THESIS

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INHERITANCE STUDY OF STARCH ACCUMULATION IN STEMS OF DRY BEANS

By

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ABSTRACT

INHERITANCE STUDY OF STARCH ACCUMULATION IN STEMS OF DRY BEANS

By

Joseph Kassian Mligo

A study of inheritance of starch accumulation was undertaken in five crosses of dry beans (Phaseolus vulgaris L.) to get information on whether the differential starch accumulation in the cultivars was due to genetic causes.

IKI solution as a starch indicator could not estimate starch levels efficiently in this study. Use of near infrared reflectance spectroscopy utilizing Neotec Grain Quality Analyzer model 41, appeared to be efficient in estimating stem starch content at 20 days after first flower.

F_2 frequency distributions showed a characteristic of transgressive quantitative inheritance. The broad sense heritability estimates proved to lie in the intermediate range with values ranging from 51% to 60%. Thus plant breeders may not have great difficulty in recognizing genetic differences among parental stocks. However, more information is needed on the extent to which the genetic differences observed can be used for selection purposes.

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INTRODUCTION

Although many plants store carbohydrate in the form of sucrose, still a great number deposit their reserve carbohydrate in the form of larger, more complex, and more insoluble molecules, the polysaccharides. Starch, in particular, serves as the reserve carbohydrate of a great number of plant species and is formed in such organs as leaves, stems, tubers, roots and seeds, where it is deposited during conditions favorable to photosynthesis.

In recent years, studies performed in dry beans at Michigan State University have been concerned with cultivar differences in carbohydrate partitioning as associated with yield and the remobilization of stored starch in the plant parts under stress conditions. In general, crop growth and yield are controlled by many environmental factors. These include light, CO_2 supply, temperature, water supply and nutrients, to name a few, which interact with the genetically determined physiological and biochemical systems of the plant. Complex control mechanisms have evolved to enable the plant to maintain a large degree of stability when external conditions change. If changes prevent the growth or yield from reaching the genetic potential of a plant then the limiting factor constitutes a stress. Thus the ability of a plant to remobilize accumulated starch under

stress conditions, as it has been found in dry beans (Adams et al 1981), such as under reduced photosynthesis, is believed to be a means by which a plant can maintain some degree of stability to external changes and hence sustain a steady rate of pod filling and ultimately a more stable yield.

Due to the fact that starch accumulation may be advantageous especially under stress conditions, incorporation of the ability to accumulate more starch into our future improved cultivars may lead to more stability of yield. The production of improved cultivars is dependent on a breeding system which allows gene exchange, and existence of heritable variation for the characters to be improved. If the cultivar differences in starch accumulation are due to genetic causes then heritable variation in starch accumulation could be used in a bean breeding program to incorporate the character into the backgrounds of desirable cultivars. The genetic basis of differential starch accumulation in beans or in any other plant is not known. The development of genotypes with high capability of accumulating starch will be facilitated by the knowledge of the inheritance of starch accumulation. Together with this, methodologies to facilitate selection which are rapid and accurate are required.

The objectives of this study were:

1. To determine the relationship of starch scores as determined by iodine potassium iodide starch staining

(IKI) and starch as quantitatively determined in the laboratory.

2. To determine whether the Grain Quality Analyzer (GQA) could be used to measure starch levels in dry beans.

3. If feasible use the GQA to study the inheritance pattern of stem starch storage in common bean (Phaseolus vulgaris).

LITERATURE REVIEW

Methodologies used for the Measurement of Carbohydrate Partitioning in Dry Beans

Methods for studying carbohydrate partitioning among strains of common beans need to be rapid, reproducible, accurate and inexpensive (Izquierdo, 1981). There are many analytical procedures relying on colorimetric measurements which have been developed for carbohydrate analysis (sugars and starch) in a number of crops (Gaines, 1971, Smith, 1969; Thivend, et al. 1966) which could be modified for beans. The drawback of these methods is that they are not suited for analyzing a large number of samples as required by plant breeders engaged in a screening program. Another drawback of these techniques is that they require considerable skill and they are time consuming and sensitive to slight variation in conditions.

In beans, carbohydrate partitioning has been estimated by using iodine- potassium - iodide (IKI) starch indicator solution (Adams, et al 1975). The indicator reaction depends upon the addition of the triodic ion to starch, yielding a brilliant blue complex (McCready et al 1943; 1970). The method involves treating freshly cut tissues with several drops of IKI starch indicator

solution and visually ranking the color development against facsimiles and varying degrees of intensity of color development observed among cultivars in stems and roots. This method has been used by other researchers, successfully, in analyzing waxy pollen mutants. Nilan (1981) reported that the IKI method permits a single observer in an eight-hour day to analyze the frequency of pollen mutants--normal (blue) and waxy (red) -- in a million pollen grains in Hordeum vulgare.

Although the method is rapid it is only semi-quantitative in respect to the estimation of quantities of starch. The correlation ($r = .85$, $df = 18$) between IKI-starch scores and in vivo starch reported by Bousalama (1977) has not been supported by subsequent studies. Izquierdo (1981), studying the applicability of IKI-starch scores and content of total soluble solids (TSS) for evaluating carbohydrate partitioning in beans, reported that correlation between IKI - starch scores and TSS contents and quantitatively determined starch were in moderate but not high agreement. Izquierdo speculated that the lack of correspondence between IKI - values and in vivo starch may have been due to differences in starch quality among cultivars. He referred to the amounts of amylose and amylopectin which are the two components of starch, the ratio of which may vary among cultivars. Amylopectin is weakly detected with IKI - starch; if a

cultivar were to have more amylopectin the IKI - starch score readings would be lower when compared to a cultivar with the same level of total starch but with starch stored primarily in the form of amylose. At present there is no information about the quality of stem or root starch among cultivars of dry beans.

In order to circumvent such possibilities and still have a rapid and reliable technique, the use of a near-infrared light reflectance (NIR) instrument to estimate percent starch needs to be explored. The NIR instrument such as the grain quality analyzer (GQA) is extremely simple to operate and requires a minimum of laboratory space (Hymowitz et al 1974). The grain analyzer does not directly measure chemical components of samples; it must be calibrated against standards. After calibration, samples in a powdered form are placed in a glass covered cup then fed into the instrument from which one can estimate the percentage of constituent being analyzed. Although the GQA will not be as rapid as the IKI starch score technique relative to chemical starch analytical methods which could be employed, NIR may prove to have advantages over the other methods.

NIR techniques have been in use for a long time. Norris and Hart (1965), investigated the water absorption bands at 0.76, 0.97, 1.18, 1.45, and 1.94 um for the

spectrophotometric measurement of water in seeds and grains. These efforts led to the development of a near infrared reflectance (NIR) technique, which originally aimed at determining the moisture content of agricultural products, but later was expanded to the measurement of protein, oil, starch and other constituents in these products.

Hymowitz et al (1974), estimated the protein and oil content in corn, soybean and oat seeds by NIR using the grain analyzer manufactured by Dicky - John Corp. These authors also studied the effect of sample grinding time on the grain analyzer readings for protein and oil content. Their most important findings were, a) the high correlation between the NIR and Kjeldahl data for protein content, and b) the lack of statistical significance between grinding time and protein or oil estimates.

Watson et al (1977), developed a regression equation for protein determination by Kjeldahl and NIR using five classes of wheat. It was found that the slope of the regression equation depended on the wheat class and that the effect of wheat class on the regression equation was not related to the particle size distribution.

Williams et al (1978), applied the NIR technique for protein and moisture testing in pulse breeding programs to improve both yield and quality of pulses in dry and tropical areas. Pulses were obtained from different research institutes throughout the world and were analyzed for protein

content by Kjeldahl and were subsequently analyzed by NIR using the Neotec Grain Quality Analyzer, model 31. They found correlation coefficients from 0.89 to 0.96 between Kjeldahl and NIR determination.

Giangiacomo et al (1981), employed the NIR technique to measure the concentration of fructose, glucose, and sucrose in model systems. Subsequently, measurements were made to estimate the same sugars in dried apple tissues. The correlation coefficients of the actual concentrations versus the predicted values were 0.995, 0.994 and 0.986 for fructose, glucose and sucrose, respectively, in the model systems comprising 20 samples. But when they tried to use the prediction equations to estimate the sugars in the dried apple samples, the corresponding correlation coefficients were 0.70, 0.55 and 0.90 for fructose, glucose, and sucrose, respectively.

Izquierdo (1981), using GQA-41, estimated the nitrogen content of several dried tissues of bean plants (seed, podwall, leafblade, petiole, stem and root) with correlation coefficients ranging from 0.873 to 0.973, between Kjeldahl and NIR.

The accuracy of NIR depends on the successful completion of the following:

1) Selection of a representative set of samples from the population.

2) Accurate laboratory analysis of the quality parameters of interest.

3) Accurate NIR data.

4) Appropriate wavelengths for the whole population.

The following are the chief sources of error in near infrared reflectance testing:

i) Selection of calibration samples

ii) Accuracy of standard chemical analysis used in calibration or monitoring.

iii) Homogeneity of ground sample.

iv) Moisture status of the sample.

v) Sample storage.

vi) Uneven or inconsistent loading of cell

Although according to literature reviewed, most work with NIR instrumentation has been with the estimation of protein and oil, it would appear that if all the above is achieved there is a possibility of being able to measure the starch content accurately and to be able to select different genotypes for use in the study of inheritance of starch accumulation in dry beans.

Genotypic Differences in Starch Accumulation and Contribution of Vegetative Carbon Reserves to Grain Yield.

The possibility of genotypic variation in starch accumulation in dry beans has been reported by Martinez et al (1975). In their study they found differences in the amount and type of reserves stored in stems of two navy bean cultivars. Whereas Nep-2 accumulated moderate to large

amounts of starch through the growing season. Seafarer accumulated little. In their study, the starch status was estimated qualitatively by the iodine-potassium iodide (IKI) starch indicator solution. The intensity of the blue color produced with IKI solution was an indication of presence or absence of starch as described in the previous section.

In a later study, starch levels in bean stems and roots as measured quantitatively (Bousalama, 1977) were found to correlate with IKI scores with a correlation coefficient of 0.85 (df = 18). According to this study, IKI scores are sufficiently accurate to show relative differences of the starch content among dry bean cultivars.

In a further study of cultivar differences in starch accumulation, Adams et al (1978) reported that the starch in roots and stems varied significantly among cultivars of dry beans. The range extended from undetectable amounts to abundant starch. Also, starch varied significantly with stage of development and a decline was noted as seed filling was completed. Although these researchers found significant stage by cultivar interactions in both root and stems the starch scores were high, starting at 50% first flower through mid-pod filling and declining when seed filling was completed. This indicates that around mid-pod filling most cultivars have maximum expression of starch accumulation in the stems and roots. This would seem then to be

the right stage for starch analysis in order to detect differences among genotypes.

In the study of patterns of partitioning and remobilization of non-structural carbohydrates in common bean and other selected grain legumes, Kabonyi (1981) observed that Redkote, Nep-2, Swedish Brown and Black Turtle Soup (BTS) often appeared as the most important starch storers of the common bean group. Seafarer was grouped as a low starch storer.

Adams et al (1978) found no clear pattern of relationship between starch accumulation in stems and roots and yield. They suggested that if there was any functional relationship, this might be expressed in a special environment such as stress. This suggestion was validated by studies conducted by Adams et al (1980) who studied cultivar differences in starch accumulation in relation to their ability to withstand a shading stress. In this study they found that Nep-2 which stores high levels of starch in the plant parts yielded more under shading stress condition during the reproductive phase than Sanilac which stores relatively little starch. Yields were not significantly different under no stress condition. They attributed the high yield under stress to differential ability to store starch in plant parts and remobilization of the stored starch reserves. Nep-2 was able to utilize its starch reserves for seed filling to compensate for the reduced

photosynthesis under stress, while Sanilac with little reserves did not have much to remobilize, hence a reduction in yield.

These findings appear to be supported by other studies of the same nature in corn and rice in which stored carbohydrates appeared to be able to support the grain growth of corn and rice at almost normal rate (Duncan et al, 1965). In these studies the opinion was offered that the stored carbohydrates can serve as a buffer to support normal grain growth despite fluctuations of weather.

Studies on wheat (Judel et al, 1982) have also shown that the depletion of non-structural carbohydrates at maturity was of much higher order in the culms and leaves of shaded plants while these parts in the control plants still contained a quantity of non-structural carbohydrates which amounted to about 10% of the grain weight. This indicates that stress conditions increase the contribution of carbohydrate reserves to grain yield.

There is also evidence which indicates that high temperature (Spiertz, 1977) or drought stress (Bidinger et al, 1977 and Gallanger et al, 1976) during the grain filling stage increases the relative contribution of vegetative carbon reserves to grain yield in wheat.

Although cultivars have been shown to vary in the amounts of carbon reserves stored, changes in the amounts of carbon reserves have been reported to be affected by

changes in seasons. Kabonyi (1981), using IKI - starch scores, observed genotypic and environmental differences with seasons in partitioning of non-structural carbohydrates in dry beans.

Thomas et al (1982) studied the seasonal changes in non-structural carbohydrate levels of wheat and oats grown in a semiarid environment. They reported that the green-leaf carbohydrate levels were relatively stable throughout the growing season and did not fluctuate in response to seasonal rainfall. Carbohydrates accumulated in the stems to a maximum of 25 to 48% (of the dry weight) at about anthesis and declined toward maturity. But, although similar trends were found in both years of their experiments, stem carbohydrate concentrations differed. Thus, while the stem water soluble carbohydrates (WSC) concentration of Pitic stems did not exceed 15% during 1978, in 1979 they reached 35%. The percentage of non-structural carbohydrates in the same wheat variety differed from 30% in 1978 to 50% in 1979. Thus it appears changes in seasons have a tremendous effect on the concentrations of the carbon reserves.

Reasoning from the findings of Gallagher et al (1976) who presented indirect evidence, based on vegetative dry weight losses, that field-grown wheat may derive more than 50% of the grain yield from pre-anthesis assimilates if subjected to drought stress, Thomas et al (1982) speculated that at least 20% of the yield may arise from vegatative reserves in semiarid environments during dry

years. They came up with a suggestion which is similar to that already given by Adams et al (1978) in dry beans, that a wheat cultivar such as 'Pitic,' which accumulates large amounts of stem carbohydrates during the period around anthesis may have an advantage if subjected to drought stress during the grain filling stage.

From the literature reviewed it suggests that cultivars with the ability to accumulate high levels of carbon reserves in the plant parts have an advantage over those which do not have the ability to accumulate more carbon reserves, especially when a condition of stress subsequently ensues. The incorporation of this character into future cultivars may improve yield stability. The genotypic variability in starch accumulation among dry bean cultivars suggests that it could be possible to select for this character if it is ascertained that much of the variation is due to genetic causes.

Genetic Control of Starch Content.

Though great differences in the degree of stem starch content occur among common bean cultivars, hardly any information has been published on the genetic control of starch content of the stems. Most of the genetic studies of starch accumulation have been done on the seeds and tubers. And much effort has been devoted to the ratio of amylose to amylopectin.

Salisbury and Ross (1969) reported that the amylose

content is genetically controlled and some waxy varieties of cereal grains have only amylopectin in their starch. The inheritance of waxy grains is controlled by a recessive gene, and only those homozygous for the gene produce amylose-free starch. They also reported that in a few other plants the starch is composed almost completely of amylose (e.g. wrinkled peas).

Akazawa (1976) reported also that it has long been recognized that the ratio between amylose and amylopectin contents in the plant is determined by genetic constitution. Thus, because of the potential industrial use of amylose, plant geneticists and breeders have attempted to develop mutants of higher amylose content. Several high amylose starches are known, such as those which occur in amylomaize and in wrinkles peas. In such cases the amylose content ranges from 50 to 80% (Akazawa, 1976).

At Washington State University, numerous mutants at the waxy locus in barley have been induced primarily to: 1) probe a particular locus in barley through inter-allellic recombination and intragenic mapping, and subsequently biochemical analysis of the mutant proteins, 2) understand pathways of starch synthesis, and 3) provide variants of starch content (amylose/amylopectin ratios) for possible livestock nutritive value (Nilan et al, 1981). From their findings with starch content, they reported that UDP-glucose glucosyltransferase has involved determining the anylose/amylopectin ratio. This

emphasizes the genetic control of the starch content. Preliminary results of these researchers showed that the waxy mutants lack detectable amounts of amylose as determined by amperometric titration. Also, analysis of mutants for soluble and bound starch synthetase activity, suggested that the waxy mutants have a lower level of bound starch synthetase activity than the parental line, 'Septoe,' used in the study, while having approximately normal levels of soluble starch synthetase activity.

Work done by Nelson and Tsai (1964) has shown that maize waxy mutants contained about one-tenth the level of bound starch synthetase activity as compared to normal maize. Nelson et al (1978) showed that this activity could be accounted for by the presence of a second bound starch synthetase having a higher affinity for substrate.

As seen from the literature review on the genetic control of starch content, it appears that the studies on the genetics of starch content have been based on the amylose and amylopectin contents. There is no report where the authors treat starch accumulation as a trait without considering its components; and it appears that amylose content is controlled by a dominant gene and amylopectin content is recessively controlled. Nevertheless, one could speculate that any differences among cultivars in starch content, no matter what fraction of starch is the more abundant, would be due to genetic effects. Moreover, the differences in the tissue studied

would not be expected to make any appreciable difference. Studies thus far performed on seeds, tuber and pollen grains have led to the same conclusions.

EXPERIMENTAL PROCEDURES

A. Chemical Quantification of Bean Stem Starch:

This experiment was performed as part of a series of experiments aimed at elucidating information on the relationship between the IKI starch staining technique and starch quantification in the laboratory in order to compare techniques for starch estimation in dry bean stems.

Five dry bean cultivars, Seafarer (determinate), Redkloud (determinate), Michigan 41228 (indeterminate), Swedish Brown (determinate) and the breeding line 61356 (indeterminate), were used in this experiment. The cultivars differed in starch accumulation capability as estimated in an earlier experiment using IKI as a starch indicator (Adams, et al, 1978).

The cultivars were grown in one row plots at East Lansing Michigan during the summer of 1981 and two row plots in 1982. In 1981 the rows were 50 cm apart and plants spaced at 15 cm within rows. Two replications were used. Because of poor accessibility to the plants during sampling in 1981, row spacing was increased to 1.0 meter in 1982 to facilitate access to the plants.

The growing point of each plant was removed by pinching when the growing point was just visible after the

primary leaves had developed. This induced two branches to form in the axil of the two primary leaves. Starch accumulation measurements were made on one of the branches while the other was left for producing seeds. Date of flowering was recorded for each plant and sampling for starch storage started 20 days after flowering, on an individual plant basis. Ten plants were sampled in each plot.

Sampling involved cutting one branch in the second internode region of the branch. Leaves and petioles were removed from the stems and the stems were cut into lengths sufficiently short to fit into sample bags. The samples were placed in plastic boxes containing dry ice for immediate stoppage of respiratory enzymes. The slant cut ends of the stem samples cut from the second internode of the stems were scored for starch with the IKI starch indicator technique in a field laboratory. Two to three drops of the IKI starch indicator solution, made by dissolving 0.3 g iodine and 1.5 potassium iodide in 100 ml. water, were applied to freshly cut section of the stem tissue. The amount of starch was rated on a five-point scale, one (least) to five (most). A color photograph containing a series of IKI - stained cross sections of stem tissues evaluated and taken from bean strains with varying amounts of stored starch were used for scoring. The remaining sample was placed back in the paper bag and stored at -3 C° for several days, then dried.

The samples were dried in a convection oven at 100 C° for one hour then at 65 C° for 72 hours. After drying, the tissue from each sample was ground to pass through a 40-mesh screen in a standard Wiley mill and stored in sealed bags for quantitative starch determination in the laboratory.

Before analyzing for starch, soluble sugars were extracted from the samples. Five hundred mg of the oven dry sample was accurately weighed on an analytical balance and placed in a 50 ml centrifuge tube. Twenty-five ml of 80% ethyl alcohol were added to each tube and samples were shaken in a water bath at 75 C° for 40 minutes. After centrifugation and discarding the supernatant containing sugars the pellets were dried at 70 C° for 12 hours, weighed and saved for starch analysis.

Starch content was determined in each sample by analyzing a 200 mg sample of dried pellet, following the procedure of Dekker and Richards (1971). A soluble starch solution was prepared by suspending 200 mg of oven-dried pellet in five ml of a 0.5 N sodium hydroxide solution for one hour. Starch solubilization proceeded by constantly vortexing the suspension for five times in one hour in a water bath maintained at 35 C°. After solubilization, the solution was neutralized by adding five ml of 0.5 N acetic acid solution. The solubilized starch was hydrolyzed to glucose using an enzyme mixture made by combining one ml of amyloglucosidase (obtained from Rhizopus spp mold), 0.2 ml alpha-amylase (Type II-A), 0.2 ml beta-amylase (Type I-B).

The enzymes were obtained from the Sigma Chemical Company, St. Louis, Missouri. The incubation of the enzymes in the starch solution was carried out for one hour by shaking in a water bath maintained at 55 C°. The glucose in the hydrolysate was determined quantitatively by a Glucostat Reagant-Assay Kit^{R} (Sigma no. 510) and absorbance read for each sample using the blank as the reference point at 450 nm. The starch concentration was calculated by reference to a standard curve (Figure 1) of soluble starch (B.D.H.). The concentrations of the soluble starch used for the standard curve ranged from 0.1 mg/ml to five mg/ml from which the equation Y = -0.052499 + 15.02647X was obtained and used in the conversion of absorbance readings into mg of starch per ml. In the equation Y = mg/ml (starch), and X = absorbance reading.

Starch (mg/ml) was converted to percentage weight of starch contained in the moisture free tissue (70 C°) by first getting the amount of starch in the total pellet then expressing this as percentage of the total tissue used.

Simple correlation coefficient was calculated between IKI-starch scores and percentage starch as determined quantitatively in the laboratory for the five cultivars.

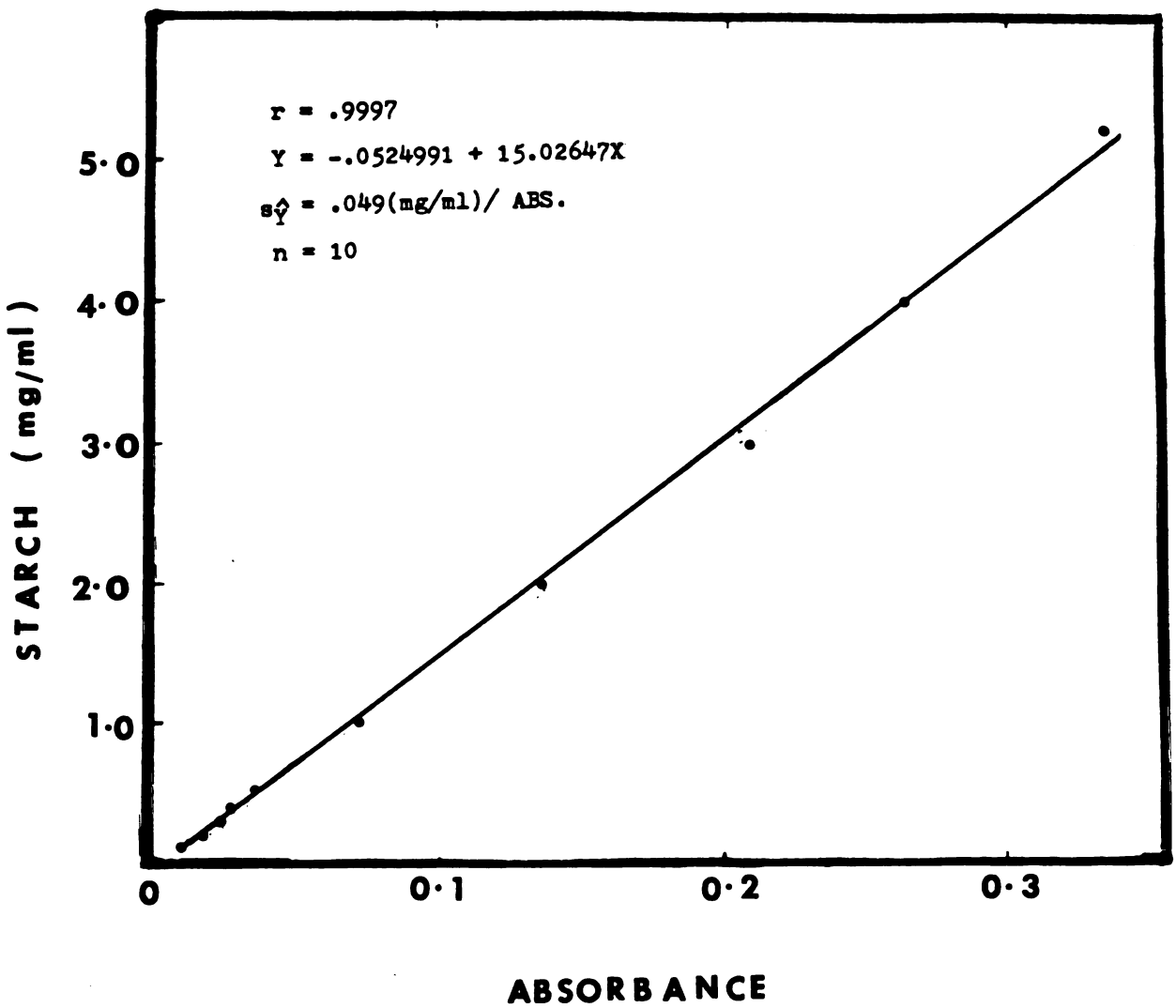

Figure 1. Starch standard curve used for the calculation of percent stem starch based upon standard quantification chemical methods.

B. Calibration of the Grain Quality Analyzer for Estimation of Starch Content in Dry Bean Stems.

The near-infrared light reflectance instrument used in this study was the Neotec Grain Quality Analyzer model 41 (GQA-41) which measures the reflectance of a finely ground sample at selected wavelengths in the near-infrared spectrum. The reflected energy is detected by lead sulfide cells sampling the reflected energy levels from each of the selected different wavelengths. The signal output is amplified, channeled through a filter synchronizer and then processed by microcomputer. In the computation process, an equation is solved, resulting in a presentation of readings on a digital readout device.

Before a NIR instrument can be used to predict the composition of a particular sample, the instrument must be calibrated. The instrument is used to obtain the reflectance readings of samples with known values which have been accurately analyzed by the traditional chemical methods from which coefficients are obtained and used for prediction of the composition of the unknown samples.

In this experiment, 40 stem samples, ground to pass a 40-mesh screen, and selected from the cultivars used in the first experiment, were used for the instrument calibration. The samples were dried at 70 C° and enzymatically hydrolyzed and percentage starch calculated from the liberated glucose as described in the above experiment.

The percentages of starch of the samples used for calibration ranged from 0.90 to 16 percent.

The remnants of the samples used for enzymatic hydrolysis were loaded into the Neotec GQA sample holder which uses a spring-loaded pressure plate to hold the sample smoothly against a clear glass window. Due to the small amounts of the samples used, a modification of the inside of the sample cup was necessary so that small samples could be loaded and readings recorded. The modification of the sample cup involved reducing the size of the cup by fitting in a piece of sponge of one cm thickness inside the sample cup and making a cut along 1.5 cm radius through the sponge. The cup so modified had a diameter of three cm and one cm depth as compared to the normal cup with five cm diameter and one cm depth. This modification reduced the amount of sample to less than half the amount required to fill the normal cup.

Reflectance readings were recorded as dR/R for each sample, where dR is the differential coefficient of a line tangent to the absorption curve peak and R is the absolute reflectance. A change in constituent amount results in a change in dR, hence the mathematical model of dR/R resulting in information relating to change of constituent.

Percentage starch as estimated by wet chemistry of each sample together with the dR/R values were entered into the computer, and a multiple regression routine using the Hal Stat 4 program was used to compute the multiplying

coefficients K_o to K_4 which gave the best fit to the following equation for the percentage starch.

$$\% \text{ starch} = K_0 + K_1\, dR/R_1 + K_2 dR/R_2 + K_3 dR/R_3 + K_4 dR/R_4$$

where K_0 is the intercept of the multilinear regression line.

K_1, K_2, K_3 and K_4 are the regression coefficients at four different wavelengths.

dR is the differential coefficient and R is the absolute reflectance value at a particular wavelength.

The K-values and the pulse points for starch were stored in the instrument and 35 samples used for calibration were run and percentage starch recorded. A bias adjustment was required to make the instrument and laboratory readings agree. Bias is defined as the average offset calculated from comparison of instrument and laboratory results. This was done by adding 0.10 to the K_0 because laboratory values tended to be a little higher than that estimated using the GQA-41.

Simple linear coefficients between laboratory and GQA-41 percentage starch and standard error of estimate (SEE) were calculated. Also, correlation between small size sample and normal size sample was calculated. This was done only with those samples which had enough material.

C. Genetic Studies:

Ten cultivars were used for the genetic studies of starch accumulation in dry beans. They were selected based on the differences in starch accumulation as estimated by the IKI starch indicator technique. They included three low starch storer cultivars, Seafarer, Michigan 41228, and Brazil-2; seven high starch storers, Nep-2, Redkloud, 61356, ICA pijao, Swedish Brown, 791515 and 790780. These parents were crossed between high and low, low and low and high by high in the greenhouse in the winter and spring of 1981 by hand pollination. F_1 seeds were advanced to the F_2 generation. When possible, characters under monogenic control were examined to insure that cross pollination had occurred.

Parents F_1 and F_2 seeds were planted in the field in the summer of 1981 at Michigan State University Crop Science Farm. Parents and F_1's were planted in single row plots. The F_2 populations were planted in two row plots, 12 meters long. In all of the populations, rows were 50 cm apart and plants spaced at 15 cm apart within rows. Parents and F_2's were grown in a randomized complete block design with two replications. The number of F_1 seeds was insufficient for a replicated experiment.

In the summer of 1982, ten F_3 seeds were planted from each of the 88 F_2 individual plants of the cross Seafarer x 61356, 36 F_2 individual plants of the cross Seafarer x Redkloud and 25 F_2 individuals from the cross

Michigan 41228 x Swedish Brown selected for F_3 family performance studies. Together with their parents these families were grown in single row plots. The design was the same as that of 1981. But because of the problems encountered in 1981 in sampling, the row spacing was increased in 1982 to one meter to allow easy accessibility to plants to be sampled.

Since the analysis of starch in stems involved cutting the whole plant, and was thus destructive, seeds from plant analyzed for starch, were advanced to the next generation. Hence, there was need to have two branches from the main stem so that one branch could be cut for starch analysis at 20 days after first flower and the other branch left to produce seeds. So the growing tips of plants were pinched as described in section A, above. A number of plants could not support the weight of branches and consequently they broke off and remained only half-intact. Because of this, a number of plants were not used for taking samples which led to reduced numbers of samples per population.

Supplemental sprinkler irrigation was applied as needed throughout the growing season. Foliage diseases, physiological disorders and insects were controlled by application of recommended chemicals. Similar field procedures were followed in 1981 and 1982.

Analysis of starch content was done on the stems of

parents, F_1, F_2 and F_3 individual plants at 20 days after first flower. This, on average, is the time of linear seed filling which coincides with the high values of starch content in the plant parts as reported by Isquierdo (1981). Sampling and estimation of starch in the field by IKI starch indicator solution were the same as described in Section A, above. The remnant tissues were dried, ground to pass 40 mesh-screen and then analyzed for percentage starch with the Neotec GQA-41 as described in Section B, above.

Initial selection of parents was based on the estimation of starch by IKI starch indicator solution. But because IKI starch scores could not correlate highly with the quantitatively analyzed starch, subsequently some of the crosses made as being high by low were in actual fact low by low as shown by the wet chemistry analysis of the parents. Thus the F_3 families of which their F_2 individual values were selected depending on IKI starch scores, were not included in the analysis because the crosses turned up to be of the low by low types. The Neotec GQA-41 showed a correlation of 0.907 (Df = 33) with the starch content as analyzed by wet chemistry. Hence the Neotec GQA-41 was used to analyze the starch content of stem samples.

Frequency distributions of the percentage starch were computed for all F_2 populations together with their parents and F_1's.

An estimate of the broad sense heritability in F_2

generation was obtained using the variances among individual plants of F_2 populations, environmental variance being estimated by the variances among individual plants of the non-segregating populations, the parents and F_1's.

RESULTS AND DISCUSSION

The results and discussion are presented in five parts: A) relationship between stem starch quantification and IKI-starch scores, B) calibration of GQA-41 (Grain Quality Analyzer model 41) for the estimation of starch content in dry bean stems, C) variation among parents in stem starch accumulation, D) evaluation of non-segregating and segregating populations and E) heritability estimates of the selected crosses for stem starch accumulation.

A) Relationship between Stem Starch Quantification and IKI-Starch Scores in Dry Bean Cultivars:

This experiment was performed to ascertain whether IKI-starch scores were sufficiently accurate in estimating quantities of stem starch in dry bean cultivars. Figure 2 shows the relationship between the stem starch quantification and IKI-starch scores at 20 days after first flower. As seen from figure 2 the two methods are not significantly correlated. The lack of high correlation between the two methods stems from the fact that: one IKI starch score is represented by several percentage stem starch values as estimated by the wet chemistry method. This implies that IKI starch scores do not accurately indicate starch concentration in this experiment.

STARCH (LAB)

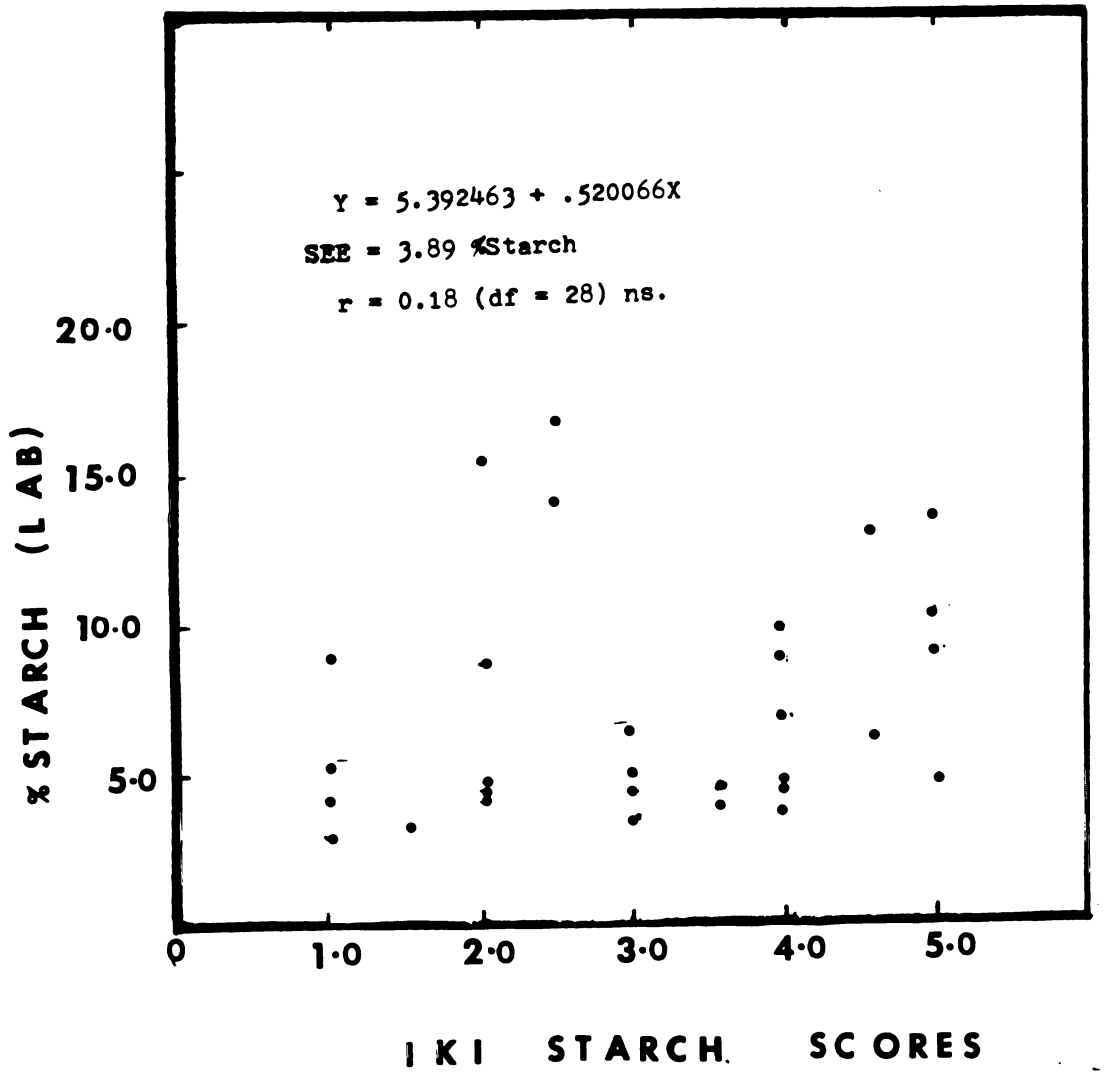

Figure 2: Relationship between IKI starch scores and percent starch as determined quantitatively at 20 days after first flower in the stems of dry bean cultivars.

Table 1 shows the percentage stem starch as quantitatively determined and IKI starch scores for 1981 and 1982 seasons for the five dry bean cultivars used in this study. The percentage stem starch in 1981 was relatively higher as compared to that of 1982 season. However, although differences among cultivars in the 1982 season were nonsignificant, the relative difference among the five cultivars remained the same in the two years (r = 0.927). IKI starch scores were also lower in the 1982 season as compared to that of 1981. Again, as with percentage stem starch, the relative differences among cultivars for stem starch estimated using IKI starch score remained the same in both years. Variation between seasons was not surprising since carbohydrate partitioning has been found to be affected by differences in seasons (Kabonyi, 1981: Thomas et al, 1982). Another aspect which might have contributed to low percentage stem starch values in the 1982 experiment could be due to sampling technique difference. In the 1982 experiment, due to the bulkness of the samples, samples could not immediately be placed into boxes containing dry ice to stop respiratory enzymes. The samples were taken to the cold room in the Crop Science Field Laboratory on an hourly basis and it took on average five hours before the samples were frozen. Hence, there is a possibility that the respiratory enzymes continued to degrade starch into sugars before the samples were frozen.

The percentage stem starch obtained for the variety Michigan 41228 (15.05%) in the 1981 season was not expected

Table 1. Stem Percentage starch as quantitatively determined and the respective IKI starch scores of five dry bean cultivars.

Cultivar	1981		1982	
	Quantitatively Determined (%)	IKI Rating	Quantitatively Determined (%)	IKI Rating
Seafarer	6.26	2.50	4.50	2.00
Redkloud	7.91	4.20	4.47	3.00
Michigan 41228	15.08	2.33	6.98	1.33
Swedish Brown	11.63	4.83	4.99	3.33
61356	4.20	4.00	3.95	3.33
LSD (.05)	5.34	0.22	ns	0.63

to be this high. This variety had the lowest value with the IKI starch indicator solution in both years of study and also it had a low IKI starch score in a previous study (Adams et al, 1978). However, Michigan 41228 had the highest percentage stem starch with the wet chemistry method in this study (Table 1). One might be led to speculate that since the IKI starch indicator solution is only sensitive to amylose, perhaps this variety (Michigan 41228) has stem starch content in appreciable amounts of amylopectin which is only weakly detected by IKI starch indicator solution. However, the highly variable response of this cultivar to IKI starch scores and laboratory starch analysis requires further investigation.

According to the results of this experiment, IKI starch scores could not be used for the estimation of starch content of the stems at 20 days after flowering for the inheritance studies of differential starch accumulation in stems of dry bean cultivars. A quantitative method easier and more rapid than laboratory chemical analysis had to be employed. The results of a search for this method are presented in the following section.

B) Calibration of Grain Quality Analyzer (GQA) for Starch Concentration Estimation in Dry Bean Stems:

Infrared reflectance spectroscopy [Neotec Grain Quality Analyzer (GQA-41)] was explored to see whether it could be used to estimate stem starch levels in dry bean cultivars. The instrument was calibrated by running a multiple regression analysis, using Hal Stat 4 program, of the wet chemistry laboratory values and the first derivative of the reflectance values $(\frac{dR}{R})$ from the calibration samples read at four different pulse point settings. The pulse points used were provided by the Neotec Company obtained from starch absorption bands. Starch has a peak at 2.10 um and Rotolo (1979) has pointed out that this peak which is provided by filter 4 can be used to determine the starch content of samples. The four pulse points provided by the company were 603 (P_1), 639 (P_2), 926 (P_3) and 970 (P_4). Product button 4 was used for the instrument calibration. Product button 4 is used for starch detection (Rotolo, 1979) and readings were recorded at the manual setting. The multiple regression analysis provided a set of K-values to be entered into the GQA-41 for the prediction of starch content of the bean stem samples. The K-values obtained for K_0 through K_4 were 7.082, -1002.939, -3355.376, -184.759 and -5225.007, respectively, with a coefficient of determination of .7313. These K-values

were entered into the GQA, then the remnant samples used for calibration were read on the GQA as unknowns for verification of calibration. The GQA readings of the 35 calibration samples corresponded closely to the laboratory analyzed values with a coefficient of linear correlation of 0.907 and a standard error computed as variation from the regression line of 1.42% starch.

The modification of the sample cup to accommodate small sample size did not affect the GQA readings. There was a very close relationship between the GQA readings using the normal sample cup size and the modified sample cup ($r = .986$). This indicated that the modified sample cup was sufficiently adequate to hold the samples for the estimation of starch content in the dry bean stems. These findings agree with those of Hymowitz et al (1974) who found no difference in percentage protein or oil in the meals between normal sample cup and the cup fitted with a false bottom which reduced by half the meal required to fill the cup.

Based on the data presented here, the GQA was used for the prediction of starch levels in dry bean stems for the selection of parents to be used in the study of inheritance of differential starch accumulation in dry bean stems. Although these findings were for stems, the applicability of GQA to other tissues will be straight forward provided appropriate samples are available for development of calibration curves.

C) Variation Among Parents

Significant differences for percentage stem starch at 20 days after first flower as estimated by the GQA-41 were observed among the ten parents evaluated in the 1981 growing season (Table 2). Michigan 41228 with 11.61% stem starch ranked the highest and 791515 with 7.07% stem starch ranked the lowest. This suggests that at 20 days after first flower cultivars could show differences in ability to store starch, hence evaluation of segregating populations for their ability to store starch in stems at 20 days after first flower will render it possible to observe differences between individuals.

From these results parents were ranked as either high or low starch storers. Parents with percentage stem starch above 10% were ranked as high and those with percentage stem starch below 9% were ranked as low starch storers. Hence Michigan 41228 (11.61%), ICIA Pijao (11.47%), Swedish Brown (10.82%) and Brazil-2 (10.40%) were ranked as high starch storers while 61356 (8.56%), Redkloud (8.45%) and Seafarer (7.47%) were ranked as low starch storers and Nep-2 (9.28%) was in the medium range. Therefore crosses of high x high, low x high, and low x low between those parents were studied for the inheritance of differential stem starch accumulation ability and the results are discussed below.

Table 2. Mean, ranges, standard deviation and coefficients of variation of percentage stem starch content 20 days after first flower of the ten dry bean cultivars grown in 1981.

Cultivar	Mean	Range	N	s	C.V.
Michigan 41228	11.61	8.81-13.86	8	1.91	16.50
ICIA Pijao	11.47	9.31-13.18	13	1.40	12.20
Swedish Brown	10.82	7.98-13.89	15	2.02	18.67
Brazil-2	10.47	7.22-14.33	12	1.79	17.10
790780	10.33	6.95-13.92	13	1.96	18.97
Nep-2	9.28	4.69-11.70	12	1.98	21.33
61356	8.56	4.80-11.37	16	2.12	24.76
Redkloud	8.45	6.71-10.68	13	1.06	12.54
Seafarer	7.47	6.40-9.17	9	0.89	11.91
791515	7.07	4.01-9.18	12	1.81	25.60
LSD (.05)	1.18				

D) Evaluation of Segregating and Non-segregating Populations for Percentage Stem Starch of Dry Bean Crosses

Low x High Crosses:

Table 3 shows the frequency distributions for percentage stem starch in parents F_1 and F_2 generations for the cross between Redkloud (low parent) and Michigan 41228 (high parent). The mean of the F_1 was between the parental values but tending towards the parent with high starch content indicating possible expression of dominant genes for high starch content.

Despite the very few individuals available for analysis in this cross, F_2 variations was continuous and extended beyond the ranges of parents indicating transgressive segregation had occurred. Starch values of about 66% of the F_2 individuals approached the parent with high percentage stem starch, which agreed with the F_1 analysis and suggested that dominance was involved in trait expression. The cross between Seafarer (low) and ICIA Pijao (high) had a similar distribution (Table 4). This may indicate that the low and high starch storer parents used in these crosses of low x high have similar allelic content for the expression of stem starch storage. This can also be supported by the results of a cross between ICA Pijao (high) and Seafarer (low) which is a reciprocal cross in terms of high and low parents. This also had a similar distribution (Table 5) as low x high crosses. Therefore, there appear to be no reciprocal effects.

Table 3. Frequency Distributions for Stem Percentage Starch Content 20 Days after First Flower in Parents, F_1 and F_2 Generations in a Cross between Redkloud and Michigan 41228.

Generations	Class centers in % starch										n	$\bar{X}$	s^2
	5.6	6.6	7.6	8.6	9.6	10.6	11.6	12.6	13.6	14.6			
Redkloud (Low)		1	3	7	1	1					13	8.43	1.13
Michigan 41228 (High)				1	1	1	1	2	2		8	11.60	3.66
F_1			1	1		1	2	1			6	10.26	4.26
F_2	1		4	3	2	5	2	7	2	3	29	10.76	6.25
Mid-Parent												10.02	

Table 4. Frequency Distributions for Stem Percentage Starch Content 20 Days after First Flower in Parents, F_1 and F_2 Generations in a cross between Seafarer and ICA Pijao.

	Class centers in % starch												
Generations	4.6	5.6	6.6	7.6	8.6	9.6	10.6	11.6	12.6	13.6	n	$\bar{X}$	s^2
Seafarer (Low)			3	3	2	1					9	7.47	0.80
ICA Pijao (High)						3	3	2	1	4	13	11.47	1.96
F_1				1		1	2		1		5	10.15	2.42
F_2	1	2	8	6	10	10	8	7	6	1	59	9.29	4.34
Mid-Parent												9.47	

Table 5. Frequency Distributions for Stem percentage Starch Content 20 Days After First Flower in Parents and F_2 Generations in a Cross between ICA Pijao and Seafarer.

Generations	Class Centers in Percentage Starch									n	X	s^2
	5.6	6.6	7.6	8.6	9.6	10.6	11.6	12.6	13.6			
ICA Pijao (High)					3	3	2	1	4	13	11.47	1.96
Seafarer (Low)		3	3	2	1					9	7.47	0.80
F_2	1	8	7	9	14	3	5	2	2	51	9.02	3.41
Mid-Parent											9.47	

Table 6. Frequency Distributions for Stem Percentage Starch Content 20 Days after the First Flower in Parents, F_1 and F_2 generations in a cross between Brazil-2 and Redkloud.

Generations	Class centers in % starch																
	1.6	2.6	3.6	4.6	5.6	6.6	7.6	8.6	9.6	10.6	11.6	12.6	13.6	14.6	n	$\bar{X}$	s^2
Brazil-2 (High)							1	1	4	1	4				11	10.47	3.20
Redkloud (Low)						1	3	7	1	1					13	8.43	1.13
F_1						2		1	1	1	4	2		1	12	10.54	5.49
F_2	1	1	1			6	5	7	4	5	8	9	1	2	50	9.66	8.16
Mid-Parent																9.45	

In the cross between Brazil-2 (high) and Redkloud (low), the F_1 mean value was slightly but not significantly higher than the high parent (Table 6). There may be therefore some degree of heterosis expressed in the F_1 of this cross. This was also observed in the growth of the F_1 plants of this cross. By visual observations, the plants in this cross showed a high vigor in growth and had very large leaves as compared to their parents. So the heterosis in stem starch content could be a reflection of high leaf area which would imply a higher source of photosynthates for transformation into starch.

High x High Cross:

The cross involving parents which were both high in percentage stem starch was that between Michigan 41228 and Swedish Brown. The difference between the cultivars in percentage stem starch was not significant. The F_2 was variable (Table 7). One thing observed in this cross was that, whereas the crosses of low x high or high x low parents indicated the F_1 and F_2 values tending towards the parent with high percentage stem starch, the situation in this cross was rather quite different. About 73% of the F_2 individuals had starch values tending towards low values. Since the cross does not involve a low parent, the variation observed would suggest that the parents used were genetically different. That is, they do not have the same allelic content for starch accumulation expression and this might have

Table 7. Frequency Distributions for Stem Percentage Starch Content 20 Days after First Flower in Parents and F_2 Generations in a Cross between Michigan 41228 and Swedish Brown.

	Class centers in % starch														
Generations	2.6	3.6	4.6	5.6	6.6	7.6	8.6	9.6	10.6	11.6	12.6	13.6	n	$\bar{X}$	s^2
Michigan 41228 (High)							1	1	1	1	2	2	8	11.61	3.66
Swedish Brown (High)						1	2	3	3	1	2	3	15	10.82	4.08
F_2	2	2	2	5	7	7	5	4	3	2	1	1	41	7.43	6.70
Mid-Parent														10.89	

led to combinations that gave values in F_2 generation which were beyond either parent. In other words, this indicates a probable transgressive segregation towards low values of percentage stem starch.

Low x Low Cross:

Table 8 shows the frequency distributions for the percentage stem starch in the parents and F_2 generations of the cross of low x low type between Seafarer and 61356. In this cross the F_2 generation was less variable. In fact, it was even less variable than one of the parents. The low variability observed in this cross may not be strange because not much variability is expected from such a cross especially if the two parents have the same genes for the expression of stem starch accumulation ability. A closer look at the distribution of the F_2 reveals observations which are rather intriguing. The mean of the F_2 generation is well below that of either parent, an observation not expected. The possibility of parents differing genetically for genes controlling starch accumulation should not be ruled out.

Summary of the Three Types of Crosses:

Starch accumulation is a complex character and it appears to be inherited in a quantitative manner. The recovery of both the parental types within the size of the F_2 populations studied, as well as the range of F_2 segregates beyond both the parental ranges in Redkloud (low)

Table 8. Frequency Distributions for Stem Percentage Starch Content 20 Days after First Flower in Parents and F_2 Generations in a cross between Seafarer and 61356.

	Class centers in % starch														
Generations	0.6	1.6	2.6	3.6	4.6	5.6	6.6	7.6	8.6	9.6	10.6	11.6	n	$\bar{X}$	s^2
Seafarer (Low)							3	3	2	1			9	7.52	0.80
61356 (Low)					1	1	3	3		3	3	2	16	8.56	4.48
F_2	6	13	18	38	41	37	16	8	2				179	4.35	2.74
Mid-Parent														8.04	

x Michigan 41228 (high) and Brazil-2 (high) x Redkloud (low) indicated that these parents had manh genes in common. However, it appeared probable that some parent combinations differed in some genes conditioning stem starch accumulation. Thus, plus and minus gene effects conditioning stem starch accumulation in different parents permitted a complementary type of gene action in the F_2 segregates. This explains the indicated transgressive segregation observed in the F_2 of the low x high and high x low crosses. In contrast, the transgressive segregation observed in crosses of the low x low and high x high cannot be explained by complementary tyep of gene action alone; since most of the segregates tended to be towafds the low side of starch values it could probably be due to other genic interactions.

Midparents Values:

Midparent values when compared to their respective F_2 values may in general indicate the type of gene action prevailing for the character of interest. Comparison between parental, midparent and F_2 values of percentage stem starch in the five dry bean crosses is presented in Table 9. In general crosses involving parents with low and high proportion of percentage stem starch produced F_2 populations with the mean values which were not significantly different from their respective midparent values. This would suggest a preponderance of additive gene action. However, this was not clearly evident for crosses involving high by high and low

Table 9. Comparison between Parental (P_1, P_2) Midparent (MP) and F_2 Values for the Dry Bean Stem Starch Content 20 Days after First Flower in five Dry Bean Crosses.

Cross	% starch			
	P_1	P_2	MP	F_2
Brazil-2/ Redkloud	10.46	8.43	9.45	9.66
Redkloud/ Mich 41228	8.43	11.60	10.02	10.76
Seafarer/ ICA Pijao	7.52	11.48	9.50	9.29
Mich 41228/ Swedish Brown	11.60	10.78	10.89	7.43*
Seafarer/61356	7.52	8.56	8.04	4.35*

*F_2 values significantly different from their respective MP values at the .05 probability level.

by low parents. In these types of crosses the F_2 values were significantly different from their respective midparent values. In both type of crosses the F_2 values were towards the low starch values. This indicates a negative departure from midparent value which may be due to genic interaction. Hence, as discussed above, this indicates some parent combinations in this study do not have the same allelic content for genes conditioning stem starch accumulation.

E) Heritability Estimates:

Effective selection of desired genotypes when conditioned by quantitative inheritance usually is difficult in segregating generations if the heritability is low. Hence, it may become desirable to determine genotypic variance in a segregating population in order to estimate the magnitude of the heritable fraction.

In this study, heritability of percentage stem starch is used in the broad sense and indicates the extent to which expression of the percentage stem starch is under genic control. The heritability for percentage stem starch was calculated as the percent genotypic variance of the total F_2 variance. Table 10 shows the heritability estimates for percentage stem starch for one high by low and two low by high crosses. In all three crosses the heritability estimates proved to lie in the intermediate range. The values ranged from 51 to 60%. These heritability values indicated that some of the differences observed in the dry bean genotypes

for stem starch accumulation levels were due to genetic influence. Plant breeders should have no great difficulty in recognizing genetic differences among parental stocks. However, more information is needed on the extent to which the genetic differences observed can be used for selection purposes. That is, heritability in the narrow sense must be determined. This can be calculated from the regression of F_3 values into F_2 values in which the heritability is largely due to additive genetic effects transmitted from parent to progeny such as $F_2 - F_3$. It was not possible in the present study to get information concerning narrow sense heritability of percentage stem starch from the regression of F_3 family values into respective F_2 values due to misclassification of parents selected for that study by the use of IKI solution as a starch indicator.

Table 10. Broad Sense Heritability (BSH) estimates for stem starch content for the F_2 progeny of three dry bean crosses.

Cross	Heritability (BSH)
Brazil-2 x Redkloud	.60
Redkloud x Michigan 41228	.51
Seafarer x ICA Pijao	.60

GENERAL DISCUSSION

As far as techniques for estimation of starch content in dry bean stem are concerned, it appeared that the IKI solution as starch indicator cannot estimate starch levels efficiently. Since IKI apparently is sensitive to only one component of starch - the amylose, the status of starch quality in beans needs to be pursued so that efficient techniques for estimation of starch content can be employed. Use of the near infrared reflectance spectroscopy utilizing Grain Quality Analyzer (GQA-41) appeared to be efficient in estimating starch content, at least as compared to the IKI starch score system, but it lacked the rapidity of the IKI starch score system.

From the inheritance studies of stem starch accumulation it was found that frequency distributions for all the segregating generations were continuous and serve to indicate that quantitative inheritance is important in determining differences in starch accumulation levels in the stems of dry bean cultivars at least for the populations studied. Transgressive segregation was indicated in both directions of low and high starch values. It is concluded that the parents probably differed in relatively few genes, with sufficient plus and minus gene effects present to account for transgressive segregation on all the three types of crosses

since all parental ranges and beyond were recovered in F_2 populations.

Although major genes were not detected, the moderately high broad sense heritability values obtained indicated that at least the stem starch content is genetically controlled, and more information is required on how much of this heritability can be used to identify desirable lines in a cultivar development program. The low x high and high x low crosses indicated that a substantial fractional variability of stem starch accumulation was additive in nature. Although results of crosses between low x low and high x high were rather intriguing, the nature of inheritance of stem starch accumulation as revealed in the current study suggests the use of recurrent selection as means of concentrating favorable alleles in a breeding population.

As noted in a previous section, the study of starch accumulation is a rather complex subject. The accumulation of stem carbohydrates as a whole results from a phase difference between the ability of the plant to produce carbohydrate and the requirement for it (assimilate sinks). Thus, although cultivars have been reported to show differential starch accumulation in stems and roots, some of these differences, in addition to genetic reasons, could be due to differences in the sink size. That is, if a cultivar has more pods much of the starch or stem carbohydrates will be reallocated to seedfilling. On the other hand, presence of fewer pods (small sink size), will mean much of the stem carbohydrates

will remain unallocated. Hence, accumulation in this case will be due to slower removal of photosynthate from the production or storage sites by translocation. Worse still, these differences can occur within a plant depending on what section of a plant is sampled. Waters et al, (1980) reported a decline in concentration of starch in the middle and upper sections of bean stems where most of the pods concentrated, whereas the concentration was raised in the lower sections of stems with few pods as pod fill proceeded. This emphasizes the consistency of the section of the plant that is sampled for study.

On the contrary, some varieties may not divert their nonstructural carbohydrate in storage to the reproductive organs because their leaf photosynthetic rate increases to match or else to exceed seed sink demand. Peet et al, (1977) showed that the leaf photosynthetic rates increased by 132, 408, 873 and 21 percent, from flowering to early pod set respectively, for Redkote, Redkloud, Swedish Brown, and Black Turtle Soup. So most of the NSC in storage for these cultivars might be of use under stress conditions. In fact, Kabonyi (1981) indicated that Black Turtle Soup appeared to be the best remobilizer of NSC in a stress situation.

Beevers (1969) discussed the biosynthesis of starch. There is indication that when provided as uridine diphosphate-glucose or adenosine diphosphate-glucose units of glucose are added to pre-existing starch or smaller molecules by starch synthetases. Starch may also be synthesized directly

from sucrose. Some cultivars may not store NSC in the form of starch, they may store it in the form of sucrose or other NSC polysaccharides. The fact that this study has singled starch accumulation does not mean that other constituents of NSC are not important. In actual fact, any form of NSC which would be the abundant carbohydrate reserve in any cultivar at any stage in the plant's ontogenetic development might serve as the source of the carbohydrate to be translocated to needy sinks, either under stress or normal growth when sink demand exceeds current photosynthate production.

APPENDIX

APPENDIX

Table 1. Multiple linear regression analysis for K-values for calibration of the neotec Grain Quality Analyzer (model 41) using Hal Stat 4 program.

```
TITLE BEAN STARCH
FILEBUILD,FREE,NV=5,DATIN=SGQA
LI=A,BB,C,D,BEAN STARCH

FIRST CASE

          A           BB           C           D    BEAN STARC
          1            2           3           4            5
   -.002450    -.004900     .012140     .003310     3.290000

* * * DATA INPUT TERMINATED BY END-OF-DATA CARD

NUMBER OF CASES READ   43  DROPPED    0  AND RETAINED   43

  BEAN STARCH
SINGLE PRECISION FILE OF   43 CASES AND   5 VARS. CREATED ON D27/M12/82

                                                          SUM OF   NON-MISSING
            VAR     MINIMUM     MAXIMUM        MEAN      SQUARES  ELEMENTS

          A   1       -.004        .002        -.002        .000         43
         BB   2       -.007       -.003        -.005        .001         43
          C   3        .009        .018         .014        .008         43
          D   4        .002        .004         .003        .000         43
BEAN STARC    5        .890      16.440        5.639     2013.925        43

ICMATRIX

  BEAN STARCH
SINGLE PRECISION FILE OF   43 CASES AND   5 VARS. CREATED ON D27/M12/82

                        1ST CASE RETAINED

          A           BB           C           D    BEAN STARC
          1            2           3           4            5
   -.002450    -.004900     .012140     .003310     3.290000

NUMBER OF CASES READ   43  DROPPED    0  AND RETAINED   43

        T A B L E  A  - -  STATISTICS ON TRANSFORMED VARIABLES

                                                    SUM OF SQUARED
          STANDARD                        SUM OF     DEVIATIONS
VAR     DEVIATIONS          SUM          SQUARES  FROM THE MEAN

  1       .001670       -.0754          .0002          .0001
  2       .001072       -.2012          .0010          .0000
  3       .002468        .5853          .0082          .0003
  4       .000649        .1349          .0004          .0000
  5      3.923906      242.4700       2013.9245       646.6756

                      SIMPLE CORRELATIONS

          VAR NO.
```

Appendix Table 1. (Continued)


```
          A    1     1.00000
         BB    2      .51798     1.00000
          C    3      .12027     -.70082     1.00000
          D    4     -.83376     -.42651     -.14042     1.00000
BEAN STARC     5     -.19474     -.68728      .59600     -.10139     1.00000
                        1           2           3           4           5
                        A          BB           C           D BEAN STARC

  BEAN STARCH
DOUBLE PREC MATRIX FOR      43 CASES AND    5 VARS. CREATED ON D27/M12/82


LS
S=P(1,2,3,4)RES

  BEAN STARCH
DOUBLE PREC MATRIX FOR      43 CASES AND    5 VARS. CREATED ON D27/M12/82

          SIMPLE CORRELATIONS OF X(1) WITH ALL OTHER VARIABLES.
                (THESE VALUES MAY BE FOUND IN THE X(1)
                 ROW AND COLUMN OF THE INPUT MATRIX.)

          A          BB           C           D BEAN STARC
          1           2           3           4           5
   1.000000    .517984     .120271    -.833761    -.194743

                  DEPENDENT VARIABLE--X(5) BEAN STARC

                       ADJ FOR OVERALL REGRESSION

                          SUM OF      DEG OF        MEAN
                         SQUARES    FREEDOM      SQUARE          F    SIG

REGRESSION              472.6913           4      118.2228   25.8508 <0.0005
(ABOUT MEAN)

ERROR                173.78430572          38       4.5733
(ABOUT MEAN)

TOTAL                646.67564106          42

 CASES                MULTIPLE CORR COEFS                STANDARD ERROR
                 R2          .R        R BAR 2     R BAR      OF ESTIMATE
    43          .7312       .8551        .7030     .8384        2.1385203

          REGRESSION       STD. ERRORS          BETA     STD ERRORS
VAR      COEFFICIENTS   OF COEFFICIENTS       WEIGHTS      OF BETAS

  0       7.08218827       3.26688791       0.00000       0.00000
  1   -1001.97907872     458.74661891       -.42686        .19535
  2   -3755.37631583     827.14458941       -.91628        .20188
  3    -134.75987936     310.29512546       -.11619        .19514
  4   -5225.00743285     930.37279829       -.84411        .15031
```

Appendix Table 1. (Continued)

	VAR	TB	FB	SIG	CORR COEFS	DELETES
CONSTANT	0	2.1679	4.6997	.036	.3318	.6980
A	1	-2.1863	4.7797	.035	-.3343	.6975
BB	2	-4.0566	16.4558	<0.0005	-.5497	.6149
C	3	-.5954	.3545	.555	-.0961	.7288
D	4	-5.6160	31.5399	<0.0005	-.6735	.5082

RM ERROR 110 ON LFN DATA
FILE IS NOT OPEN.

RC = 0B,ADDRESS= 22735

BEAN STARCH
SINGLE PRECISION FILE OF 43 CASES AND 5 VARS. CREATED ON D27/M12/82

R E S I D U A L S

CASE	BEAN STARC Y = X(5)	ESTIMATED Y	Y - ESTIMATED Y
1	3.2900000	6.4429739	-3.1529739
2	5.2200000	6.9142518	-1.6942518
3	10.2800000	11.0527812	-.7727812
4	9.2300000	9.2515501	-.0215501
5	8.6700000	9.6330161	-.9630161
6	5.8200000	7.3959583	-1.5759583
7	16.4400000	12.7271453	3.7128547
8	15.0600000	11.1558600	3.9041400
9	13.7300000	10.9619794	2.7680206
10	12.8600000	9.1366152	3.7233848
11	13.1200000	10.9291641	2.1908359
12	8.9000000	9.0321835	-.1321835
13	4.6800000	9.0863533	-4.4063533
14	3.9100000	6.9066370	-2.9966370
15	4.0200000	7.8539412	-3.8339412
16	4.4400000	6.4326937	-1.9926937
17	2.8400000	3.7000394	-.8600394
18	6.2300000	7.4121489	-1.1821489
19	4.6500000	5.5706205	-.9206205
20	4.4000000	4.5642530	-.1642530
21	4.3500000	6.5560706	-2.2060706
22	8.6100000	4.8497352	3.7602648
23	8.2300000	6.4730652	1.7569348
24	4.5100000	4.2611823	.2488177
25	6.7800000	8.4931410	-1.7131410
26	3.5000000	1.9059187	1.5940813
27	4.6800000	3.3270573	1.3529427
28	3.9600000	2.4471251	1.5128749
29	2.7000000	2.4420625	.2579375
30	2.4900000	.9860227	1.5039773
31	1.1000000	-.9745289	2.0745289
32	5.7900000	3.3603502	2.4296498
33	3.3200000	3.2283263	.0916737
34	2.2800000	2.0224668	.2575332
35	2.8000000	3.9385724	-1.1385724
36	4.9900000	5.4175214	-.4275214
37	1.8600000	2.6831720	-.8231720
38	2.3000000	2.8970701	-.5970701
39	1.1400000	.7[illegible]4357	.3975643
40	3.9600000	3.33[illegible]3660	.6219340

Appendix Table 1. (Continued)

41	1.1700000	3.1067735	-1.9367735
42	3.2700000	3.8553736	-.5853736
43	.8900000	.9528546	-.0628546

NUMBER OF CASES READ 43 DROPPED 0 AND RETAINED 43

SUM Y		SUM RES
242.47000000		.00000000
SS OF Y	SS(Y - MEAN Y)	SS RES
2013.92450000	646.67564186	173.78430572
PROP. OF VARIATION EXPLAINED BY COEFS	DURBIN WATSON STATISTIC	SUM OF SQUARES (RES - PREV RES)
.73126511	.87165575	151.48008900

END STAT

READY 15.33.02
CATALOG.SG0A.JOF30.
CATALOG.SG0A.JOE30.

READY 15.34.01

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