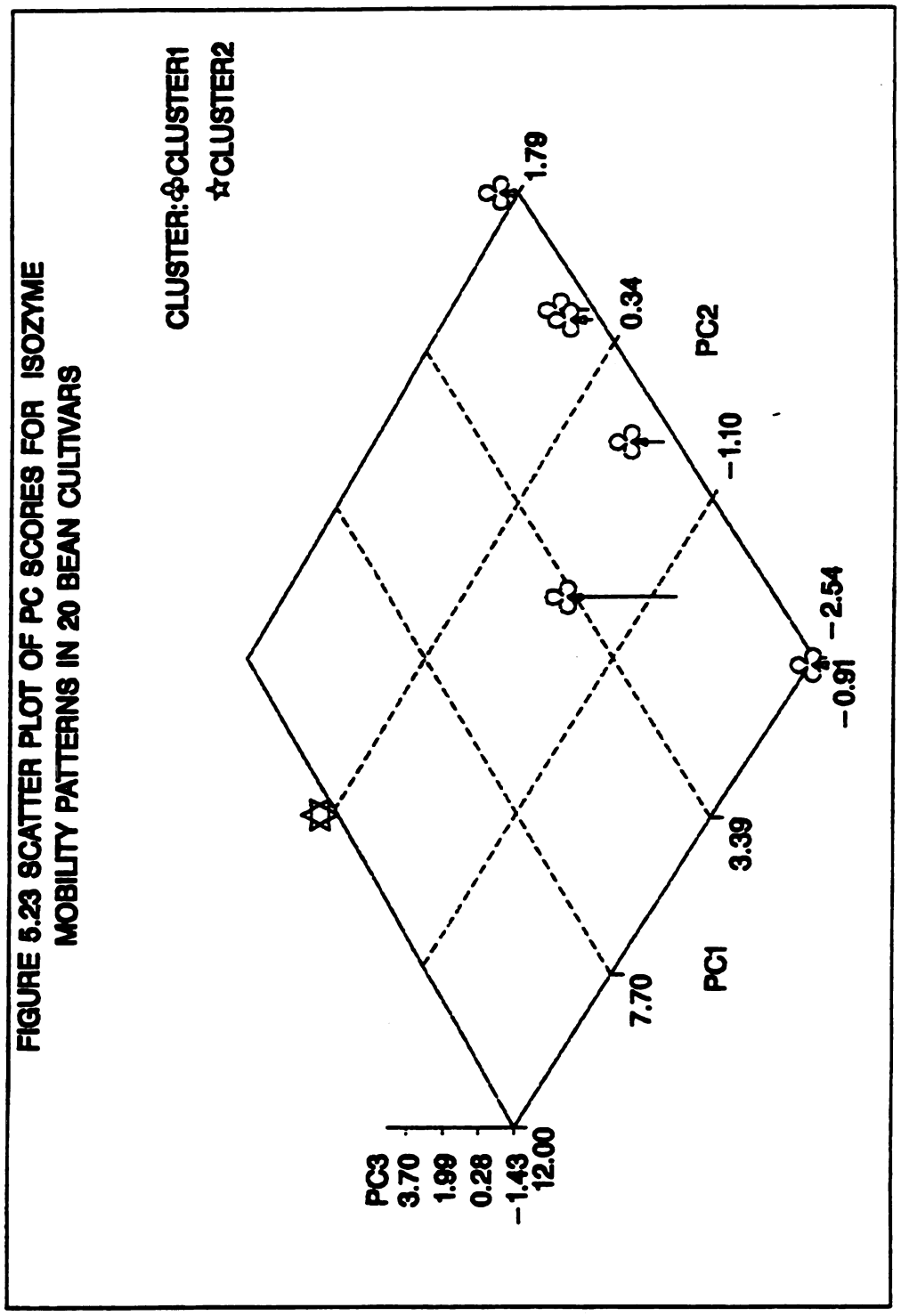


FIGURE 5.23 SCATTER PLOT OF PC SCORES FOR ISOZYME MOBILITY PATTERNS IN 20 BEAN CULTIVARS

Figure 5.20) were equally relatively susceptible to several races than the groups in Clusters I, II or III (Table 5.27, Figure 5.18). In general, there appeared to be an indication of an association between seed class clusters with similarity for reaction to rust isolates.

The cluster outcome on the basis of isozyme mobility patterns as fast (F) and slow (S) for twelve enzyme systems resulted into two major cluster groups with the small to medium-seeded cultivars forming one group (Cluster I with nineteen cultivars, Table 5.29) and the single-member cultivar Montcalm (large kidney) forming the second cluster group. A similar clustering pattern (with two cluster groups) was achieved when the same data was converted into Nei's genetic identities or distance on the basis of allelic frequency of enzyme loci using the UWPGMA method of clustering. The clustering of the 20 bean cultivars, whether by major storage protein (phaseolin) or Nei's genetic identities/distances resulted into two major groups confirming previous results (Gepts et al., 1986; Sprecher, 1988). The usefulness of isozyme mobility patterns for establishing or substantiating cultivar similarity established by disease reaction data was constrained by, perhaps, the non-representativeness of the enzyme loci assayed or the total number of loci involved is a minor and/or non-representative portion of the complete genome that the overall genetic relationship is only approximately predicted (Bassiri and Adams, 1978). Nevertheless, the subset of cultivars included in clusters III, IV, V, VII and VIII of the 1976 IBRN by Ghaderi et al. (1984) were recreated without change albeit their being in a single, large cluster (Cluster I). One difference was the hierarchy in cultivar ordering within this large cluster that appeared to have separated cultivars randomly. Clustering of the bean cultivars by isozyme mobility patterns, as occurred also for cultivar clustering by agrophysiological traits, separated the entries into groups that predominantly gave the appearance of a "gene-pool" cluster. Unlike the clustering pattern by agrophysiological traits, however, in which cultivars cluster-grouped by a certain commercial class designation (tropical, small blacks for example), also exhibiting a preponderance of a single reaction

phenotype (all cultivars in the group predominantly exhibiting resistance or susceptibility for several races), the clustering pattern with isozyme mobility score did not show such a pattern.

E. Combined trait measurements

1. Cluster analysis of 16 bean cultivars on the basis of combined measurement for agrophysiological, disease reaction and isozyme mobility scores

Scores on varying scales for six agrophysiological traits, disease reaction grades to nine rust races and isozyme mobility scores for 12 enzyme systems combined for 16 cultivars are presented in Table 5.30. Three clustering methods were used in producing three, two and four groups for complete linkage, average linkage and Ward's minimum variance methods, respectively (Figure 5.24, Table 5.31).

Average linkage and complete linkage with two and three groups each produced identical cluster-grouping with similar ordering of cultivars in the hierarchy in clusters II and III, respectively. Four cluster groups were produced by Ward's method. Because of that, the clustering pattern in this method was different (Anderberg, 1973). However, it had the same cultivars in Cluster III as in Cluster II of the complete linkage method. The formation of the four clusters in Ward's method separated resistant cultivar clusters that produce reaction with uredinia of pustule sizes less than 0.3 mm in diameter (R) such as LaVega, Mexico-309, Mexico-235, Ecuador-299, B-190, CNC-2 and Rico-Bajo-1014 and with resistance to several races such as Mexico-235 and Ecuador-299 (Cluster I) from those that produce the hypersensitive resistance (HR) group with non-sporulating pustules of size less than 0.3 mm in diameter (2,2+) such as Cuilapa-72, Aurora and Nep-2 (Cluster II). This indicates also that variables (attributes) for disease reaction scores as a group has influenced the clustering outcome (Anderberg, 1973) to a greater extent than either agronomic or isozyme mobility scores. The grouping of cultivars in clusters III and IV of Ward's method also separates the

Table 5.30: Scores for combined data for agrophysiological (6), disease reaction (9) and isozyme mobility (12) patterns in 16 bean cultivars

	PC	SC	SS	D	GS	PP	R38	R39	R40	R41	R43	R46	R49	R52	R53	ACP	DIAP-1	DIAP-2	EST-1	EST-2	MDH	ME	PRX-1	PRX-2	RBCO	SP	SKDH
LaVega	1	1	1	1	3	1	4	4	7	2	4	5	4	4	2	2	2	2	2	1	2	1	1	1	1	2	1
Mexico-235	2	2	1	1	3	1	1	1	2	4	4	4	2	2	2	2	2	2	1	1	2	1	1	1	1	2	1
CNC-2	1	1	1	1	3	1	1	1	4	4	2	4	2	4	4	2	2	1	2	1	2	1	1	1	1	2	1
C-49-242	1	1	1	1	3	1	5	5	7	6	7	6	6	4	6	2	2	1	2	1	2	1	1	1	1	2	1
Cuilapa-72	1	1	1	1	2	1	2	1	2	2	7	4	6	2	2	2	2	1	2	1	2	1	1	1	1	2	1
Mexico-309	1	1	2	1	4	1	2	2	4	4	4	4	7	3	3	2	2	2	2	1	2	1	1	1	1	2	1
RB-1014	2	3	1	2	1	1	2	2	4	4	4	4	4	4	2	2	1	2	1	2	1	1	1	1	1	2	1
Bcu.-299	2	2	1	1	4	1	1	1	2	2	4	4	4	2	2	2	2	2	2	1	2	1	1	1	1	2	1
Nep-2	3	4	1	1	2	1	2	2	2	2	2	7	7	2	2	2	2	1	2	1	2	1	1	2	1	2	1
Aurora	3	4	1	1	3	1	2	2	2	2	7	7	7	2	2	2	2	1	2	1	2	1	1	2	1	2	1
ICA-Pijao	1	1	1	1	2	1	2	2	7	7	7	7	4	7	7	2	2	1	2	1	2	1	1	1	1	2	1
KW-780	3	4	3	1	4	2	3	3	3	7	7	7	3	7	7	2	2	2	2	1	2	1	2	1	1	2	2
UI-111	1	5	2	1	2	1	2	2	7	7	7	7	7	7	2	2	2	2	1	1	2	1	1	1	1	2	1
GN-1140	3	4	3	1	3	1	2	1	7	4	6	4	4	7	7	2	2	2	2	1	2	1	1	1	1	2	1
M/WhtRnr	3	4	2	1	4	1	3	3	3	7	7	7	3	7	7	2	2	2	2	1	2	1	1	2	1	2	1
B-190	1	1	1	1	3	1	2	2	4	4	4	4	7	4	4	2	2	1	2	1	2	1	1	1	1	2	1

PC = Flower color
SC = Seed color
SS = Seed size
D = Determinacy
GS = Guide size
PP = Phaseoline protein

R38-R53 = Rust races
ACP = Acid phosphatase
DIAP-1 = Diophorase 1
DIAP-2 = Diophorase 2
EST-1 = Esterase 1
EST-2 = Esterase 2
MDH = Malic dehydrogenase

ME = Malic enzyme
PRX-1 = Peroxidase 1
PRX-2 = Peroxidase 2
RBCO = Rubisco
SP = Seed protein
SKDH = Shikimic dehydrogenase

Table 5.31: Cluster analysis of combined scores for agrophysiological, disease reaction and biochemical traits on 16 bean cultivars using three clustering methods

Cluster Methods			
CI	CII	CIII	CIV
	Complete Linkage		
LaVega	Cuilapa-72	C-49-242	
Mexico-309	Aurora	ICA-Pijao	
B-190	Nep-2	UI-111	
Mexico-235		GN-1140	
Ecuador-299		KW-780	
CNC-2		M/WhfRnr	
RB-1014			
	Average Linkage		
LaVega	C-49-242		
Mexico-235	ICA-Pijao		
Ecuador-299	UI-111		
CNC-2	GN-1140		
RB-1014	KW-780		
Mexico-309	M/WhfRnr		
B-190			
Cuilapa-72			
Aurora			
Nep-2			
	Ward's Method		
LaVega	Cuilapa-72	C-49-242	KW-780
Mexico-309	Aurora	ICA-Pijao	M/WhfRnr
B-190	Nep-2	UI-111	GN-1140
Mexico-235			
Ecuador-299			
CNC-2			
RB-1014			

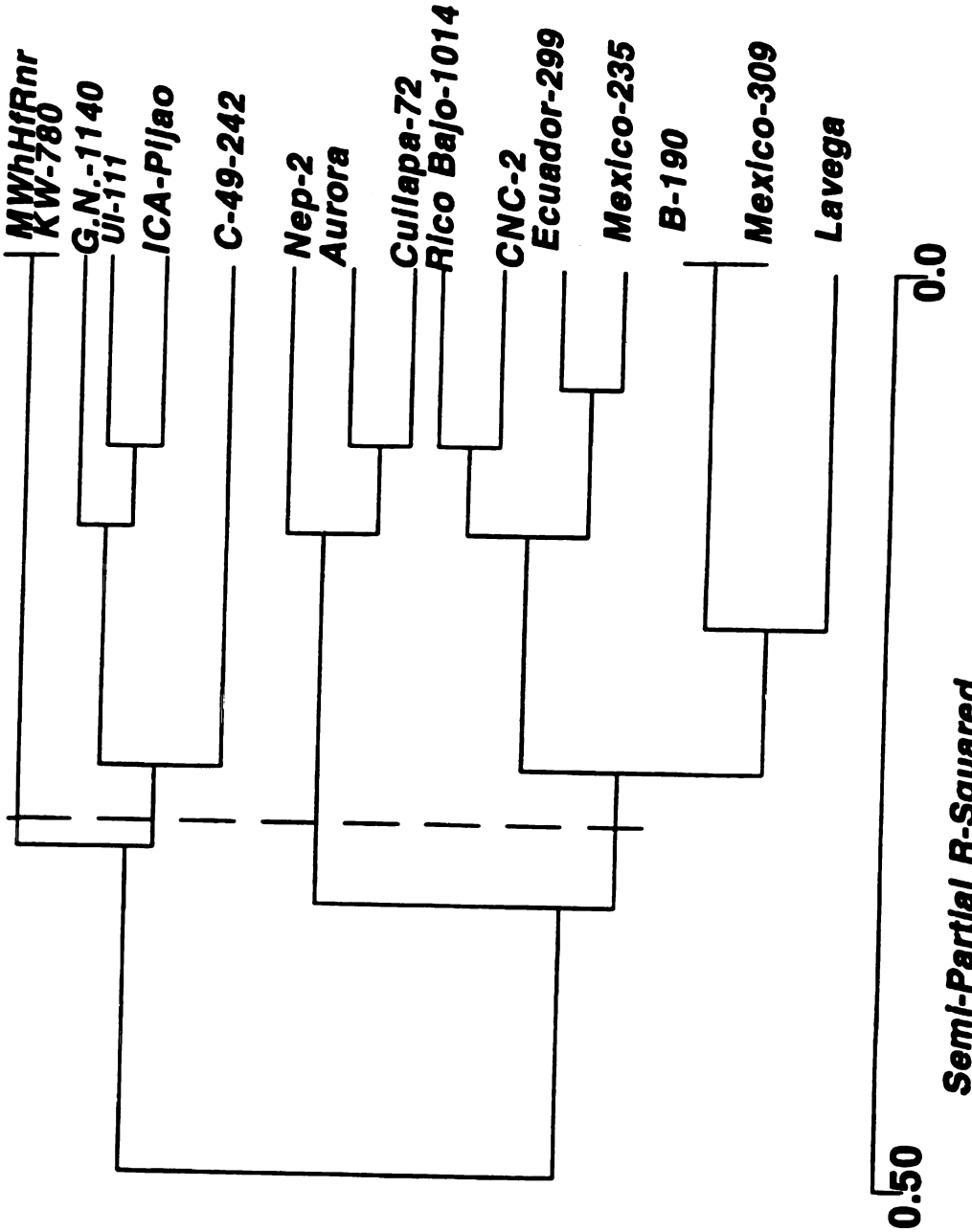


Figure 5.24 Ward's clustering of combined disease reaction isozyme banding and agrophysiological traits.

cultivars into the variability behaving group composed of C-49-242, ICA-Pijao, Pinto-111 and GN-1140 and the similarity behaving cultivars KW-780 and M/WhfRnr. Average linkage also produced two reaction phenotype categories by separating the cultivars into the predominantly resistant group (Cluster I) and the predominantly susceptible groups (Cluster II).

PCA of the combined variables showing differences in PC scores among cluster members for the first, second and third principal axes and accounting for only 58.3 percent of total variation is presented in Figure 5.25. Due to singularity problems, it was impossible to determine Mahalanobis's distance (D^2).

The clustering of the 16 cultivars using data from six agrophysiological traits, isozyme mobility patterns on 12 enzyme systems and disease reaction for nine described rust isolates by Ward's minimum variance method, produced four clusters that separated the cultivars into four reaction phenotype categories. The cluster outcome clearly indicated the influence of a variable or variables (Anderberg, 1973), i.e., disease reaction variables, that dominated the outcome of the cluster grouping. The grouping procedure separated the predominantly small pustule type resistance (R) group comprising LaVega, Mexico-309, B-190, Mexico-235, Ecuador-299, CNC-2 and Rico-Bajo-1014 from the hypersensitivity resistant (HR) types consisting of Cuilapa-72, Nep-2 and Aurora probably by one or few rust isolates that elicit such reactions. The last two groups consisted of the identically behaving cultivars KW-780 and Mountain White Half-Runner, which were separately grouped from the variably behaving cultivar groups C-49-242, ICA-Pijao, GN-1140 and the susceptible cultivar UI-111. The cultivars Mexico-309 and Rico-Bajo-1014 (Cluster V of Ghaderi et al., 1984) and Nep-2 and Aurora (Cluster VII of Ghaderi et al., 1984) clustered together as in the 1976 IBRN with one cultivar in each missing from the old group.

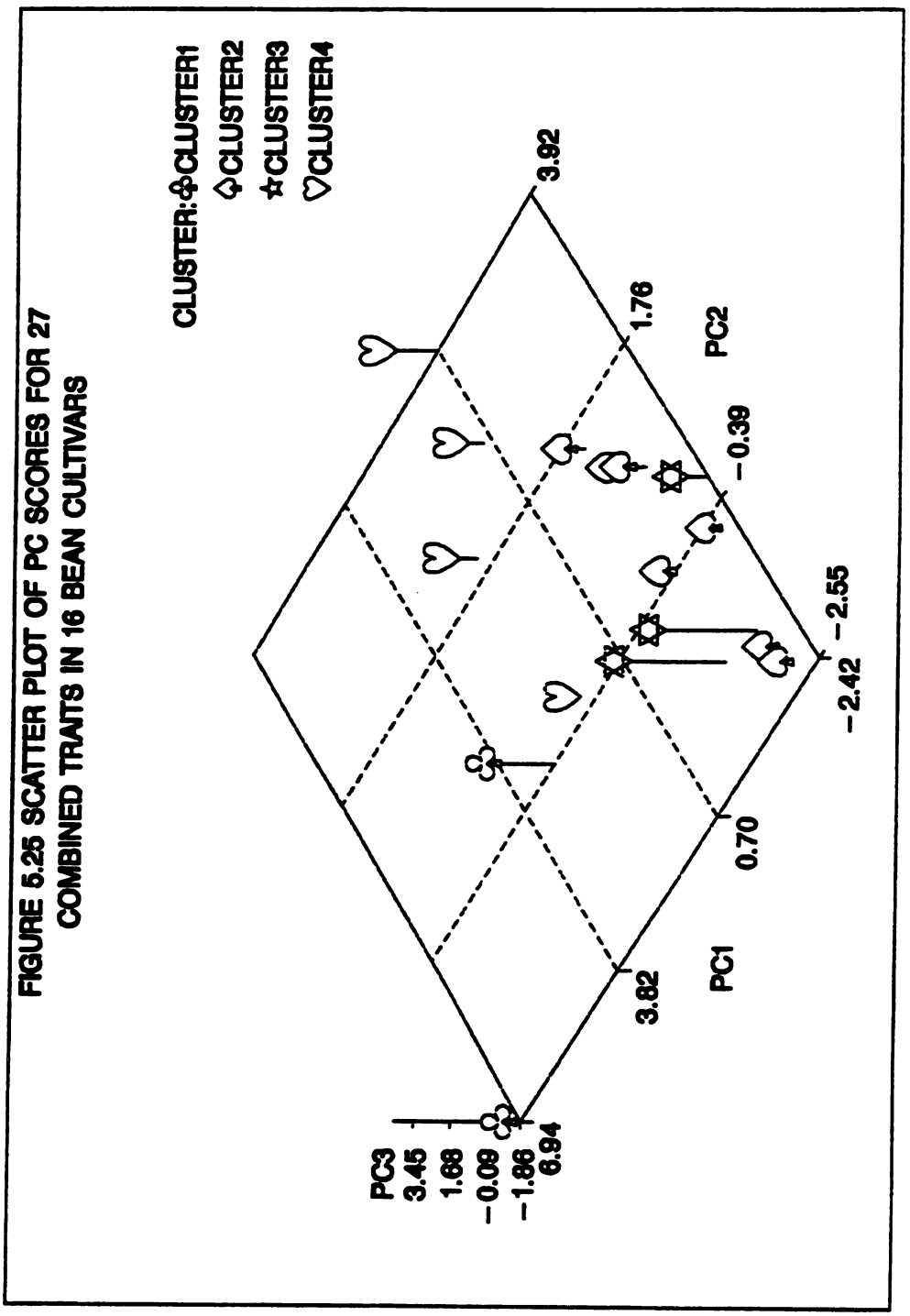


FIGURE 5.25 SCATTER PLOT OF PC SCORES FOR 27 COMBINED TRAITS IN 16 BEAN CULTIVARS

Discussion

Results from cluster analysis are known to indicate phenetic similarities (Molina-Cano, 1979; Ghaderi et al., 1982; Michener and Sokal, 1958), the similarities of which could emanate either from a common pedigree or from being a member of a common gene pool (Adams, 1977; Singh et al., 1991a). Field reaction data on 88, 52 and 46 bean lines that were tested in 16, 6 and 14 locations, respectively in 1975, 1976 and 1977 IBRNs were subjected to cluster and other multivariate statistical analysis. Of these, only a few of the bean lines in the Redland series, and the Puerto Rico (PR) series and probably a few others may contain lines that are presumably related through common ancestry. The majority of the entries in these IBRNs, however, contain ordinary (non-pureline) varieties that are accessions from plant introductions (PI), old landraces or selections from landraces that do not trace to a common pedigree source (Adams, M.W.; Freytag, G.; Silbernagel, M.J.; and McClean, P., personal communications). The view that most of these varieties of beans that do not trace to a common pedigree source draw their similarities from shared genes from belonging to a common gene pool was supported by the outcome of the analysis of coefficient of parentage (r) that resulted in a preponderance of non-integer (zero) r values that indicated lack of pedigree relationships for most of these bean lines.

Comparison of cluster analysis results of 88 bean cultivars for field reactions to population races in the 1976 IBRN using three systems programs (CLUSTAN, SPSS-X and SAS) and three clustering methods (complete linkage, average linkage and Ward's minimum variance method) produced the same general pattern of cultivar clusters as in the grouping results of the same data by Ghaderi et al. (1984). In spite of the expected differences for cultivar clusters from the different cluster algorithms (Afifi and Clark, 1984; Romesburg, 1984), the results, in particular, for the subset of cultivars in the original clusters by Ghaderi et al., 1984 (clusters III, IV, V, VII and VIII) were consistently the same irrespective of system

programs or the cluster algorithm used. The same pattern was also observed for the majority of the bean lines that clustered together irrespective of program and cluster methods.

Cultivar identity established from cluster analysis of similar reaction response patterns to rust disease in the field was further supported by similar findings in controlled environments using the same cluster and multivariate analysis methods. Cultivars neatly fell into three or four homogeneous reaction phenotype categories that express correct classification of reaction phenotypes that in turn reflect similarity of genes or gene complexes for reaction to the races. These similarities could be traced to common ancestry among some of these clusters. However, similarities for traits not established by co-ancestry (identity by descent) for most of these cultivars have been attributed to gene-pool membership (identity by state). Genetic similarities among these cultivar was further indicated by F_2 non-segregation among the different cultivar pair crosses within and between cluster groups. Moreover, close to 50% of the crosses showed linkage/pleiotropic relationships lending further evidence to cultivar relationships.

The cluster study revealed similar pathotypes among test locations on the basis of eliciting similar reaction patterns on many bean cultivars. New cluster formations indicative of occurrences of new pathotypes were observed as well as identification of broadly resistant cultivars with presumably several genes for resistance to the races that allow them to behave similarly from season to season.

Further characterization of cultivars on the basis of isozyme banding patterns for twelve enzyme systems revealed a different dimension of cultivar interrelationships that bear little similarity to relationships on the basis of reactions to rust disease. Bean cultivars were recognizable into the Mesoamerican (small- to medium-seeded) and Andean (large-seeded) types on the basis of phaseolin seed protein (Singh et al., 1991a; Sprecher, 1988; Gepts et al., 1986). Further examination of clusters yield seven sub-groups that reveal distinct

morphology, seed classes and disease resistance. Interestingly, the downsizing of the clusters to three on the basis of Ward's clustering of isozyme banding patterns further revealed the Mesoamerican (small-seeded), the Andean (large-seeded, example Montcalm) and a hybrid (introgressant, KW-780) between the Mesoamerican and Andean lines (Singh et al., 1991a, 1991b).

Cultivar characterization by agrophysiological traits resulted predominantly in the clustering of cultivars by commercial classes (Ghaderi et al., 1982). The outcome appears to suffer from scaling which influenced the cluster outcome. Similarly, there was no gain of information by the procedure of combining various different traits (disease reactions, isozyme banding patterns and agrophysiological traits) to cluster bean cultivars. The clustering appears also to suffer from and be influenced by the differential weights inherent in the character traits.

The ability of the different system programs and cluster algorithms to produce the same clustering pattern of bean lines composed of varied germplasm sources (landraces, PIs, etc.) indicates the basic similarities that exist among the various programs and cluster algorithms that also suggest the possible use of one or the other available methods for such studies. Moreover, the behavior of cultivars within clusters to produce, on the average, similar reaction response patterns for endemic population races from location to location and clustering together as a group for disease reactions underscores the usefulness and relevance of disease reaction data as a biological yardstick to display inherent genetic relationships among bean cultivars. The similarities among bean lines for reaction response patterns are thus reflections of underlying similar genes or genic complexes for reactions to these races. Theory of similarity of genes or genic complexes conditioning similar reaction response patterns in various bean cultivars has been noted by several investigators (Stavely, 1984a, 1984b; Stavely et al., 1989; Ballantyne, 1978; Kardin and Groth, 1985; Ghaderi et al., 1984; Singh et al., 1991a, 1991b; Adams, 1977). These patterns are known to change contingent upon changes in

the genetic make-up of the opposite interacting units, i.e., the pathogen, the corresponding host genetic make-up and the environment milieu in which this takes place in accordance with the gene-for-gene system (Person, 1958; Flor, 1971; Christ and Groth, 1982a; 1982b; Groth and Roelfs, 1982a) in a perpetual cycle of competition between the interacting units.

It is customary to use cluster analysis alone in exploratory data analysis or for estimating similarities among objects (Liang and Cassady, 1966; Akinola and Whiteman, 1972; Johnson, 1977; Ghaderi et al., 1979, 1980; Brown et al., 1983; Carver et al., 1987; Romesberg, 1984; Miles and Steadman, 1989) or as an adjunct with other methods including pedigree (Adams, 1977; Janoria et al., 1976; Morishima, 1968; Molina-Cann, 1975; and Murphy et al., 1986), or to verify preliminary grouping obtained by such methods as canonical variate analysis, Mahalanobis's distance and PCA (Adams, 1977; Acquaah, 1987; Singh et al., 1991b; Vairavan et al., 1973; Morishima, 1969; Narayan and Macefield, 1976; Ghaderi et al., 1982, 1984; Lee and Kaltiskes, 1972; and Martinez et al., 1983).

The cluster grouping from field reaction data on 88, 46 and 52 genotypes in the 1975, 1976 and 1977 IBRN respectively were also examined by PCA and computing Mahalanobis's distance (D^2) to confirm cluster results. Total variability accounted for by the first three principal component axes retained for the 1975, 1976 and 1977 IBRN were generally low at 75.1%, 54.6% and 61.0%, which were less than desirable in separating the clusters into distinct groups. However, total variability accounted for by the first three principal component axes of PCA in controlled environments of: 1) disease reaction studies of 23 pureline cultivars to four described rust isolates; 2) isozyme mobility patterns for 12 enzyme systems; and 3) agrophysiological score was much higher at 95.4%, 87.7% and 89.2%, respectively.

Mahalanobis's distance (D^2), often used to estimate genetic divergence among groups (Lee and Kaltiskes, 1973; Martinez et al., 1983; Vairavan et al., 1973; and Narayan and Macefield, 1976), furnished distance values between groups that gave an indication of

intercluster relationships. The intercluster relationships on the basis of Mahalanobis's distance (D^2) for disease reaction response patterns clearly shows the relative closeness of a pair of clusters as compared to another pair in the array of clusters. This is evident in the disease reaction data on 23 bean cultivars tested against four and nine described rust isolates in the greenhouse.

GENERAL SUMMARY AND CONCLUSIONS

I. Clustering by disease reaction attributes in the field

A. 1976 International Bean Rust Nursery (IBRN)

The application of cluster analysis to agronomic, morphological disease reaction data and biochemical attributes singly or as a procedure to augment other univariate and multivariate statistical methods has become increasingly useful in helping sort out various agronomic crops for a cursory look at cultivar interrelationships (Akinola and Whiteman, 1972; Ghaderi et al., 1980, 1982, 1984; Molina-Cano, 1975).

Cluster analysis of bean cultivars for field reaction to endemic races of the bean rust fungus *Uromyces appendiculatus* (Pers. Unger) in the 1976 IBRN using three systems programs (CLUSTAN, SPSS-X and SAS) and three different cluster analysis algorithms (complete linkage, average linkage and Ward's minimum variance method) resulted in the same general pattern of cultivar cluster grouping as in the cluster grouping results of the same data by Ghaderi et al. (1984). Seven, five and six cluster groups were obtained for complete linkage, average linkage and Ward's minimum variance methods respectively, when Romesburg's criterium (1984) was applied to cut the cluster dendogram (tree). This is in contract to the eight clusters obtained by Ghaderi et al. (1984) using CLUSTAN with Ward's minimum variance method. The number of clusters at eight was chosen by Ghaderi et al. (1984) as optimal because this gave the greatest contrast of within-cluster to between-cluster mean squares in the analysis of variance. The differences in the number of cluster groups produced by each cluster method slightly affected both cultivar membership and hierarchy of

relationship for few of the cultivars tested. However, the clustering together of several of the bean cultivars on the basis of their field reaction response patterns and in particular, the subset of clusters from the original clustering by Ghaderi et al. (1984) (Clusters III, IV, V, VII and VIII); irrespective of the systems program, or the cluster algorithm used, while indicating the basic similarities that exist among the various programs and fusion techniques, also underscores the usefulness and relevance of disease reaction data to explain inherent genetic relationships among cultivars. On the other hand, the similarities in cluster outcomes in general, irrespective of methods used, also indicate the possible use of one or the other available methods for such studies.

B. 1975 IBRN

The purpose of including field reaction data in the 1975 IBRN and the 1977 IBRN was to observe outcomes of cluster membership from data that were obtained one season earlier and one season later than the 1976 IBRN. In 1975, of the fifteen test locations used, only six were retained for cluster analysis purposes as these contained 52 cultivars that were uniformly tested in these locations. Of the six locations, four were common test sites for both the 1975 and 1976 IBRN. With regard to the bean lines, there were 38 cultivars that were also common to both 1975 and 1976 testing.

Five, five and four cluster groups were obtained for complete linkage, average linkage and Ward's minimum variance methods respectively on SAS. Considering only the cluster outcome from Ward's method (Table 8), several cultivars in the 1975 IBRN showed the same tendency to cluster together, which for the most part, retained their old cluster membership with cultivars that they were with in the 1976 IBRN. This time, however, they were clustered as members of a new but larger group that also included other cultivars from different clusters in the 1976 IBRN. Examples of this are cultivars such as 4961-54-1, Porillo-70, Porillo

Sintetico and PI 207824, which were in Cluster I of the 1976 IBRN but clustered again as one group in Cluster I of the 1975 IBRN along with other cultivars form other groups. This feature of clustering of a set of cultivars, that do not necessarily share a common parentage, based on similar reaction response patterns to endemic rust races in one set of test condition is an indication of possession of similar genes or genic complexes for reaction to these races (Ghaderi et al., 1984; Stavely et al., 1989; Ballantyne, 1978). In contrast, some of the bean lines have been observed to have broken up from their old cluster grouping and formed entirely new clusters based on their new reaction response patterns in the 1975 IBRN. Examples of these include cultivars C-49-242, ICA-Pijao, KW-780, CNC-2 and Ecuador-299, to mention a few of those that are common to both 1975 and 1976 test seasons. Although it is rather difficult to extrapolate the effect of the new test condition imposed by the test season in 1975 and for that matter the 1977 test season from the cluster outcomes alone in the absence of precise information on racial spectra during the test season, it nevertheless serves to elucidate the important role that this variable plays in the host parasite system (Van der Plank, 1968).

By the same token, while genetic similarities among cultivars belonging to a cluster in one set of test conditions is attributed to a set of similar genes or genic complexes for reaction to endemic rust races contingent on the existence of corresponding interacting genes in both the host and the pathogen in accordance with the gene-for-gene concept (Flor, 1971), similarity based on reaction response patterns by the same cultivars along with other new cultivar members forming new cluster groups in a different test condition is attributed to a different set of similarity genes or genic complexes that are governed by the same basic principle of the gene-for-gene system (Person, 1969; Ghaderi et al., 1984).

The presence of diverse pathogenic potential as indicated by location specificities and differences in racial composition by time of planting was reported for test sites during the

1975, 1976 and 1977 IBRN tests (CIAT, 1979), which substantiates the above assertion. In addition, bean lines have been observed that cluster together in two or more different test seasons regardless of differences in test conditions, indicating the existence of broadly resistant cultivars with presumably several genes for resistance to multiple races that enable them to behave (cluster together) similarly from season to season. This is particularly true for such cultivars as Cuilapa-72 and Mexico-309, Nep-2 and Aurora from this study and several other such cultivars identified by CIAT (1979). Stavely et al. (1984) reported the existence of several cultivars with broad resistance genes.

C. 1975 and 1976 IBRN combined

The purpose of cluster analyzing the combined data common to both the 1975 and 1976 test season was made with the objective of clustering the cultivars on a large number of attributes. Four cluster groups were obtained by Ward's minimum variance method (Table 14) in which old cluster membership alignments as in the 1976 IBRN were literally forced to reappear because of the dominant influence of a number of attributes (Anderberg, 1973) (16 test sites in the 1976 IBRN) on the cluster outcome. It appears it is inappropriate to put together biological data from two different test seasons for classification purposes. A definitive comparison between the two seasons (test conditions) could have been achieved by extracting field reaction data for similar test location of a common set of cultivars. Even this is not without difficulty as one must be provided with precise racial composition for each test condition.

D. 1977 IBRN

Clustering of the 46 bean cultivars in the 1977 IBRN by Ward's minimum variance method resulted in five cluster groups (Table 14). The clustering pattern for the cultivars in

the 1977 IBRN was also remarkably similar to the clustering pattern in the 1975 and 1976 IBRNs. For example, five cultivars from a total of seven that were clustered together in the 1976 IBRN in Cluster I were also clustered together in the 1977 IBRN in Cluster V. These included cultivars 4691-54-1, Porillo-70, M/WhfRnr, Epicure and Veracruz-1A6. Similarly, of the four cultivars that were common to both 1976 and 1977 IBRN and which were clustered together in Cluster II of the 1976 IBRN, three (Redlands Pioneer, Redlands GLB and Redlands GLC) were also clustered together in Cluster II of the 1977 IBRN. Cultivars PI226883, Cacahaute-72, Redlands Autumn Crop and Brown Beauty also common to both 1976 and 1977 IBRNs clustered together in Cluster III of the 1977 IBRNs. In general, the same tendencies in clustering behavior that were apparent in the clustering pattern of the cultivars common to 1975 and 1976 IBRNs were also observed in the clustering of the cultivars common to both 1976 and 1977 IBRN. Furthermore, an apparent tendency by cultivars with known pedigree relationships to cluster together was noted in the 1977 IBRN.

Considering the cluster outcomes form all three test seasons (1975, 1976 and 1977 IBRNs) and all clustering methods used, three-bean cultivar pairs (4961-54-1 and Porillo-70, Mexico-309 and Cuilapa-72, Nep-2 and PR-3) were clustered together regardless of test condition or clustering method used. In particular, the cultivars Mexico-309 and Cuilapa-72 along with cultivars CNC-3, Ecuador-299, Mexico-235 among several others were noted as being the most widely resistant entries in the 1975, 1976 and 1977 IBRN (CIAT, 1979).

II. Clustering by disease reaction attributes in the greenhouse

There is an obvious triplefold advantage in using pureline cultivars, described rust isolates and controlled test conditions over tests using landrace cultivars, population rust races and field test conditions whether characterizing the host cultivars or the pathogenic races and the interactions therefrom.

Twenty-three pureline bean cultivars were uniformly tested against four described rust races in the greenhouse in East Lansing, Michigan and against nine described rust races, which included the above four races, in Beltsville, MD. In another test, 19 pureline cultivars were also tested against 26 described rust races that included the races used in East Lansing and Beltsville, Maryland. The reaction scores were used in separate runs to cluster the 23, 23 and 19 observations, respectively.

A. Cluster analysis of disease reaction of 23 pureline bean cultivars to four described rust races

Ward's minimum variance method resulted in producing three cluster groups that coincided with the separation of the cultivars into three major reaction phenotype categories: (1) cultivars with predominantly small pustule type resistance, i.e., pustules less than 0.3 mm in diameter (Cluster I, Table 20); (2) cultivars with predominantly hypersensitive (necrotic) reaction, i.e., non-sporulating necrotic spots less than 0.3 mm to 0.5 mm in diameter (Cluster II, Table 25); and (3) cultivars with moderately to highly susceptible reaction phenotypes (Cluster III, Table 20).

The cultivar clusters in this particular analysis resemble the subset of cultivar clusters in Clusters III, IV, V, VII and VIII of the 1976 IBRN by Ghaderi et al. (1984). In particular, the groups formed by cultivars LaVega, Mexico-235 and CNC-3 (Cluster I, Table 20), Mexico-309 and Cuilapa-72 (Cluster II, Table 20), Nep-2 and Aurora (Cluster II, Table 20) and KW-780 and ICA-Pijao (Cluster III) were clustered together as in the 1976 IBRN. The new grouping, however, based on distinct reaction phenotypes that reflect similar genes for reaction to the races excluded the cultivars Rico-Bajo-1014 from the original grouping with Nep-2 and Aurora. The cluster group that included C-49-242 and CNC-2 was dissolved in this analysis because of divergent reaction responses to the races between C-49-242 and

CNC-2 that joined their respective groups consistent with their reaction phenotypes. The clear separation of the 23 cultivars into groups that reflected correct classification into precise reaction phenotypes was achieved by establishing test conditions that utilized pureline cultivars, and described rust isolates that were allowed to interact and express correct phenotypes in controlled environments. In addition, a strong tendency was observed for cultivars with a known common pedigree to cluster together.

B. Cluster analysis of disease reaction of 23 pureline cultivars to nine described rust races

The same 23 pureline cultivars that were tested for reaction to four races (41, 46, 49 and 53) in the greenhouse in East Lansing were tested for reaction to nine described rust races that included the above four races. Clustering by Ward's minimum variance method produced three clusters as in the previous study using the four rust races. There was a slight difference in the cluster outcome, particularly in the ordering of the cultivar int he hierarchy and the inclusion of the cultivar GN-1140 in Cluster III (Table 20). Despite this difference, the separation of cultivars into three major reaction phenotype categories were recreated in the clustering step using the nine described rust races. Here again, the separation of cultivars into groups that express correct classification into reaction phenotypes reflect similar genes for reaction to races whether four described races or nine described races are used. In both cases, the subset of cultivars from the 1976 races that were grouped in clusters III, IV, V, VII and VIII by Ghaderi et al (1984) were clustered into just three clusters constituting the three major reaction phenotypes. Test conditions involving the use of pureline varieties and described rust isolates in controlled environments were helpful in this study.

C. Cluster analysis of 19 pureline bean cultivars for reaction to 26 described rust races

Cluster analysis of disease reactions of 19 bean cultivars to 26 rust races was subjected to three clustering methods (complete linkage, average linkage and Ward's minimum variance method). Considering the cluster outcome from Ward's method, four groups were formed comprising cultivars LaVega, C-49-242, Nep-2, Aurora, C-20, Mexico-309, B-190, Cuilapa-72 and 51051 in Cluster I; Mexico-235, Ecuador-299, CNC-2, CNC and Olathe in Cluster II; KW-780 and M/WhfRnr in Cluster III and Pindak, Pinto-111 and Seafarer in Cluster IV. In this study, cultivar pairs Nep-2 and Aurora (Cluster VII of Ghaderi et al., 1984) and Cuilapa-72 and Mexico-309 (Cluster V of Ghaderi et al., 1984) which were included in Cluster I along with other cultivars did cluster together as in the 1976 IBRN. It also appears in this study that the clustering step has separated the most susceptible cultivars (Clusters III and IV) from the most resistant cultivars (Clusters I and II, Table 23).

Observation of the cluster outcomes from using 4, 9 or 26 described rust races on 23, 23 and 19 pureline cultivars in controlled test conditions resulted in three or four groups that separated the cultivars into three or four reaction phenotype categories of homogeneous groups that more or less express correct classification of reaction phenotypes that in turn reflect similarity of genes or genic complexes for reaction to the races in question.

Overall, it may be worthwhile noting the following:

i) that the use of pureline cultivars along with described rust races in controlled environments has allowed cultivar separation on the basis of correct reaction phenotypes that can be interpreted in terms of the gene-for-gene host-parasite system.

ii) although the clustering procedure selected for comparing (Ward's minimum variance method) cultivar relationship on variable attributes is reported for producing "compact" clusters with few cluster numbers, the procedure appears

to have been particularly constrained by the inadvertent use of few number of observation in the controlled test conditions.

ii) The selection of the subset of cultivars from clusters III, IV, V, VII and VIII of Ghaderi et al. (1984) for further studies, although random, may have been biased towards selection of predominantly resistant entries (nine bean lines were reportedly highly resistant to several rust races as compared to four bean lines with variable reaction to several of the races). This bias is evident in the consistent clustering of these same cultivars together in many instances regardless of test conditions or clustering method used.

D. Clustering of rust races by their ability to elicit similar reaction on pureline bean differentials

Rust isolates were clustered by their ability to elicit similar reaction responses on 19 bean cultivars in two separate cluster steps. The first set consisted of 26 described rust races which were applied on 19 bean cultivars while the second set consisted of 33 described races which were tested on 19 standard bean differentials. Of these, nine bean cultivars and 26 rust races were common to both data sets. The second test set with 33 isolates contained seven more isolates (44, 54, 55, 62, 68, 69 and 70) that were not included in the first test set.

Four racial cluster groups were produced for each test set when Ward's minimum variance method was used on each data set separately. The pattern of clustering and assignment of rust isolates within clusters for each test set were basically similar. The clustering pattern, however, displayed groups reflecting less than complete agreement. This difference emanated from differences in the composition of the differential cultivars within each test set (nine common differentials from a total of 19) and the differences in the number of isolates in each test set (26 vs 33 isolates). Nevertheless, the clustering of the rust isolate

identified four racial groups and, by extension of the same theory from cultivar clusters, each group belongs to the same virulence/pathogenicity group possessing similar genes or genic complexes for eliciting similar pathogenic reactions phenotypes on the sets of differential cultivars.

E. Cluster relationships among bean cultivars by isozyme mobility patterns and agrophysiological traits

Six seed traits were scored on variable scales as agrophysiological traits for 22 bean cultivars. Similarly, 12 enzyme systems were studied to monitor isozyme mobility patterns for 20 bean cultivars. Clustering of the 22 observations produced six clusters, the pattern of which was strongly influenced by certain variables. The clustering pattern gave the impression of a "gene-pool" type of cluster. This is shown by the clustering of tropical small blacks together as a group (Cluster I, Table 30) and in general the tendency for cultivars in the same commercial class designations to cluster together (Ghaderi et al., 1982). The clustering by agrophysiological traits which pooled certain seed classes together revealed that most cultivars within these classes were resistant to several races of the rust fungus. A good example may be seen in Cluster I (Table 30) which included LaVega, CNC-2, CNC-3, CNC, B-190, Mexico-309 (all tropical small blacks), Ecuador-299 and Mexico-235 (both small reds). The clustering by agrophysiological traits also produced two groups that clustered cultivars LaVega, Mexico-235 and CNC-3 (Cluster III of Ghaderi et al., 1984) together and Nep-2 and Aurora (Cluster VII of Ghaderi et al., 1984) together as in the 1976 IBRN. It is also interesting to note that three cultivars with white seed coat color (KW-780, GN-1140 and M/WhfRnr) and seed of medium- to large-size that clustered together in Cluster IV (Table 35, Fig. 21) were relatively more susceptible to several races than the cultivars in Clusters I, II or III (Table 35, Figure 21).

The cluster outcome on the basis of isozyme mobility patterns as fast (F) and slow (S) for 12 enzyme systems resulted in two major groups with the small- to medium-seeded cultivars forming one group (Cluster I with 19 cultivars, Table 33) and the single member cultivar Montcalm (large, kidney) forming the second group. A similar clustering pattern (with two clusters) was achieved when the same data were converted into Nei's genetic identities or distance on the basis of allelic frequency of enzyme loci using the UWPGMA method of clustering. Clustering of the bean cultivars by isozyme mobility patterns, as was true for cultivar clustering by agrophysiological traits, resulted in several cultivars being cast into groups that predominantly gave the appearance of a "gene-pool" cluster. Unlike the clustering pattern by agrophysiological traits in which cultivars cluster-grouped by a certain commercial class designation (tropical, small blacks for example), also exhibiting a preponderance of a single reaction phenotype (all cultivars in the group predominantly exhibiting resistance or susceptibility for several races), the clustering pattern with isozyme mobility scores did not show such a pattern (Sprecher, 1988).

F. Cluster relationships among 16 bean cultivars for combined measures of three different characteristics

Data from six agrophysiological traits, isozyme mobility patterns on 12 enzyme systems and disease reaction for nine described rust races were combined for cluster analysis of 16 cultivars that were uniformly tested for these traits. Ward's minimum variance method produced four clusters that separated the cultivars into four categories. The cluster procedure separated the predominantly small pustule type resistance (R) group comprising LaVega, Mexico-309, B-190, Mexico-235, Ecuador-299, CNC-2 and Rico-Bajo-1014 from the hypersensitivity resistant (HR) types consisting of Cuilapa-72, Nep-2 and Aurora. The last two groups consisted of identically behaving cultivars KW-780 and Mountain White Half

Runner, which were separately grouped from the variably behaving cultivar groups C-49-242, ICA-Pijao, GN-1140 and the susceptible cultivar Pinto-111. The cultivars Mexico-309 and Rico-Bajo-1014 (Cluster V of Ghaderi et al., 1984) and Nep-2 and Aurora (Cluster VII of Ghaderi et al., 1984) clustered together as in the 1976 IBRN with one cultivar in each missing from the old group.

G. Cultivar relationship based on disease reaction data in the field compared to gene differences for reaction to four rust races in the greenhouse

Differences on the average of almost 15% greater genetic identity or similarity for cultivars within-clusters as compared to cultivars between-clusters, as judged from mendelian genetic tests with four isolates (41, 46, 49 and 53), provided support to the position taken in this study that cultivars within-clusters were genetically more similar than cultivars between-clusters (Tables 10 and 11, Chapter 4). However, in spite of the finding that 57.1% of the within-cluster crosses showed allelic identity (no segregation in the F_2) over the four races, and 43% of between-cluster crosses showed allelic identity over the same four rust races thus favoring the above hypothesis, we need to ponder over this question and, in particular, we need to explain: 1) why 43% of the within-cluster crosses showed segregation at one or more loci and 2) why 43% of the between-cluster crosses displayed no segregation in the F_2.

It is perhaps appropriate to recreate the scenario that led to the postulation of the above hypothesis ahead of proposing possibilities in statistical terms as to why the genetic outcome observed occurred as it did in this study. In particular: a) the hypothesis of greater identity or similarity among cultivars within clusters than cultivars between-clusters was based on the outcome of a cluster analysis of field reaction data on 88 bean lines (genotypes) that were tested in 16 locations in the 1976 IBRN. For that particular analysis, eight clusters were arbitrarily considered as optimal using Ward's minimum variance method of cluster analysis,

among several other methods. The decision on the optimal number of clusters (eight clusters) chosen, tacitly assumes internal homogeneity of cultivars within clusters and therefore greater genetic similarity or identity of cultivars.

However, it should be remembered that cluster analysis is only useful as an exploratory analysis to reveal natural or biological groups among observations. As the criterion for selecting the optimum number of cluster groups is arbitrary, its utility is limited by its subjectivity as a yardstick for assessing cultivar relationships. Inasmuch as this limitation exists, the postulates drawn from such a subjective criterion is bound to show relative inaccuracies in its forecast of cultivar genetic identities or similarities. Thus, the observation of a certain amount of non-segregation in the between-cluster crosses and about the same amount of segregation in the within-cluster crosses may not totally be eliminated.

b) It is also worthwhile to consider the manner with which a cluster analysis method is run in order to shed more light on the cluster outcomes. In cluster analysis, a number of attributes are used in aggregate to cluster the observations. Assuming absence of influence on the cluster outcome by any particular attribute, cluster groups will be produced based on these aggregate attributes, the number of groups formed depending ultimately ont he investigator and the inherent characteristics of the cluster method selected. In this situation, the cluster outcome, for example, of cultivars clustered together by a number of rust races precisely reflects the similarities of the cultivars within a cluster on an aggregate number of races and not for a particular race. If this is the case, it would be unrealistic to expect genetic identities or similarities within-cluster crosses on a scale of total uniformity of cultivars as if it were from a single attribute (single rust race) cluster grouping, or not to expect a certain amount of genetic identity in the between-cluster crosses where genetic analysis is based on a single race.

c) It has been observed in this study that a number of cultivars exhibited broad resistances for a number of rust races. These cultivars clustered together regardless of

differences in test conditions (test seasons, field or greenhouse conditions) or the type of cluster method employed for clustering them. This stability in clustering has an obvious bearing on the outcome of observed genetic identity within- and between-cluster crosses.

Other than subjectivity of cluster analysis, and the existence of broadly resistant cultivars to account for the discrepancies observed in the within-cluster and between-cluster crosses, there are two more important possibilities that could also account for discrepancies observed in the hypothesis:

1) that the four races used in this study may not necessarily be very representative of the rust races encountered in the field by the 88 bean lines screened in the 16 locations and reported in the 1976 IBRN, on which the initial clustering was done.

2) The bean lines selected from the subset of clusters (Clusters III, IV, V, VII and VIII by Ghaderi et al., 1984) from a total of eight clusters for crossing in the within-cluster and between-cluster crosses may have been too few in number to adequately represent the genetic situation as originally postulated.

Hence, for reaction to specific races, as races 41, 46, 49 and 53, the cultivars within-clusters, assuming the field races encountered in the 1976 tests were distinct from these four races in their genes for virulence/pathogenicity, could easily be genetically different at one or more loci, and would therefore display segregation in F_2 when tested against one or more of the four specific races, thus accounting for the 43% within-cluster crosses showing segregation in these results. Furthermore, cultivars belonging to different clusters on the basis of the 1976 data don't necessarily have to be genetically different for reaction to specific races (41, 46, 49 and 53), again assuming the field races encountered in the 1976 tests were genetically distinct from the four specific races used in this study. This would then account for the lack of segregation observed in the 43% of the between-cluster crosses.

LIST OF REFERENCES

Adams, M.W. 1977. An estimation of homogeneity in crop plants with special reference to genetic vulnerability in the dry bean, *Phaseolus vulgaris* L. Euphytica 20:665–679.

Acquaah, G. 1987. Genetic and multivariate statistical evaluation of a phenotypic recurrent selection program for recombining erect architecture and large seed size in *phaseoli vulgaris* L. Ph.D. dissertation. Michigan State University, East Lansing, Michigan.

Afifi, A.A. and V. Clark. 1984. Computer aided multivariate analysis. Lifetime Learning Publications. Belmont, California.

Akinola, J.D. and P.C. Whiteman. 1972. A numerical classification of Cajanus Cajun (L.) Mellsp. accessions based on morphological and agronomic attributes. Aust. J. Agric. Res. 23:995–1005.

Anderberg, M.R. 1973. Cluster analysis for applications. Academic Press. New York.

Ballantyne, B. 1978. The genetic basis of resistance to rust caused by *Uromyces appendiculatus* in bean (*Phaseolus vulgaris*). Ph.D. thesis, University of Sydney, Australia. 262 pp.

Bassiri, A. and Adams, M.W. 1978. Evaluation of common bean cultivar relationships by means of isozyme electrophoretic patterns. Euphytica 27: 707–720.

Brown, K.D., M.E. Sorrels and W.K. Coffman. 1983. A method for classification and evaluation of testing environments. Crop Sci. 23(5): 889–893.

Broich, S.L. and Palmer, R.G. 1980. A cluster analysis of wild and domesticated soybean phenotypes. Euphytica 29: 23–32.

Carver, B.F., E.L. Smith and H.V. England, Jr. 1987. Regression and cluster analysis of environmental response of hybrid and pureline winter wheat cultivars. Crop Sci. 27(4): 659–664.

Christ, B.J. and V.J. Groth. 1982a. Inheritance of resistance in three cultivars of beans to the bean rust pathogen and the interaction of virulence and resistance genes. Phytopathology 72:771–773.

Christ, B.J. and V.J. Groth. 1982b. Inheritance of virulence to three bean cultivars in three isolates of the bean rust pathogen. Phytopathology 72: 761–770.

CIAT. 1979. International Bean rust Nursery results 1975–1976. CIAT, Pub. No. 20EB–1, 19 p.

CIAT. 1979. International Bean Rust Nursery results 1977–78. CIAT, Pub. No. 20EB–1–77/78.

Cox, T.S., G.L. Lookhart, D.E. Walker, L.G. Harrell, L.D. Albers and D.M. Rogers. 1985. Genetic relationships among hard red winter wheat cultivars as evaluated by pedigree analysis and gliedin polyacrylamide gel electrophoretic patterns. Crop Sci. 25(6): 1058–1062.

Decker, D.S. 1985. Numerical analysis of allozyne variation. *Cucurbita pepo*. Econ. Bot. 39(3): 300–309.

Flor, H.H. 1971. Current status of the gene–for–gene concept. Ann. Rev. Phytopath. 9: 275–269.

Gepts, P., T.C. Osborn, K. Rashka and F.A. Bliss. 1986. Phaseoline protein variability in wild forms and landraces of the common bean (*P. vulgaris L.*): evidence for multiple centers of domestication. Eco. Bot. 40(4): 451–68.

Ghaderi, A., M.W. Adams and A.W. Saettler. 1984. A quantitative analysis of host–pathogen–environment in International Bean Rust Nurseries (IBRN). Mimeograph. Crops & Soil Sci Dept., MSU, East Lansing, Michigan 48824.

Ghaderi, A., M.W. Adams and A.W. Saettler. 1982. Environmental response patterns in commercial classes of common beans (*Phaseolus vulgaris L.*). Theor. Appl. Genet. 63:17–22.

Ghaderi, A., E.H. Everson and C.E. Cress. 1980. Classification of environment and genotypes in wheat. Crop Sci. 20(6): 707–710.

Ghaderi, A., M. Shishegar, A. Rezai and B. Ehdaie. 1979. Multivariate analysis of genetic diversity for yield and its components in mung bean. J. Amer. Soc. Hort. Sci. 104(6): 728–731.

Groth, J.V. and A.P. Roelfs. 1982a. Genetic diversity for virulence in bean rust collections. Phytopathology 72: 982–983.

Janoria, M.P., A.M. Rhodes, and M.N. Shrivastava. 1976. Phenetic similarity among dwarf rice cultivars grown in two environments, as estimated by numerical taxonomy (NT) methods. Ann. Bot. 40: 845–849.

Johnson, G.R. 1977. Analysis of genotypic similarity in terms of mean yield and stability of environmental response in a set of major hybrids. Crop Sci. 17(6): 837–842.

Johnson, S.C. 1967. Hierarchical clustering schemes. Psychometrica 33:24–54.

Kardin, M.K. and J.V. Groth. 1985. The inheritance of resistance in two white-seeded dry-bean cultivars to seven bean rust isolates. Phytopathology 75: 1310 (Abstr.).

Lee, J. and P.J. Kaltiskes 1973. The application of Mahalonobis's generalized distance to measure genetic divergence in Durum wheat. Euphytica 22: 124–131.

Liang, G.H.L. and A.J. Cassady. 1966. Quantitative presentation of the systematic relationship among twenty-one sorghum species. Crop Sci. 6:76–79.

Martinez, W.D.J., M.M. Goodman and D.H. Timothy. 1983. Measuring racial differentiation in maize using multivariate distance measures standardized by F_2 populations. Crop Sci. 23:775–781.

Michener, C.D. and R. R. Sokal. 1957. A quantitative approach to a problem in classification. Evolution 11:130–162.

Miles, R. and J.R. Steadman. 1989. Cluster analysis of bean rust (*U. appendiculatus*) isolates collected in Nebraska and Colorado, 1979–1986. Ann. Rep. Bean Improv. Coop. 32: 128–129.

Miles, R. and J.R. Steadman. Cluster analysis of bean rust (*U. appendiculatus*) isolates collected in the Dominican Republic, 1982–85. Ann. Rep. Improv. Coop. 32: 130–131.

Molina-Cano, J.L. 1976. A numerical classification of some European barley cultivars (*Hordeum vulgare L.S.I.*) Z. Pflanzensuchtz 76:320–333.

Morishima, H. 1969. Phenotic similarity and phylogenetic relationships among strains of Oryza perennis, estimated by methods of numerical taxonomy. Evolution. 23: 429–443.

Murphy, J.P., T.S. Cox and D.M. Rodgers. 1986. Cluster analysis of red winter wheat cultivars based upon coefficients of parentage. Crop Sci. 26(4): 672–676.

Narayan, R.K.J. and A.J. Macefield. 1976. Adaptive responses and genetic divergence in a world germplasm collection of chickpea (*Cicer arietinum L.*) Theoretical and Applied Genetics 47: 179–187.

Person, C. 1959. Gene-for-gene relationships in host-parasite systems. Can. J. Bot. 37: 1101–1130.

Romesberg, H.C. 1984. Cluster analysis for researchers. Lifetime Learning Publications, Belmont, CA.

SAS Institute. 1982. SAS users guide: Statistics. SAS Institute, Cary, NC.

Singh, S.P., R. Nodari and P. Gepts. 1991a. Genetic diversity in cultivated common bean: Allozymes. Crop Sci. 31: 19–23.

Singh, S.P., J.A. Gutierrez, A. Molina, C. Urrea and P. Gepts. 1991b. Genetic diversity in cultivated common bean: II. Marker-based analysis of morphological and agronomic traits. Crop. Sci. 31: 23-29.

Smith, J.S.C. 1984. Genetic variation within US commercial hybrid maize: Multivariate analysis of allozyme data. Crop Sci. 24:1041-1046.

Smith, J.S.C., M.M. Goodman and C.W. Stuber. 1985. Genetic variability within US maize germplasm. I. Historically important lines. Crop Sci. 25(31): 550-555.

Smith, J.S.C., M.M. Goodman and C.W. Stuber. 1984. Variation within Teosinte III. Numerical analysis of allozyme data. Econ. Bot. 38(1): 97-113.

Sneath, P.H.A. and Sokal, R.R. 1973. Numericla taxonomy. W.H. Freeman 7 Co., San Francisco.

Sneath, P.H.A. and R.R. Sokal. 1962. Numerical taxonomy. Nature 193 (4818): 855-67.

Sokal, R.R. 1974. Classification: Purposes, principles, progresses, prospects. Science 185(4157): 115-1123.

Sokal, R.R. and Sneath, P.H.A. 1963. Principles of numerical taxonomy. 359 pp. W.H. Freeman & Co., San Francisco.

Sprecher, S.L. 1988. Allozyme differentiation between gene-pools in common bean (*P. vulgaris L.*) with special reference to Malawian germplasm. Ph.D. dissertation. Michigan State University, East Lansing.

Stavely, J.R. 1984a. Pathogenic specialization in *uromyces phaseoli* in the United States and rust inheritance in beans. Plant. Dis. 68: 93-99.

Stavely, J.R. 1984b. Genetics of resistance to *Uromyces phaseoli* in a *phaseolus vulgaris* line resistant to most races of the pathogen. Phytopathology 74: 339-344.

Stavely, J.R., Steadman, J.R. and McMillan, R.T., Jr. 1989. New pathogenic variability in *Uromyces appendiculatus* in North America. Plant Disease 73: 428-432.

Turner, B.J. 1974. Application of cluster analysis in Natural Resources research. Forest Science 20(4): 343-349.

Vairavan, E., E.A. Siddiq, V. Arunathalam and M.S. Swaminathan. 1973. A study on the nature of genotypic divergence in rice from Assam and North East Himalayas. Theor. Appl. Genet. 43:213-221.

Van der Plank, J.E. 1968. Disease resistance in plants. New York and London. Academic Press.