

CERTAIN GENETICAL AND MORPHOLOGICAL
ASPECTS OF A BICARPELLATE FORM OF
GARDEN BEAN, PHASEOLUS VULGARIS L.

by

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INTRODUCTION

The recorded history of the common, lima, and runner beans begins at about the time of the discovery of America. Early explorers reported a diversity of kinds occurring on the American continental areas and also on the outlying islands. This suggests a long period of cultivation.

Since these early times, beans have been given much consideration by botanists and horticulturists and today are a crop of considerable economic importance. In recent years the development of the sciences of genetics and cytology has provided much information concerning the development of particular characters in the bean plant. This has made it possible to provide plants of high quality and of high yield which are resistant to several of the more serious bean diseases.

The present study is concerned with the morphology and genetics of an unusual form of the common garden bean (Phaseolus vulgaris L.) which produces twin-podded fruits. In studying the inheritance of any character it is of value to know as much as possible

about the origin, expression, and interaction of the character with its environment. With these aspects in mind, it was considered essential to describe the general morphology of this bicarpellate race, particularly of the flowers giving rise to these twin-podded fruits, and to determine whether or not this character could be transmitted to a progeny resulting from a cross with a single-podded bean.

The plant material to be discussed in this paper comes under the classification of teratological material, regarded by some investigators as curiosities and by others as reversions to a primitive condition or as mutant forms. As a consequence, very little is known, or has been published, regarding this phenomenon in the beans.

The chromosome number of Phaseolus vulgaris L. is remarkably stable as compared with many other horticultural crops. It, therefore, seemed possible that a plant exhibiting such a character as polycarpelly might differ in chromosome number, chromosome morphology, or possess certain characters different from those of a normal bean plant and be useful as a breeding tool. It has been stated in the literature

that the single carpel in the Leguminosae is actually the sole surviving member of a former whorl of carpels. If this be so, and the carpels formerly present no longer appear in present-day strains, it seemed entirely possible that a race exhibiting this polycarpellate condition might also retain other genetic characteristics which may have been lost in the processes of evolving a single carpellate form. With these possibilities in mind, an investigation of this material appeared to be worthwhile. Apart from these considerations, some merit might exist for a plan to increase production of certain shelled beans by increasing the number of pods per plant.

REVIEW OF LITERATURE

The common garden bean has been in cultivation for a rather long period and has been the subject of much investigation. It is appropriate that literature pertinent to the broader aspects of this investigation be presented as an aid in evaluating and interpreting the present work.

Morphology

According to Eames (9, p.176) there is considerable taxonomic, morphological, and anatomical evidence that the solitary carpel of the Leguminosae represents the sole surviving member of a former whorl of carpels.

In discussing the Leguminosae in her "Illustrations of Carpel Polymorphism," Saunders (19, pp.255-258) mentions that the residual vascular tissue available for the gynoecium represents elements which once furnished the midrib cords of two pentamerous carpel whorls in some ancestral form. She states also that, in Arachis and Scorpiurus, the ground plan of the gynoecium is still traceable.

In other genera of the Leguminosae, the remaining elements, which once developed into the vascular system of ten carpels, still survive in whole or in part, but

are now fashioned into the venation systems of a median pair of carpels.

Aside from the indirect evidence above, there have appeared from time to time, according to Worsdell (29, p.92), polycarpellate forms among the Leguminosae. He states that in this family there has been very great reduction in the gynoecium resulting in a single carpellate structure. Reversions, partial or complete, would be expected to occur from time to time. Mimosa has occasionally produced five carpels: a case of complete reversion to the number of members possessed by the other whorls. Flowers of Trifolium repens L. have been reported with two and three carpels respectively, and those of the scarlet runner bean, Phaseolus multiflorus Willd., with two.

Reference to a variety of Phaseolus vulgaris L., named Baptisia alba, producing flowers with twin pods is made by Saunders (21, pp.189-190). She explains this condition as "arising from a bifurcation of the axis above the level of origin of the perianth and stamen whorls, but below that of the carpels." This interpretation involves a complete halving of the residual vascular cylinder at this level resulting in the formation of two new cylinders. This duplication is depicted as closely

resembling the normal course of development in some rosaceous forms.

An extensive search of the literature reveals no opposition to the view that the legume represents the sole surviving member of a former whorl of carpels.

Differences of opinion do exist on whether the legume carpel is terminal, pseudo-terminal or lateral on the axis. Saunders (19, p.254) holds to the view that the solitary ovary is terminal in the strict sense and is similar in this respect to the single drupe of Prunus.

Eames (9, p.176) states that the position of the carpel in the Leguminosae is a pseudo-terminal one. In discussing Prunus with five traces and Actea with three, he states that "the cylinder continues on beyond the exit of the traces and the fact that the traces are not terminal is clear." According to Eames, in most leguminous genera the cylinder does not extend beyond the trace origins. There are, however, many other genera in the family in which considerable arcs of the cylinder are dropped out at that level. Eames considers that the morphological condition present in these genera is similar to the situation found in Prunus and Actea, but with greater specialization the vestigial, vascular tip has disappeared in these forms.

That the development of the carpel is perfoliate is the conclusion of Moore (14, p.289), who describes the early development of the gynoecium in the papilionaceous Leguminosae. With the beginning of development of the gynoecium, the stele rapidly tapers off and linear development of the axis ceases, its elements swing in markedly and seem pinched off. In more mature flowers bits of provascular traces can be seen beyond the departure of the carpel traces. Thus, from a strict anatomical standpoint, Moore concludes that the gynoecium is not terminal.

In discussing phyllody in beans, Dana (6, p.173) reports that, occasionally, instead of an accessory blossom being formed, the axis was extended into a shoot which was normal in appearance. This extension of the axis into a shoot is in harmony with the findings of Eames, that the legume carpel is lateral rather than terminal, as held by Saunders.

The early development of the pistil in the normal flower of the soybean is described by Guard (11, p.101) as a "leaflike carpel folded on its midrib with margins scarcely touching." Protuberances, later giving rise to ovules, are produced alternately on the inner surface of these carpel margins prior to the time these margins become fused or coalesce.

Detailed anatomical studies of Phaseolus vulgaris L. variety Black Valentine, have been made by Doult (8, pp. 19-20). She describes the traces to the pistil as consisting of portions of the central stele remaining after the traces have been supplied for the bracts and other floral whorls. These remaining traces divide to form a somewhat continuous circle in the stele which, at the base of the pistil, consists of twelve bundles. Six of these twelve bundles differentiate toward the posterior side and six toward the anterior side. Later in development the pistil becomes separated from the staminal ring and the vascular supply of the carpel consists of one large crescent shaped dorsal bundle and two posterior bundles which form the ventral suture. These ventral bundles alternately give off traces to the ovules. Vascular tissues continue into the style in the same arrangement, but become reduced. Vascular tissue in the carpel walls are formed as branches from the dorsal bundle similar to the way in which lateral veins branch from the midribs of a leaf.

A study of the anatomy of the carpel in the bean by Woodcock (28, p. 270) substantiates the work of Miss Doult. He indicates that the carpel walls are

furnished with vascular material from the eight bundles at the base of the pistil and from a compound dorsal bundle.

Reviews on the subject of vascular floral anatomy considering the various theories of carpel initiation and development are given by Arber (3), Bancroft (4), and Wilson and Just (27).

Effects of Environment

According to Allard and Zaumeyer (1, pp.3-9), Phaseolus vulgaris L. and other Legumes are responsive to length of day. Although some varieties of P. vulgaris L. normally are persistent in exhibiting a certain growth form in response to all photoperiods, other varieties display great modification of this, depending upon variations in light intensity, temperature, humidity and length of the photoperiod. Most beans of the species P. vulgaris L. are said to be day neutral; however, ten of twenty varieties of this species classed as pole beans were found to be of the short day class and ten of the day neutral class. Of bush varieties, twenty-four green-podded and eleven wax-podded showed day neutral behavior. The flowering of some was delayed. Of five Refugee varieties classified as indeterminate in growth habit, four were short day and one day neutral.

That environment may affect the number of carpels differentiated in certain plant materials is indicated by the report of Philps (16, pp.815-820) who has described an abnormal development of double and malformed fruits in the midseason varieties of sweet cherry, Prunus avium L. This was thought to be associated with unusually high temperatures during fruit bud formation and differentiation the previous year.

Cytogenetical Aspects

A number of studies have been made regarding the inheritance of particular characters in the bean. Currently there is considerable interest in the inheritance of resistance to many of the virus diseases affecting garden beans. A careful review of the literature available disclosed no information on the inheritance of a character such as polycarpelly.

According to Norton (15, pp.550-560), plant habit in beans is largely determined by the presence or absence of three characters designated by letters A, L, & T. A is a dominant character for the presence of axial inflorescence permitting an indefinite growth of the main stem and a, its recessive allele, resulting in a terminal inflorescence causing definite growth. The

length of the axis is determined by a factor L controlling plant habit and probably governed by a series of two or more factors L_1 , L_2 , etc. which behave as quantitative characters. Climbing habit is due to a factor for circumnutation. This factor Norton calls T. The factors A, L, & T may be present in any possible combination and give rise to various habit types of beans. The dominant factors and their recessive alleles are associated with the following growth response: A--Axial position of pods, a--terminal position of pods; L--long plant axis, l--short plant axis; T--circumnutation, t--absence of circumnutation. Thus, ALT represents the pole beans, ALt runner beans, ALT, alT, and alt, the bush types.

The inheritance of colors and pod characters have been described for certain bean varieties by Kooiman (13), Prakken (17), and Sirks (23). Differences of opinion as to factors present and designation of factors responsible for particular color effects exist. The scope of the present investigation does not include a study of the genetics of color inheritance.

Cytological studies on Phaseolus vulgaris L. by Weinstein (26, p.258) reveal that the diploid chromosome number for P. vulgaris L. is 22. This chromosome count was made by examining preparations of root tips

from twenty seedlings of four varieties. From sixty equatorial plates of chromosomes counted, the diploid number was found to be 22 in all cases.

A check of the chromosome numbers listed in the Chromosome Atlas of Cultivated Plants by Darlington and Ammal (7, p.171) designates 22 as the diploid chromosome number for the species vulgaris. Within the genus Phaseolus the basic chromosome number is 11 and 12, and only one tetraploid of 44 chromosomes is listed for the entire genus; this in the species aureus. Thus, the genus Phaseolus can be considered as a relatively stable one.

MATERIALS

Seeds of the bicarpellate race of Phaseolus vulgaris L. were made available by Quentin B. Zielinski, Associate Professor of Horticulture at Oregon State College. The exact origin of the material has not been determined. The original seed was secured in 1948 from the H. L. Price family in Blacksburg, Virginia. Plants of this particular race have been grown by the Price family for many generations for home canning purposes.

Mature plants produced from this seed are medium to large in size when grown under optimum conditions. Plants are 4-5 feet or more tall, with a spread at the base of 1-1 $\frac{3}{4}$ feet, of good climbing habit, but rather open in growth; vigor is moderate, bearing period is long, yield good; branches are few and green throughout, stems medium-rough; veining is medium, and of medium thickness, leaflets 4 $\frac{1}{2}$ inches long by 3 $\frac{1}{2}$ inches at the base, laterals are equal, margins rounding at the base and acute at the tip; pistils are medium to short, flowers are white. The inflorescence is a raceme borne in the axils of the leaves; peduncle is short, pedicels are of medium length. The flower exhibits variations

from that of the normal species in that it develops two or more carpels and slight variations in number of stamens and petals sometimes occur. Carpels are green throughout and usually fused along the ventral margins. Average length is five inches, width three-quarter inches, smooth, rounding tapering to a point at ends. Mature seeds are a mustard-tan mottle with greyish-white ground color. Hilum ring is light brown, hilum is white. Average seeds are 1.33 centimeters long by .69 centimeters wide, ends are somewhat blunt or blocky.

Seeds of Phaseolus vulgaris L., U. S. No. 5 Refugee, were supplied by B. F. Dana, Plant Pathologist, U. S. Department of Agriculture, Oregon State College Experiment Station, Corvallis, Oregon. No. 5 Refugee was released by the U. S. Department of Agriculture in 1935 as a mosaic-resistant bean of the stringless Refugee type. This variety has been described by Wade and Zaumeyer (25, pp.1-2) and is a dwarf bush type bean resistant to the seed borne virus of yellow bean mosaic.

METHODS

Plants for this study were grown in the greenhouses at Oregon State College and also at the horticultural farm. Most of the material was grown under long day conditions with an average temperature of 65° F. Some plants of the bicarpellate bean were grown under short day conditions and lower temperatures to observe the effects of environments on the expression of the bicarpellate character.

Material for the morphological study of the flowers of the bicarpellate race of P. vulgaris L. consisted of flower buds collected before and after anthesis. The buds were killed and fixed in Randolph's Modified Navashin fluid (18, pp.95-96). Standard procedures of dehydration and embedding to prepare the material for paraffin sectioning were used.

Serial sections of the embedded material were cut at 15-20 microns. Sections were stained with a safranin-fast green combination. Both transverse and longitudinal sections were made.

Reciprocal crosses between the bicarpellate forms of P. vulgaris L. and U. S. No. 5 Refugee were made to determine whether the bicarpellate character could be transmitted to the progeny resulting from such crosses.

Sixteen plants, eight bicarpellate and eight of U. S. No. 5, were used for this purpose; about one-hundred crosses were made. Flowers were carefully emasculated and examined before crosses were made in order to guard against self-pollination.

Progeny resulting from these crosses were grown and selfed systematically for four generations under outdoor growing conditions. Thus, it was possible to observe certain differences in growth response which might be attributed to photoperiodic and temperature conditions existing at various times of the year.

In order to determine the possibility of virus effects or the effects of cytoplasmic materials on the formation of double pods, two flats of twenty-five plants each of the common bean, P. vulgaris L., variety Black Valentine, were inoculated with the extracted juices from the pods, flowers, stems and leaves of the bicarpellate form of bean plant. These inoculations were made by scratching the upper surface of the heart-shaped leaves of young bean plants with carborundum dust and then applying the extracted juices to these scratched surfaces. These plants were then observed periodically for evidence of bicarpellate flowers.

Aceto-carmine smears of pollen mother cells and very young leaf tips were made in order to determine the chromosome number present in the bicarpellate form of P. vulgaris L.

The observations and interpretations which follow were made from an examination of these sections, smears, and the progeny resulting from the hybridization program indicated above.

RESULTS

Gross Morphology of Twin Carpels

An examination of the flowers of the bicarpellate strain of P. vulgaris L. reveals that occasional variations occur in regard to the number of petals and stamens formed. In general, however, these floral whorls tend to approximate the conditions in the normal legume flower. The chief difference in floral structure is the presence of two carpels which, under proper environmental conditions, develop and mature normally. These two carpels exhibit varying degrees of fusion throughout their length, at times being almost completely fused and sometimes nearly separate. The ventral sides of the carpels are adjacent as shown in Fig. 1 (p. 20). This bicarpellate bean may be termed a true breeding race by reason of the continuity of the bicarpellate condition for many generations. Tricarpellate and tetracarpellate bean fruits have also been formed on occasions within the duration of this investigation. One such tricarpellate flower is shown in cross section Fig. 2 (p. 20), revealing the young developing carpels at a stage prior to the fusion of the carpel margins to form the ventral

Figure 1. Pods of bicarpellate bean showing fusion along ventral sutures.

Figure 2. Cross section of a tricarpetlate flower showing carpels prior to fusion of the ventral margins.



sutures. Developing ovules can be seen at the ventral margins of these young carpels similar to the situation Guard (11, p.101) described for the soy bean.

Anatomy of Twin Carpels

A study of the serial sections of young flowers prepared from paraffin mounts reveals a vascular system similar to that described by Doult (8, pp.19-20). The vascular supply in the pedicel consists of twelve bundles as seen in Fig. 3 (p. 23). These give rise to the vascular supply for the bracts and the remaining floral whorls, calyx, corolla, and stamens as in Fig. 4 (p. 23). The bundles remaining form the traces for the two carpels as seen in Fig. 5 (p. 25). At this level the central stele is just beginning to separate from the stamen disc and is made up of twelve bundles forming an ellipsoidal cylinder with the long axis of the cylinder in the anterior-posterior plan. At successively higher levels, the following changes occur: large compound vascular bundles are formed at both ends of the long axis of the cylinder, these constitute the dorsal or midrib bundles for the two diametrically opposed carpels. At a point approximately mid-distant from the two extremities of the central cylinder, four major vascular bundles form the ventral bundles for the

Figure 3. Cross section of pedicel showing twelve vascular bundles.

Figure 4. Cross section of bicarpellate bean flower showing vascular supply of floral whorls below the carpels.

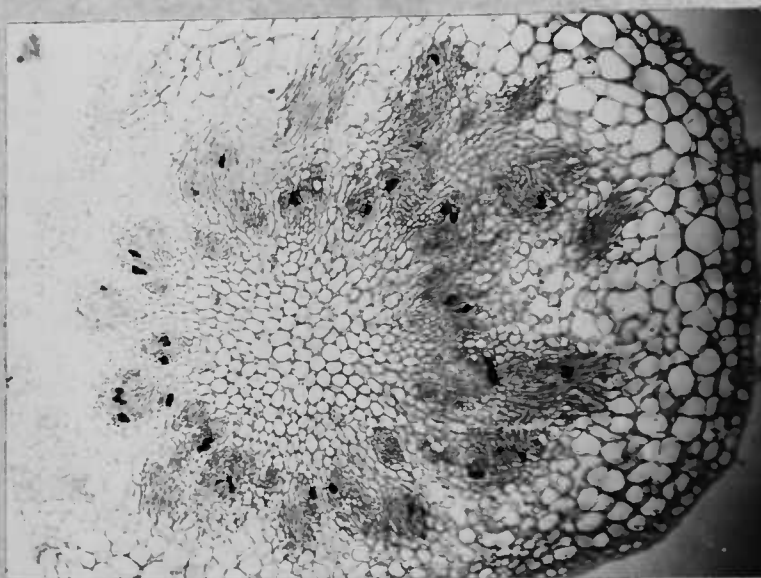
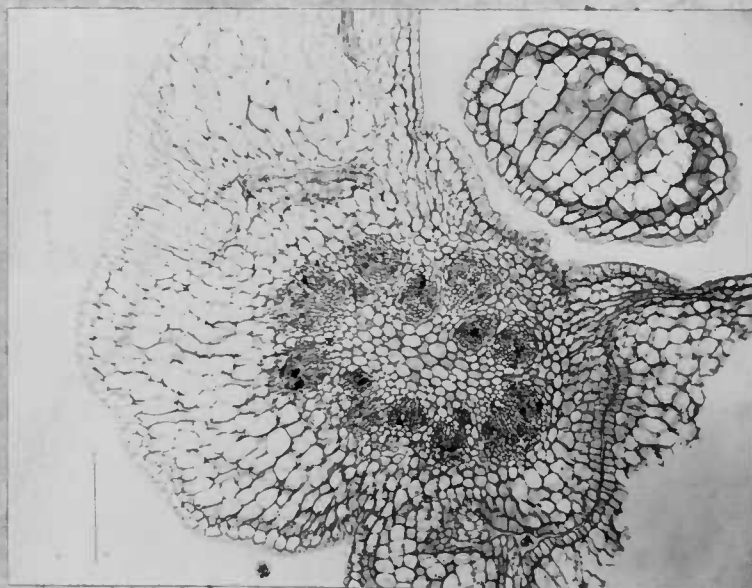
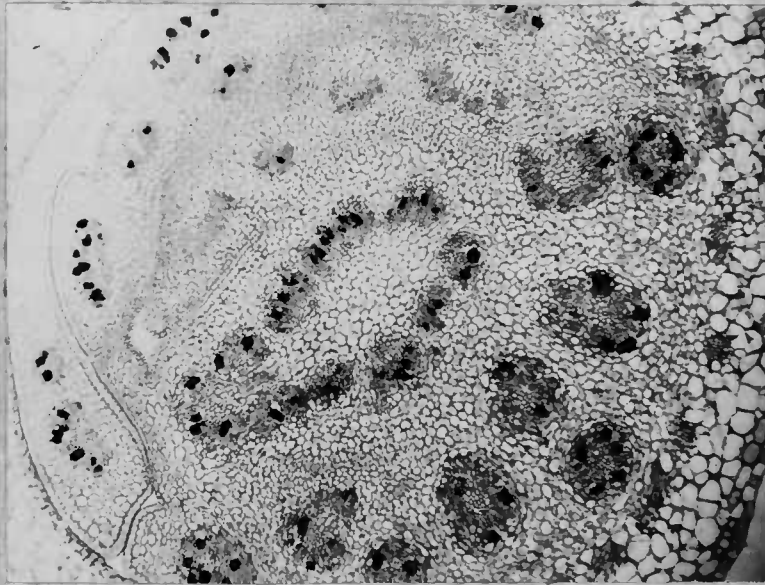


Figure 5. Cross section of bicarpellate bean flower showing central vascular cylinder which gives rise to two carpels.

Figure 6. Cross section of bicarpellate bean flower showing development of two carpels. End of axis can be seen persisting between four ventral bundles.



two carpels. Near the base of the pistil the remaining vascular elements are utilized in the vascular system of the carpel walls and undergo anastomosis and reduction. Some of this can be seen in Fig. 6 (p. 25). At this stage, the central axis still persists and can be seen in the area between the four ventral bundles. Seen also is a trace of an anterior stamen just departed from the vascular bundles forming the midrib cords of one of the carpels. The tissue appearing between the staminal ring and the carpels is a portion of the stamen disc remaining at this level. Locules are also visible at this stage. At successively higher levels, Fig. 7 & 8 (p. 28) and Fig. 9 (p. 30), the central axis disappears and the locules increase in size. In Fig. 8, the central axis has disappeared and the two carpels share a common locule. Fig. 9 shows the carpels fused along their ventral margins, each with a developing ovule in the ovarian cavity, and each carpel now entirely free from attached stamens.

The development of the carpel in this bicarpellate race would appear to be lateral and in agreement with the findings of Moore (14, p. 289, Eames (9, p. 176), and Dana (6, p.173). The lateral development of the carpel seems evident from Fig. 6 (p. 25) and Fig. 7 which indicates that a portion of the central axis

Figure 7. Cross section of bicarpellate bean flower showing enlargement of locular cavity. End of axis is seen tapering off between the four ventral bundles.

Figure 8. Cross section of bicarpellate bean flower showing a common locular cavity prior to fusion of the ventral margins.

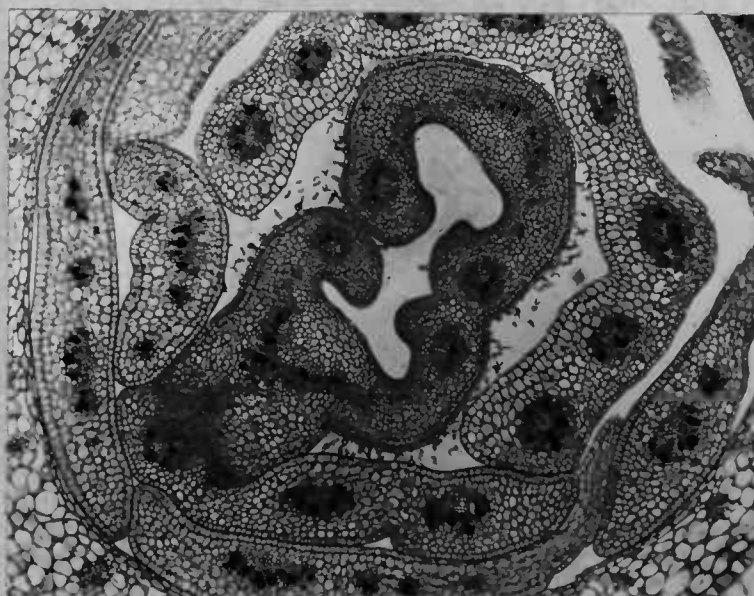
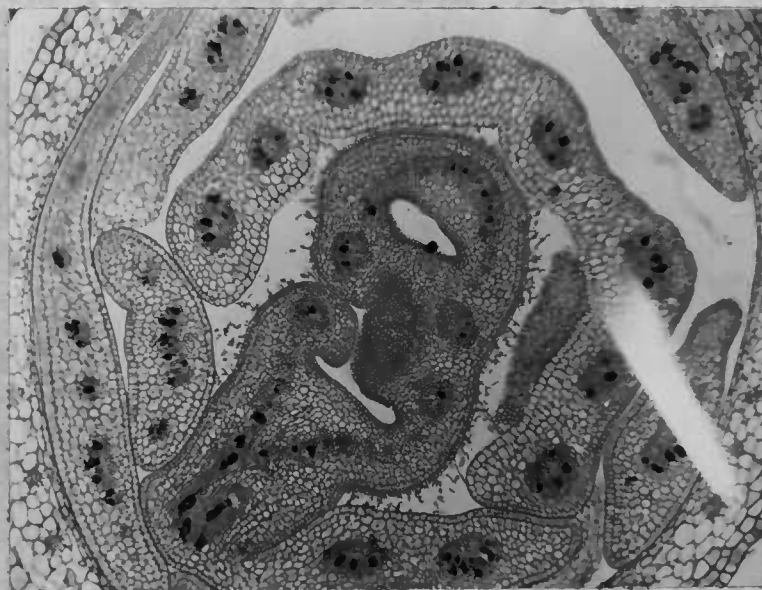
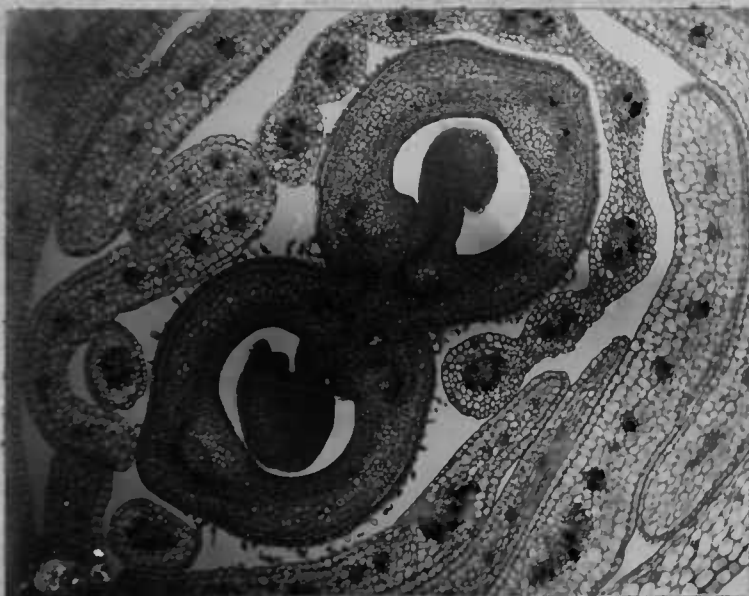


Figure 9. Cross section of bicarpellate bean flower showing two carpels fused along their ventral margins and young developing ovules within each ovarian cavity.



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persists between the ventral bundles of the two carpels. That each carpel is normal and functional appears evident from Fig. 9 (p. 30). Seeds produced in either carpel germinate readily and reproduce the race.

Presence of Three Carpels

Figure 10 (p. 33) shows the vascular supply of a central stele which gives rise to three carpels. These carpels can be seen in Fig. 2 (p. 20), each separate from the other, margins not yet coalesced, with developing ovules appearing from a ventral margin in each carpel.

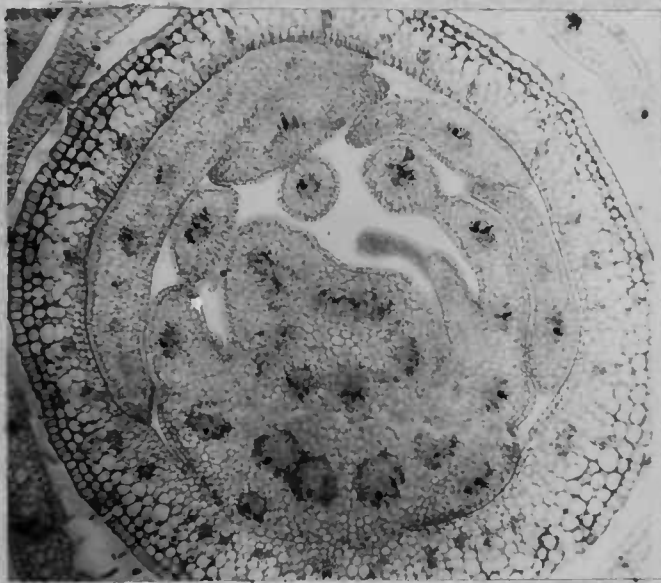
Environmental Effects

As mentioned in the literature, Allard & Zaumeyer (1, pp. 3-9), beans may be sensitive to photoperiod and, from their response to length of day, can be conveniently classified as day neutral, short day, or long day classes.

The bicarpellate race discussed is particularly sensitive to photoperiod, and perhaps to temperature, or to both. The photoperiodic and thermal sensitivity of these plants is evident from the growth responses observed when length of day and temperature conditions are varied.

Figure 10. Cross section of a bean flower showing the central cylinder which gives rise to the three carpels seen in Figure 2.

Figure 11. A polar view showing the diploid number of chromosomes in the bicarpellate form of Phaseolus vulgaris L. at metaphase.



Under low temperature, short-day periods such as occur in April or September, these plants produce nearly all double-pods. When days are longer and higher temperatures are encountered, a low percentage of pods are double, in fact, it is often difficult to determine the bicarpellate nature of the plant under these conditions. This situation is not unlike that which Philps (16, pp. 815-820) described in sweet cherry the season following a particularly warm summer.

This condition and a lack of facilities for controlling these factors made it difficult to select for bicarpellate genotypes among the progeny when crosses were made.

Chromosome Number in the Bicarpellate Bean

As mentioned earlier, it was thought that this double-podded bean might possibly differ in chromosome number from the normal Phaseolus vulgaris L. A number of acetocarmine leaf tip smears and smears of dissected anthers were made to determine any difference in chromosome number. In six polar views of mitotic metaphase, in which the chromosomes were separated well enough to count, the total number of chromosomes in each case was 22, the accepted number for Phaseolus vulgaris L.

An attempt was made to study the morphology of the chromosomes of the bicarpellate race from some of the meiotic figures, but this was not pursued sufficiently to produce any new evidence.

The chromosomes in the bean are rather small as noted in Fig. 11 (p. 33) in which the magnification is 1,350 times.

Hybridization

From a total of one-hundred reciprocal cross pollinations between the bicarpellate, or double-podded, form of garden bean and the U. S. No. 5, or single-podded, selection, twenty-two crosses were effected. Seventeen crosses were from pollinations in which U. S. No. 5 was the female parent and five were from crosses in which the bicarpellate bean was the female parent. The F_1 progeny from the double-pod x single-pod type of cross consisted of twelve plants which were single-pod and three which were mostly single-pod but developed occasional double-pods. The F_1 progeny from the single-pod x double-pod type of cross consisted of thirty-seven plants which were single podded. These F_1 plants were grown under long day conditions in which the day temperature sometimes exceeded 90° F.

Seeds representing the F_2 generations were field grown under long day conditions. In the F_2 plants resulting from selfed F_1 plants of a single-pod x double-pod type of cross, one plant in a population of approximately seventy-five plants developed double-pods on one branch of the plant and the remaining branches gave rise to single-pods.

In the F_2 plants resulting from selfed F_1 's of crosses from a double-pod x single-pod type of cross, one plant produced double-pods with an occasional single pod occurring, three plants produced pods which were nearly all single but with occasional double-pods occurring. The remaining plants (approximately forty) were all single-podded.

Seeds from all these F_2 plants were harvested, but only a few selections were planted for the F_3 generation. Seed of one such F_2 single-podded selection (double x single line) gave rise to F_3 plants which were single-podded and some which were single and double. The F_3 plants which were both single and double-podded produced plants which were both single and double-podded; among fifteen F_4 plants, only two were found which were single-podded.

From an F_2 plant (double x single line) which produced pods which were nearly all of the double-podded

type, the F_3 plants were double-podded and bush type. Seed from these F_3 plants produced bush type double-podded plants in the F_4 generation. Nineteen of these double-podded plants were grown in the F_4 generation.

Seeds from an F_2 partially double-podded type plant, which resulted from selfing an F_1 of single-podded type, gave rise to F_3 plants which were all single-podded. The F_4 generations of this line, represented by twenty-four plants, were also single-podded.

Inoculations

From fifty Black Valentine bean plants which were inoculated with the extracted juices from leaves, stems, and pods of the double-podded bean, one flower was found to have two pistils.

DISCUSSION

A number of reciprocal crosses were made between the bicarpellate bean and U. S. No. 5 Refugee. These parents differ in regard to carpel number, growth habit, and resistance to common bean mosaic. U. S. No. 5 is an erect bush-type, monocarpellate bean, which is resistant to common bean mosaic. The bicarpellate bean is a pole type climbing bean susceptible to this type of virus-caused mosaic. The two parents differ also in color of seeds produced. U. S. No. 5 produces a dark-brown, mottled seed; the bicarpellate bean produces a mustard-tan mottled seed.

As mentioned under "environmental effects," difficulty was encountered in evaluating progeny in regard to the bicarpellate character. The F_1 progeny was grown under environmental conditions which later were found not to be particularly favorable for development and expression of the double-pod character.

Sufficient information was gained to indicate that the bicarpellate condition was transferrable both when the bicarpellate form was the female parent, and also when it was the male parent. Occasional double-pods appeared on some plants, which were otherwise monocarpellate, regardless of which way the cross was made.

This would indicate that the transmission of the bicarpellate character is not entirely due to maternal effects.

F₂, F₃, and F₄ progeny were grown and the seeds were gathered. All plants were classified as accurately as possible in regard to number of carpels present. Among the selections made in the F₂ was one plant classified as bush type double-pod with seed color similar to U. S. No. 5. Seed from this plant produced only bush type double-podded progeny. Thus indicating a combination of characters from both parents.

Difficulty in detecting the bicarpellate character due to environmental effects, which are not thoroughly understood, made it impossible to determine the exact mode of inheritance or to calculate Mendelian ratios.

It seems likely from the results obtained that monocarpellate and bicarpellate beans differ by a factor or factors capable, under proper environmental conditions, of causing the central cylinder to differentiate two or more carpels. That this character can be transmitted through male or female gametes was also indicated.

One possible explanation of some of the manifestations observed in the bicarpellate parent and among the progeny might be the synthesis of a growth-regulating substance under gene control. The presence of

certain branches of a single plant producing double-pods, while the remaining branches on the plant produced only single-pods indicates that some substance influencing carpel differentiation is present in insufficient amounts to cause double-pods in the entire plant.

On two occasions, a bicarpellate fruit has appeared on plants of the Black Valentine variety of bean which were growing in close proximity to a bicarpellate bean plant; in each instance the plants were infested with red spider-mites. This situation would indicate the possibilities of the transfer of some substance from the bicarpellate bean capable of causing a double-podded fruit to form on the Black Valentine. One flower having two pistils has been found among fifty plants which were inoculated with juices from the bicarpellate bean.

In addition to these observations, a flower with two carpels has appeared on one of twelve plants which were treated in the heart-shaped leaf stage of development by injecting a solution of boron, colchicine, and indoleacetic acid into the petioles and into the first internodes adjacent to the shoot tip.

While these observations are interesting, it is recognized fully that these suggestions relative to possible roles of growth regulators, or perhaps even

viruses, are based on insufficient data; much additional work would be required to prove or disprove the hypothesis.

The possibility that environmental conditions may interfere with the full expression of the bicarpellate character, and the fact that not all the seed available from the F_2 and subsequent generations could be grown, has made it difficult to determine the exact mode of inheritance of this bicarpellate character.

The author has attempted to show the differences in the morphology, and cytogenetics between a bicarpellate garden bean and the customary monocarpellate form.

CONCLUSIONS

It has been shown that flowers of the bicarpellate race produce twin-pods, both of which are normal and functional. These pods are sometimes entirely fused along the ventral margins and at times nearly separate. The vascular supply for these two carpels is differentiated from a common central cylinder without a bifurcation of the axis. The development of the carpel is shown to be lateral on the axis. Certain figures indicate and support this contention and also show young developing ovules on the ventral margins of open carpels.

From a limited number of chromosome counts, it seems likely that the chromosome number in the bicarpellate form is 22. Details of chromosome morphology were not determined.

From studies of the bicarpellate parent and the progeny resulting from reciprocal crosses with a monocarpellate bean, it seems likely that the two forms differ by a factor or factors capable, under proper environmental conditions, of causing the central cylinder to differentiate two or more carpels. That this may be due to a growth-regulating substance under gene control is a suggested hypothesis.

The nature of the bicarpellate bean would make it unacceptable as a commercial canning bean. There might be some merit in producing double-podded forms of dry or shell beans if this character could be uniformly fixed for a particular environment. In this way it could provide a means of increasing production.

The chief value of this bicarpellate bean material probably lies in affording an opportunity to study in more detail the fundamental relations between genes and environment. The present investigation constitutes a preliminary study, which would be of much value to a broader more detailed study made under controlled environmental conditions.

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