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Starch Grain Characters, Amylose, and Amylo-Pectin Contents in Relation to Pollination Time and Formation of Cotyledons in Phaseolus Lunatus L. and Phaseolus Polystachyus L.

Amrik Singh Dhaliwal
Utah State University

STARCH GRAIN CHARACTERS, AMYLOSE, AND AMYLO-PECTIN CONTENTS IN RELATION TO POLLINATION TIME AND FORMATION OF COTYLEDONS IN *PHASEOLUS LUNATUS* L. AND *PHASEOLUS POLYSTACHYUS* L.

by

Amrik Singh Dhaliwal

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Amrik Singh Dhaliwal

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INTRODUCTION

Beans are an important source of carbohydrates, proteins, minerals, and vitamins for millions of vegetarians in the world, especially in Asia. The quality and harvesting time of beans depend upon the purpose for which the beans are used. For example, for canning and freezing the beans should be harvested when they are neither starchy nor watery but should be sugary. The processing industry in Asia is not highly developed. Hence, the food is usually preserved by sun drying. Sun dried beans are often consumed the year around.

Pod setting is a problem in lima beans and in various other crops. One of the most important studies on fruit setting of lima beans was made by Lambeth (11) who classified unfruitfulness as associated with various internal and external factors. In addition to the internal factors, it has been recently established that certain other physiological factors such as internal nutritive conditions, plant growth regulators, and chemical composition influence fruitfulness. Successful pollination and fertilization require favorable environmental conditions which influence the ripening of the crop. The harvesting time is directly related to the pollination time--the earlier the pollination, the earlier the harvest. However, in most of the varieties of lima beans indeterminate types of pod sets have been observed. In addition, the number of pod sets depends upon the night temperature and relative humidity (17). Under such conditions the quality and chemistry of beans are not recognized.

The starch of beans is often considered as a single substance which is made up of two components, namely amylose and amylo-pectin. Amylose content is considered to be a more important constituent of starch than amylo-pectin, because of its industrial uses in the manufacturing of edible casings, in the paper industry as a binding agent, and in textiles as a permanent finish where other hydroxylated polymers have been used (23).

Genera and species differ in their contents of amylose and amylopectin as well as in many other important characteristics which, if present in one, could be of great value to growers. This encourages breeders and geneticists to endeavor to improve their cultivated species by making inter and intra-specific crosses.

The purpose of this investigation was to present the studies on the starch grain characteristics (size, shape, hylum, and lamella development), amount of amylose, amylo-pectin and total starch in relation to pollination time and size of beans in certain varieties of P. lunatus L., P. polystachyus L., and F_1 cross (P. lunatus L. var. Fordhook X P. polystachyus L.).[1]

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[1]Cross was kindly made by Dr. A. P. Lorz, Horticulturist, University of Florida, Gainesville, Florida. In further discussion instead of mentioning P. lunatus L. var. Fordhook X P. polystachyus L., there would be mentioned P. lunatus L. X P. polystachyus L.

REVIEW OF LITERATURE

The literature on starch contains more than 6,000 references, but only a fraction of them pertaining to lima beans is being cited in this thesis.

Blossom drop in lima beans

Davis (7) found that high temperature influenced the set of pods more than any other factor and the percent of pod set could be predicted from maximum temperature with fair accuracy. The study of this nature could be of great value in determining what areas might be best suited for crops with a critical environmental requirement.

Wolf (33) studied 66 good and poorly set Henderson lima bean vines for nitrate, nitrogen, available phosphorous, potassium, calcium, and magnesium contents. Well set plants had higher concentrations of nitrate nitrogen, potassium, available calcium, and magnesium but less available phosphorous as compared to poorly set plants. Seed set was closely related to the concentration of nitrate nitrogen and available calcium in the main stems.

The study of Cordner (6) showed that the blossom abscission in lima bean variety Henderson Bush was associated with high air temperature and a dry atmosphere. The critical time was in the early part of the blooming period of the raceme. Racemes that failed to set at that time were characterized by a low final set.

Inter and intra-specific crosses

Buisland (5) explained that in crosses of Phaseolus species with

P. vlugaris failure might occur at three stages of development: (a) If part of style or stigma were injured in crossing, the flower was dropped within 2 to 3 days. (b) In many cases pods attained the size of 2 to 3 cm. but they were dried up afterwards. (c) Many times it happened that the pods practically reached the normal length but they did not produce seeds. With P. dumosus a number of pods were produced with normally coated seeds but no contents (5). Likewise, Honma (10) reported a cross between P. vlugaris and P. acutifolus. Crossed flowers did set pods but none of them reached maturity. The abortion of the embryos occurred within 24 days following pollination. F_1 plants were obtained by culturing the excised embryos in vitro. The reciprocal cross using P. acutifolius as a female parent was not successful. The object of this cross was to transfer a character of common bean blight resistance from P. acutifolius var. Tepary to the P. vulgaris var. Great Northern.

Wester and Jorgensen (32) in crossing study with limas concluded that controlled pollination without emasculation was more successful than conventional methods of making crosses. Hundred percent success was possible in non-emasculated flowers as compared to emasculated flowers.

Gates (9) investigated that the epigeal and hypogeal germinations in P. vulgaris and P. multiflorus, respectively, possessed a pair of interspecific genes. He had no evidence as to why the zone of growth was above the attachment of cotyledons in one species and below it in the other. Wall and York (31) found that the cotyledons position on central axis of Phaseolus seedlings was controlled by multiple factors and there was no evidence of dominance.

Lorz (12) attempted the P. polystachyus L. X P. lunatus L. cross 50 times with no success. The reciprocal cross was made 100 times which resulted in only 7 F_1 plants. The object of this cross was to incorporate into the P. lunatus L. species the hypogeal germination of the P. polystachyus L. to solve the emergence problem in lima beans. Very often in a compact soil seedlings due to epigeal germination could not protrude through the soil with a heavy load of cotyledons and resulted in a low percentage stand of the crop. Hence, the cross was made in an attempt to avoid this difficulty.

According to Small (26) the habitate of P. polystachyus L. extends as far as Florida, Minnesota, Ontario, Maine, Texas, and Nebraska, so it seemed apparent that this root stock had some degree of cold resistance. Keeping this in view, Lorz (12) quoted that P. polystachyus L. might be of importance to the knowledge of plant breeders.

Breeding for high amylose content

Zuber, Grogan, Deatherage, Hubbard, Schulze, and MacMasters (34) studied two high amylose parents, ha_1 and ha_2 of corn, and F_1, F_2, and F_3 generations of the cross ha_1 X ha_2. The factors responsible for high amylose were not allelic in the two parents. It was possible to identify high amylose F_1 kernals very easily by selecting for a tarnished wrinkled grain character. F_3 ears gave amylose determination in the range from 25.4 to 70.3 percent. The amylose content of 70.3 percent was higher than both the parents.

Vineyard, Bear, MacMaster and Deatherage (29) concluded that the highest amylose content was the result of the various multiple recessive combinations of the genes affecting endosperm composition. On the contrary, the homozygous recessive condition of a single recessive gene,

wx, produced starch with 100 percent amylo-pectin content. They also analyzed 21 mutants consisting of genes which affected starch synthesis and evaluated for their effects on the amylose fraction of the corn starch. They pointed out that three of these, ae, su_2 and du, showed sufficient increase in amylose development.

Bear, Vineyard, MacMasters, and Deatherage (3) reported that the contents of amylose and amylo-pectin present in corn was 1 and 3 percent respectively. This ratio of amylose content of the starch was increased to 82 percent by U.S.D.A. researchers from crosses by combining two genes each known to increase amylose. In addition they found that the high amylose corn had starch granules smaller in size and irregular in shape than regular corn.

Chemistry of starch

Frey-Wyssling (8) reported that amylose, which has linear molecules, and amylo-pectin, which has branched molecules, gave different colorations with iodine. Longer amylose chains gave typical blue iodine color and molecules with branched chains produced red or even brown coloring with iodine. It was believed that these colorations were due to the fact that iodine molecules were arranged along the center of the chain helix.

McCready and Hassid (13) described a method for separation of potato starch into amylose and amylo-pectin which was different in comparison to the method described by McCready et al. (14). They also emphasized that the enzymatically synthesized starch resembled the amylose fraction of potato starch in its properties.

Waldt and Kehoe (30) found that in general starch occurred in the form of white granules. Each granule was made up of two polymers, amylose and amylo-pectin. It was insoluble in water and resistant to

naturally occurring hydrolytic agents. The two polymers were different in molecular weight and chemical structure. Amylose (linear polymer) consisted of 200 to 1000 glucose residues in a single long molecule linked together by oxygen bridges, whereas the amylo-pectin (branched polymer) was made up of chains of 1500 glucose residues.

Rahman (16) found that commonly the quality of lima beans decreased as the stage of maturity increased. The most practical method by which quality of lima beans could be evaluated was the refractive index which was mentioned by Rahman (16).

Starch grains under microscope

Bonner (4) indicated that starch was stored in large amounts in many seeds, fleshy tubers, roots, woody twigs, leaves, and many micro-organisms in the form of grains, 1 to 150 microns in diameter depending on the plant source.

He further explained that the starch granules under the microscope were minute structures built up of molecules arranged in layers, usually concentrically. The shape of starch granules from different plants also varied from small polygons (corn) to large spherical granules (wheat and rye). The hylum was usually a dark spot located in the granule.

Van de Sande-Bakhuyzen (28) was the first who described that the lamellae formation in the starch grains was due to the external environmental conditions. Working on wheat, he found that when the external conditions, such as illumination and temperature, were constant lamellae of wheat starch grains did not occur. Roberts and Proctor (18) investgated that starch grains formed in potato tubers from the plants grown under constant light and temperature (63° F.) showed lamellation clearly indistinguishable from starch grains formed in tubers grown under normal

field conditions.

Sjostrom (25) observed that species differ greatly in starch granules with respect to size, shape, and other characteristics. The rice starch granules were the smallest and their diameter was from 3 to 8 microns. The average corn starch granules were of medium size, 10 to 25 microns, but deviations in both directions were common. The tapioca starch granules were of about the same average size as corn starch granules. Sweet potato starch granules were similar to corn starch granules in appearance but they were larger; the average size was about 1 1/2 to 2 times that of the corn starch granules. The wheat starch granules varied in size. The largest granules had a diameter of 35 microns. He further stated that rye starch resembled wheat starch but the granules were usually larger and thicker. His further investigations on sago and potato starch showed that they belonged to the group of large starches. The granules varied in size; the largest were 50 to 60 microns and the smallest ones were 15 to 25 microns in diameter.

Barham, Wagner, Williams, and Reed (2) found that varieties differed in starch grain size, structure, and their other properties like gelatinization and viscosity. Furthermore, they explained that starch grain size and structure might be influenced by environmental factors.

Salunkhe and Pollard (20) investigated a rapid and quick method to determine the stage of maturity and quality of lima beans by microscopic examination of starch grains. They found that as the size of beans increased, the starch grains' size increased and the hylum of the starch grains became larger, thicker, and progressively ramified. When the hylum of the starch grain began to thicken along with its ramification, then it might be estimated that the field was ready to harvest without

much loss in quality. Salunkhe and Pollard (21) in further studies reported that various groups of sizes of starch grains in different varieties of lima beans had different structures. They further found that elongation of the hylum always occurred in the direction of longer axis of the starch grains. Salunkhe and Pollard (19) also noted that the starch grains in the potato tubers having high specific gravity showed more distinct lamellae and hyla than those possessing low specific gravity. In addition, their investigations showed that the lamellae and hyla of the starch grains in early plantings were more distinct than those from late plantings.

Sharma (24) found that various sizes of starch grains in potato tubers were related to the specific gravity. The starch grains above 75 microns and below 25 microns in diameter affected the specific gravity of the potatoes, but between 25 and 75 microns had no effect. That is the higher the percentage of large starch grains above 75 microns the higher the specific gravity and the higher the percentage of starch grains below 25 microns the lower the specific gravity of the potato tubers.

Alsburg (1) reported that mechanically injured starch grains swelled in cold water as much as non-injured grains increased in size in boiling or hot water.

MATERIALS AND METHODS

Five varieties of lima beans, namely Burpee Bush, Clark's Bush, Utah 16, Wasatch Bush, and Concentrated Fordhook of species P. lunatus L. were planted at Utah State Experimental Farm at Farmington, Utah, on June 1, 1958. The plots were 32 feet long and 2 1/2 feet wide. The crop was well grown. Weedings and irrigations were done at the interval of 7 days throughout the growing season.

Sixty-six days after seeding, opened flowers of each variety were tagged (August 6, 1958). Flowers which were opened on a particular date and before noon were tagged. Similarly, at 7-day intervals, the flowers were tagged on August 13, 1958, and August 20, 1958. As the beans of the species P. polystachyus L. and of F_1 Cross were kindly obtained from Dr. A. P. Lorz, Horticulturist, University of Florida, Gainesville, Florida, they were not tagged at these dates of pollination.

The pods formed were harvested at one time at full maturity on October 10, 1958. Number of days and degree days (27) were calculated for lima bean plants for each pollination time as shown in appendix table 8. They were computed according to the technique established by Seaton (22).

$$\text{Degree days} = \frac{\text{maximum temperature} + \text{minimum temperature}}{2} - 50^{\circ}\ \text{F.}$$[1]

The harvested beans from each date of pollination were classified into three groups by visual observations (figure 1). These groups were

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[1]The 50° F. base line temperature below which growth in lima beans does not take place has been established by Seaton, H. L. Metal Division Research and Development Department, Continental Can Company, Inc. 1350 West 76th Street, Chicago 20, Ill.

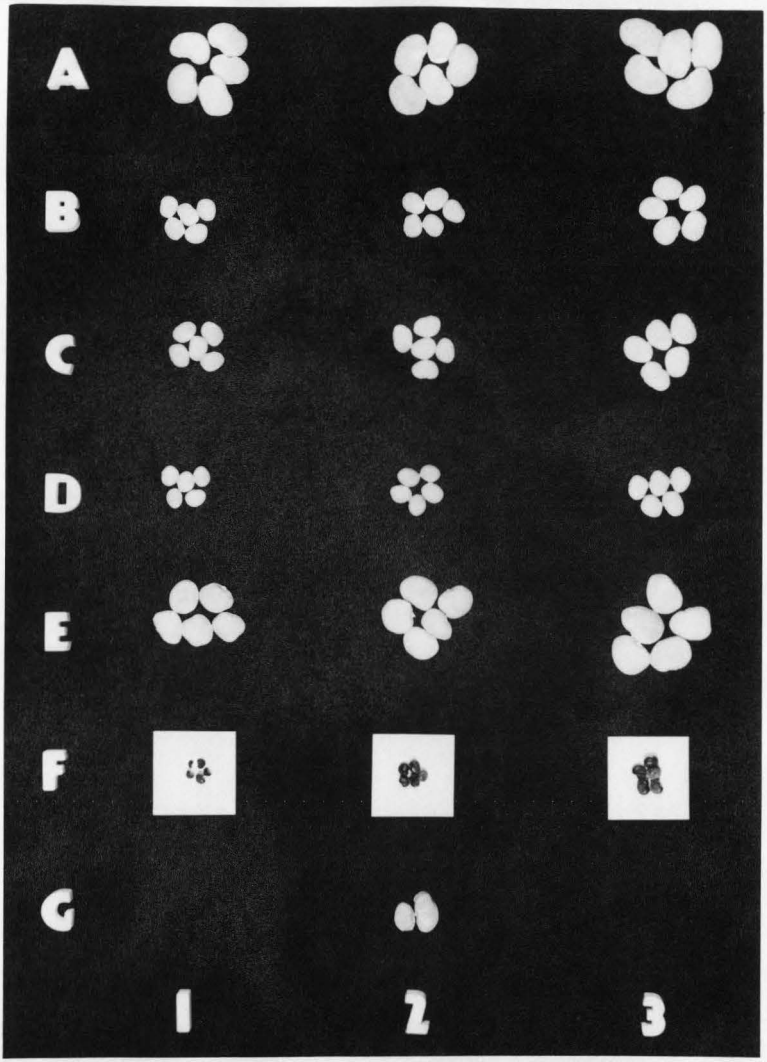


Figure 1. Arbitrary size classification of beans of Phaseolus species. Species and varieties: A = Burpee Bush; B = Utah 16; C = Clark's Bush; D = Wasatch Bush; E = Concentrated Fordhook; F = P. polystachyus L.; and G = F_1 Cross--P. lunatus L. x P. polystachyus L. Size of beans: 1 = small beans; 2 = medium beans; and 3 = large beans.

small, medium, and large. In each classification five beans were selected. All the beans in each group were dried at 158° F. for 24 hours and each individual bean was weighed separately on a gram scale to two decimal places. In addition to this, the beans from species P. polystachyus L. were also classified into three groups, as mentioned above, with 5 beans in each group regardless of dates of pollinations. Seeds of this species were very small and, therefore, could not be weighed individually. All 5 beans were weighed together in each group of classification. As the supply of species Cross F_1 beans was limited, one bean was taken at random from 10 beans for study.

Dried and weighed beans were individually ground with a pestle and mortar. The ground material with a drop of distilled water on a slide was studied under the microscope for size, shape, hylym (hilum) and lamella development of starch grains with a combination of an eye piece 10X and an objective 43X. The characteristics, shape, hylum, and lamella were further classified into 2 to 3 sub-classes. They were: for shape--circular, oblong, and irregular; for hylum--unhylumed and hylumed; for lamella--unlamellated and lamellated starch grains. In each microscopic field of a slide only one starch grain was considered as a part of the observation. In total, 45 seeds and 4500 starch grains in each variety were examined for the specific characteristics mentioned above.

Starch analysis

Amylose, amylo-pectin, and total starch were determined according to the procedure standardized by McCready and co-workers (14), which is summarized below.

Extraction of sugars.--About 0.2 gram of flour from the beans was weighed in the centrifuge tubes and washed thoroughly with 80 percent

alcohol to remove sugars. The sugar-free residue was stirred into 6.5 ml. of 52 percent perchloric acid and 5 ml. water, while the tubes were kept in ice cold water. Then 20 ml. of water were added. This solution was stirred and then centrifuged. Similarly, this procedure was followed three times to be certain that both amylose and amylo-pectin were extracted from the residue.

Determination of amylose.--Five ml. of the sugar-free starch solution was diluted with 495 ml. of water. Five ml. of Iodin-potassium Iodide reagent was added to it and mixed thoroughly. After about 15 minutes the determination of the intensity of the blue colors were made with light of wave length near 6600 Å. in a Baush and Lomb colorimeter.

Determination of starch.--Five ml. of starch solution was diluted to 500 ml. Five ml. of the diluted solution was put into borosilicate glass tubes, cooled in a water bath, and 10 ml. of fresh anthron reagent were added into them and thoroughly mixed. The tubes were heated together for 8 minutes at 212° F. and cooled rapidly to 77° F. in a water bath. After this process the color intensities were determined using light of wave length near 6300 Å.

The data were analyzed statistically following the methods described by Ostle (15).

RESULTS AND DISCUSSION

The present study was conducted to find out if there was difference in starch grain characteristics, amylose, and amylo-pectin contents for 3 size groups of beans and 3 subsequent pollinating dates in species and varieties of genus phaseolus L. The results obtained during the course of this study are presented and discussed below.

Burpee Bush variety

This is a large seeded variety which grows well under Utah conditions. Table 1 shows that in general weight of the bean and size of the starch grain varied from 1.38 to 0.62 grams and 34.82 to 21.46 microns respectively. Oblong, slightly hylumed, and lamellated starch grains were more common than irregular, unhylumed, and unlamellated starch grains, irrespective of the size groups of beans and dates of pollination. Unhylumed starch grains were rare in this variety, but slightly hylumed starch grains were commonly found. Salunkhe and Pollard (20) found that the maturity could be determined by examining the hylum of the starch grains. They noted that as the maturity advanced the hylum of the starch grain began to thicken and was ramified. Results obtained in this experiment did not agree with their results, as it is clear from table 1 and figure 2 which show about 75 percent slightly hylumed starch grains from fully matured beans. Hence, it seems that the hylum development may be a varietal character. It is, however, well known that the hylum development is a generic character.

Size of the starch grains was closely associated with weight and

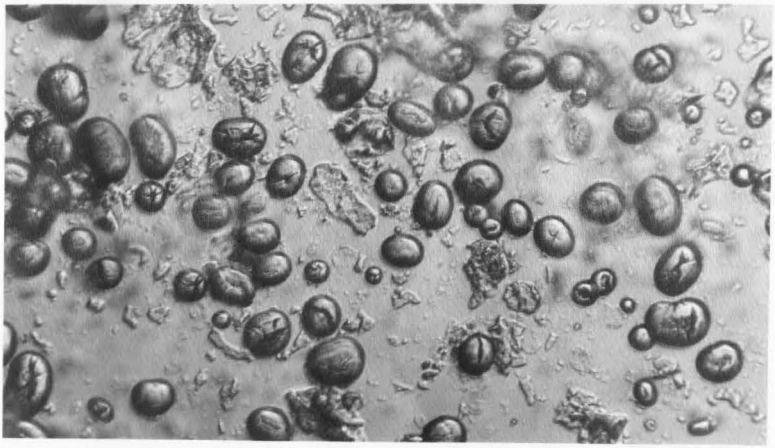
Table 1. The effect of pollination time, size, and weight of beans on the size, shape, hylum, and lamella development of starch grains in Burpee Bush variety

Pollination time	Size[a] of beans	Weight of beans	Size of starch grain	Shape of starch grains			Hylum[b] of starch grains			Lamella[c] of starch grains	
				Circular	Oblong	Irregular	Unhylumed	Slightly hylumed	Highly hylumed	Unlamellated	Lamellated
		grams	microns	percent	percent	percent	percent	percent	percent	percent	percent
Aug. 6, 1958	Large	1.38	34.82	44.2	43.8	12.0	0.6	77.2	22.2	3.6	96.4
	Medium	0.90	30.80	38.6	47.2	14.2	4.6	80.4	15.0	15.0	85.0
	Small	0.76	30.75	44.0	45.0	11.0	0.6	85.2	14.2	4.0	96.0
Aug. 13, 1958	Large	1.24	35.23	37.0	51.0	12.0	1.4	68.2	30.4	7.4	92.6
	Medium	0.78	29.64	45.5	45.5	9.0	0.0	80.0	20.0	0.2	99.8
	Small	0.62	27.64	31.0	61.0	8.0	0.0	73.2	26.8	1.2	98.8
Aug. 20, 1958	Large	1.23	31.29	31.4	56.2	12.4	0.0	62.2	37.8	1.2	98.8
	Medium	0.80	24.26	38.8	49.2	12.0	1.0	67.0	32.0	21.4	78.6
	Small	0.68	21.46	39.2	52.8	8.0	0.0	79.5	20.5	29.0	71.0

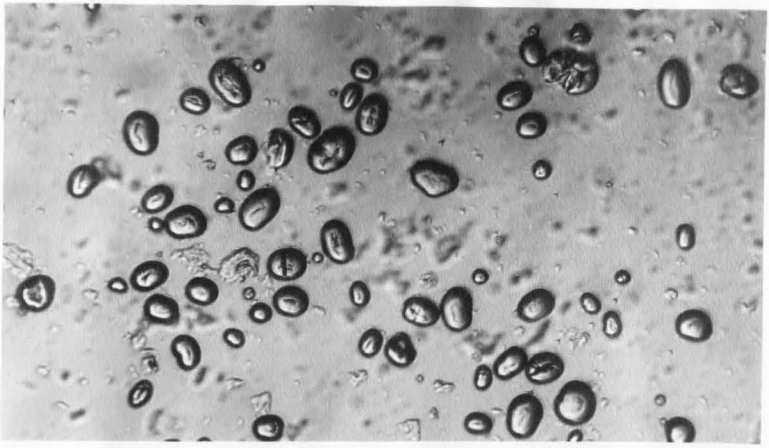
[a]Arbitrary classification.

[b]Hyla = The nuclei around which starch layers are deposited or perhaps the formation of nuclei as a result of progressive dehydration of starch grains as beans advance in maturity.

[c]Lamela = The concentric and/or eccentric layers of starch deposited around the hylum.



Starch grains (x 430) from large beans



Starch grains (x 430) from small beans

Figure 2. The effect of size of beans on starch grain characters in Burpee Bush variety, pollinated on Aug. 13, 1958. (Compare size, shape, and structural development of hyla of starch grains of large and small beans.)

size of beans. Sharma (24) observed the close association of size of starch grains with specific gravity of potatoes. Table 1 shows that in three dates of pollination the average size of starch grain was directly related to the size of bean. It can also be noted that with the advancement of the date of pollination the starch grain size decreased with respect to the weight and size of bean. This might be due to the availability of nutrition and heat units, where earlier formed beans received more than the latter formed beans.

The regression analysis in appendix table 9 clarifies that for the three pollinating dates the F values were significant at one percent level, which means that a large share of the variation of starch grains was due to regression. The r^2 values showed high percentage of variation in starch grain size which was accounted by weight of beans. The variations in starch grain size on three pollinating dates were 85.5 percent, 87.9 percent, and 81.7 percent respectively. These results can further be confirmed by figure 3, which was based on the regression equations $Y = 23.34 + 8.28\ X$, $Y = 20.67 + 11.68\ X$, and $Y = 9.12 + 18.34\ X$. In general, if the regression equation $Y = a + b$ was discussed, then "a" represents the population value of Y (starch grain size) when X (weight of the bean) is equal to zero. The "b" indicates the change which occurs in Y for a unit change in X. The quantities "a" and "b" are the sample estimates of the population parameters "A" and "B".

The comparison of "b" values in three pollination times can be seen in appendix table 16, which shows that b_1 (first pollination) was significantly lower than b_2 (second pollination) and b_3 (third pollination) at 5 percent and 1 percent levels respectively. The value of b_2 was significantly lower than b_3 at the 5 percent level. These progressively

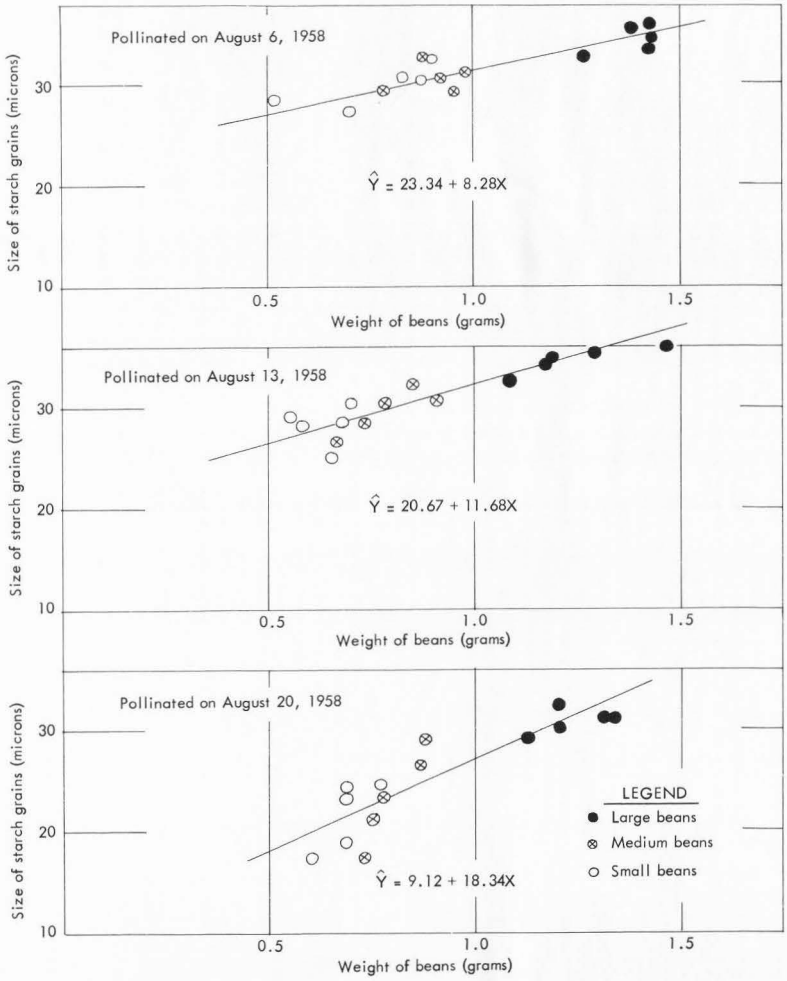


Figure 3. Relationship between weight and size of beans and size of starch grains in Burpee Bush variety, pollinated on August 6, August 13, and August 20, 1958

increased "b" values with advanced pollination times might be due to the effect of heat units, nutritive, irrigational, and other environmental factors which may have played roles during the development of the beans.

Utah 16 selection

This selection has small beans with white cotyledons. It may be observed in table 2 that size and weight of beans and size of starch grains was smaller in this selection as compared to the variety Burpee Bush.

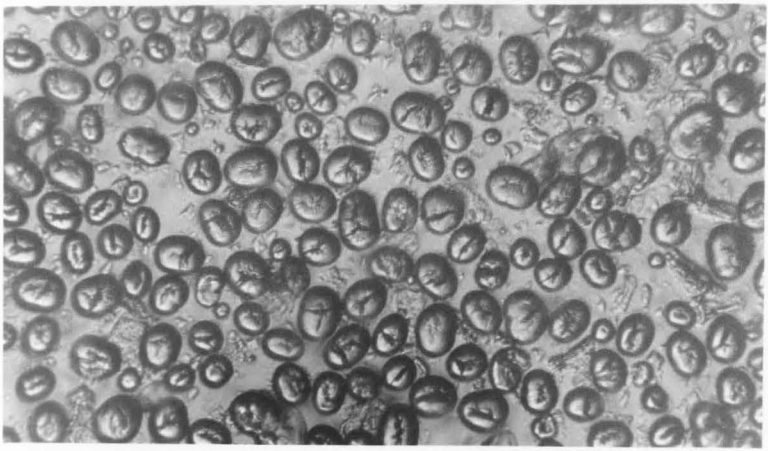
There was a direct relationship of weight and size of beans and the size of starch grains. The same pattern was followed in three pollination dates. It can also be noted that in each pollination date the size and weight of the bean affected the size of the starch grains in more or less the same manner as in the variety Burpee Bush, which conforms with findings of Salunkhe and Pollard (21). The shape, hylum, and lamella development of starch grains were also similar to the above mentioned variety.

The characteristics (shape, hylum, and lamella) of starch grains from figure 4 can be well compared with the starch grains of Burpee Bush variety in figure 2. The starch grain characteristics of these two varieties were identical except for size, which was smaller in Utah 16 selection than Burpee Bush variety.

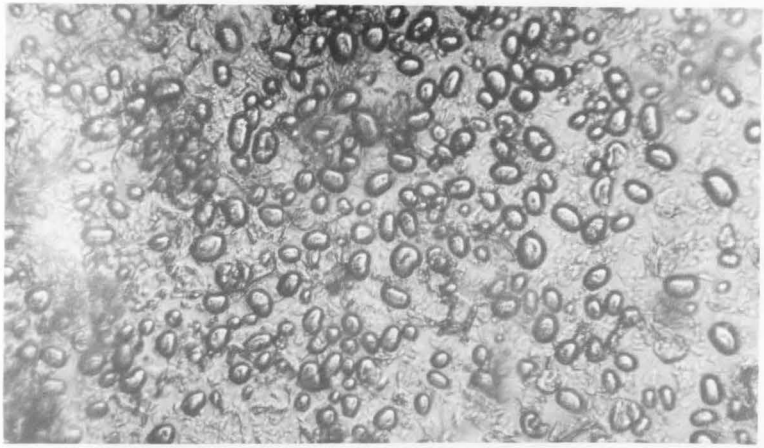
Figure 5 represents a good linear relationship between weight and size of beans and size of starch grains. The trend in the increase of size of starch grains per unit increase of weight of beans was the same in the three dates of pollination except for slight differences in the slope of the regression lines. The "b" values were 20.13, 24.16, and 23.75, which in comparison with each other were non-significant. The percentage of variation in starch grain sizes which was accounted by

Table 2. The effect of pollination time, size, and weight of beans on the size, shape, hylum, and lamella development of starch grains in Utah 16 Selection

Pollination time	Size of beans	Weight of beans	Size of starch grain	Shape of starch grains			Hylum of starch grains			Lamella of starch grains	
				Circular	Oblong	Irregular	Unhylumed	Slightly hylumed	Highly hylumed	Unlamellated	Lamellated
		grams	microns	percent	percent	percent	percent	percent	percent	percent	percent
Aug. 6, 1958	Large	0.72	30.29	57.0	36.8	6.2	0	49.4	50.6	8.0	92.0
	Medium	0.23	21.89	49.4	45.2	5.4	0	76.6	23.4	6.8	93.2
	Small	0.12	17.96	37.2	57.6	5.2	0	89.4	10.6	45.6	54.4
Aug. 13, 1958	Large	0.57	28.34	44.0	49.6	6.4	0	46.4	53.6	11.6	88.4
	Medium	0.31	24.08	33.2	58.8	8.0	0	59.4	40.6	24.0	76.0
	Small	0.21	19.01	28.8	63.4	7.8	0	50.0	50.0	41.4	58.6
Aug. 20, 1958	Large	0.46	26.57	38.8	55.6	5.6	0	36.4	63.6	15.8	84.2
	Medium	0.30	24.88	34.6	61.2	4.2	0	40.2	59.8	7.6	92.4
	Small	0.21	21.27	48.0	48.0	4.0	0	61.6	38.4	24.2	75.8



Starch grains (x 430) from large beans



Starch grains (x 430) from small beans

Figure 4. The effect of size of beans on starch grain characters in Utah 16 Selection, pollinated on August 13, 1958. (Compare size, shape, and structural development of hyla of starch grains of large and small beans.)

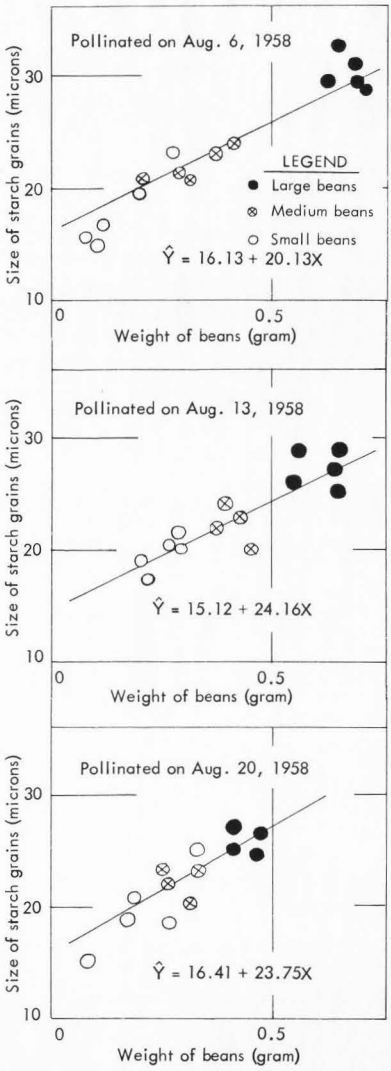


Figure 5. Relationship between weight and size of beans and size of starch grains in Utah 16 Selection, pollinated on August 6, August 13, and August 20, 1958

the weight of the beans in three dates of pollination were 90.7 percent, 87.8 percent, and 78.6 percent respectively, which can also be seen from appendix table 10.

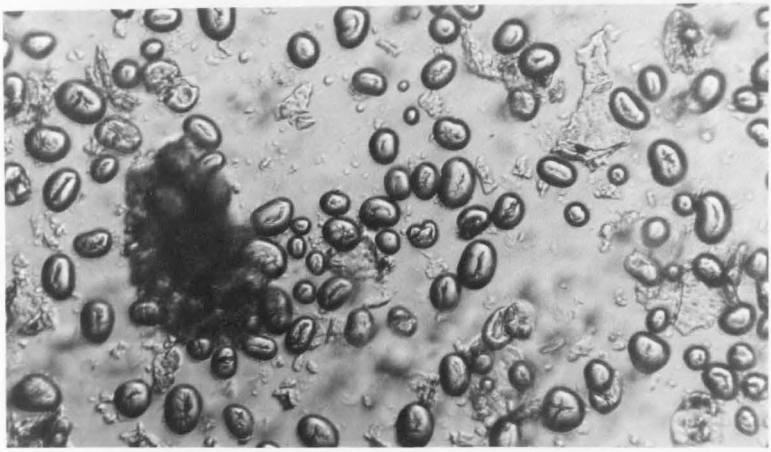
Clark's Bush variety

This variety is small seeded with green cotyledons. Table 3 shows that on the average the size of starch grains was similar to Utah 16. Oblong starch grains were more frequently present than other types. Unhylumed starch grains were absent. Unlamellated starch grains were almost absent in pollination date of August 6, 1958, but were present to the extent of 18 percent in the pollination dates of August 13 and August 20, 1958. Frey-Wyssling (8) explained that the lamellae were due to the alternation of day and night, with the dense, highly refrecting part of each layer deposited during the day. Van de Sande Bakhuizen (28) showed, if external conditions were constant, lamillation did not occur because nutritive material was then always available in the same concentration. On the contrary, Roberts and Proctor (18) investigated that starch grains formed in potato tubers from the plants grown under constant light and temperature (63° F.) showed lamellation clearly indistinguishable from starch grains formed in tubers grown under normal field conditions. Keeping the above discussion in view, the conclusion could be drawn that lamellae did occur in lima beans but were not visible under the microscope with the combination of 10X and 43X lenses, due to the lesser difference of refrective index of deposited starch during the day as well as night times in comparison to other varieties.

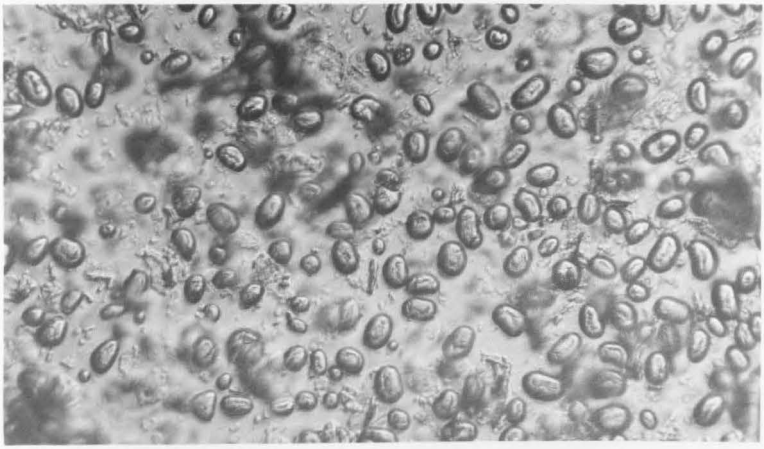
Figure 6 indicates that the difference in starch grain size of large and small beans was not remarkable but was quite proportionate to the variation in weight and size of beans.

Table 3. The effect of pollination time, size, and weight of beans on the size, shape, hylum, and lamella development of starch grains in Clark's Bush variety

Pollination time	Size of beans	Weight of beans	Size of starch grain	Shape of starch grains			Hylum of starch grains			Lamella of starch grains	
				Circular	Oblong	Irregular	Unhylumed	Slightly hylumed	Highly hylumed	Unlamellated	Lamellated
		grams	microns	percent	percent	percent	percent	percent	percent	percent	percent
Aug. 6, 1958	Large	0.59	25.60	27.4	67.0	5.6	0	60.4	39.6	7.0	93.0
	Medium	0.39	23.88	32.8	63.0	4.2	0	40.0	60.0	4.4	95.6
	Small	0.37	25.87	28.0	64.2	7.8	0	37.4	62.6	0.0	100.0
Aug. 13, 1958	Large	0.54	25.06	29.6	63.2	7.2	0	48.4	51.6	0.0	100.0
	Medium	0.39	22.85	28.8	66.6	4.6	0	43.8	56.2	1.6	98.4
	Small	0.28	19.30	35.0	58.8	6.2	0	70.4	29.6	23.8	76.2
Aug. 20, 1958	Large	0.47	23.81	27.8	62.4	9.8	0	35.2	64.8	7.6	92.4
	Medium	0.28	22.34	27.6	62.0	10.4	0	45.0	55.0	17.8	82.2
	Small	0.24	21.43	28.4	62.0	9.6	0	53.0	47.0	19.2	80.8



Starch grains (x 430) from large beans



Starch grains (x 430) from small beans

Figure 6. The effect of size of beans on starch grain characters in Clark's Bush variety, pollinated on August 13, 1958. (Compare size, shape, and structural development of hyla of starch grains of large and small beans.)

Appendix table 11 shows that in pollination date August 6, F value was not statistically significant. However, for pollination dates August 13 and August 20, 1958, F values were significant at 1 percent level and 5 percent level respectively. The same picture would be more clear by looking at the graphical presentation in figure 7. The percentage of variations in starch grain size which were accounted by weight and size of beans in three pollination dates were 6.7 percent, 62.8 percent, and 38.5 percent, which are presented by r^2 values in appendix table 11. The comparisons of degree of increase in starch grain size per unit weight of beans in three polination dates were not identical. Values for b_1 were significantly lower than b_2 at 1 percent level but b_1 vs. b_2 and b_2 vs. b_3 were not significant.

Wasatch Bush variety

Variety Wasatch Bush is small seeded with green cotyledons and was developed in Utah. The variation in weight and size of beans and size of starch grains was greater than Clark's Bush. Oblong, highly hylumed, and lamellated starch grains were common as compared to other characteristics (table 4). Figure 8 shows that hylum was highly ramified and generally formed along the long axis of the starch grains, which confirms the findings of Salunkhe and Pollard (21). However, in a few cases hylum was formed along the short axis, which did not agree with their findings. The starch grains of this variety were distinguishable from other varieties by the formation and prominence of hyla and lamellae (table 4 and figure 8).

Appendix table 12 shows that F values in the three pollination dates were significantly different at 1 percent level, which can be confirmed by looking at the scattered diagram and regression line (figure 7).

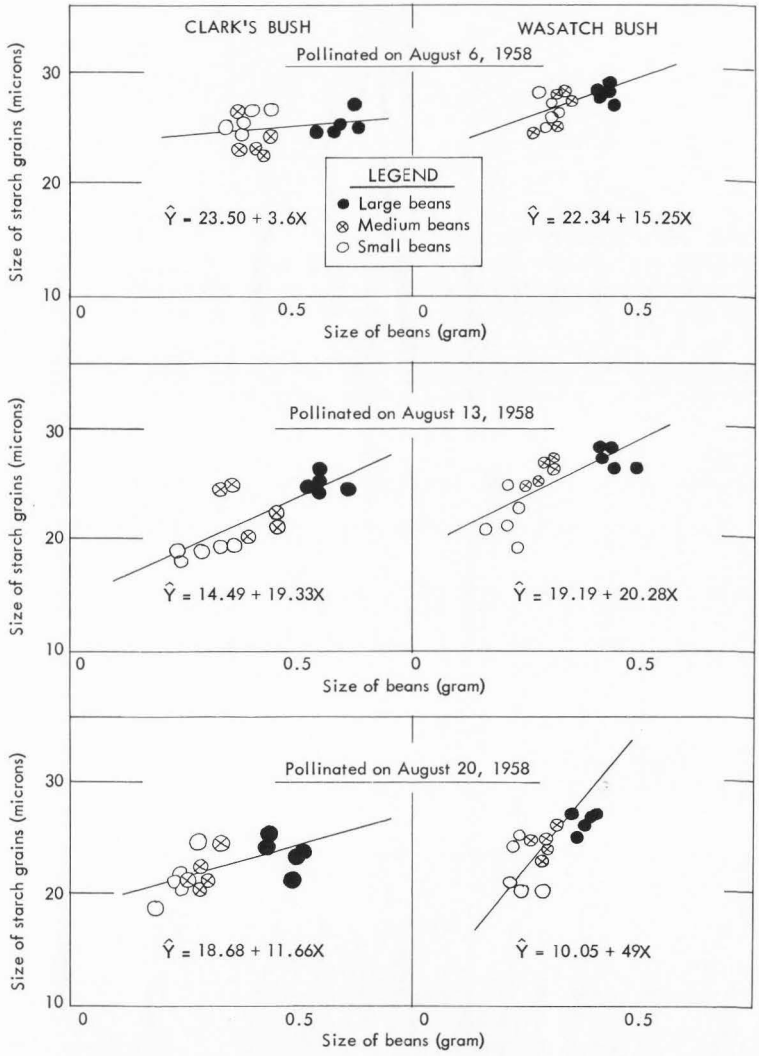
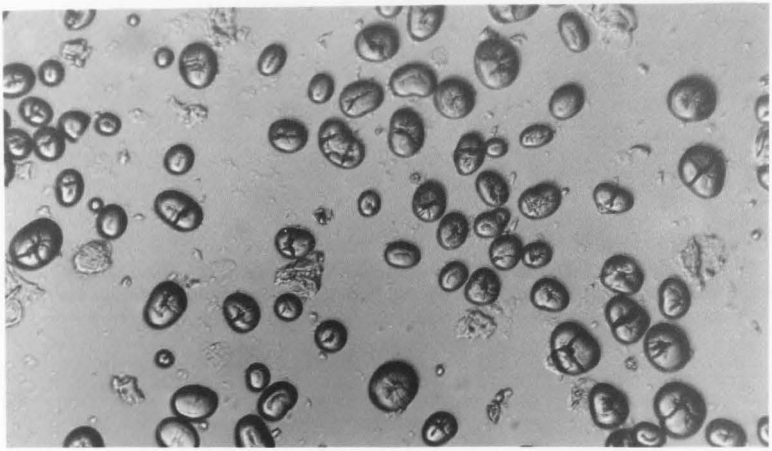


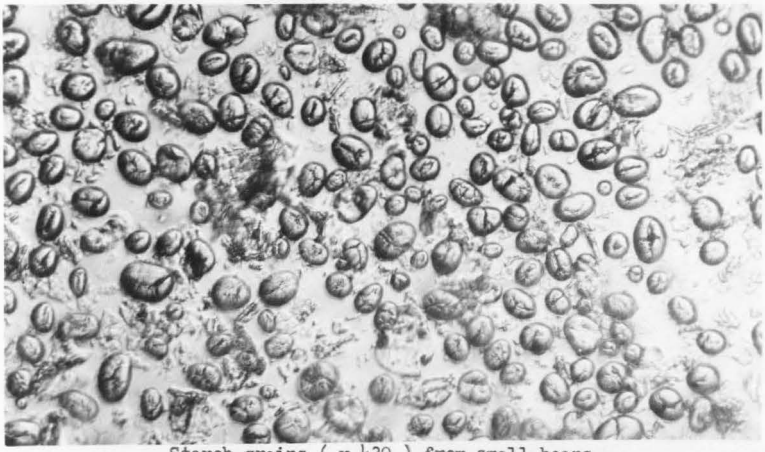
Figure 7. Relationship between weight and size of beans and size of starch grains in Clark's Bush and Wasatch Bush varieties pollinated on August 6, August 13, and August 20, 1958

Table 4. The effect of pollination time, size, and weight of beans on the size, shape, hylum, and lamella development of starch grains in Wasatch Bush variety

Pollination time	Size of beans	Weight of beans	Size of starch grain	Shape of starch grains			Hylum of starch grains			Lamela of starch grains	
				Circular	Oblong	Irregular	Unhylumed	Slightly hylumed	Highly hylumed	Unlamellated	Lamellated
		grams	microns	percent	percent	percent	percent	percent	percent	percent	percent
Aug. 6, 1958	Large	0.41	28.43	36.8	50.4	12.8	0	8.8	91.2	1.8	98.2
	Medium	0.31	27.35	26.8	57.4	15.8	0	20.0	80.0	3.4	96.6
	Small	0.29	26.76	24.4	63.0	12.6	0	11.4	88.6	0.4	99.6
Aug. 13, 1958	Large	0.43	27.59	40.4	52.4	7.2	0	20.8	79.2	0.6	99.4
	Medium	0.28	26.58	29.4	58.2	12.4	0	17.2	82.8	1.4	98.6
	Small	0.20	22.23	45.4	44.8	9.8	0	36.2	53.8	9.8	90.2
Aug. 20, 1958	Large	0.37	26.74	31.6	51.8	16.6	0	12.6	87.4	22.6	77.4
	Medium	0.28	24.96	34.4	50.8	14.8	0	25.4	74.6	13.6	86.4
	Small	0.24	22.53	37.6	48.0	14.4	0	22.2	77.8	29.2	70.8



Starch grains (x 430) from large beans



Starch grains (x 430) from small beans

Figure 8. The effect of size of beans on starch grain characters in Wasatch Bush variety, pollinated on August 13, 1958. (Compare size, shape, and structural development of hyla of starch grains of large and small beans.)

As the pollination date advanced the b and r^2 values increased. This means with the advancement of pollination dates the weight and size of beans and size of starch grains became closely associated. Hence, it was the character of the variety (2).

Appendix table 16 indicates that b_1 and b_2 were significantly lower than b_3 at 1 percent level but there was no significant difference between b_1 and b_2.

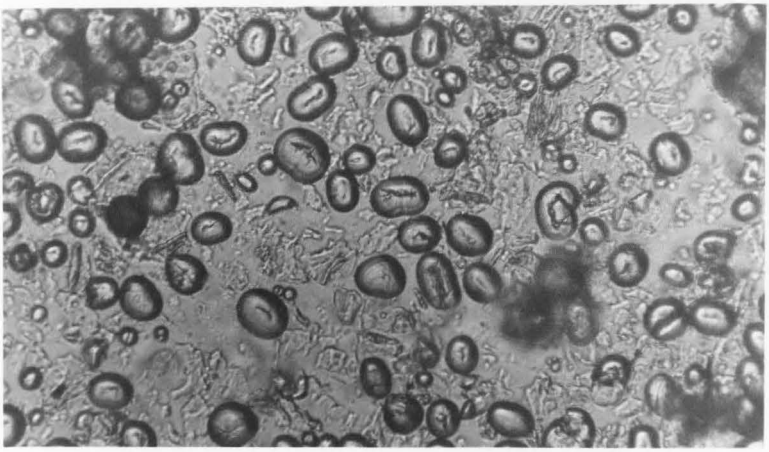
Concentrated Fordhook variety

This large seeded variety with white cotyledons was the selection from common Fordhook variety grown in eastern states of America. Table 5 shows that the size of starch grains was directly related to the weight and size of beans. That is, as the weight and size of beans increased, the size of their starch grains increased progressively (21). The size of starch grains was very nearly the same as the size of starch grains of Burpee Bush but greater than the size of starch grains of Utah 16, Clark's Bush, and Wasatch Bush. Oblong starch grains were common (21). Slightly hylumed starch grains were greater in number than highly hylumed starch grains. Figure 9 indicates that the hylum development of the starch grains also occurred along the short axis, but the majority of the hylum development was found along the long axis.

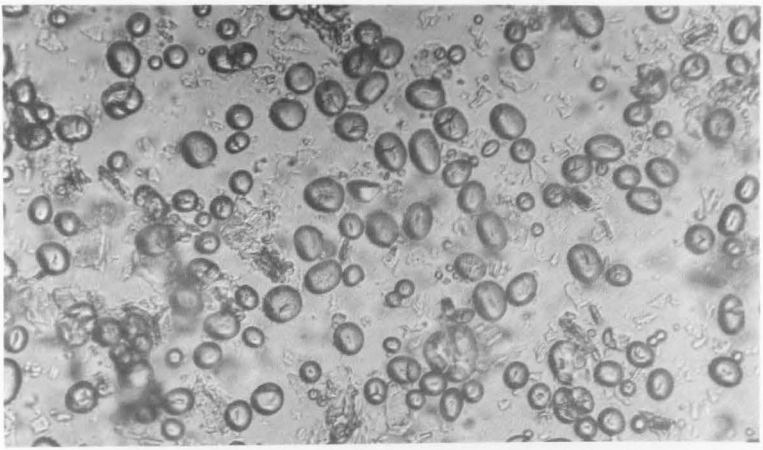
The regression analysis of appendix table 13 shows that the F value in first date of pollination was significant at 5 percent level but the F values in other two dates were significant at 1 percent level of significance. This shows that as the pollination time advanced the size of starch grains and weight and size of beans, like the variety Wasatch Bush, became more closely associated with each other. In other words, as the weight and size of beans increased, the size of starch grains

Table 5. The effect of pollination time, size, and weight of beans on the size, shape, hylum, and lamella development of starch grains in Concentrated Fordhook variety

Pollination time	Size of beans	Weight of beans	Size of starch grain	Shape of starch grains			Hylum of starch grains			Lamella of starch grains	
				Circular	Oblong	Irregular	Unhylumed	Slightly hylumed	Highly hylumed	Unlamellated	Lamellated
		grams	microns	percent	percent	percent	percent	percent	percent	percent	percent
Aug. 6, 1958	Large	1.50	30.66	35.4	55.4	9.2	0	85.0	15.0	0.0	100.0
	Medium	0.75	26.82	32.2	51.6	6.2	0	50.4	49.6	21.0	79.0
	Small	0.50	20.64	56.4	39.4	4.2	0	74.2	25.8	20.8	79.2
Aug. 13, 1958	Large	1.47	31.40	43.6	47.0	9.4	0	77.4	22.6	4.6	95.4
	Medium	0.78	27.25	31.2	58.2	10.6	0	69.4	30.6	4.8	95.2
	Small	0.60	23.93	47.8	45.2	7.0	0	80.2	19.8	9.4	90.6
Aug. 20, 1958	Large	1.50	30.66	35.8	55.0	9.2	0	85.0	15.0	0.0	100.0
	Medium	0.75	26.82	42.2	51.6	6.2	0	50.4	49.6	21.0	79.0
	Small	0.50	21.96	56.4	39.4	4.2	0	74.2	25.8	20.8	79.2



Starch grains (x 430) from large beans



Starch grains (x 430) from small beans

Figure 9. The effect of size of beans on starch grain characters in Concentrated Fordhook variety, pollinated on August 13, 1958. (Compare size, shape, and structural development of hyla of starch grains of large and small beans.)

also increased (figure 10). The percentage of variation in starch grain size which was accounted by the weight and size of beans in three pollination times was 40.2 percent, 72.6 percent, and 64.0 percent, which are indicated by r^2 values in appendix table 13.

Appendix table 16 shows that all the three combinations of b values were non-significant at 5 percent level. That is, the rate of increase in starch grain size per unit weight of beans was the same in three pollination times.

Phaseolus polystachyus L. species

P. polystachyus L. is commonly grown in southeastern states of America. It has purple or whitish corolla and scimitar shaped pods, about 4 to 8 centimeters long. The stem is of climbing habit. P. lunatus L. is grown widely in the United States. It has white corolla and broad scimitar shaped pods. P. polystachyus L. has hypogeal germination, whereas P. lunatus L. has epigeal germination (12).

Table 6 shows that the behavior of size of starch grains and other starch grain characteristics was similar to that of previously mentioned varieties of P. lunatus L. The weight and size of beans were smaller as compared to P. lunatus L. In general, the unlamellated starch grains were more frequent in this species than in P. lunatus L. Furthermore, the percentage of unlamellated starch grains was higher in small groups of beans as compared to medium and large groups of beans. In addition, starch was more highly developed in large beans as compared to the development of starch in small beans (figure 11).

As mentioned before, the lamellation depends on the deposition of starch during day and night, where denser and more highly refrecting part of each lamella is deposited during the day (4 and 8). The

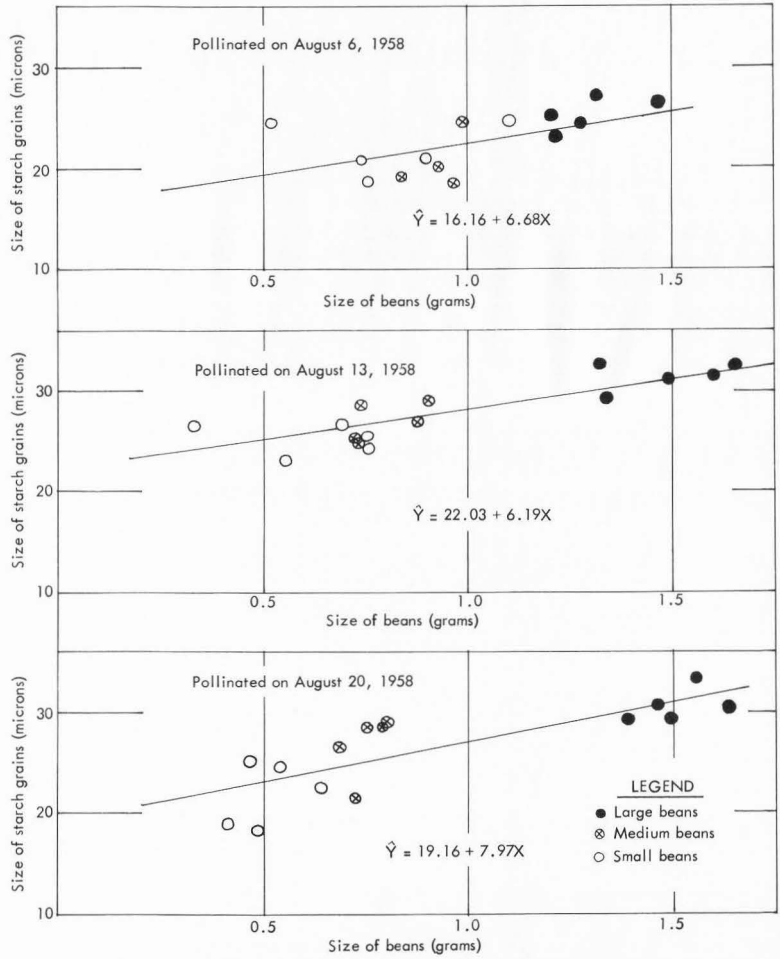
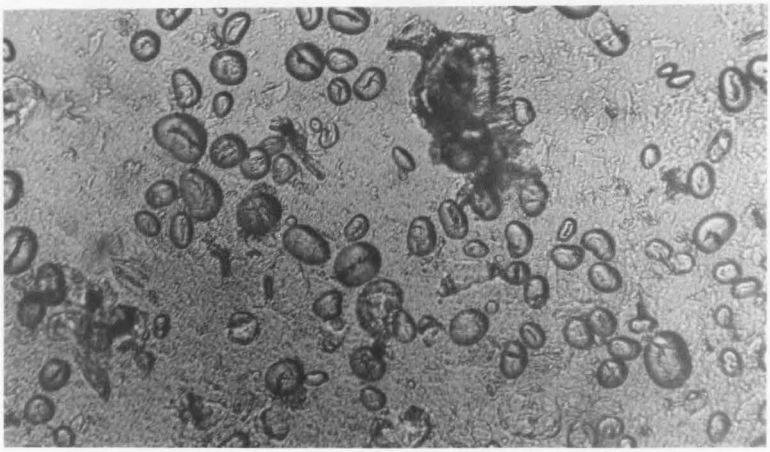


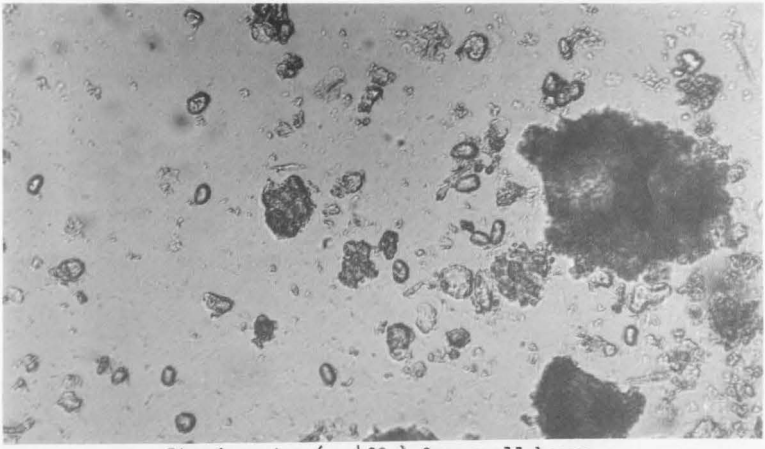
Figure 10. Relationship between weight and size of beans and size of starch grains in Concentrated Fordhook variety, pollinated on August 6, August 13, and August 20, 1958

Table 6. The effect of size and weight of beans on the size, shape, hylum, and lamela development of starch grains in species P. polystachyus L. and in F_1 Cross (P. lunatus L. x P. polystachyus L.)

Species	Size of beans	Weight of beans	Size of starch grain	Shape of starch grains			Hylum of starch grains			Lamella of starch grains	
				Circular	Oblong	Irregular	Un-hylumed	Slightly hylumed	Highly hylumed	Unlamel-lated	Lamel-lated
		grams	microns	percent	percent	percent	percent	percent	percent	percent	percent
P. poly-stachyus L.	Large	0.40	21.64	29.0	65.0	6.0	0	47.0	53.0	42.0	58.0
	Medium	0.25	18.48	24.0	73.0	3.0	0	79.0	21.0	43.0	57.0
	Small	0.11	14.45	16.0	81.0	3.0	0	93.0	7.0	94.0	6.0
P. lunatus L. x P. polystachyus L.		0.84	31.58	63.0	37.0	0.0	0	21.0	79.0	4.0	96.0



Starch grains (x 430) from large beans



Starch grains (x 430) from small beans

Figure 11. The effect of size of beans on starch grain characters in P. polystachyus L. species. (Compare size, shape, and structural development of hyla of starch grains of large and small beans.)

results from table 6 show that the starch deposition in P. polystachyus L. was less effected during the daytime. This seemed that nearly the same amount of starch was deposited during the day as at night.

The regression analysis of variance in appendix table 14 shows that F value was significant at 1 percent level. This indicates that the weight and size of beans were closely associated with the size of starch grains. As the weight and size of beans increased, the size of starch grains also proportionately increased (figure 12). The variation in starch grain size which was accounted by the weight of the beans was 99.1 percent. This is represented by r^2 values in appendix table 14.

F1 Cross (P. lunatus L. var. Fordhook x P. polystachyus L.)

This cross was made by Lorz (12) in Gainesville, Florida. The purpose of this cross was to transfer a character of hypogeal germination from P. polystachyus L to P. lunatus L. to solve the emergence problem in lima beans. Likewise, the author feels that the frost injury to the seedlings occurs in the northern states which can be eliminated if a variety of lima beans could be developed which has characters similar to those of P. lunatus L. and P. polystachyus L. Furthermore, the beans of this variety might be easily digestible due to its high amylose content which might be of value to the people with weak digestive systems. Lorz (12) explained that it was difficult to get F_2 seeds from F_1 plants due to the high percentage of gametic sterility which was caused by the chromosomally unbalanced female gametes. This was also true in crosses of other species (10).

Table 6 shows that the weight and size of bean and size of starch grain were similar to P. lunatus L. and much higher than P. polystachyus L. parent. Other starch grain characteristics (shape, hylum, and

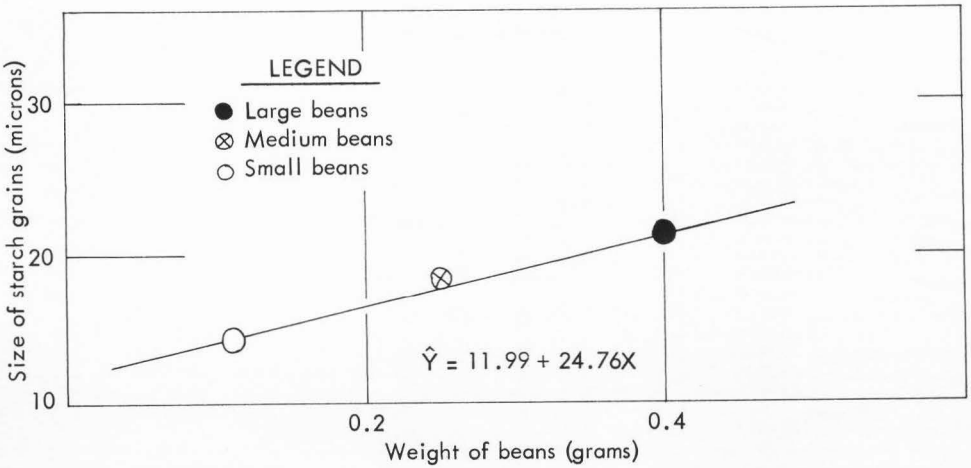


Figure 12. Relationship between weight and size of beans and size of starch grains in P. polystachyus L. species

lamellation) were also not identical to both the parent species. Circular shape, highly hylumed, and lamellated starch grains were common (figure 13).

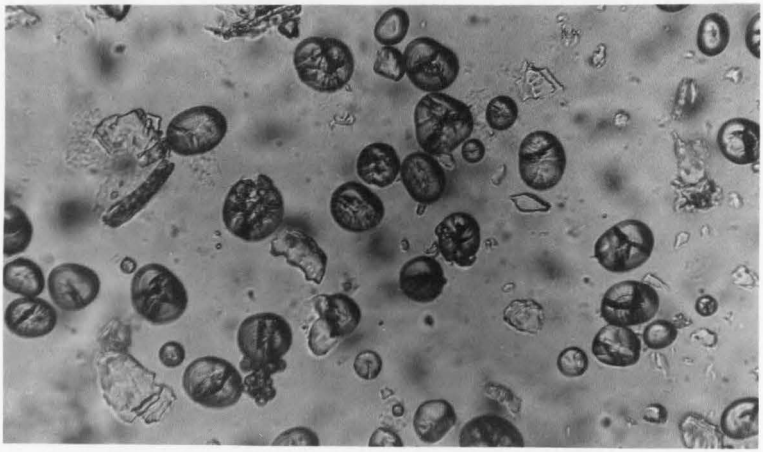
Amylose and amylo-pectin contents

Table 7 indicates that amylose content in the two species was similar and the variable factor was amylo-pectin. Utah 16 and Clark's Bush possessed the same amount of amylose regardless of the size groups of beans. Wasatch Bush, Concentrated Fordhook, and P. polystachyus L. showed the trend as if they contained a lower percentage of amylose in the large beans. In the previous discussion it was found that large beans had large sized starch grains and nearly regular in shape, whereas small beans had small sized starch grains which were slightly irregular in shape. Hence, these results confirmed the results found in corn, that the high amylose corn hybrids possessed higher percentage of small and irregular starch grains (Senti, 23).

The cross between P. lunatus L. var. Fordhook and P. polystachyus L. showed 15.06 percent amylose in F_1 beans which was about one-half of both the parent species. The bean from which amylose was determined was large, weighing 0.84 gram with average starch grain size of 31.58 microns and had 63 percent circular starch grains. Keeping the above mentioned statement in view, it was concluded that one reason of low percent amylose content might be of circular and large starch grains (23) and another might be of genetic nature. At this stage it is difficult to conclude the exact genetic behavior, but it is possible that complementary factors might be responsible.

Sampling analysis

The analysis of variance in appendix table 15a was computed with



Starch grains (x 430)

Figure 13. Starch grain characters in F_1 cross P. lunatus L. x P. polystachyus L. (Compare with the size, shape, and structural development of hyla of starch grains of P. lunatus L. var. Concentrated Fordhook (figure 9) and of P. polystachyus L. (figure 11).)

Table 7. The effect of size and weight of beans on the amylose, amylopectin, and total starch content of species and species F_1 cross (*P. lunatus* L. x *P. polystachyus* L.)

Species	Pollination time	Size of beans	Weight of beans	Amylose	Amylopectin	Total starch
			grams	percent	percent	percent
Utah 16	Aug. 6, 1958	Large	0.72	24.44	21.27	45.71
		Medium	0.23	24.44	8.34	32.78
		Small	0.12	24.44	9.13	33.57
Clark's Bush	Aug. 6, 1958	Large	0.59	24.44	4.31	28.75
		Medium	0.39	24.44	17.62	42.06
		Small	0.37	24.44	20.68	45.12
Wasatch Bush	Aug. 6, 1958	Large	0.41	23.85	23.24	47.09
		Medium	0.31	24.44	25.03	49.47
		Small	0.29	24.44	21.29	45.74
Concentrated Fordhook	Aug. 6, 1958	Large	1.50	22.47	13.50	35.97
		Medium	0.75	24.44	28.08	52.52
		Small	0.50	24.44	16.94	41.38
Phaseolus polystachyus L.	-	Large	0.40	24.44	2.21	26.65
		Medium	0.25	24.45	3.27	27.72
		Small	0.11	24.45	6.00	30.45
P. lunatus L. x *P. polystachyus* L.	-	-	0.84	15.06	29.75	44.81

5 beans, 10 slides prepared for each bean, and 10 starch grains studied per slide. F value showed that the variation among starch grain sizes was significant at 1 percent level.

The appendix table 15b shows the variances computed with different combinations of slides prepared and starch grains studied per slide. The idea of computing these variances was as to study whether or not this design with 10 slides per bean and 10 starch grains per slide was more or less efficient and economical than a similar design employing certain combinations of slides per bean and starch grains studied per slide.

In addition, table 15b indicates that as the number of slides per bean and starch grains studied per slide increased, the variances decreased and the efficiency of the design, time, and expense increased. For example, to prepare a slide it took two minutes and to examine a starch grain it took 15 seconds, and the costs for preparing each slide and for examining each starch grain were 8 cents and 1.5 cents respectively. Therefore, if the time and expense were calculated, keeping variance nearly constant, it appears that 4 slides and 8 starch grains per slide were the least expensive and minimum time consuming among the combinations examined in appendix table 15b. Further, it depends upon an individual, how much efficiency he wants in his experiment and how much he wants to spend. Hence, the experiment can be designed accordingly.

SUMMARY AND CONCLUSIONS

Species P. lunatus L. and P. polystachyus L. were studied. Five varieties of P. lunatus L. were grown at U.S.U. experiment station at Farmington, Utah. Pods were tagged at three different dates of pollination (August 6, August 13, and August 20, 1958). These varieties were harvested at the full maturity. Species P. polystachyus L. and F_1 cross (P. lunatus L. var. Fordhook x P. polystachyus L.) were furnished by Dr. A. P. Lorz, Horticulturist, University of Florida, Gainesville, Florida. The F_1 seeds and the parents together with five varieties of P. lunatus L. were examined under the microscope for the starch grain characteristics (size, shape, hyla, and lamella) and were analyzed for the amylose and amylo-pectin content.

The results obtained indicated that the species and the varieties of P. lunatus L. differed in starch grain size and other characteristics. Varieties Burpee Bush and Concentrated Fordhook were large seeded and possessed large starch grains. But varieties Utah 16, Clark's Bush, and Wasatch Bush were small seeded and possessed smaller starch grains as compared to the large seeded varities.

Species P. polystachyus L. had small seeds containing small starch grains which were more or less like small seeded varieties of P. lunatus L. The F_1 seeds (P. lunatus L. var. Fordhook x P. polystachyus L.) and starch grain size were similar to that of large seeded varieties of P. lunatus L.

As the pollination time advanced, the size of the starch grains

decreased. The size and weight of beans had linear correlation with the size of starch grains.

The amylose content in both the species was similar (24.44 percent), but the F_1 cross (P. lunatus L. var. Fordhook x P. polystachyus L.) decreased in amylose content (15.06 percent).

The sampling analysis indicated that as the number of slides per bean increased and number of starch grains per slide decreased with constant variance (efficiency) the time and expenses increased. More starch grains per slide and less slides per bean seemed desirable.

LITERATURE CITED

(1) Alsburg, C. L. Studies upon starch. Ind. Eng. Chem. 18:190-193. 1926.

(2) Barham, H. N., Wagoner, J. A., Williams, B. M., and Reed, G. N. A comparison of the viscosity and certain microscopical properties of some Kansas starches. Jour. Agr. Res. 68:331-345. 1944.

(3) Bear, R. P., Vineyard, M. L., MacMasters, M. M., and Deatherage, W. L. Development of "amylomaize" - Corn Hybrids with high amylose starch: II. Results of breeding efforts. Jour. Amer. Soc. Agron. 50:598-602. 1958.

(4) Bonner, J. Plant biochemistry. Academic Press Inc., New York 10, N. Y. (1st ed.) 48-64. 1950.

(5) Buisland, T. J. The crossing of beans (Phaseolus spp.) Euphytica. 5:41-50. 1956.

(6) Cordner, H. B. External and internal factors affecting blossom drop and set of pods in lima beans. Proc. Amer. Soc. Hort. Sci. 30:571-576. 1933.

(7) Davis, J. F. The effect of some environmental factors on the set of pods and yield of white pea beans. Jour. Agr. Res. 70(7): 237-249. 1945.

(8) Frey-Wyssling, A. Submicroscopic morphology of protoplasm. Elsevier Publishing Company, New York. (2nd ed.) 310-326. 1953.

(9) Gates, R. R. Epigeal germination in the Leguminoseae. Bot. Gaz. 113:151-157.

(10) Honma, S. A bean interspecific hybrid. Jour. Hered. 47:217-220. 1956.

(11) Lambeth, V. N. Some factors influencing pod set and yield of lima beans. Mo. Agr. Exp. Sta. Res. Bull. 466. 1950.

(12) Lorz, A. P. An interspecific cross involving the lima bean Phaseolus lunatus L. Science 115:702-703. 1952.

(13) McCready, R. M. and Hassid, W. Z. The separation and qualitative estimation of amylose and amylo-pectin in potato starch. Jour. Amer. Chem. Soc. 65:1154-1157. 1943.

(14) McCready, R. M., Guggolz, J., Silviera, V., and Owens, H. S. Determination of starch and amylose in vegetables. Anal. Chem. 22:1156-1158. 1950.

(15) Ostle, B. Statistics in research. The Iowa State College Press, Ames, Iowa. (2nd ed.) 117-231. 1956.

(16) Rahman, A. R. A. The effect of stage of maturity on the quality of lima beans for processing (Master's thesis, Utah State University, Logan, Utah). 1954.

(17) Rappaport, L. and Carolus, R. L. Effect of night temperature at different stages of development on reproduction in the lima bean. Proc. Amer. Soc. Hort. Sci. 67:421-428. 1956.

(18) Roberts, E. A. and Proctor, B. E. The appearance of starch grains of potato tubers of plants grown under constant light and temperature conditions. Science 119:509-510. 1954.

(19) Salunkhe, D. K., and Pollard, L. H. Microscopic observations of starch grains in relation to maturity of potatoes. Proc. Amer. Soc. Hort. Sci. 64:331-334. 1955.

(20) _____ and _____ A rapid and simple method to determine the maturity and quality of lima beans. Food Technol. 9:45-46. 1955.

(21) _____ and _____ Further studies on the microscopic examination of starch grains in relation to maturity of lima beans. Food Technol. 9:521-522. 1955.

(22) Seaton, H. L. Scheduling plantings and predicting harvest maturities for processing vegetables. Food Technol. 9:202-209. 1955.

(23) Senti, F. R. High amylose corn. Jour. Agr. and Food Chem. 6:641-642. 1958.

(24) Sharma, K. N. Studies on starch grain sizes and specific gravity in potato varieties (Ph.D. thesis, Michigan State University, East Lansing, Michigan). 1956.

(25) Sjostrom, O. A. Microscopy of starches and their modifications. Jour. Ind. Eng. Chem. 28:63-64. 1936.

(26) Small, J. K. Manual of the south eastern flora. The Science Press Printing Co., Lancaster, Pa. (1st ed.) 723-725. 1933.

(27) United States Weather Bureau. Climatological data. Government Printing Office, Washington 25, D. C. 9:No.6-10. 1958.

(28) Van de Sande-Bakhuyzen, H. L. Proc. Soc. Exp. Biol. Med. 23:302. 1926.

(29) Vineyard, M. L., Bear, R. P., MacMasters, M. M., and Deatherage, W. L. Development of "amylomaize" - Corn hybrids with high amylose starch: I. Genetic considerations. Jour. Amer. Soc. Agron. 50:595-598. 1958.

(30) Waldt, L. M., and Kehoe, D. Starch chemistry for the food technologist. Food Technol. 13(1):1-4. 1959.

(31) Wall, R. J. and York, T. L. Inheritance of seedling cotyledon's position in phaseolus species. Jour. Hered. 48:71-74. 1957.

(32) Wester, R. E.,and Jorgensen, H. Emasculation unnecessary in hybridizing lima beans. Proc. Amer. Soc. Hort. Sci. 55:384-390. 1950.

(33) Wolf, R. Chemical factors influencing the set of Henderson lima beans. Jour. Amer. Soc. Agron. 34:646-650. 1942.

(34) Zuber, M. S., Grogan, C. O., Deatherage, W. L., Hubbard, J. E., Schulze, W. E., and MacMasters, M. M. Breeding high amylose corn. Jour. Amer. Soc. Agron. 50:9-12. 1958.

APPENDIX

Table 8. Total number of days and degree days calculated from sowing date to three pollination dates for Burpee Bush, Utah 16, Clark's Bush, Wasatch Bush, and Concentrated Fordhook varieties

Pollination time	Days	Degree days
August 6, 1958	66	1493.0
August 13, 1958	73	1685.0
August 20, 1958	80	1887.0

Table 9. Regression analysis for the comparison of weight and size of beans with size of starch grains in Burpee Bush variety, pollinated on August 6, August 13, and August 20, 1958

Pollination time	Source	Degrees of freedom	Sum of squares	Mean sum of squares	F value	r^2
Aug. 6, 1958	Total	14	94.56			
	Regression	1	80.89	80.89		
	Error	13	13.67	1.05	77.04**	0.855
Aug. 13, 1958	Total	14	172.35			
	Regression	1	151.55	151.55		
	Error	13	20.80	1.60	94.72**	0.879
Aug. 20, 1958	Total	14	353.83			
	Regression	1	289.17	289.17		
	Error	13	64.66	4.97	58.18**	0.817

**Significant at 1 percent level

Table 10. Regression analysis for the comparison of weight and size of beans with size of starch grains in Utah 16 selection, pollinated on August 6, August 13, and August 20, 1958

Pollination time	Source	Degrees of freedom	Sum of squares	Mean sum of squares	F value	r^2
Aug. 6, 1958	Total	14	464.87			
	Regression	1	421.61	421.61		
	Error	13	43.26	3.33	126.61**	0.907
Aug. 13, 1958	Total	14	239.32			
	Regression	1	210.25	210.25		
	Error	13	29.07	2.24	93.86**	0.878
Aug. 20, 1958	Total	14	143.40			
	Regression	1	112.80	112.80		
	Error	13	30.60	2.35	48.00**	0.786

**Significant at 1 percent level

Table 11. Regression analysis for the comparison of weight and size of beans with size of starch grains in Clark's Bush variety, pollinated on August 6, August 13, and August 20, 1958

Pollination time	Source	Degrees of freedom	Sum of squares	Mean sum of squares	F value	r^2
Aug. 6, 1958	Total	14	28.89			
	Regression	1	1.93	1.93		
	Error	13	26.96	2.07	$0.932^{n.s.}$	0.067
Aug. 13, 1958	Total	14	107.09			
	Regression	1	67.27	67.27		
	Error	13	39.82	3.06	21.98**	0.628
Aug. 20, 1958	Total	14	52.96			
	Regression	1	20.40	20.40		
	Error	13	32.56	2.50	8.16*	0.385

$^{n.s.}$Not significant at 5 percent level

**Significant at 1 percent level

*Significant at 5 percent level

Table 12. Regression analysis for the comparison of weight and size of beans with size of starch grains in Wasatch Bush variety, pollinated on August 6, August 13, and August 20, 1958

Pollination time	Source	Degrees of freedom	Sum of squares	Mean sum of squares	F value	r^2
Aug. 6, 1958	Total	14	19.54			
	Regression	1	9.25	9.25		
	Error	13	10.29	0.79	11.71**	0.473
Aug. 13, 1958	Total	14	105.99			
	Regression	1	57.57	57.57		
	Error	13	48.42	3.72	15.47**	0.543
Aug. 20, 1958	Total	14	77.56			
	Regression	1	72.00	72.00		
	Error	13	5.56	0.43	167.44**	0.928

**Significant at 1 percent level

Table 13. Regression analysis for the comparison of weight and size of beans with size of starch grains in Concentrated Fordhook variety, pollinated on August 6, August 13, and August 20, 1958

Pollination time	Source	Degrees of freedom	Sum of squares	Mean sum of squares	F value	r^2
Aug. 6, 1958	Total	14	112.21			
	Regression	1	44.62	44.62		
	Error	13	67.59	5.19	8.58*	0.402
Aug. 13, 1958	Total	14	124.65			
	Regression	1	90.57	90.57		
	Error	13	34.08	2.62	34.57**	0.726
Aug. 20, 1958	Total	14	277.21			
	Regression	1	177.60	177.60		
	Error	13	99.61	7.66	23.18**	0.640

*Significant at 5 percent level

**Significant at 1 percent level

Table 14. Regression analysis for the comparison of weight and size of beans with size of starch grains in *P. polystachyus* L. species

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F value	r^2
Total	2	26.04			
Regression	1	25.82	25.82		
Error	1	0.22	0.22	115.37**	0.991

**Significant at 1 percent level

Table 15a. Analysis of variance of the size of starch grains in first date of pollination and small size group with five beans, ten slides prepared per bean, and ten starch grains studied per slide

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F value
Total	499	1276.24		
Seeds	4	113.90	28.47	6.86**
Slides	45	187.01	4.15	
Starch grains	450	975.30	2.16	

**Significant at 1 percent level

$s^2 = 0.199$

Table 15b. Variances of treatment means of combinations of number of slides and number of starch grains examined per slide

Starch grains per slide	Number of slides						
	2	4	6	8	10	15	20
1	1.2000	0.6000	0.4000	0.3000	0.2400	0.1600*	0.1200*
2	0.6500	0.3250	0.2166	0.1625*	0.1300*	0.0866	0.0650
4	0.3750	0.1875	0.1250*	0.0937	0.0750	0.0500	0.0375
8	0.2375	0.1187*	0.0791	0.0594	0.0475	0.0316	0.0237
10	0.2100	0.1050	0.0700	0.0525	0.0420*	0.0280	0.0210
15	0.1733	0.0866	0.0577	0.0433	0.0346	0.0231	0.0173
20	0.1550*	0.0775	0.0516	0.0387	0.0310	0.0206	0.0155

*Constant variance for computing the time and expense in a particular combination of slides and starch grains per slide. Cost to obtain final variance = 190 cents.

Table 16. The comparison of the degree of increase of starch grain size per unit weight of beans in five varieties for three pollination times (August 6, August 13, and August 20, 1958)

Variety	b value			Combination of b values for comparison	t value
	b_1	b_2	b_3		
Burpee Bush	8.28	11.68	18.34	b_1 vs. b_2	+2.38*
				b_1 vs. b_3	-4.19**
				b_2 vs. b_3	-2.73*
Utah 16	20.13	24.16	23.75	b_1 vs. b_2	-1.30 n.s.
				b_1 vs. b_3	-0.94 n.s.
				b_2 vs. b_3	+0.10 n.s.
Clark's Bush	3.60	19.33	11.66	b_1 vs. b_2	-2.94*
				b_1 vs. b_3	-1.52 n.s.
				b_2 vs. b_3	+1.23 n.s.
Wasatch Bush	15.25	20.28	49.00	b_1 vs. b_2	-0.61 n.s.
				b_1 vs. b_3	-6.42**
				b_2 vs. b_3	-3.26**
Concentrated Fordhook	6.68	6.19	7.96	b_1 vs. b_2	+0.92 n.s.
				b_1 vs. b_3	-0.45 n.s.
				b_2 vs. b_3	-0.92 n.s.

*Significant at 5 percent level

**Significant at 1 percent level

n.s. Not significant at 5 percent level

b_1 Pollinated on August 6, 1958

b_2 Pollinated on August 13, 1958

b_3 Pollinated on August 20, 1958