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thesis entitled

GENETIC, PHYSIOLOGICAL AND ANATOMICAL STUDIES
OF A NARROW-LEAFLET MUTANT IN THE DRY BEAN

presented by

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has been accepted towards fulfillment
of the requirements for

Ph.D degree in Crop Science

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Date Feb. 23, 1978

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GENETIC, PHYSIOLOGICAL AND ANATOMICAL STUDIES OF A NARROW-LEAFLET MUTANT IN THE DRY BEAN (*PHASEOLUS VULGARIS L.*)

by

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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

1978

ABSTRACT

GENETIC, PHYSIOLOGICAL AND ANATOMICAL STUDIES OF A NARROW-LEAFLET MUTANT IN THE DRY BEAN (*PHASEOLUS VULGARIS L.*)

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A narrow-leaflet mutant was observed in the dry bean, variety 'Seafarer", which had been treated with fast neutrons. The mutant is characterized by small and narrow leaflets, sterility, many slender weak branches, a dwarf and bushy appearance, and prolonged vegetative growth. Since the narrow leaflet mutant is sterile, the gene for this trait has been maintained in the population through heterozygotes, recognizable by their intermediate-type leaflets. Genetic, physiological and anatomical studies were undertaken to investigate the mode of inheritance, the nature of induced mutation and obtain additional information concerning the physiological-agronomic potential for narrow-leaflet mutants.

A series of experiments was conducted in the greenhouse as well as in the laboratory from summer 1975 to winter 1977. The intermediate leaflet plants were out-crossed to six other unrelated varieties. Genetic study was made on plants of the F_1 to F_3 generations. Plants of the F_3 generation were used for physiological study. Anatomical study was made on plants resulting from selfed seeds of the Original Intermediate.

The intermediate-leaflet types, upon selfing, yielded three classes of progenies according to leaf shape. They were normal, intermediate, and narrow-leaflet plants with a ratio of 1:2:1. Upon crossing the intermediate to six other unrelated varieties, the F_1 progenies of every cross could be classified into two classes: normal and intermediate types with a ratio 1:1. Upon selfing the F_1 intermediate leaflet types, they yielded plants classifiable in the three previously mentioned categories in the same ratio. The gene controlling leaf shape, therefore, follows a monogenic segregation with incomplete dominance.

Narrow-leaflet plants of the F_2 generation were associated with sterility, short stature, many branches and prolonged vegetative growth. The whole complex was inherited as a single mendelian unit along with the narrow-leaflet character. In the F_2 generation of two crosses, a few narrow-leaflet plants were found to produce seeds. When seeds were planted, they bred true for narrow leaflet and fertility. The data can be interpreted on the basis of either of two hypotheses as regards genetic regulation of this complex. According to the first hypothesis, the original mutation affected two closely linked genes, one responsible to leaf shape, the other affecting fertility. In the crosses to Montcalm and 31908, these effects were separated by intra-chromosomal recombination to produce a few fertile plants with narrow leaves. The second hypothesis specifies that the mutated gene is pleiotropic for shape, stature, branching and fertility, but that in the crosses the normal genotypes of Montcalm and 31908 contributed modifier genes to the F_1 and F_2 which restored fertility to certain recombinants without affecting leaf shape or other components of the complex.

There is little evidence that would favor one of these hypotheses over the other; if the first interpretation is the correct one, then the recombinant products should have included a sterile-normal, which was not obtained. However, the populations were quite small and that recombination could have been missed for reasons of insufficient sampling.

Physiological studies conducted on F_3 plants of two crosses revealed that the photosynthetic rate, translocation rate, stomatal resistance and stomatal density, the narrow-leaflet plant did not show any significant difference from either the normal or the intermediate.

Anatomical study of the normal and narrow-leaflet phenotypes did not show any significant difference in cell size or cell thickness. The narrow-leaflet gene is believed to control leaflet length and width by increasing or decreasing the number of cells rather than changing tissue components.

To

my Mother

my brother, Sangar Wilawan

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my advisor, Dr. M. W. Adams for his valuable guidance, moral support, patience, kindness, encouragement and understanding at all times during my study and thesis preparation. I am indebted to him for all those things which made my study possible and rewarding.

My grateful acknowledgement is extended to the members of my committee: Dr. S. Honma, Dr. G. J. Hogaboam and Dr. W. Tai for their kindness in serving on my committee, valuable suggestions and available help throughout my study at this University.

Special thanks are due to Dr. J. Wiersma for his continual assistance, encouragement, suggestions and friendship during my study and conduction of experiments.

Thanks are also extended to Dr. I. W. Knowbloch for his help during my specimen preparation for anatomical studies, to J. L. Taylor for his assistance in the greenhouse experiment, to Miss V. Imamee for her help in the greenhouse during Summer, 1976, to Miss S. Vajrabul, Miss K. Urasyanandana, and R. H. Huang for their help in various ways during my thesis preparation.

A grateful acknowledgement is also extended to Kasetsart University for financial support throughout my study at Michigan State University.

Finally, my sincere thanks go to my husband, DHAVORN, for his constant love, understanding and sacrifies given to me for the entire three years of my study. Without his dedication, my graduate study could never have been possible.

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INTRODUCTION

Narrow-leaflet mutants of two classes were found in the M_2 generation of the dry bean variety 'Seafarer' which had been treated with fast neutrons. Plants of the first class had long, semi-narrow (intermediate) leaflets; otherwise they exhibited normal vegetative growth and fertility. The narrow-leaflet mutants of the second class had long, narrow leaflets from the first trifoliolate leaves onward. The plants were sterile and did not produce complete or fertile flowers. Bud formation occurred, but the buds subsequently degenerated. However, the narrow-leaflet genotype has been maintained from generation to generation through heterozugotes (class-1 mutants).

Three phenotypes were characterized with respect to leaf type, i.e. normal (Seafarer type), intermediate, and narrow-leaflet types (Figures 1-3). The heterozygote could be distinguished from the normal (Seafarer type) by the reduction of the width of the middle leaflet and slightly reduced sizes of adjacent leaflets. The leaf-shape differences were clear enough that usually the true narrow-leaflets (class-2) could be detected among the segregating phenotypes without difficulty.

Narrow-leaflet mutants are of interest, since the small leaf character has been implicated as a desire component of an ideotype of dry beans (Adams, 1975). A small leaf also orient its leaves more vertically during daylight hours. As a result, light penetration into the canopy should be enhanced and the more basal leaves function more efficiently.

Although the true narrow-leaflet mutants in this study were sterile, the heterozygotes were fertile, with normal growth and seed production. The present experiment was aimed at understanding the mode of inheritance as well as to evaluate some physiological characteristics attributable to this narrow-leaflet phenotype. The findings would be helpful in determining whether this gene(s) is responsible for any valuable agronomic characters for future breeding purposes. Moreover, this study might also provide additional information on the nature of radiation-induced mutation in crop plants.

REVIEW OF LITERATURE

After the discovery that X-ray and other ionizing radiation (gamma ray and neutron), as well as chemical mutagens, could induce mutation in organisms, intensive work has been done in many countries to induce mutations in crop plants. The large mutant collections established in many institutions were used in the following ways:

(1) As sources for theoretical studies in the field of genetics, cytology, histology, embryology, physiology, developmental biology and biochemistry of plants of all kinds (Spike, 1961; Mericle and Mericle, 1969).

(2) As a 'key' for the study of taxonomical and evolutionary problems. The value of induced mutations in phylogenetic analysis has been demonstrated in some crops (Swaminathan, 1963; Gottschalk, 1969; Majid, 1973).

(3) As a source of valuable agronomic characters for breeding now implies that this method has come of age as a leading adjunct to plant breeding. A number of important crop varieties have been released commercially (Sigurbjornsson and Micke, 1969).

Mutation is the process of change from one hereditary state to another, Auerbach (1976) mentioned three main types of nuclear change which leads to phenotypic changes: (a) changes in the number of chromosones, (b) changes in the number and arrangement of whole genes (intergenic or structural changes) and (c) changes in individual genes (intragenic changes or gene mutation).

Changes in chromosome number result in polyploidy, haploidy, polysomy, monosomy or nullisomy. They are not included in the term mutation. All of these phenomena have played a great role in plant evolution and are used by plant breeders for the development of new strains and the analysis of existing ones. Intergenic changes include deletion, duplication of individual genes or sequences of genes; insertation of a chromosome segment into a new position; and exchange of segments between chromosomes. Intragenic changes or "point" gene mutation are changes in individual genes. Both intergenic and intragenic changes are produced by the same agents. In many cases, it is difficult or impossible to distinguish between these two. The term "point mutation" covers both true gene mutation (intragenic changes) and rearrangements that mimic them (Auerbach, 1976).

Genetic studies:

Characteristics of the leaf have been considered as the most dramatically affected in mutation research. Several types of leaf shape have been induced in jute (Singh et al., 1973; and Kundu et al., 1961; Singh, 1974) after different radiation treatments. Several leaf mutants were crossed with the normal leaf type in white jute. All gave normal looking F_1 plants. The F_2 generations of all the crosses exhibited a monogenic ratio of 3 normal: 1 mutant type, indicating that each mutant leaf characters was governed by a single recessive gene (Singh, 1974).

In sweet clover, Melilotus alba, the cutleaf mutant was reported as a monogenic trait with pleiotropic characters (Kirk and Armstrong, 1934). The other leaf mutant "rugose" with extremely large and wrinkled leaves was found to be controlled by a single recessive gene with major pleiotropic efforts. The pleiotropy affected leaf size, leaf morphology, flower morphology, leaf:stem ratio, and female fertility (Goplen, 1962). The two leaf mutants were chosen to study the mode of inheritance by Gengenbach et al. (1969). They were curled leaf and multifoliate leaf. In the cross between the curled leaf mutant and the normal plant, the F_1 plants were normal in morphology. Plants in the F_2 generation segregated into 3 normal: 1 curled leaf. In the cross of the multifoliate leaf mutants with normal plants, the multifoliate character was found to be controlled by a single dominant gene. Goplen (1967) reported the other leaf mutant in sweet clover. The mutant was unifoliate leaves with cauliflower inflorescences. Genetic analysis showed that this abnormal complex was conditioned by a single recessive gene with pleiotropic effects.

The 'ivy leaf' was a mutant resulting from X-irradiation of tuber-eye-pieces of the potato. The 'ivy leaf' was dominant without any pleiotropic effects (Van Harten et al., 1973).

In tomato, Lycopersicon esculentum, two mutants for leaf shape were reported by Mathan and Jenkins (1962). The lanceolate phenotype (La/La+) was found to be determined by a single gene in heterozygous condition. It differed from the normal in that it had simple, entire leaves rather than the odd-pinnately compound leaf of the normal tomato. The homozygous lanceolate (La/La) was expressed in 1 of 3 forms: reduced, modified, or narrow. The reduced form grew into a column of tissue up to 5 cm in height and 0.2 cm in diameter and was completely devoid of cotyledons or other foliar structures. The modified forms produced a cotyledon-like structure and a bud that failed to develop. The narrow form produced a cotyledon-like structure and a bud that developed into a shoot with a very small simple leaves but without flowers (Mathan and Jenkins, 1962).

Percival et al., (1976) reported on the genetic analysis of a round-leaf mutant in cotton (Gossypium hirsutum L.). The plant had several modified morphological features. The leaf edges rolled slightly downward; the leaf lobes were short, and rounded. Plants were upright, medium stature, and had shorter than the normal internodes. Inheritance and linkage tests showed that the mutation was conditioned by a dominant gene. In the homozygous state the mutant was functionally lethal. They proposed the mutant gene by assigned the gene symbols Rl_2Rl_2.

The pimiento pepper, (Capsicum annuum L.), the segregation ratios in the F_2 of crosses between a round leaf and normal was controlled by an allele recessive to normal. There were no obvious deleterious effects. Leaf length was reduced but not leaf width (Greenleaf and Hearn, 1976).

Broad variability with regard to leaf structure has been known within the Leguminosae (Gottschalk, 1968 and 1969). According to Gottschalk, one of his narrow leaf mutants (NO. 180A) was associated with a number of deviating characters such as small narrow leaflets and stipules, female sterility, a reduction of the internode length, and increase of internode number. He believed that the whole complex of deviating features was not due to one single pleiotropic gene but to at least two closely linked but different genes. In Vicia faba, three unifoliate mutants, two induced and one spontaneous, were studied morphologically and genetically (Sjodin, 1964). He found that three different loci were involved in expressing the unifoliate character. In black gram (Phaseolus mungo L., now Vigna mungo) radiation-induced mutants affecting shape and size of the leaf are known (Jana, 1962) and most of the mutants had reduced fertility. Four leaf mutants, crinkled leaf, waxy leaf, narrow leaf, and unifoliate were induced in black gram following treatments with X-rays and/or ethyl methane sulfonate (EMS). The crinkled leaf and waxy leaf mutants had normal fertility, whereas the narrow-leaf mutant was partially sterile. All the mutants except unifolilate behaved as monogenic traits, recessive to the normal (Roa and Jana, 1976).

Physiological studies:

Genetic control of photosynthesis can be exerted on both the CO_2 - fixing system and the CO_2 - transport system of the leaf. Biochemical capacity to fix CO_2 is a function of the enzyme complement of the chloroplast, which in turn is under the control of this organelle's own genes. Since the chloroplast can transmit their own genetic information independently, this provides an additional opportunity for genetically based variation in photosynthesis (Leopold and Kriedeman, 1975).

Differences in photosynthetic efficiency have been demonstrated widely by several investigators. Varietal differences in photosynthetic rates have been observed in oats (Crisswell and Shibles, 1971); maize (Heichel and Musgrave, 1969; Duncan and Hesketh, 1968); sugarcane (Irvine, 1967; Pendleton et al., 1968); soybeans (Dornhoff and Shibles, 1970; Ojima, 1972); tall fescue (Asay et al., 1974) and dry beans (Izhar and Wallace, 1967; Wallace et al., 1976).

Izhar and Wallace (1967) measured the rate of net carbon dioxide exchange (NCE) in five varieties of beans. They found that these varieties differed significantly from each other. The variety 'Red Kidney' had the lowest net NCE. They believed that the basis for varietal differences in NCE rate was quantitiative, and there might be few genes involved, and there was some dominance for the low photosynthetic efficiency of Red Kidney.

Selection of genotypes for high photosynthetic rates in order to increase yield has been practiced by many workers. In dry beans, it has been suggested that the ideal type should carry genes which favor the higher rates of net CO_2 fixation (Adams, 1975). Researchers have sought higher photosynthetic efficiency by selecting cultivars for high photosynthetic rates per unit of leaf area and by developing the most efficient canopy. However, the high photosynthetic rates per unit leaf area are often not correlated with high yield. In sugarcane it was found that photosynthetic rates per leaf unit area did not increase yield (Irvine, 1975). High photosynthetic rates per unit leaf area were not correlated with yields of tall fescue (Nelson et al., 1975); maize (Ariyanaya, 1974; Victor, 1975).

However, a positive correlation was found between the rates of photosynthesis from flowering to pod set of nine varieties of dry bean and their final seed yields (Peet et al., 1977). They also found that in one variety high seed yields were also associated with very low photosynthetic rates. This variety has a high harvest index and high malate dehydrogenase and glucolate oxidase activities. They suggested that high seed yields were not necessarily associated with high photosynthetic rates but might result from a more efficient utilization of photosynthate.

Ashley et al., (1977) reported that single leaf apparent photosynthesis (AP) rates of soybean cultivars in the greenhouse were not related to single leaf AP rates obtained under field conditions. Neither greenhouse nor field AP rates on single leaves were consistently related to field canopy AP rates or to seed yield.

Rate of photosynthesis can be affected by several factors. For example, internal regulation consists of the photosynthetic enzyme system, leaf resistance, and leaf age. The environmental factors are, for example, light intensity, carbon dioxide concentration, oxygen concentration, and temperature around the plant (Crookston et al., 1974; Austin and Mclean, 1972; Collatz, 1977; Taylor et al., 1972; Taylor and Rowley, 1971).

Only a few studies have been conducted in relation to leaf mutation and physiological processes. In soybean (Glycine max L.), it was reported that the narrow-leaflet Harosoy had a 28% greater mean daily net carbon dioxide exchange (NCE) on a leaf basis than normal-leaflet Harosoy (Egli et al., 1970). Another experiment in relation to the comparison between normal and narrow leaflets was reported by Hiebsch et al., (1976). In their experiment, two isogenic soybean lines with normal and narrow leaflets were compared in the field for differences in net carbon dioxide exchange rates, water use, and water-use efficiency with various combinations of population and row spacing. They found that leaflet type did not significantly affect net carbon dioxide exchange, water use, or water-use efficiency.

In cotton (Gossypium hirsutum L.) plants with okra or superokra leaves have several agronomic characteristics which could make them better adapted to narrow-row culture than plants with normal leaves. Buxton and Stapleton (1970) described a cotton leaf model in which the superokra leaf shape was predicted to have a photosynthetic advantage per unit leaf area over normal leaf shape. Elmore et al., (1976), however, found similar rates between superokra leaf plants and several normal leaf lines. Moreover, Baker and Mylure (1969) compared canopy carbon dioxide exchange rate (CER) per unit ground area of 'Rex' normal and okra leaf cotton communities planted in standard row width and found no significant difference. Pegelow et al., (1977) investigated the effect of these leaf types on canopy photosynthesis and transpiration of narrow row cotton. They found that normal leaf plants had CO_2 exchange rate higher than superokra leaf plants. Leaf type effects on transpiration were small and inconsistent. Differences in photosynthesis to transpiration ratios were normal > okra > superokra. Thus, the small leaf types did not appear to be associated with efficiency of water use.

The yield of crop plant is determined not only by its efficiency of light utilization, but also by its ability to translocate its assimilates to growing or storage tissues. As early as 1896, Ewart suggested that photosynthesis was influenced by the degree of translocation of photo-assimilates from leaves. Since then, many claims have been made that the utilization of assimilates by 'sink' affects the photosynthesis of leaves, presumably through the assimilate transport system.

Sugarcane was found to translocate about 80% of an initial pulse of assimilated $^{14}CO_2$ in 4 h (Hartt and Kortschalk, 1967) and in corn translocated almost 80% in 2.5 h (Hofstra and Nelson, 1967). Sugarbeet translocated about 60% in 3 h (Mortimer, 1965), soybean a maximum of 45% in 2 h (Thrower, 1967), tobacco about 22% in 5.5 h (Shiroya _et al_., 1961) and pine seedlings about 15% in 7 h (Shiroya _et al_., 1962). Hofstra and Nelson (1969a) reported that species which were known to have high photosynthetic rates, such as grasses, sorghum and millet, exported 70% or more of the assimilated ^{14}C during the first 6 h after assimilation, compared to values of 45 to 50% for tomato, castor bean, and soybean.

In corn, between 80 and 90% of the assimilated ^{14}C was translocated from the fed area of the leaf in 24 h with 50% moved out in the first 30 m (Hofstra and Nelson, 1969b).

In pea (*Pisum sativum* L.), translocation of ^{14}C- assimilate was achieved within 24 h: there was no significant secondary movement of ^{14}C within the subsequent 24 h (Harvey, 1973).

Pattern of translocation of pulse label was shown by Liu *et al.*, (1973). In bean (*Phaseolus vulgaris* L.), two cultivars, Michelite-62 and Red Kidney, were studied. Data on translocation indicated that export of a pulse of photosynthetically assimilated ^{14}C from the source leaf of either Michelite-62 or Red Kidney followed an exponential pattern and showed an initial rapid phase followed by a second slower phase. Rate of translocation of pulse label of Michelite-62 was higher than that of Red Kidney. Only 38% of the ^{14}C remained in the leaf of Michelite-62 after 8 h, while Red Kidney retained up to 60% of the label. Michelite-62 was also found to have a higher photosynthetic rate than Red Kidney. They concluded that there was a positive correlation between photosynthetic efficiency and translocation efficiency in these two varieties.

In *Pisum sativum* L., there are a number of leaf mutants available. Harvey (1974) compared three types of leaf mutants with normal-leaf varieties in translocation potentials. The mutants differed markedly in foliar morphology: genotype *afafTlTl* had leaflets converted to tendrils; *AfAftltl* had tendrils converted to leaflets; *afaftltl* had relatively minute leaflets on branched petioles. The finding was that in translocation terms the leaf and pod had a well defined source and sink relationship that was independent of leaf morphology. The foliar mutant genotypes *afafTlTl* and *afaftltl* were comparable to normal (*AfAfTlTl*). Therefore, fundamental changes in pea leaf morphology could be made genetically without a marked effect on the photoassimilate export potential of the leaf.

Stomates are scattered on both sides of leaf surface in dicotyledonous species having netted venation, while in monocotyledonous, they are arranged in parallel rows on the adaxial surfaces (Ketellaper, 1963; Northern, 1958). Greater stomatal frequency was reported on abaxial surfaces in alfalfa (Cole and Dobrenz, 1970), wheat (Teare *et al.*, 1971) and creeping bentgrass (Shearman and Beard, 1972). Similar stomatal frequency was reported on both surfaces of the leaf of barley (Miskin and Rassmusson, 1970) while blue panicgrass (Dobrenz *et al.*, 1969) and maize (Heichel, 1971) had higher stomatal number on abaxial than adaxial surfaces.

Stomatal density usually ranges from 50 to 500 mm^{-2} (Keteller, 1963; Slatyer, 1967). Stomata may be separated by no more than one or two epidermal cells at high densities. Creeping bentgrass (*Agrostis palustris* Huds.) has been reported to have a ratio of one to two stomata to two epidermal cells (Meusel, 1964).

A number of workers (Eckerson, 1908; Miller, 1938; Ormrod and Renny, 1968) have shown differences in stomatal frequency between plant genera. Varietal differences in stomatal frequency within the single plant species have been reported in alfalfa (Cole and Dobrenz, 1970), barley (Miskin and Rassmusson, 1970) and wheat (Teare, 1971).

Environmental conditions under which plants are grown have been shown to influence stomatal frequency. Shading has reduced stomatal frequency in a number of plants (Brown and Rosenberg, 1970; Knecht and O'Leary, 1972; Penfound, 1931). In a bush bean (*Phaseolus vulgaris* L.), the abaxial density of stomates remained relatively constant throughout development of the plant while the adaxial density of stomates decreased from 70 stomates mm^{-2} during the early stage of growth to 15 stomates mm^{-2} during the late stage of growth (Davis *et al.*, 1977).

Developmental and genetical effects have been reported to affect stomatal number and density. Kazemi *et al.*, (1977) found that in 12 cultivars of spring wheat, there was significant variation among cultivar means for stomatal frequency. However, variation among plants within cultivars was also significant. The adaxial surface was more variable than the abaxial. They suggested that directional selection for stomatal number would be difficult.

Transpiration and photosynthesis are possibly influenced by stomatal frequency. Heichel (1971) reported that a maize cultivar with lower stomatal frequency had faster net photosynthesis than a cultivar with greater stomatal frequency. However, Miskin *et al.*, (1972) found that stomatal frequency did not influence rate of photosynthesis in barley, but did influence transpiration and stomatal diffusion resistance. Reducing stomatal width with phenyl mercuric acetate led to significantly less evaporation from a red-pine forest (Turner and Waggoner, 1968; Waggoner and Bravdo, 1967).

Stomatal response can be expressed in terms of the changes in resistance to diffusion of water vapor and carbon dioxide. Peet *et al.*, (1977) reported that stomatal resistances differed significantly among varieties and developmental stages in dry bean varieties. They found that average resistance was lowest at early pod set, being 5% lower than at late pod set. They also found that varieties with high seed yield had lower stomatal resistance at pod set.

Anatomical studies:

A number of investigations have been conducted in relation to leaf anatomy and some physiological processess, for example, photosynthesis. Most photosynthesis occurs in the leaves. Thus, investigators have looked for an association between leaf physical characteristics and photosynthesis in order to select plants for high CO_2 fixation rates without having to do a direct measurement of photosynthesis.

El-Sharkawy (1965) found that among species, the ratio of internally exposed cell surface to volume of cells was positively correlated with photosynthetic rate. Carlson _et al._, (1970) reported that photosynthesis in alfalfa was associated with thickness of palisade and mesophyll layers. Plants with high SLW (specific leaf weight = ratio of leaf weight to ratio of leaf area) had thick palisade cells, and more mesophyll cells per unit leaf area are also observed on plants with high SLW. Delaney and Dobrenz (1974) found that plants with small leaves had the greatest SLW, palisade tissue thickness. A positive association was observed between palisade tissue thickness and photosynthesis expressed on a leaf basis.

In soybean leaves, SLW, and leaf thickness itself were correlated with carbon dioxide exchange rate (CER). The thickness differences were most strongly expressed in the upper palisade and paraveinal mesophyll layers (Dornhoff and Shibles, 1976).

The cross-section of the primary leaves of plants grown at high, medium and low light intensity showed an increasing thickness of the leaf with increasing light. The leaf thickness was caused by thicker layer of both palisade and spongy parenchyma cells (Louwerse and Zweerde, 1977).

In comparative leaf anatomy of two compact apple mutants and their normal forms, Liu and Eaton (1970) found that the compact mutants had thicker palisade parenchyma and greater total leaf thickness than did normals. The anatomical differences observed tended to favor the compact mutants with regard to photosynthetic efficiency, since photosynthetic rate could be a function of their density-thickness in g/cm^2 fresh weight (McClendon, 1962).

Tal et al., (1974) reported that the wilty mutant of pepper had a much greater portion on intercellular space than the normal. The mutant contained fewer and smaller mesophyll cells than the normal plant. The anticlinal walls of the epidermis of the mutant were almost straight whereas those of the normal were wavy. Transpiration per unit leaf area of whole plants, percentage of stomata open both day and night, and water loss from detached drying leaves were all higher in the mutant.

Anatomical analysis was used to detect differences in a large number of induced mutants in jute. Eight different antomical criteria; number of cell layers, length and breadth of fiber bundles, number of fiber cells per bundle, length and breadth of fiber cells, lumen size and fiber wall thickness were studied. Thirty-four out of 38 radiation mutants exhibited significant variation from the control for one or more of the criteria studied. Mutants with drasitc morphological changes exhibited variability for a large number of anatomical criteria (Abraham and Joshua, 1974).

MATERIALS AND METHODS

Genetic studies:

Plant material for this study consisted of seven lines of dry beans (*Phaseolus vulgaris* L.), which were divided into two groups on the basis of seed size:

Lines	Seed size and color
1. Original Intermediate	Small (184.0 mg/seed), white
2. Seafarer	Small (178.3 mg/seed), white
3. Nep-2	Small (108.8 mg/seed), grey-white
4. Jamapa	Small (98.8 mg/seed), purple-black
5. 2114-R	Small (156.8 mg/seed), white
6. Montcalm	Large (356.4 mg/seed), red
7. 31908	Large (488.2 mg/seed), white

The intermediate leaflet type was the class-1 mutant, which yielded on self fertilization three classes of progeny: normal, intermediate and narrow leaflet. Seeds of the Original Intermediate were obtained from bean collections in the Department of Crop and Soil Sciences, Michigan State University. The Original Intermediate was one of several mutants phenotypes, including both intermediate and narrow-leaflet plants, taken from a large field of beans planted in June 1973 to M_2 generation seed following fast neutron irradiation of the parental variety, Seafarer (dosage 400, 700, and 1,000 RAD given to air dry seeds in the laboratory of the International Atomic Energy Agency, Vienna, in November, 1972). The M_1 generation had been grown at Vicosa, Brazil, under the supervision of Dr. Clibas Veiera during Jan-April, 1973. The fertile intermediate had been selfed and maintained as a genetic stock since 1973. Therefore, the three leaflet types which segregated upon selfing of the intermediate differed from each other only by gene(s) controlling leaf shape (or by factors linked to the leaf shape gene(s)).

Experiments were conducted in a greenhouse at Michigan State University from Summer, 1975 to Winter, 1977. The first experiment involved a study on the mode of inheritance of this leaflet mutation.

Selfed seeds of the Original Intermediate plants were sown in the greenhouse and plants were classified according to the leaflet shapes they displayed. The normal leaflet plant was similar to the Seafarer parent variety (Figure 1). The intermediate-leaflet plant had long, semi-narrow leaflets, and exhibited normal growth and fertility (Figures 2, 4). The narrow-leaflet plant had short internodes, and many weak, thin branches which gave it a bushy appearance. The leaflets were long and narrow, but were thick and dark green in color (Figures 3, 5). Narrow-leaflet types did not exhibit normal reproductive growth. Flower bud formation did occur, but the bud subsequently degenerated before anthesis (Figure 6). Anatomical observations of the flower bud showed that microsporogensis did occur, producing normal microspore mother cells. Apparently, in the reproductive process, megasporogenesis was distributed, resulting in the sterility of the flower bud.

Classification of phenotypic differences among plants were made many times during plant growth to assure they have been classified correctly. The narrow-leaflet plants were detected as early as the first fully expanded trifoliolate leaf. The intermediate type could be separated from the normal leaf type from the third trifoliolate leaf onward. Four experiments were made with this objective. The data were pooled and a X^2-test of fitness to a genetic hypothesis was applied.

To study the pattern of narrow-leaflet gene(s) displayed in other genetic backgrounds, the intermediate types were crossed to the following lines: Seafarer, Nep-2, Jamapa, 2114-R, Montcalm and 31908. Seeds obtained from these crosses were planted in the greenhouse in randomized complete block designs. F_1 plants segregated into two groups, normal (Figure 7) and intermediate. They had good vegetative growth and fertility. Leaflet width, length and weight as well as petiole length, diameter and weight were measured on fifth trifoliolate leaves. Height and number of nodes were also recorded. Seeds from the intermediate types from every cross were planted in a completely randomized design in the greenhouse during Summer, 1976. About 50 seeds were used in each cross. Classification based on phenotypic differences was made several times during the growing period. Three distinct groups were found in all crosses: the normal, the intermediate and the narrow-leaflet types. Although the sizes of the leaflets were different, the shape of leaflets was similar in all crosses (Figures 8, 9, 10). Measurements were made at first flowering stage; e.g. height; number of branches; length; width and weight of leaflets on the third trifoliolate leaf. Data for phenotypic differences were pooled and a X^2-test of fitness to the hypothesis was calculated. The data collected at the first flowering were analyzed using the F-test and Tukey's LSD $_{.05}$ to detect differences among means of parental varieties as well as the F_2 progenies of each cross.

<u>Physiological studies</u>:

F_3 seeds from the F_2 intermediate-leaflet type were chosen from two crosses, the Original Intermediate phenotype to Nep-2 and 31908.

The seeds were planted in a greenhouse in a completely randomized design during Winter, 1977. F_3 plants in this experiment were used for genetic analysis as well as physiological studies.

1. *Photosynthesis*. F_3 plants from the crosses of Nep-2 X Original Intermediate segregated into the three previously described classes. F_3 plants and the parental lines were grown in the greenhouse where supplemental artificial light was given 16 hours daily. The photosynthetic measurement was made on the first flowering day. Five randomly selected plants from each class of each cross were used for the study. Two samples from each plant were collected, one from the middle leaflet of the third trifoliolate and other from the middle leaflet of the fourth trifoliolate leaf. Carbon dioxide uptake of the leaf was measured and analyzed using the procedure described by Hatfield (1975) and Nalor and Tearl (1975). A leaf section was exposed to labeled CO_2 ($10.2\ \mu Ci^{-2}\ {}^{14}CO_2$) for 20 seconds. Immediately, the exposed area was excised with a leaf punch and put in a scintillation vial containing 1 ml of NCS (solubilizer). The leaf discs dosed with ${}^{14}CO_2$ were left in solubilizer for at least 48 hours, then bleached with 1 ml of a solution of 1 g benzoyl peroxide in 5 ml toluene. Eighteen ml of a scintillation fluid were added to the vials after the addition of the bleach. The composition of the bleach was PPO:POPOP:toluene in the proportion 6g:75mg:1 litre. After addition of the scintillation fluid, the vials were allowed to set for 24 hours to reduce the effect of the chemoluminescence of the fluid. All samples were counted for 1 minute on a Beckman liquid scintillation counter. Then the carbon dioxide uptake of the leaf section was calculated, as described by Hatfield, 1975; Naylor and Tearl, 1975.

2. Translocation. Measurement of translocation of pulse-labeled $^{14}CO_2$ was made at the same time on the same plant as for the measurement of photosynthesis. On the third and fourth trifoliolate leaves, the two remaining leaflets were used. Labeled $^{14}CO_2$ was administered to the source leaf for 20 seconds. The leaf sections were collected at 30 minutes and 60 minutes after exposure time. The measurement of radioactivity left in the leaf sections was made using the same procedure as described for the measurement of photosynthesis. Using data collected for photosynthesis as time 0, translocation was estimated by calculating the percentages $^{14}CO_2$ left in the leaves at 30 minutes and 60 minutes.

3. Stomatal density. Silicone rubber impressions were made of the adaxial and abaxial surfaces at the widest portion of the middle leaflet of the third trifoliolate leaf, using a modification of the method reported by Sampson (1961). The same sets of plants from previous experiments were used. Silicone rubber was mixed with the requisite amount of catalyst and immediately poured over the leaf surfaces on both sides. The silicone rubber hardened in about 5 minutes and it was lifted away with forceps. This was the negative replica. It was left to dry in a dessicator containing phosphorus pentoxide for 3-4 days. To make a positive replica, clear fingernail polish was poured over the dry undisturbed surface. It was allowed to dry completely, then the transparent replica was separated and mounted on a slide. Stomatal counts were made in microscopic fields from each impression. For each impression one picture was also taken. The area observed was 0.377 mm^2, with the counts being converted to a mm^2 basis. Some parts of this study were done using the scanning electron microscope (SEM). The

positive replica was mounted on the stub, gold coated and viewed with the SEM. The area observed under the SEM was 0.175 mm^2. The counts were converted to a mm^2 basis. The two techniques yielded the same result. However, the light microscope procedure was much easier and less time consuming.

4. Stomatal resistance. Stomatal resistance was determined by measuring the rate per unit time of voltage change across the humidity-sensitive resister. Two points (30-70) on the output meter scale were selected and the time necessary for the circuit output to traverse these points (30-70) was measured with a stop watch. This voltage change was related to diffusion resistance by calibration with a series of plexiglass plates of known resistance at temperatures 17.5, 25.5 and 35 C in a growth chamber at a relative humidity of about 45 percent. A calibration curve at 25 C was constructed. The procedure used in this study was similar to that described by Kanemasu, Thurtell, and Tanner (1969). The diffusion resistance porometer used in the study is commercially available from Lambda Instrument Corporation, Lincoln, Nebraska. Then data from the greenhouse experiment were converted to the known resistance on the calibration curve. Five randomly selected plants from each group were used to measure stomatal resistance. These plants were not the same plants as in the three previous experiments. The reason was that this experiment was conducted about a week after the others, all plants were not in good condition due to some damage of the leaves caused by leaf punching and replica making. However, these plants were grown at the same time and were treated with the same environment as those mentioned plants. Data were collected from the middle leaflets of the third and the fourth trifoliolate leaves.

Anatomical study:

Seeds from the Original Intermediate line were planted in the greenhouse in the Fall, 1976. After the plants had the first and the third fully expanded trifoliolate leaves, ten randomly selected normal plants and ten randomly selected narrow-leaflet plants were used to perform the study. Small sections of the middle leaflets of the first and the third trifoliolate leaves were excised from the plants. They were immediately fixed in Craff III, and prepared for anatomical study using the procedure described by Sass (1966) and Wilkinson and Beard (1975). The sections were cut as a thickness of 10 microns and stained in Fast Green and Safranin. The mounted slide was examined under a light microscope. Pictures were taken and used for quantitative analysis. Photographs were cut out and weighed to determine the relative proportions and sizes of each tissue. The thickness of the leaflet and size of cells were also measured with the help of an occular micrometer.

Shoot tips of these plants were also collected for the anatomical study. They were collected about 25 days after planting. Shoot tips were fixed in FAA, and followed the same procedure as described for the study of leaf anatomy. However, the sections were observed under the light microscope to provide a general idea about cell size and flower bud formation as well as branch formations. No quantitative analysis was performed.

RESULTS

Genetic studies:

Seeds collected from intermediate-leaflet plants obtained from the bean collection of the Department of Crop and Soil Sciences, were planted five times in the greenhouse, from July, 1975 to October, 1975. Plants in all experiments segregated into three phenotypic groups: the normal, the intermediate and the narrow-leaflet types. The ratio of the normal to the intermediate to the narrow-leaflet was approximately 1:2:1 (Table 1). The X^2-value and statistical probability suggested the strong likelihood that a single mendelian unit, without dominance, governed the inheritance of this trait. Segregation of progenies for leaf type showed that the intermediate leaflet type was heterozygous. The intermediate plant carried an allele for normal leaflets and an allele for narrow leaflets. Progenies from normal plants bred true and were normal, indicating that the normal leaflet plant carried the gene for normal-leaflets in a homozygous condition. In all of these experiments, the narrow-leaflet plants did not set seed. Proof of the homozygous condition of the gene for narrow leaflets in these experiments, therefore, was not possible. The intermediate possessed the characters of both the normal and the narrow-leaflet, e.g. semi-narrow leaflets, fertility (Figures 2, 4).

When comparing total leaf area from three trifoliolate leaves of ten randomly selected plants of each group, it was found that differences in leaf area were significant. The normal-leaflet phenotype had a larger leaf area than both the intermediate and the narrow-leaflet phenotypes.

Among all three phenotypes the narrow-leaflet had the smallest leaf area. The intermediate type was also significantly different from the narrow-leaflet (Table 5).

Differences in leaflet weights were not significant. Non-significant differences were also found in specific leaf weight (SLW). The narrow-leaflet phenotype was the highest one of the three groups. The intermediate phenotype had the lowest value for specific leaf weight. However, the differences among them were not significant at the 5% level by the F-test. From these data (Table 5), these three phenotypes differed significantly in leaflet size but not in weight. Therefore, the narrow-leaflet gene not only caused plants to differ in leaflet shape, but also in leaflet size.

Narrow-leaflet gene in different genetic background:

Plants obtained from crosses of the intermediate leaflet plants to six unrelated (normal) varieties segregated into two distinct classes: normal, and intermediate-leaflet types. Classification was not difficult, since the differences between them were clear cut. In all crosses, the intermediate plant types exhibited the same pattern of leaf shape regardless of leaflet size. Expected ratio between classes was 1 to 1. The values of X^2 and probability are shown in Table 2. It was found that the data provided enough evidence to accept the hypothesis that the ratio 1:1 was reasonable ($p = 0.10 - 0.25$). The number of plants in each cross was small, and likely to cause deviation from the expected ratio. In the cross of Original Intermediate X Montcalm, the number of plants in the normal group was higher than in the intermediate group, with $X^2 = 5.34$ and $P = .025$. This was probably caused by the smaller number of plants rather than true differences between them.

By this experiment, the narrow-leaflet 'gene' was introduced into other genetic backgrounds. The mode of inheritance followed the single gene pair (monogenic trait) with incomplete dominance. Inheritance followed the same pattern in all crosses.

In the F_2 generation, segregation of the 'gene' for narrow leaflet occurred. In all crosses, F_2 plants segregated for leaf shape into three classes: normal, intermediate and narrow-leaflet types. The expected ratio was 1:2:1. F_2 plants in this study were derived from the intermediate-leaflet F_1 parents. In selfing a heterozygote, it is expected that progenies will occur in the ratio 1:2:1 for normal: intermediate: narrow-leaflet plants. The probability values are given for each class in Table 3. The data show that in all crosses segregation ratios of 1:2:1 were found. The narrow-leaflet 'gene' showed the same expression in all genetic backgrounds (Figures 8-10). Regardless of difference in other factors, F_2 plants in these crosses reproduced the same pattern of segregation for leaf shape. Segregation found in F_2 of the cross between the intermediate and Montcalm fit the expected ratio (with $X^2 = .00$, $P = 1$).

To follow the pattern of segregation in the F_3 generation, the cross between the intermediate and Nep-2 was selected for study. In F_3 generation, plants obtained from the intermediate F_2 parents could be divided into three distinct classes: normal, intermediate and narrow-leaflet plants (Table 4). The expected ratio was observed among the three classes. This finding provided evidence that the gene governing leaflet shape followed the same pattern as it did in the F_2 generation. What happened to the progenies of normal F_2 and narrow F_2's, in the F_3 generation:

The narrow-leaflet plant possessed some associate characteristics beside being narrow leaflet. It had numerous to many branches, was short in stature, had sterile flower buds, prolonged vegetative growth and a bushy appearance (Figures 5, 6). All deviating characters were transmitted from generation to generation along with narrow leaflet from the Original Intermediate genetic background. The whole complex behaved as if it were controlled by a single gene. But, in contrast to the intermediate appearance of leaves in the plant shown to be heterozygous, the appearance of the heterozygote relative to other traits of the complex was completely normal. If the mendelizing unit, termed here 'the narrow-leaflet gene' is, in fact, a single gene in the modern sense, then in terms of gene action, the action is co-dominance for the leaf shape effect, and completely recessive to normal for the other traits of the complex.

In observing the pattern of these deviating characteristics in other genetic background, it was found that at least in two crosses, the complex was partially broken down. In the F_2 population of the cross between the intermediate and Montcalm red kidney, one out of six narrow-leaflet plants had a pod. There were six seeds in this pod. When these were planted, they bred true for narrow-leaflet type (Figues 11-13), and produced normal appearing flowers. Later on, the plants were attacked by leafblight and most pods abscised. Few seeds could be collected. Not only were the plants of the narrow-leaflet phenotype, they were also fertile. The narrow-leaflet plants with fertility still retained other characteristics of the complex: short stature, numerous branches, prolonged vegetative growth. The cross Original Intermediate X 31908 also provided the same result. In the Original Intermediate X 31908 cross, one narrow-leaflet plant was fertile. It had four pods with 14 seeds. Upon planting these seeds, they bred true for narrow-leaflet plants (Figure 28). However, leaf sizes varied among them. Some of them had slightly larger leaflets than others. With regard to leaflet shape, all were similar. Five of them produced flowers and good seed set. The rest of them produced flowers at a very late stage of development. At the end of the growing period, some plants were infested with insects and diseases, therefore, I did not collect seed from them. They were discarded before seed setting. Only seeds from healthy plants were collected.

F_2 population studies:

F_2 plants along with their parents were studied in many aspects beside leaflet shapes. The data are summarized in Table 6.

1. Height: Among parents, 2114-R was the tallest. This variety was semi-vine, small white-seeded type with pale green leaflets. Nep-2 was the shortest parent. This trait was measured at first flowering day. Nep-2 under the greenhouse condition continued growing in height after the first flowering day. Variety 2114-R differed significantly in height from 31808, Montcalm and Nep-2 (following Tukey's LSD .05). Among the F_2 plants of the cross between the Seafarer and the Original Intermediate, the normal-leaflet plant was the tallest. The intermediate type lay between the normal and the narrow-leaflet types in height. The narrow-leaflet allele was incorporated into both the intermediate and the narrow-leaflet plants. Being incorporated it caused plants not only to change in leaf shapes but also in height. However, the differences were not significant. This situation was also found among F_2 plants of the crosses of Nep-2 X Original Intermeidate, Jamapa X Original Intermediate, 31908 X Original Intermediate and 2114-R X Original Intermediate (Figures 14-19). In the Nep-2 X Original Intermediate classes, the normal and the intermediate plants were significantly taller than the Nep-2 parent. At present, it is not determined

whether this character is controlled by the same gene governing leaf shape or by the other genes that have mutated at the time of irradiation.

2. Number of branches. The number of branches on the main stem was counted on the first flowering day and recorded on an individual plant basis. On narrow-leaflet plants most of which were sterile, the number of branches were counted when 50% of plants in the cross showed evidence of flowering. Table 6 indicates that, among parental varieties, the Original Intermediate bore the highest number of branches on the main stem with a mean value of 5. Seafarer, Nep-2, Jamapa, Montcalm, 31908 and 2114-R had mean values of 4, 3, 1, 4, 4, and 3, respectively. In all crosses, the narrow-leaflet types had the highest mean number of branches as compared to the other two phenotypes. When compared with its parents, it had a higher number of branches than either. In the present study it is not determined whether the gene governing leaf shape is also responsible for the branching or whether other mutated genes are responsible for the increase in number of branches.

3. Number of nodes. Data were collected on the first flowering day on the number of nodes on the main stem. The same rule was applied to the narrow-leaflet as with the other trait, i.e. data were collected when 50% of plants in the cross had flowered. Number of nodes ranged from 6 to 14. Variety 2114-R, being the tallest in the group, also had the highest number of nodes. Variety 31908 had the lowest number of nodes and differed significantly in node number from most of the parents, except Montcalm. In the Seafarer X Original Intermediate cross F_2 plants which belonged in the same class according to leaf shape had the same number of nodes. A different situation was found in the cross Nep-2 X Original Intermediate. In this cross, the intermediate plant had the

lowest number of nodes and differed from either the normal or the narrow-leaflet types. In the cross Jamapa X Original Intermediate, the normal had the highest node number. However, node number was likely to be a more or less constant factor among these populations (Table 6).

4. Weight of leaflets. The average weights of three leaflets of the third trifoliolate leaf are shown in Table 6. Among parental varieties, Seafarer had the lowest leaflet weight. The highest parent was 31908. Variety 31908 differed significantly from Seafarer, Original Intermediate, 2114-R and Jamapa. In all crosses, the three leaflet phenotypes (normal, intermediate and narrow) did not differ significantly from each other. However, the trends for leaflets were normal, intermediate, narrow, except in the progenies of the 31908 X Orignial Intermediate. In this cross, the intermediate had the lowest leaflet weight. Therefore, on the basis of the third trifoliolate leaf weight, there were no significant differences among the three segregating leaflet phenotypes of all crosses examined.

5. Length of petiole: Data on the length of petioles are shown in Table 6. Original Intermediate had the shortest petiole among parental varieties. Nep-2 was recorded as having the longest petiole. In all crosses, the lengths of petiole were more or less the same. The mutant gene for leaf shape, therefore, is not likely to cause any significant change in the length of petiole.

6. Diameter of petiole. Data on the diameter of petioles are present in Table 6. Among parents, the Original Intermediate had the smallest diameter of petiole, whereas variety 31908 had the largest diameter. The narrow-leaflet type from the cross of Montcalm X Original Intermediate had the smallest diameter (.20 cm) and was significantly different from the normal and the intermediate types of

the same cross. In the cross 31908 X Original Intermediate, the narrow-leaflet types was also significantly shorter than that of the normal phenotype of the same cross. The narrow-leaflet phenotype is likely to have smaller diameter than the other phenotypes of the same cross.

7. Petiole weight. Data on the petiole weight are present in Table 6. Variety 31908 had the highest petiole weight among parents, whereas Original Intermediate had the lowest petiole weight. In all crosses, the three leaflet phenotypes did not show any statistical significant difference in petiole weight.

8. Length of leaflets. Average lengths of three leaflets of the third trifoliolate leaf are shown in Table 6. The greatest length was found in variety 31908, whereas the shortest length was recorded in Seafarer. Variety 31908 differed significantly in leaf length from Seafarer and Original Intermediate. In all crosses, the narrow-leaflet had the longest leaf length as compared to the other two phenotypes. However, statistically significant differences were not obtained as far as leaflet phenotype was concerned.

9. Width of leaflets. The average widths of three leaflets of the third trifoliolate leaf are shown in Table 6. Variety 31908 differed significantly in width from the Original Intermediate. Montcalm was also significantly different from Original Intermediate. In all crosses, the narrow-leaflet phenotypes had the narrowest width as compared with the other two phenotypes. The trends were normal, intermediate, narrow. This is a reversion of the trend for length. Therefore, narrow-leaflet genes are clearly associated with an increase in leaf length and a reduction in leaf width of the plant carrying them.

10. Leaf area. The averages of three leaflets are shown in Table 6. Variety 31908 had the greatest area among all parents. It also differed

significantly from all other parents on the basis of the F-test and the Tukey's LSD. In all crosses the narrow-leaflet had the smallest area among all phenotypes of the same cross. However, the three leaflet phenotypes within a given cross did not differ significantly from each other.

11. Specific leaf weight (SLW): The specific leaf weight is the ratio of the dry weight of leaf in milligrams to the leaf area in cm^2. The averages of SLW of three leaflets of the third trifoliolate leaf are present in Table 6. The SLW varied among varieties and phenotypes in this study. No statistically significant difference could be obtained on the basis of the F-test.

Physiological studies:

1. Photosynthesis. Photosynthesis rate data were collected on the date of first flowering for the normal and intermediate phenotypes. In progeny from the Original Intermediate group, the narrow-leaflet types did not flower, the data were collected when 50% of sister plants (normal and intermediate) were flowering. The same rule was applied to the narrow-leaflet in the Nep-2 X Original Intermediate cross. However, since the narrow-leaflet plants in the cross 31908 X Original Intermediate flowered, data were collected at first flowering day. The results are presented in Table 7.

The average photosynthetic rates were highest in the normal-leaflet type of the F_3 generation of the cross Nep-2 X Original Intermediate, and lowest in the normal-leaflet type of the cross 31908 X Original Intermediate. Nep-2 and 31908 had almost the same photosynthetic rate. Among selfed progeny of the Original Intermediate group, rates were highest in the narrow and lowest in the normal-leaflet phenotypes, although not significant using Tukey's LSD.

In the F_3 generation of the cross 31908 X Original Intermediate, the intermediate-leaflet phenotype had a significantly higher rate than the normal. The photosynthetic rate in the narrow-leaflet type did not differ significantly from either the normal or the intermediate phenotypes. Therefore, the narrow-leaflet phenotype was neither higher nor lower than the normal-leaflet phenotype in its rate of photosynthesis, on a per-unit leaf area basis.

2. <u>Translocation</u>. Data on the translocation of pulse label are presented in Table 8 as the percentage of ^{14}C remaining in the source leaf at 30 minutes and 60 minutes after labeling. Nep-2 translocated significantly faster than 31908. In Nep-2, 41% of a pulse of photosynthetically-assimilated ^{14}C was translocated within 30 minutes after labeling. The rate of translocation was markedly slower after 30 minutes. This can be seen from the data which shows that only about 12% of the pulse label was translocated during the second half hour. Variety 31908 translocated photo-assimilated ^{14}C at the same rate at 30 minutes and 60 minutes after labeling.

In selfed progeny of the Original Intermediate, the normal-leaflet phenotype translocated significantly faster than the narrow-leaflet phenotype during the first 30 minutes. The intermediate phenotype translocated at a faster rate than the narrow-leaflet phenotype. However, the differences between them were not significant. At 60 minutes, the three leaflet phenotypes had almost the same amount of ^{14}C remaining in the leaf.

In the progeny of the cross between Nep-2 X Original Intermediate, the three phenotypes did not differ significantly in their rates of translocation. However, the narrow leaflet type translocated slightly faster than the normal and the intermediate phenotypes.

At 60 minutes, there were no significant differences among phenotypes. The highest rate of translocation was found in the narrow-leaflet phenotype of the Nep-2 X Original Intermediate cross. Variety 31908 had the lowest rate of translocation. All phenotypes resulting from the Nep-2 cross were more efficient in translocating label at 60 minutes than any of the progeny of the cross involving 31908, in keeping with the differences between Nep-2 and 31908. The averages of the cross population are very nearly what would be expected if translocation rate is heritable and the genes responsible are behaving additivity, since the mean of the population from Nep-2 X Original Intermediate is about 49% at 60 minutes which is nearly the same as the mid-parent ((Nep-2 + Original Intermediate) /2 = 48.50) and mean of the F_3 population from 31908 X Original Intermediate is about 58% at 60 minutes which is nearly the same as the mid-parent ((31908 + Original Intermediate) /2 = 58.7).

The percentages of ^{14}C remaining in the source leaf following pulse labeling are plotted semi-logarithmically against time (Figures 20 to 23). The patterns of translocation follow straight lines in almost all phenotypes. The slope for Nep-2 is steeper than for 31908, indicating that Nep-2 has a faster rate of translocation (Figure 20). In the Nep-2 X Original Intermediate cross, the three leaflet phenotypes have parallel slopes (Figure 22). The narrow-leaflet phenotype shows the fastest rate of translocation, then the normal, followed by the intermediate phenotypes. For the F_3 progenies of the cross 31908 X Original Intermediate, the intermediate had a faster rate than either the normal or the narrow-leaflet phenotypes (Figure 23). The data suggest that translocation probably was not disturbed by the change in leaf morphology.

3. Stomatal density. The number of stomates per unit leaf area was measured with the silicone impression method, similar to that described by Sampson (1961). The data on stomatal density for adaxial and abaxial surfaces are presented in Table 9.

Variations in stomatal densities among phenotypes were noted. However the F-test did not show significant differences. The adaxial (upper) surface has lower stomatal density than the abaxial (lower) suraface (Figures 24 and 25). The highest number of stomates per mm^2 on the adaxial surface was 65, and the lowest was 42. Nep-2 had the highest number of stomates (= 299) on the abaxial surface; the Original Intermediate was shown to have lowest number (= 221). The narrow-leaflet phenotypes from two crosses (Nep-2 X Original Intermediate, 31908 X Original Intermediate) had the lowest stomates per mm^2 as compared with the other two phenotypes. The differences were not significant by the F-test.

4. Stomatal resistance. The data for stomtal resistance are shown in Table 10. Stomatal resistances differed significantly between Nep-2 and 31908. Nep-2 had the lowest resistance whereas 31808 had the highest among genotypes examined. Within the Original Intermediate group, the stomatal resistances were 1.66, 2.00 and 2.21 for the normal, the intermediate and the narrow-leaflet phenotypes respectively. Three leaflet phenotypes of the Nep-2 X Original Intermediate cross had more or less the same level of resistance. The intermediate phenotype of the 31808 X Original Intermediate cross had the highest stomatal resistance. The normal and the narrow-leaflet phenotypes were more or less the same in the degree of resistance. Therefore, the three leaflet phenotypes do not appear to be associated with significant differences in stomatal resistance.

Anatomical studies:

Leaf thickness, width and length of upper epidermis, and width and length of lower epidermis were measured with the help of an occular micrometer. The percentages by volume of upper and lower epidermis, palisade and spongy mesophyll layers were determined by photographic methods. Photographic sections were cut and weighed, then converted to percentage by volume of each increment. The measurement made on the middle leaflet of the first trifoliolate leaf are shown in Table 11. The data on the middle leaflet of the third trifoliolate leaf are presented in Table 12.

At the first trifoliolate leaf, the leaf of the narrow-leaflet phenotype was thicker than the leaf of the normal phenotype. The normal-leaflet phenotype had longer epidermal cells than the narrow-leaflet phenotype. The length of lower epidermal cells was also greater in the normal than in the narrow-leaflet phenotype. Width of lower epidermal cells was greater in the narrow than in the normal phenotype, but not significantly. This also found when comparison were made by volume between increments. The normal and the narrow phenotypes consisted of various components with essentially the same percentage by volume (Figures 26-27).

The middle leaflet of the narrow phenotype was found to be thicker than that of the normal phenotype. The length of upper epidermal cells of the normal-leaflet phenotype again was greater than of the narrow phenotype. When the lower epidermal cells were compared, the normal was larger than the narrow-leaflet phenotype in this aspect. However, the F-test did not show any significant difference between these two phenotypes on the various components. Widths of upper and lower epidermal cells for both phenotypes were slightly different. When percentages by volume of various components were compared, there were no significant differences that could be detected by the F-test.

On the basis of anatomical studies, these two leaflet phenotype did not exhibit any significant differences in the various tissue studied.

Table 1. Phenotypic segregation observed in offsprings (1:2:1 ratio) of the Original Intermediate population resulting from the radiation induced mutation of Seafarer.

Date of planting	Observed Phenotypes Normal	Inter	Narrow	X^2	Probability
1. July 21, 1975	45	124	49	2.22	0.25 - 0.50
2. September 26, 1975	4	29	15	7.12	0.025- 0.05
3. October 3, 1975	15	23	16	1.03	0.50 - 0.75
4. October 11, 1975	9	31	7	4.16	0.10 - 0.25
5. October 21, 1975	44	89	49	0.07	0.975- 0.99
Pooled data	177	276	136	2.66	0.25 - 0.50

Table 2. Phenotypic segregation observed in F_1 progenies (1:1 ratio) resulting from the crosses between the Original Intermediate with some other varieties.

Crosses	Observed Phenotypes Normal		Observed Phenotypes Intermediate	X^2	Probability
1. Intermediate X Seafarer	6		4	0.41	0.50 - 0.75
2. Intermediate X Nep-2	4		7	0.81	0.25 - 0.50
3. Intermediate X Montcalm	10		2	5.34	0.025- 0.05
4. Intermediate X Jamapa	8		4	1.34	0.10 - 0.25
5. Intermediate X 31908	6		3	1.00	0.10 - 0.25
6. Intermediate X 2114-R	4		7	0.81	0.25 - 0.50
Pooled data	38	:	27	1.86	0.10 - 0.25

Table 3. Phenotypic segregations observed in F_2 offsprings (1:2:1 ratio) from F_1 intermediate plant types of crosses between Original Intermediates with some other varieties.

Crosses	Observed Phenotypes Normal	 Inter	 Narrow	X^2	Probability
1. Intermediate X Seafarer	11	17	10	0.47	0.95 - 0.975
2. Intermediate X Nep-2	9	24	12	0.64	0.75 - 0.90
3. Intermediate X Montcalm	6	12	6	0.00	1.00
4. Intermediate X Jamapa	14	21	12	0.69	0.50 - 0.75
5. Intermediate X 31908	15	17	11	2.63	0.25 - 0.50
6. Intermediate X 2114-R	14	28	11	0.51	0.75 - 0.90
Pooled data	69	119	63	0.956	0.51 - 0.75

Table 4. Phenotypic segregations observed in F_3 progenies (1:2:1 ratio) from F_2 intermediate plant types of the cross between the Original Intermediate and Nep-2.

Date of planting	Observed Phenotypes Normal	 Inter	 Narrow	X^2	Probability
1. January 3, 1977	9	13	7	0.57	0.75 - 0.90
2. February 17, 1977	12	22	10	0.18	0.90 - 0.95
Pooled data	21	35	17	0.55	0.75 - 0.90

Table 5. Leaf size, weight and specific leaf weight of three trifoliolate leaves among three segregating phenotypes of the Original Intermediate variety at 30 days after planting.

Genotype	Leaf size (cm^2)	leaf weight (mg)	specific leaf weight (mg/cm^2)
1. Normal	305.31	465	1.528
2. Intermediate	294.49	443	1.503
3. Narrow	244.58	395	1.614
Tukey's LSD	35.77	NS	NS

Table 6. Means of characters measured in the F_2 population and their parents on the first flowering day.

Population	Height (cm)	Node	Length petiole (cm)	Diameter petiole (cm)	Wt. of petiole (mg)	No. of branch	Wt. of leaflet (mg)	Lt. of leaflet (cm)	Wd. of leaflet (cm)	Area leaflet (cm^2)	Specific leaf weight (mg/cm^2)
Seafarer	72.40	8	7.20	.23	20.96	4	67.90	9.14	6.93	34.04	2.26
Original Intermediate	96.40	10	6.80	.22	19.34	5	69.81	10.21	5.42	32.70	2.14
Nep-2	31.17	10	16.90	.28	84.43	3	138.27	12.79	8.49	69.98	2.01
Jamapa	64.60	9	11.46	.28	43.82	1	121.19	12.22	9.56	61.72	1.99
Montcalm	49.00	7	14.46	.27	79.50	4	147.62	14.28	7.70	60.76	2.49
31908	57.00	6	14.18	.40	107.82	4	223.99	16.23	10.31	106.98	2.17
2114-R	129.80	14	14.32	.27	47.86	3	144.86	11.95	6.70	47.50	2.38
Seafarer X Intermediate											
Normal	118.40	9	6.00	.24	15.86	4	77.68	9.76	6.24	36.63	2.06
Intermediate	108.40	9	6.82	.21	17.90	5	70.60	10.38	5.84	35.55	1.96
Narrow	78.60	9	6.84	.21	17.44	9	58.41	12.04	4.20	25.98	2.22
Nep-2 X Intermediate											
Normal	127.00	12	10.14	.27	51.34	5	117.21	12.25	8.17	62.62	2.04
Intermediate	107.20	10	9.88	.26	37.50	4	97.22	14.08	7.07	57.59	1.68
Narrow	79.20	12	9.98	.24	38.62	8	84.74	16.43	5.23	49.66	1.73
Jamapa X Intermediate											
Normal	106.20	12	8.98	.28	45.04	5	104.09	10.74	6.79	44.88	2.32
Intermediate	75.20	10	9.04	.26	37.08	5	94.74	11.00	6.13	40.61	2.39
Narrow	37.80	10	10.02	.25	43.94	8	92.45	14.69	4.67	37.60	2.51
Montcalm X Intermediate											
Normal	82.00	8	9.92	.28	58.22	6	156.18	13.65	7.64	63.32	2.54
Intermediate	72.80	7	8.90	.28	41.10	5	101.50	12.85	6.50	48.58	2.11
Narrow	67.60	8	9.14	.20	34.90	8	90.90	13.30	5.06	47.48	2.17
31908 X Intermediate											
Normal	96.04	7	12.05	.34	77.87	3	191.78	14.28	9.45	83.26	2.56
Intermediate	97.00	8	14.88	.31	67.20	4	171.30	14.17	9.44	83.65	2.10
Narrow	80.33	7	11.10	.28	76.20	5	178.32	16.79	6.67	69.15	3.01
2114-R X Intermediate											
Normal	128.39	10	10.15	.26	42.21	5	112.29	11.47	7.27	50.96	2.27
Intermediate	130.72	11	10.48	.26	42.74	5	120.23	13.03	7.14	54.73	2.28
Narrow	97.00	9	9.70	.25	40.60	9	98.88	15.08	4.84	35.68	2.62
Tukey's LSD	72.61	1.98	4.11	.05	38.30	5	95.76	5.15	4.11	43.70	NS

Table 7. Rate of photosynthesis (in mg CO_2 $dm^{-2}hr^{-1}$) of parents and their F_3 progenies with contrasting leaf types.

Population	Photosynthesis
Nep-2	6.67
31908	6.95
Original Intermediate	
Normal	8.23
Intermediate	10.07
Narrow	10.48
Nep-2 X Original Intermediate	
Normal	11.39
Intermediate	8.21
Narrow	6.77
31908 X Original Intermediate	
Normal	5.23
Intermediate	8.80
Narrow	6.87
Tukey's LSD	3.09

Table 8. Percentage of translocation at 30 minutes and 60 minutes of parents and their F_3 progenies with contrasting leaf types.

Type of plant treated	Translocation (Percentages of ^{14}C left in leaf)	
	30 min	60 min
Nep-2 (normal)	58.64	46.85
31908 (normal)	82.70	66.03
Original Intermediate		
Normal	66.90	49.73
Intermediate	71.52	49.23
Narrow	81.86	51.53
Nep-2 X Original Intermediate		
Normal	69.63	49.32
Intermediate	71.29	51.62
Narrow	61.83	46.06
31908 X Original Intermediate		
Normal	81.96	64.69
Intermediate	64.85	54.90
Narrow	77.34	58.67
Tukey's LSD	20.10	N.S.

Table 9. Stomatal density (stomates/mm^2) of bean classes with contrasting leaf types.

Population	Stomatal density	
	Adaxial	Abaxial
Nep-2	48	299
31908	42	249
Original Intermediate		
Normal	42	246
Intermediate	45	221
Narrow	46	230
Nep-2 X Original Intermediate		
Normal	51	264
Intermediate	53	289
Narrow	47	242
31908 X Original Intermediate		
Normal	65	293
Intermediate	59	278
Narrow	58	260

Table 10. Stomatal resistance (sec/cm^{-1}) of bean classes with contrasting leaf types.

Population	Stomatal resistance
Nep-2	1.14
31908	2.85
Original Intermediate	
Normal	1.66
Intermediate	2.00
Narrow	2.21
Nep-2 X Original Intermediate	
Normal	1.66
Intermediate	1.27
Narrow	1.65
31908 X Original Intermediate	
Normal	1.64
Intermediate	2.57
Narrow	1.80
Tukey's LSD	1.36

Table 11. Leaf anatomy measurements on the middle leaflet of the first trifoliolate of the normal and the narrow-leaflet phenotypes in the population resulting from selfing intermediate plants.

Varables	Phenotypes	
	Normal	Narrow
1. Thickness of leaf (Mm)	178.83	197.29
2. Width of upper epidermis (Mm)	19.65	19.91
3. Length of upper epidermis (Mm)/cell	29.92	25.63
4. Width of lower epidermis (Mm)	14.19	17.76
5. Length of lower epidermis (Mm)/cell	18.76	16.05
6. Upper epidermis by volume (%)	10.07	10.02
7. Lower epidermis by volume (%)	8.88	8.88
8. Palisade layer by volume (%)	36.55	36.26
9. Spongy layer by volume (%)	43.50	44.73

Table 12. Leaf anatomy measurements on the middle leaflet of the third trifoliolate leaf of the normal and the narrow-leaflet phenotypes in the population resulting from selfing intermediate plants.

Variables	Phenotypes	
	Normal	Narrow
1. Thickness of leaf (µm)	143.30	155.80
2. Width of upper epidermis (µm)	17.20	17.84
3. Length of upper epidermis (µm)/cell	23.94	19.09
4. Width of lower epidermis (µm)	13.07	13.99
5. Length of lower epidermis (µm)/cell	17.78	14.11
6. Upper epidermis by volume (%)	11.12	11.02
7. Lower epidermis by volume (%)	11.02	10.03
8. Palisade layer by volume (%)	39.08	39.33
9. Spongy layer by volume (%)	38.75	39.60

Figure 1. Normal leaflet type from ozalid paper drawing.

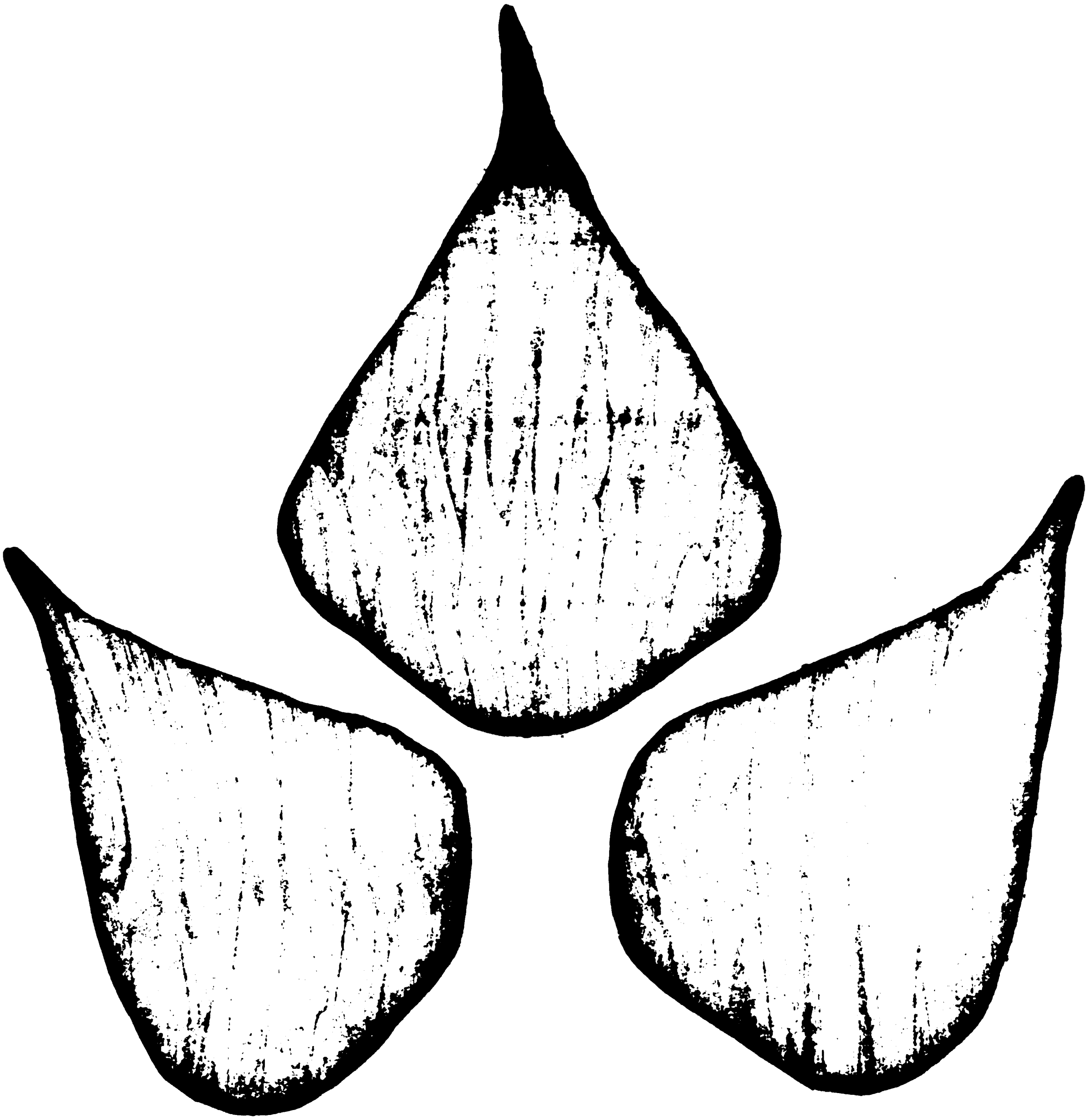


Figure 1.

Figure 2. Intermediate leaflet type with semi-narrow leaflets from ozalid paper drawing.

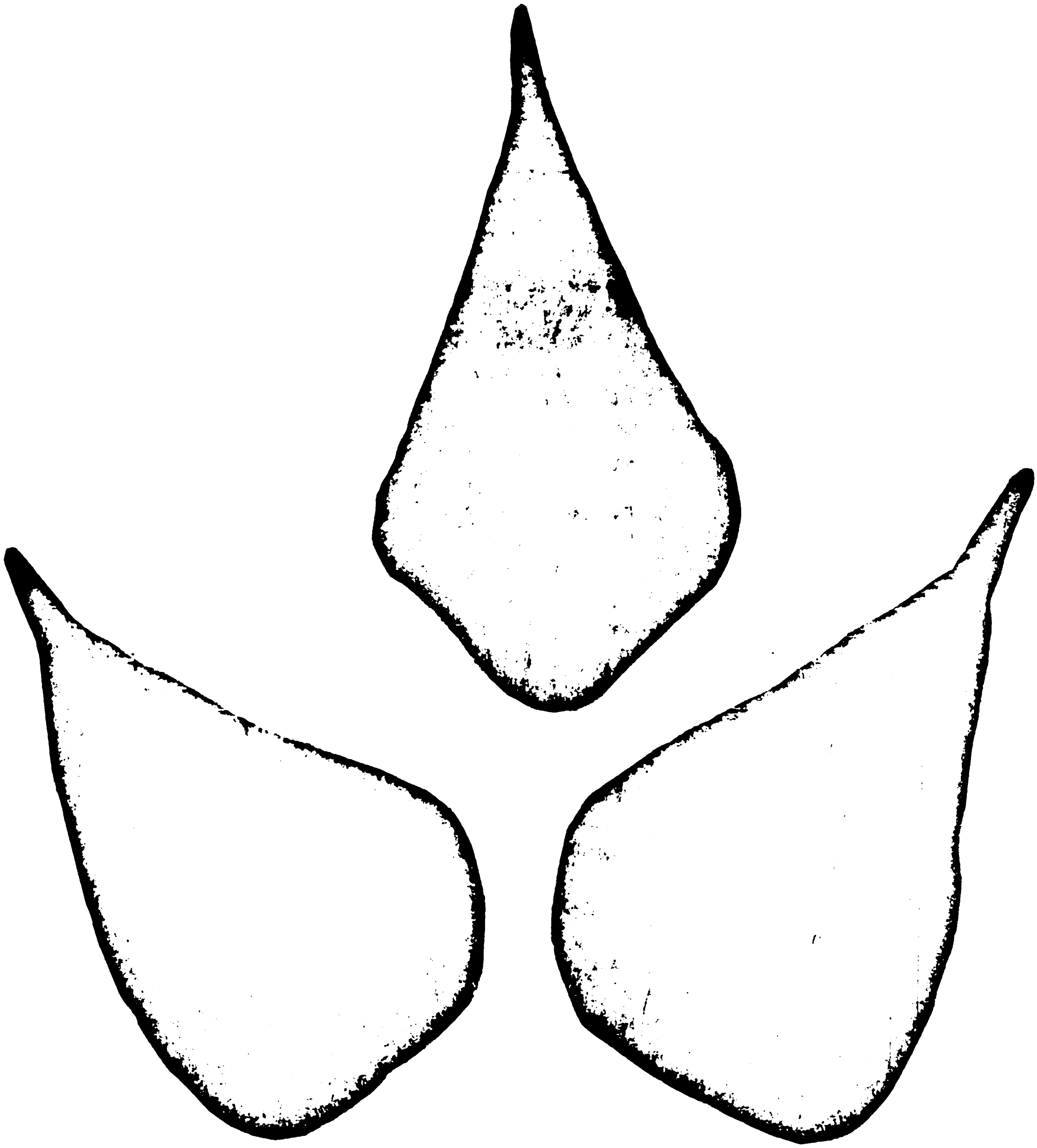


Figure 2.

Figure 3. Narrow-leaflet type from ozalid paper drawing.

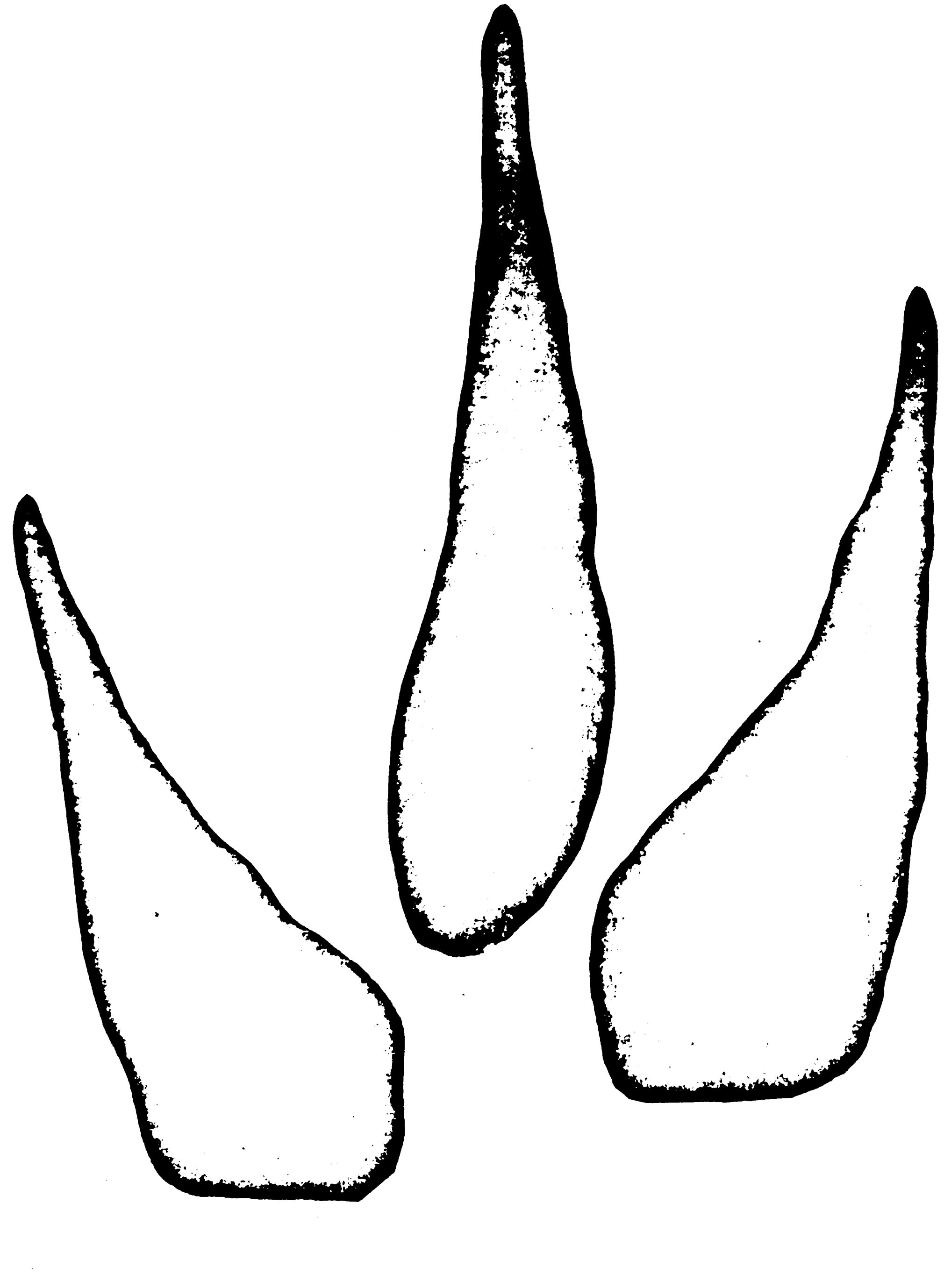


Figure 3.

Figure 4. The Original Intermediate plant with semi-narrow leaflets and fertility.

Figure 5. The narrow-leaflet plant with narrow leaflets, short stature and sterility.

Figure 4.

Figure 5.

Figure 6. Sterile flower bud of narrow leaflet mutant.

Figure 6.

Figure 7. Normal leaflet phenotype of the F_2 generation of the Nep-2 X Original Intermediate cross at early stage of growth.

Figure 8. Narrow leaflet type of the F_2 plant resulting from the cross Nep-2 X Original Intermediate at early stage of development.

Figure 7.

Figure 8.

Figure 9. The F_2 plant of narrow-leaflet phenotype resulting from the cross Montcalm X Original Intermediate at early stage of growth.

Figure 10. The narrow-leaflet plant of the F_2 generation of the cross Jamapa X Original Intermediate at early stage of growth.

Figure 9.

Figure 10.

Figure 11. Narrow-leaflet plant with flowers and pod of the F_3 generation of the cross Montcalm X Original Intermediate.

Figure 12. Narrow-leaflet plant with pod of the F_3 generation of the cross Montcalm X Original Intermediate.

Figure 11.

Figure 12.

Figure 13. The narrow-leaflet plant of the cross Montcalm X Original Intermediate at early pod set.

Figure 28. The narrow-leaflet plant of the F_3 generation of the cross 21908 X Original Intermediate at flowering stage.

Figure 28.

Figure 13.

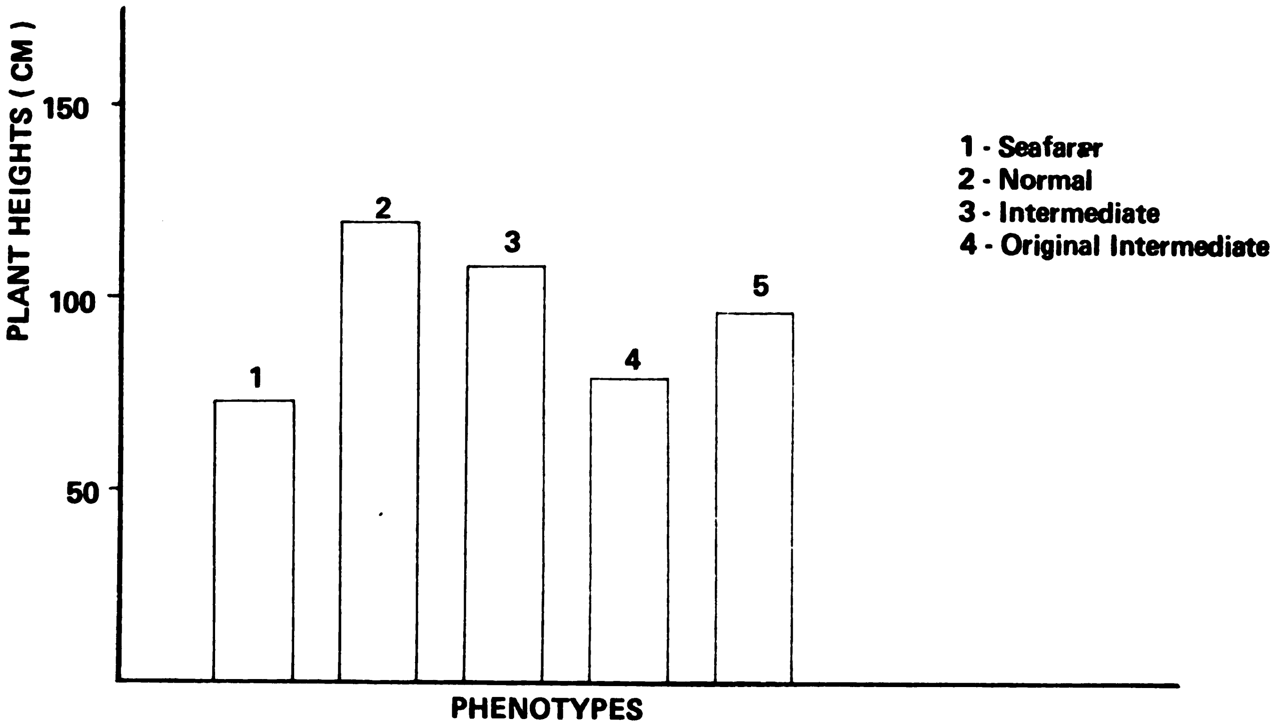


Fig. 14 Heights of Seafarer, Original Intermediate and their F_2 progenies with contrasting phenotypes.

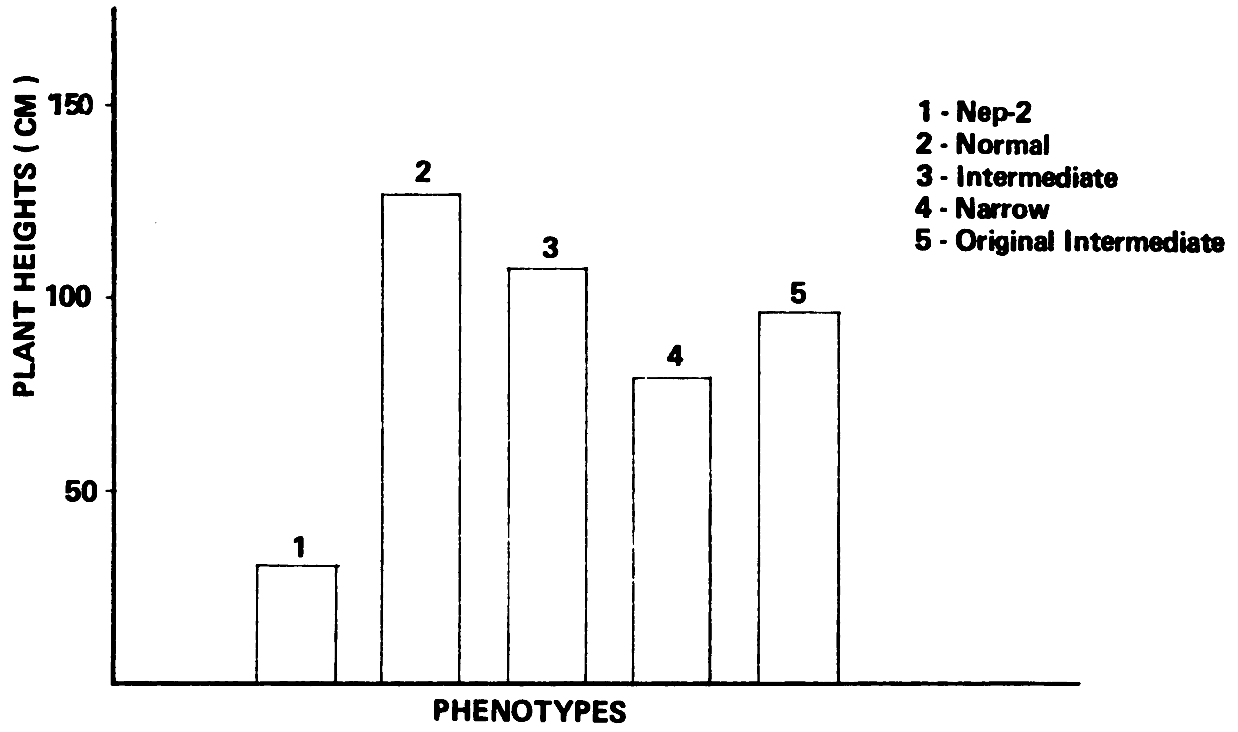


Fig. 15 Heights of Nep-2, Original Intermediate and their F_2 Progenies with three contrasting phenotypes.

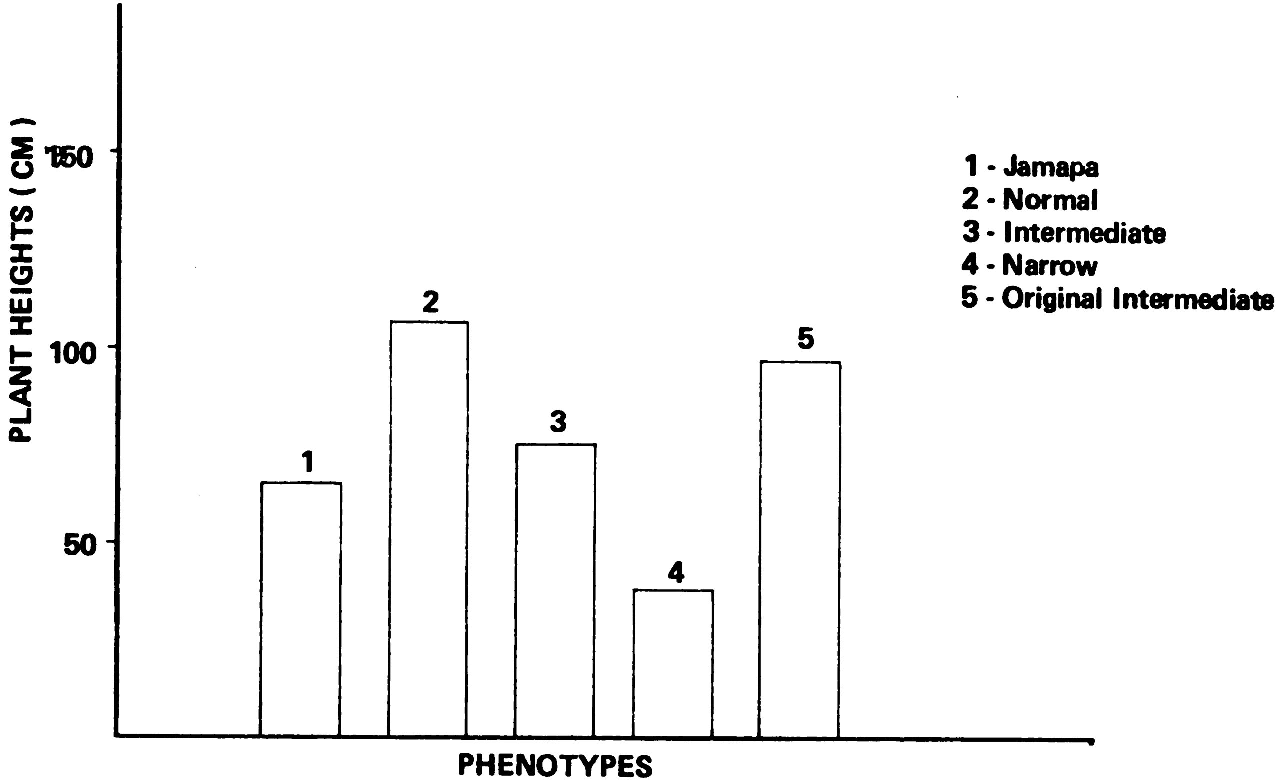


Fig. 16 Heights of Jamapa, Original Intermediate and their F_2 progenies with three contrasting phenotypes.

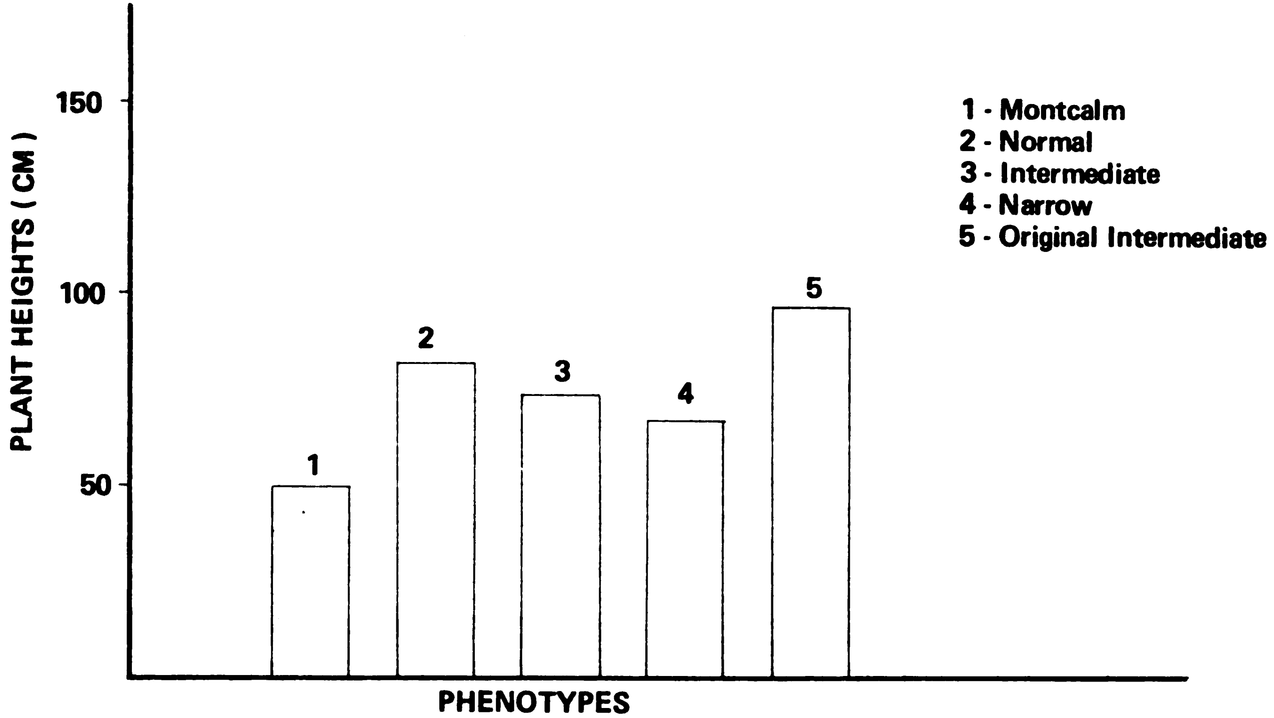


Fig. 17 Heights of Montcalm, Original Intermediate and their F_2 progenies with three contrasting phenotypes.

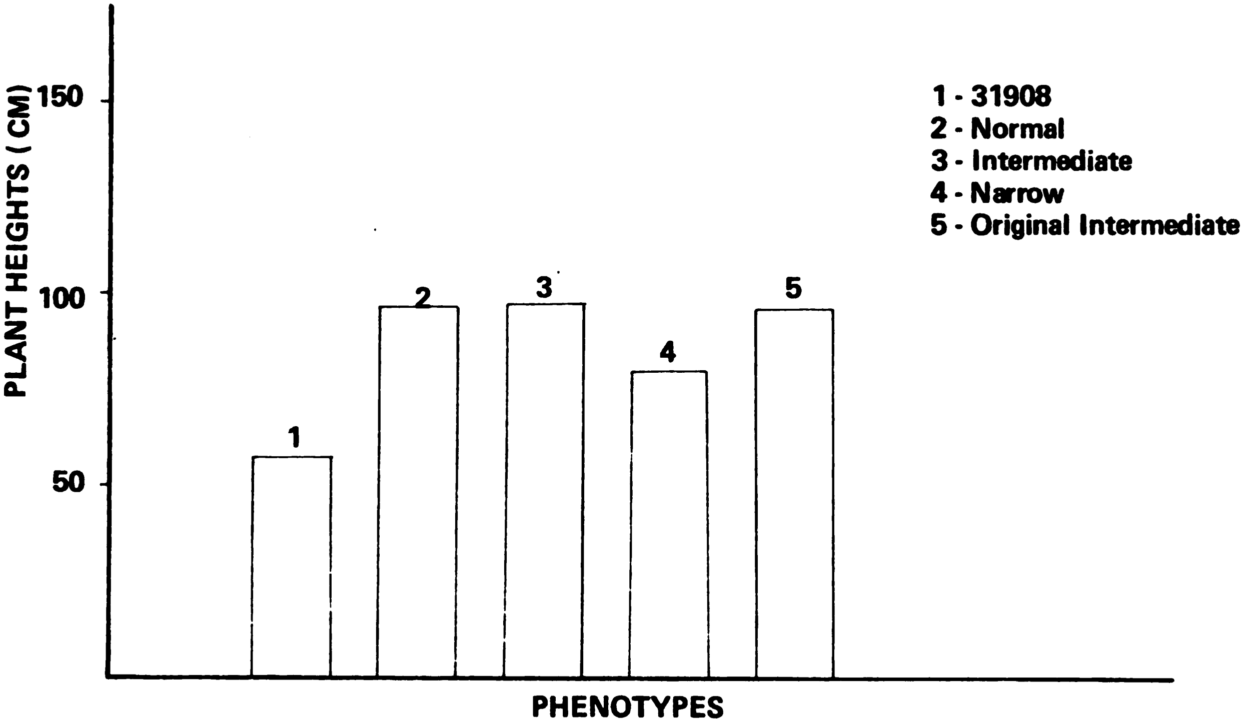


Fig. 18 Heights of 31908 , Original Intermediate and their F_2 progenies with three contrasting phenotypes.

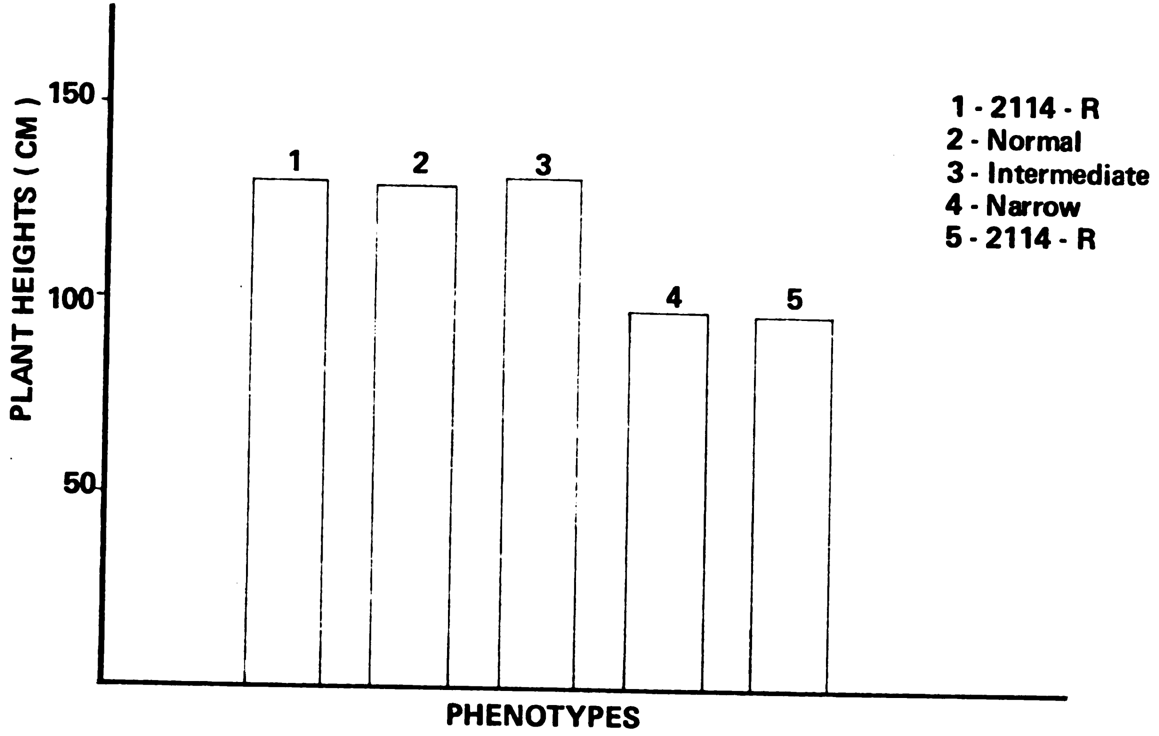


Fig. 19 Heights of 2114 - R , Original Intermediate and their F_2 progenies with three contrasting phenotypes.

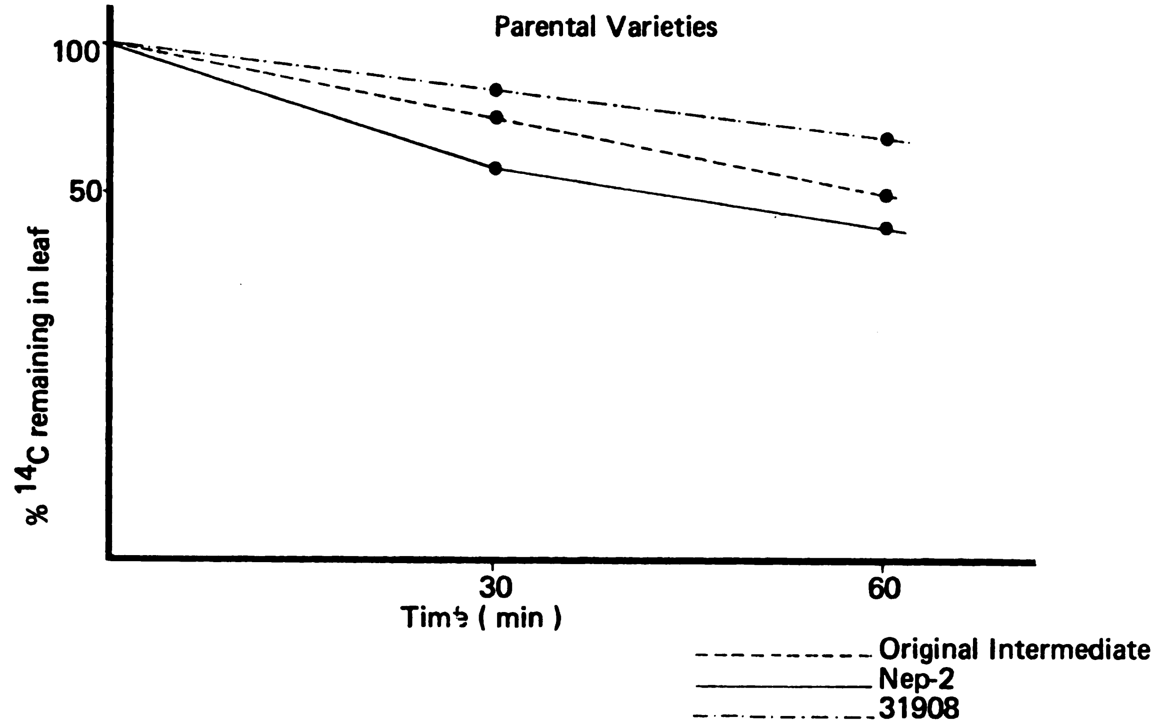


Fig. 20 Translocation of ^{14}C-pulse out of the source leaves of Nep-2, 31908 and Original Intermediate derived from the desrease in ^{14}C-remaining in the leaf after 30 min and 60 min, after labelling with $^{14}CO_2$.

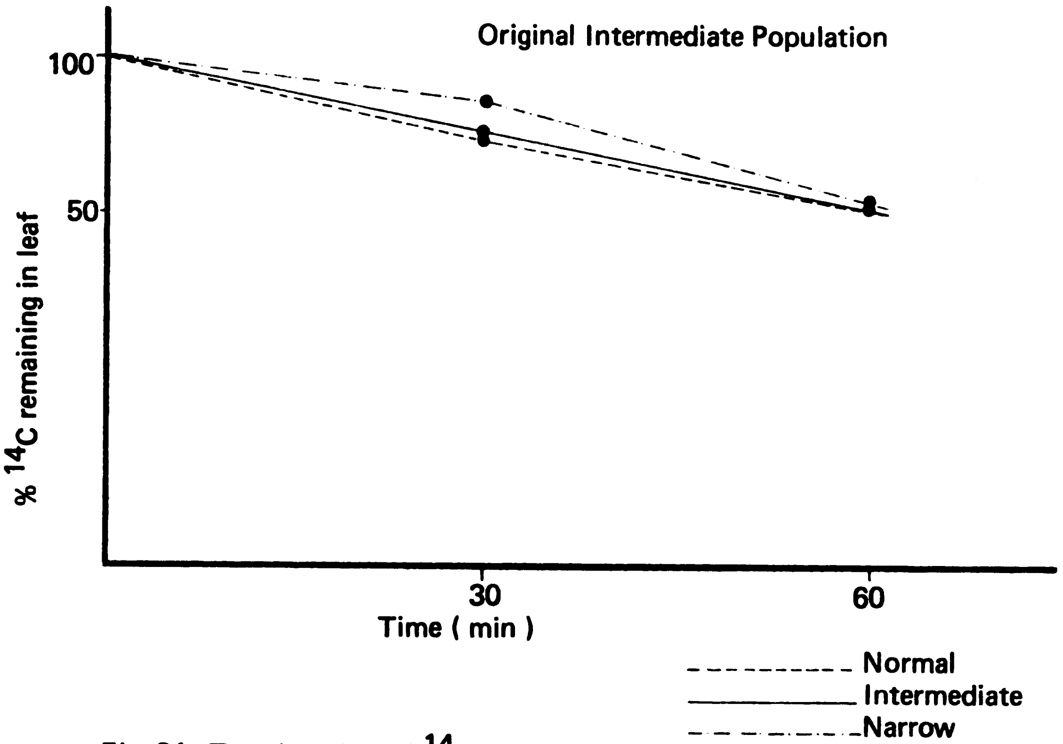


Fig. 21 Translocation of ^{14}C-pulse out of source leaves of the normal, intermediate, and narrow leaflet phenotypes in the Original Intermediate group.

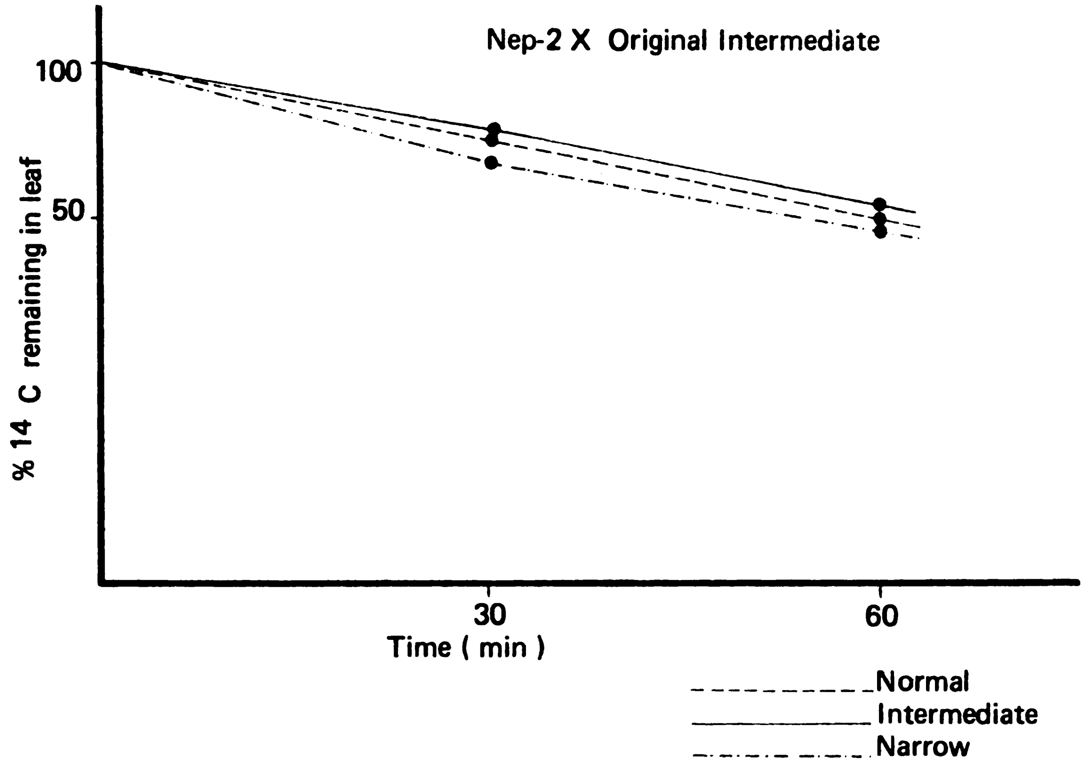


Fig. 22 Translocation of ^{14}C-pulse out of source leaves of the normal, intermediate, and narrow leaflet phenotypes of the F_3 progenies of the Nep- 2 X Original Intermediate cross.

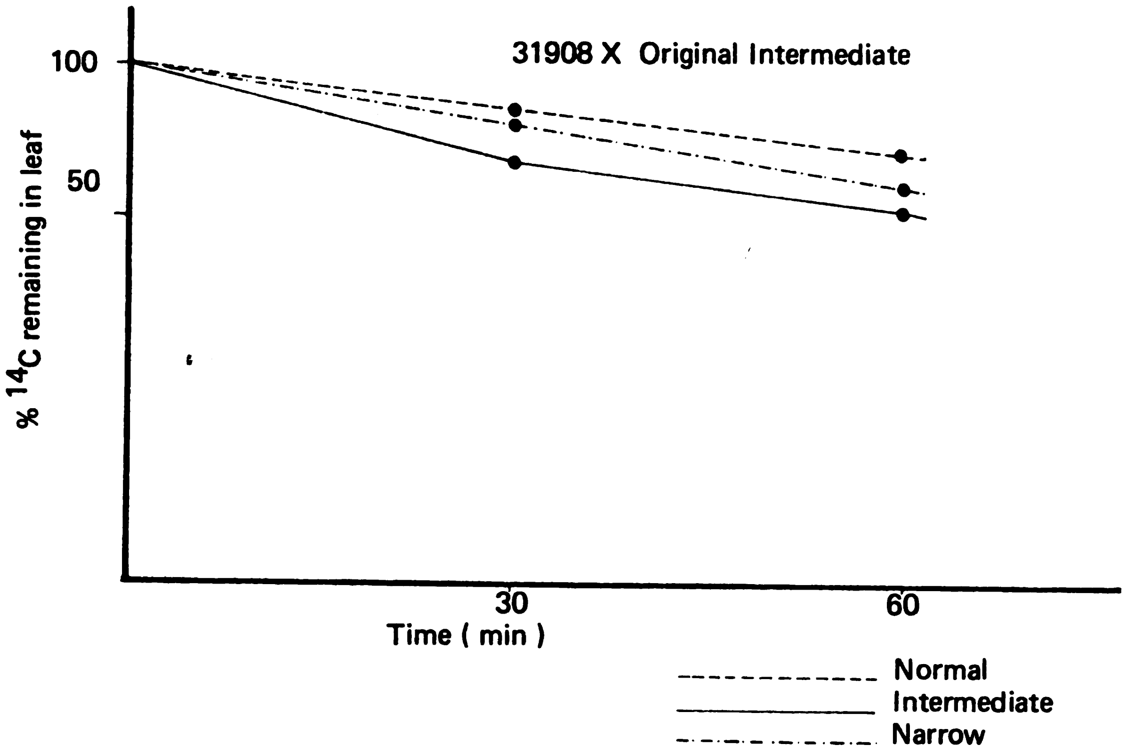


Fig. 23 Translocation of ^{14}C-pulse out of source leaves of the normal, intermediate and narrow leaflet phenotypes of the F_3 progenies of the 31908 X Original Intermediate cross.

Figure 24. An abaxial (upper) surface of bean leaf.

Figure 25. An adaxial (lower) surface of bean leaf.

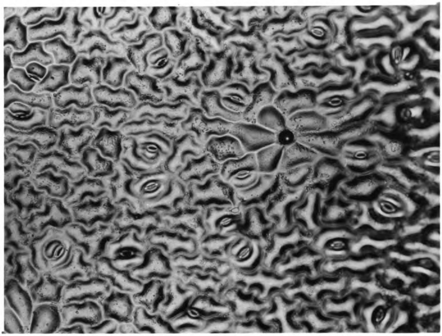


Figure 24.

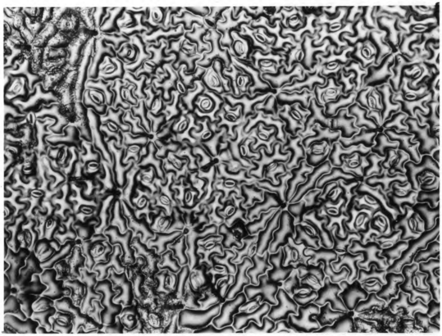


Figure 25.

Figure 26. Leaf cross section of the normal-leaflet phenotype.

Figure 27. Leaf cross section of the narrow-leaflet phenotype.

Figure 26.

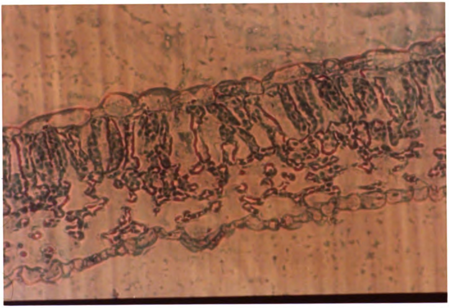


Figure 27.

DISCUSSION

Pleiotropy is commonly regarded as one of the fundamental properties which a gene may possess. Numerous examples described in the literature show that a single gene may have pleiotropic effects.

There are four possible interpretations that a mutant organism regularly shows a specific group of distinct anomalies and transmits this complex from one generation to the next as a whole:

(1) A single mutant gene is responsible for the whole complex: (classical pleiotropy);

(2) A small portion of a chromosome containing several genes has been lost;

(3) Several closely linked or neighboring genes have mutated;

(4) The regulator gene regulating a series of other functional genes has mutated.

Monogenic segregation can be expected in all these cases but only the first one can be interpretated as being a true example of pleiotropic gene action. The other three events simulate a pleiotropic effect of one gene although several genes are lost, changed, or de-regulated.

The Original Intermediate population, upon selfing, segregated into three distinct classes: normal, intermediate and narrow-leaflet types with a 1:2:1 ratio. The narrow-leaflet mutant possessed the whole complex of deviating characters: small and narrow leaflets, sterility, dwarf growth habit, many weak thin branches, prolonged vegetative growth. From the segregating ratios, it was believed in the beginning that this complex was controlled by a single pleiotropic gene. It was assumed that the gene for narrow-leaflet was incompletely dominant over the gene for normal leaflet. Therefore, in heterozygous condition, it gave rise to the intermediate leaf type. If we designate the normal-leaflet type as AA and the narrow-leaflet type as aa, therefore, Aa is the intermediate type. Selfing the intermediate, results in the following ratio:

Aa X Aa
↓
1 AA : 2 Aa : 1 aa
Normal: Intermediate: Narrow

Upon crossing the intermediate-leaflet type with six other unrelated varieties, the F_1 progeny was classified into two groups: normal and intermediate, with a 1:1 ratio. This is what is expected in crossing the normal-leaflet type with the intermediate-leaflet type if a single locus is responsible.

However, the postulation that the whole complex is due to the action of one single pleiotropic gene could not be upheld in the F_2 generation. In the F_2 generation, some plants of two crosses (Montcalm X Original Intermediate, and 31908 X Original Intermediate) had narrow leaflets but were also fertile. The narrow-leaflet plants still had the other components of the complex: bushy appearance, many branches and short in stature.

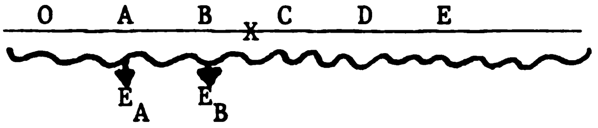
From this finding, it would appear that there may be two possibilities to explain the genetic regulation of this complex. One is that at least two genes are involved, one gene responsible for narrow leaflet and the other for sterility. These two closely linked genes mutated simultaneously during the fast neutron treatment. These two closely linked genes would be transmitted as a single unit of heredity in the Original Intermediate genetic background. This would continue generation after generation unless or until crossing over shifted them from one homologue to the other. Unfortunately, it would appear that no crossing over has ever taken place between these two loci in the Original Intermediate genetic background. We have never found narrow-leaflet plants with fertility in this population. Upon crossing the intermediate-leaflet plants with normal-leaflet varieties, these two linked genes were brought into new genetic backgrounds. In the F_1 generation, segregating 1 normal: 1 intermediate, the intermediate plants would have one set of genes from the Original Intermediate-leaflet parent and the other from one of the six normal parents. For example, in the F_1 of the cross Montcalm X Original Intermediate, the intermediate plant would have one set of chromosomes from the Original Intermediate and one set from Montcalm. When sporogenous cells of the hybrid intermediate plant underwent meiosis, crossing over might have taken place and separated the gene for narrow leaflet from the gene for sterility. At the same time, the gene for fertility was inserted adjacent to the narrow-leaflet gene. This small percentage of crossing over gave rise to a new gene recombination. This is why one narrow-leaflet plant with fertility could occur in the cross, Montcalm X Original Intermediate. The same mechanism can be used to explain the occurrence of the narrow-leaflet plant with fertility in the cross 31908 X Original Intermediate. If narrow-leaflet plant with sterility could occur as the product of crossing over, we also would expect to have the normal-leaflet plant with sterility. In population studied, we did not see any normal-leaflet plant associated with sterility. However, the small number of F_2 plants (about 50 plants) in each cross is probably insufficient to allow the recovering of the normal-leaflet plant with sterility. That may be why we did not obtain any normal-leaflet plant with sterility as would be expected if crossing over had occurred between genes for the narrow-leaflet and for fertility.

The second possibility is that this complex is controlled by a single pleiotropic gene. This gene controls leaf shape as well as fertility. The occurrence of narrow-leaflet plants with fertility in F_2 generation of two crosses (Montcalm X Original Intermediate and 31908 X Original Intermediate) probably was under the influences of modifier genes contributed by Montcalm and 31908. In the F_3 generation, narrow-leaflet plants bred true and gave rise to narrow-leaflet progenies with varying degree of fertility. This suggests that a number of modifier genes were involved and in this F_3 generation there were segregations of these genes which resulted in plants with different degree of fertility.

I rule out the possibility that the whole complex of characters (at least narrow leaflets and sterility) was the result of deletion of a small portion of chromosome containing several genes (or at least two genes). If this were the true situation, there would be no crossing over taking place at deleted loci. So we would never have had the narrow-leaflet plant with fertility. If we do accept that a regulatory gene controlling several functional genes (or at least two genes) has mutated, it must be assumed that regulatory gene controls the functioning of a gene for leaf shape, and a gene for fertility as well as genes for other complex complex traits. When the regulatory gene mutated, it de-regulated all functional genes under its control resulting in pleiotropy or a complex of traits. However, if this were the true situation, a plant with narrow-leaflets as well as fertility would never have occurred. This is because if these two traits are regulated by the same regulatory gene, the narrow-leaflet trait will always be associated with sterility. As far as leaf shape and sterility are concerned, it appears that this assumption (mutation of a regulatory gene) does not hold true.

At present, it is not possible to state that which assumption (two closely linked genes or a single pleiotropic gene) is the best to explain the genetic regulation of this complex. To prove that two closely linked genes (leaf shape and fertility) are involved. I should have a large number of F_2 plants in order to recover the normal-leaflet plant with sterility as another crossover product. If I could obtain a normal-leaflet plant with sterility, I would be very confident in particulating that at least two closely linked genes are involved in this complex trait. If in back crossing the fertile narrow-leaflet plant to one of its parent (Montcalm or 31908), it should happen that I obtain the narrow-leaflet plant with high fertility. I would probably assume that modifier genes are involved in giving rise to narrow-leaflet plant with fertility.

The means of the plant height in F_2 progenies resulting from crossing intermediate leaflet types to six other varieties, suggest that the narrow-leaflet plants were shorter than the normal and the intermediate-leaflet plants. This suggests, as one possibility, that a gene(s) controlling height of plant is (are) closely linked with the narrow-leaflet gene. They are transferred together as a single unit to the offspring. The other possibility is that the gene controlling narrow leaflets has pleiotropic effects. Auerbach (1976) stated that the majority of mutations have pleiotropic effects. This is the expected consequence of the fact that the biochemical pathways starting from different genes intersect in many places, reinforcing, inhibiting, deflecting and variously modifying each other. Haslot (1969) gave an explanation for the existence of pleiotropy in higher plants. He pointed out that if we assume that transcription units (operons) do exist, composed of several genes involved in different biosynthetic sequences, they will be transcribed and give rise to a single m-RNA molecule. If transcribed unit consists of genes OABCDE, when the ribosome attach to this m-RNA molecule and move away in the same direction, each enzyme being synthesized in the order A, B, C... Then a mutagenic alteration in gene C, say, could result in an increase probability for the ribosome to detach from m-RNA at the point of mutation. In this case, genes located further on the right would not give rise to the corresponding enzymes E_C and E_D and E_E. Pleiotropy would then result for effects controlling by these genes.



In the present case, it is not possible to determine which possibility is likely to be true, Back crossing to the parents or out crossing to the other variety many times and observing the phenotypes of the offspring should be helpful in final judgment of this complex.

The same data show that plants with narrow-leaflets also had the highest number of branches recorded on the first flowering day. Number of branches lay mostly between 8-9 in the narrow-leaflet plants of the F_2 generations. The normal phenotypes possessed 3-6 branches on the first flowering day. According to Tonguthaisri (1976), genetic interaction played a great role in controlling this trait. In the present study, it is not determined whether the number of branches is controlled by the same gene governing narrow leaflet or by the other gene(s) which has (have) mutated at the time of mutagenic treatments.

Length of petiole varied among the three leaflet phenotypes. No definite pattern was associated with any leaflet phenotypes. This is not the case in the diameter of the petiole. The narrow-leaflet types had the smallest diameter among other two phenotypes of the same cross. It is not known at present that whether this trait is governed by other gene(s) closely linked with gene governing leaf shape or by gene governing leaf shape itself.

The study on the photosynthetic rates among three leaflet phenotypes reveals that the narrow-leaflet is not superior to the normal or the intermediate types in photosynthetic efficiency. Similar findings have been reported in soybean (Heibsch et al., 1976) and cotton (Pegelow et al., 1977), where the narrow leaflets did not prove to be more efficient than the normal leaflets in relation to photosynthesis. The data also show inconsistency in measured photosynthetic rates as far as leaflet phenotypes are concerned. For example, the narrow-leaflet plants of the Original Intermediate population had the highest photosynthetic rate within that group, whereas the narrow-leaflet phenotypes of Nep-2 X Original Intermediate cross had the lowest photosynthetic rate among other phenotypes of the same cross. A similar situation was also found for the normal-leaflet phenotype. This suggests that the gene(s) governing photosynthesis very probably differ from those governing leaf shape. This study was carried out on plants of the F_3 generation of two crosses. In the F_3 generation, there might be segregation of genes for phtosynthesis independently of genes for leaf shape. Number of plants used in this study may not be sufficient to provide randomness for genes affecting photosynthesis in plants of the selected population. Moreover, techniques of photosynthesis measurement might render rate measurements inconsistent due to uncontrollable variations in light, temperature, handling and machine efficiency. Lastly, it is noted that genetic control photosynthesis can be exerted on both the CO_2-fixing system and the CO_2-transport system. Biochemical capacity to fix CO_2 is a function of the enzyme complement of the chloroplast, which in turn is under the control of this organelle's own genes. Chloroplasts can transmit their own genetic information independently from the nuclear genes. Leaflet shapes are controlled by nuclear genes. This evidence was supported by the phenotypes of F_1 plants, in which reciprocal crosses yielded similar results. The genes governing shape of leaflet act independently from genes governing photosynthesis. Therefore, leaflet morphology can be changed without any change in photosynthetic rates.

The yield of crop plants is determined not only by efficiency of light utilization, but also by ability to translocate assimilates to growing or storage tissues. In this study, narrow-leaflet plants were compared with normal and intermediate plants in translocation efficiency. No significant differences were found among the three leaflet phenotypes. The factors responsible for translocation of photosynthates are probably not related to factors governing leaf shapes. This finding agrees well with the recent report on translocation efficiency of leaf mutants by Harvey (1974). He stated that in respect of translocation the leaf and pod had well defined source and sink relationship that was independent of leaf morphology.

Plants had about five times as many stomates on the abaxial surface of the leaf as on the adaxial surface. Although a statistically significant difference was not obtained in terms of stomatal density, Nep-2 had a greater number of stomates than 31908 on either side of the leaf. When stomatal resistance was measured, Nep-2 had significantly lower stomatal resistance than 31908. This is what was expected since it is logical to believe that the increase in stomatal resistance is associated with a decrease in stomatal density. In two crosses having the Original Intermediate as a common parent, the three leaflet phenotypes did not show any significant differences either in stomatal density or stomatal resistance. I, therefore, believe that factors governing these two traits are independent of factors governing leaf shapes.

From the anatomical study, it was found that plants that differed in leaf shape did not show any significant difference in leaf anatomy. Leaf thickness was found to vary with leaf position. The middle leaflet of the first trifoliolate leaf was thicker than the third trifoliolate leaf.

The narrow-leaflet gene caused plants to have significantly smaller leaf area than the normals and have similar SLW (specific leaf weight = the ratio of the leaf weight to leaf area) to the normals (Table 5). It is logical to think that the narrow-leaflet gene causes a change in cell number rather than cell size. If the narrow-leaflet phenotype is associated with the increase or decrease in cell size, it would have caused the plant to change in SLW. This is not the case because both normal and narrow-leaflet phenotypes have similar SLW's. Therefore, the number of cells, is probably responsible for the differences in leaf shapes.

At outset of this study, it had not been determined whether the narrow-leaflet mutant has any valuable agronomic characteristic. In terms of physiological characteristics such as photosynthetic and translocation efficiencies, the narrow-leaflet mutant did not show that it was superior or inferior to the normal-leaflet plant. This outcome suggests the desirability of further study in a field experiment to measure the amount of light penetrating into the plant canopy as well as canopy photosynthesis in comparison between the normal and the narrow-leaflet types. A subsequent study would probably yield the final judgment to this problem.

SUMMARY AND CONCLUSIONS

A series of experiments was conducted in the greenhouse at Michigan State University from Summer 1975 to Winter 1977 to study the mode of inheritance as well as to evaluate physiological characteristics attributable to the narrow-leaflet mutant of dry bean variety 'Seafarer'. Narrow-leaflet mutants (homozygotes) in our collection are sterile; the intermediate-leaflet types (heterozygotes) are fertile with normal growth and seed production. Therefore, the intermediate-leaflet types were used to maintain the mutant allele. The intermediate-leaflet types were crossed to six other unrelated varieties. Plants of the F_1 to F_3 generations were used for genetic study. Physiological study was made on plants of the F_3 generation. Anatomical study was performed on plants resulting from selfed seeds of the Original Intermediate. The results are summarized as follows:

1. The Original Intermediate plant, upon selfing, segregated into three distinct classes: normal, intermediate and narrow-leaflet phenotypes with a 1:2:1 ratio. This was interpreted as monogenic segregation with incomplete dominance.

2. Upon crossing the Original Intermediate-leaflet type with six other unrelated varieties, the F_1 progency could be classified according to leaf shape into two classes, normal and intermediate with a 1:1 ratio.

3. Upon selfing the F_1 intermediate-leaflet types of every cross, I obtained F_2 progenies with three classes: normal, intermediate and narrow-leaflet types. The segregation ratio was 1:2:1.

4. The narrow-leaflet mutant possessed the whole complex of characters: small and narrow leaflets, sterility, dwarf growth habit, many weak thin branches, prolonged vegetative growth. The data suggest two possible mechanisms regulate this complex. One assumption is that this complex is controlled by at least two closely linked genes which mutated during fast neutron treatments. One gene is believed to govern the leaflet shape and the other the fertility. The evidence came from the occurrence of narrow-leaflet plants with fertility in the F_2 generation of two crosses (Montcalm X Original Intermediate and 31908 X Original Intermediate). It is assumed that a small percentage of crossing over must have taken place at meiosis in the F_1 generation. This crossing over is responsible for breaking tight linkage between the gene for narrow leaflet and the gene for fertility. The other possibility is that this complex is controlled by a single gene with pleiotropic effects which mutated during fast neutron irradiation. The occurrence of narrow-leaflet mutants with fertility would probably be due to the effect of modifier genes which contributed to the offsprings by variety 31908 or Montcalm.

5. Narrow-leaflet plants of these two crosses bred true and gave rise to narrow-leaflet progenies in the F_3 generation. Narrow-leaflet progenies were fertile and produced seeds.

6. From the data on means of characters measured in the F_2 population, the narrow-leaflet plants were always shorter than either the normal or the intermediate-leaflet plants. This suggests two possibilities of genetic regulation of this character. One is that gene(s) controlling height is (are) closely linked with narrow-leaflet gene. The other possibility is that the gene governing leaflet shape has pleiotropic effects; it also controls the height of plant.

7. In the F_2 generation, three leaflet phenotypes of each cross followed the same pattern as far as length and width of leaf were concerned. For length of leaf, the trend is narrow : intermediate : normal and for the width of leaf, the trend is normal: intermediate : narrow leaflet.

8. Number of nodes of three leaflet phenotypes were more or less the same in the F_2 generation of every cross. This is probably due to gene(s) controlling node number being independent from gene governing leaf shape.

9. Number of branches varied among three leaflet phenotypes. The narrow-leaflet phenotype had the highest number of branches among all three phenotypes. At present, it is not clear whether the number of branches is controlled by a gene for narrow-leaflet or by a gene for fertility or whether by one or two neighboring genes that have also mutated.

10. The three leaflet phenotypes had similar specific leaf weights.

11. When the intermediate plants of the F_2 generation of the Nep-2 X Original Intermediate were selfed, they produced the progenies of three classes: normal, intermediate and narrow-leaflet types in a ratio 1:2:1. This confirms the hypothesis of monogenic segregation.

12. Rate of leaf photosynthesis was measured in plants of the F_3 generation of two crosses as well as in the parental varieties. The photosynthetic rate in the narrow-leaflet type did not differ from either the normal or the intermediate phenotypes. Therefore, the narrow-leaflet phenotype did not prove to be better than the normal phenotype in the rate of photosynthesis.

13. In respect to photo-assimilate translocation, these three-leaflet phenotypes of the F_3 generation did not differ significantly at

either 30 minutes or 60 minutes, the data suggest that genes governing translocation have additive effects.

14. In plants examined, the number of stomates per unit area on the abaxial surface was five times greater than the number of stomates on the adaxial surface. No significance could be obtained among three leaflet phenotypes in terms of stomatal density.

15. In the F_3 generation of two crosses involving the Original Intermediate as the common parent, the three leaflet phenotypes did not show any significant difference in stomatal resistance.

16. It is assumed that gene(s) controlling either stomatal density or stomatal resistance are not closely linked with that governing leaf shape. Change in one direction could be made without change in the other.

17. Anatomical study was made on the leaf sections of the normal and the intermediate plants which resulted from selfing the Original Intermediate plants. The data show that the two leaflet phenotypes did not exhibit any significant difference in the various components studied.

18. Therefore, the narrow-leaflet gene is likely to control leaf width and leaf length by decreasing number of cells in one direction and increasing in the other, rather than through changes in tissue components.

19. According to results obtained in the present investigation, it was not proved that the narrow-leaflet mutant was either superior or inferior to the normal-leaflet type in terms of physiological characteristics. This suggests the need of further study on succeding generation of fertile narrow-leaflet mutant progenies in field experiments. Measurement of light penetrating into the plant canopy, and canopy photosynthesis, as well as translocation should be performed in the field. This probably will help in arriving at a final judgment as to whether the

narrow-leaflet is responsible for any valuable agronomic characteristics for further breeding purposes.

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