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INTERSPECIFIC HYBRIDIZATION  
BETWEEN PHASEOLUS VULGARIS L. AND  
PHASEOLUS COCCINEUS LAM.

by

Aly M. Ibrahim

A DISSERTATION

Presented to the Faculty of  
The Graduate College of the University of Nebraska  
In Partial Fulfillment of Requirements  
For the Degree of Doctor of Philosophy  
Department of Horticulture and Forestry

Under the Supervision of Professor Dermot P. Coyne

Lincoln, Nebraska

May, 1974

**TITLE**

INTERSPECIFIC HYBRIDIZATION BETWEEN PHASEOLUS

VULGARIS L. AND PHASEOLUS COCCINEUS LAM.

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To my late parents

PREVIEW

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## Introduction

Miranda (1967a) found primitive varieties of Phaseolus vulgaris L. (common bean) in Mexico and wild varieties of other Phaseolus species in the Sierra Madre Occidental. A great amount of genetic diversity was observed. Gentry (1969) reported multiple origins of cultivated beans in Mesoamerica.

Phaseolus vulgaris and Phaseolus coccineus Lam. are considered closely related species. Two major genes are considered to be species specific and are used to separate these two species; a gene controlling cotyledon position at time of germination (hypogeal versus epigeal), and a second gene stigma shape (extrorse versus introrse). One allele of one gene determines hypogeal germination in P. coccineus and its allele epigeal germination in P. vulgaris. One allele of the second gene controls the extrorse stigma shape in P. coccineus and its allele introrse stigma shape in P. vulgaris. These species are interesting for the study of the genetic basis of the barriers to species crossability and inter-specific gene transfer. Meiosis in the interspecific hybrid is not disturbed and recombination between different traits occurs readily except for species-specific alleles mentioned previously by Lamprecht (1941).

Phaseolus coccineus, commonly called Scarlet Runner, has been utilized extensively in breeding programs because it is resistant to most root rot pathogens that attack



Phaseolus vulgaris (Cardwell, 1961). A native species, such as P. coccineus, having a comparatively wide geographic distribution, may possess yet unrecognized resistances to diseases and pests of cultivated beans and may also possess physiological traits of adaptive value. Richardson and Evans (1972) reported that P. coccineus showed a greater degree of resistance than P. vulgaris to Colletotrichum lindemuthianum, causal pathogen of anthracnose.

Crosses between P. vulgaris and P. coccineus are generally successful when P. vulgaris is used as a maternal parent (Honma and Heeckt, 1959, and Al-Yasiri and Coyne, 1966). Crosses with P. coccineus as female were successful in a few genetic combinations (Lamprecht, 1941). Embryo abortion was reported also when P. coccineus was used as female parent (Honma and Heeckt, 1959, and Al-Yasiri and Coyne, 1966). This was explained on the basis of chromosomal aberration (Honma and Heeckt, 1959), or disturbance in the development of both embryo and endosperm (Kroh, 1962, Brink and Cooper, 1940, Johanson and Smith, 1956, Thomas, 1964, and Smartt, 1971). One of the objectives of the present investigation was to develop a method to prevent embryo abortion.

The introduction of the hypogeal germination trait into P. vulgaris would reduce injury to seedlings emerging in crusted soils. Lamprecht (1941) described cotyledon inheritance as monofactorial and reported that this trait could not be transferred from one species to the other. Gates

(1951), Wall and York (1957) and Honma and Heeckt (1958, 1962) reported that cotyledon position (hypogeal versus epigeal) was multifactorial in inheritance and that the hypogeal germination trait could be transferred to P. vulgaris.

Another objective of this research was to try to develop cross-pollinating lines of P. vulgaris by transferring genes controlling the mechanism for cross-pollination in P. coccineus. It was proposed to investigate if the cytoplasm of either species had a significant effect on the success of transferring the nuclear genes for the cross-pollination mechanism from P. coccineus into the P. vulgaris genome.

P. coccineus, according to Gates (1951), was considered self-sterile. Cooper, according to Cardwell (1961), indicated that the papillar layers on the stigma caused a mechanical barrier for selfing in P. coccineus. Lamprecht (1941) reported that the cross-pollination of P. coccineus was due to the presence of the extrorse stigma. Cooper, according to Cardwell (1961), and Hawkins and Evans (1973) showed the dehiscence of pollen on the style hairs. Wall (in a personal communication, 1971) reported that P. coccineus was cross-pollinated but not self-incompatible (it will self if the protected stigma is brushed with self pollen). Cardwell (1961) used a toothpick, covered by an abrasive material, to place pollen on the stigma surface. He obtained 52 percent success in selfing P. coccineus.

In order to transfer the cross-pollination mechanism from P. coccineus to P. vulgaris, we should know why selfing

does not occur normally in P. coccineus.

Cross-pollinating lines of P. vulgaris could be used in different mating schemes to break up linkages and facilitate increased gene recombination. There is a grave need to introduce much new germplasm into modern bean cultivars. A great genetic vulnerability to pests and diseases exists because of the narrow-based germplasm utilized in bean variety development. Also, the utilization of new breeding approaches which a cross-pollinating system would permit, might permit breeders to move off the yield plateau and increase yields of this highly nutritive crop for the benefit of mankind.

PREVIEW

## Literature Review

### Interspecific Hybridization.

Interspecific hybridization within the genus Phaseolus has been attempted over the past century; however, the crossing behavior between cultivars of different species has not been fully resolved (Smartt, 1971). Mackie and Smith (1935) reported the occurrence of hybridization between cultivars in the field in the following species, Phaseolus vulgaris L., P. acutifolius A. Gray, P. multiflorus L., P. coccineus L. and P. lunatus L. Miranda (1967a) reported a natural hybrid between the putative parents corresponding to the two Phaseolus species P. vulgaris and P. coccineus. Strand (1943) reported that all species of Phaseolus are considered difficult to cross. According to Barrons (1938) and Allard (1953) the percentage of natural crossing in beans was affected by degrees of isolation. Magruder and Wester (1939) and Magruder (1943) found that the percentage (out-crossing) varies not only between species but also among cultivars of the same species.

The effect of environment on the hybridization has also been reported (Down 1935, Strand 1943, and Smith and Richard 1962). Artificial pollination under field conditions has been unsuccessful, whereas it has succeeded in the greenhouse when (a) the temperature is maintained between 20°C and 24°C, and (b) the relative humidity is kept over 80 percent.

Down (1935) stressed the importance of emasculation of the flowers 1 to 2 days before crossing. He noted that care must be taken to avoid mutilation of the flower parts which cause their drying out. Wade (1943) improved the percentage of fruit set from 17.5 percent to 52.5 percent by a new technique in which the banner of the male flower was placed on the pollinated flower after being crushed in his mouth. Cross pollination was done either at the time the female flowers were opening or when the female flower buds had reached their maximum size (Wester and Jorgensen 1950). A few years later Buishand (1956) classified the crossing methods into two categories: (1) with emasculation, which includes: (a) the rubbing method, (b) the hooking method, and (c) repeated pollination; or (2) without emasculation, which includes: (a) the rubbing method, and (b) the clamping method. Honma (1956) modified Wade's technique by keeping the stigma of the male parent attached to the stigma of the female parent after removing a small portion of female keel.

Lamprecht (1941) reported success in crossing when certain species are used as maternal parents. In crosses between P. vulgaris with P. multiflorus, success varied with the parental line used. With certain strains as parents no seeds were obtained, with others non-viable seeds, while others produced two to three germinable seeds. Kroh (1962) found significant differences in the fertility of four P. coccineus clones. Two of the clones produced exclusively normal plants when used as the male parent in crosses with

P. vulgaris; the remaining two clones yielded exclusively crippled plants. These differences were attributed to genotypic clonal differences. Lamprecht (1948b) related species differences to: (1) differences in genotype constitution, (2) chromosome structure, and (3) polyploidy. Species might differ from each other in two different ways: (a) A gene may be present in one species and not in the other. This may cause incompatibility with the genome of the other species. (b) Two genes may differ in the alleles of the same gene. However, Lamprecht (1945) reported the possibility of producing types that combine the most valuable character in both P. vulgaris and P. coccineus except for those which control cotyledon position and stigma shape. He suggested that the cotyledon position in both P. vulgaris and P. coccineus is controlled by two different alleles of the same "interspecific gene." He applied the same situation to the stigma shape. He said the two genes are not self-reproducing, but for each gene there is progen and that fails in the foreign plasmon. Additionally, the established genic-plasmic mechanism forms a barrier between the species. Wall and York (1957), on the other hand, reported that the position of the cotyledon in Phaseolus is controlled by multiple factors. In attempts to incorporate the hypogeal germination habit of P. polystrachus into P. lunatus, so as to avoid the high mortality among the seedling as a result of breaking the hypocotyl, Lorz (1952) made 50 crosses using P. polystrachus L. as female and P. lunatus as male.

He had no success. Out of 100 reciprocal crosses he obtained seven  $F_1$  plants. Honma and Heeckt (1958, 1959) failed to produce the  $F_1$ , P. coccineus with P. lunatus. However, when they sprayed the flowers with N-M-tolylphthalamic acid at 200 to 1000 ppm at the time of pollination a few pods set, but they aborted within 2 weeks. On the other hand, when a piece of cotton soaked with 500 ppm of the same substance was attached to the pedicel of the pollinated flower, some pods did develop and reach maturity. Honma and Heeckt (1958), Coyne (1964), and Kammerman and Bemis (1960) stated that interspecific hybridization might be achieved by using parents possessing a broad genetic base. Wall and York (1960) and Wall (1966) suggested that introgression may be more effectively achieved through the female gametes than through the male gametes. Al-Yasiri and Coyne (1966) divided the crossability between different species into compatible, partially compatible, and incompatible. Kammerman and Bemis (1960) and Bemis and Kedar (1961) identified two types of dwarfness when they crossed different P. vulgaris cultivars as female with P. coccineus: B dwarfs which were lethal in the seedling stage and T dwarf plants which produced flowers and viable pollen.

There was no uniformity in the  $F_1$  P. vulgaris with P. multiflorus. Three different types of plants were produced. Multiflorus-like giants, intermediate (1 meter high) and dwarfs with sickly appearance. The stigma shape, cotyledon position and flower color were intermediate in the  $F_1$  while

inflorescences and pods resembled P. multiflorus Lamprecht, (1941). Lamprecht suggested mono-factorial inheritance for the cotyledon position and stigma shape, and multifactorial inheritance for color inheritance. Honma and Heeckt (1962) obtained two F<sub>1</sub> plants from crossing P. coccineus as female with (P. vulgaris with P. coccineus) as male. One of the two plants was dwarf and failed to set pods, and the other resembled P. coccineus. Honma and Heeckt (1959) used heterozygous parents in crosses between P. vulgaris with P. lunatus and found that among the 10 F<sub>1</sub> plants produced, eight resembled the female parent in morphology, while the other two failed to resemble any parent. Similar results were obtained by Honma and Heeckt (1958) in crossing P. coccineus with P. lunatus in which the hybrids resembled P. coccineus. According to Miranda (1967a) hybrids between P. vulgaris with P. coccineus showed epigeal germination similar to P. vulgaris. Contrary to that, Caspari (1948) reported that hybrids between P. vulgaris with P. multiflorus resembled the male parent. Smartt (1971) in describing the F<sub>1</sub> P. vulgaris with P. acutifolius, stated that the primary leaf petiole was large like P. vulgaris, and the inflorescences were similar to those of P. acutifolius.

#### Embryo Resorption.

Many explanations have been suggested for the embryo resorption phenomena. If complete abortion occurs early in crosses, it seems likely that the cross will not succeed.



Embryo abortion usually occurs in the early stage of pod development. Honma and Heeckt (1959) found that embryo abortion took place 8 to 13 days after pollination of P. coccineus with P. lunatus with or without hormonal spray. Smartt (1971) found in the  $F_1$  P. vulgaris with P. acutifolius the pods usually collapsed and dried out rapidly after they reached their maximum length.

Smartt (1971) attributed the failure of interspecific hybridization to, (1) the failure of the pollen to permeate the stigma, (2) introduced pollen failed to penetrate the style, (3) introduced pollen failed to effect fertilization, (4) zygote failed to develop or embryo aborted, (5) seed produced fail to germinate. Lamprecht (1948b), Honma and Heeckt (1958), Coyne (1964), Kammerman and Bemis (1960), and Bemis and Kedar (1961) reported that certain genotypes might be involved in the species crossability barriers. Smartt and Haq (1972) found that it is possible to select cross-compatible individuals which produce normal hybrids. According to Miranda (1967b) genetic flow has occurred from P. vulgaris into P. coccineus by means of natural interspecific hybridization and the constant backcross of the hybrid to the P. coccineus. Honma (1968) related difficulties in obtaining favorable recombinants from intraspecific crosses in P. vulgaris to a chromosomal aberation, such as an inversion. Kroh (1962) showed that the cause of embryo abortion was related to disturbances in development and in differentiation. He reported that

the abnormal development of both embryo and endosperm was caused by disturbances in nutrition. Similar results were obtained by Brink and Cooper (1940), Johanson and Smith (1956), Thomas (1964), and Smartt (1971). Brink and Cooper (1940), studied 36 species of angiosperms. They found that the ovules contained low food reserves at the time of fertilization. Embryo development depended on food translocation from normal tissue by means of the endosperm. Brink and Cooper (1940, 1947), Lamprecht (1941), and Caspari (1948) reported that the cause of embryo abortion was due to plasmon-gene interaction and indicated a different response of the same gene in different cytoplasm. The plasmon has the ability to produce more or less effective crossing barrier which may affect single genes, gene groups, chromosomes, or whole genomes. The effect of cytoplasm can be detected in the reciprocal differences in the  $F_1$  and unchanged transmission to the  $F_2$  generation. It is suggested that the reason for reciprocal differences in species hybrids is that hybrid genomes in general are unstable.

#### Improving Fruit Set.

One of the goals for most plant breeders is to increase crop yields. Brinkley (1932) studied the amount of blossom and pod drop in six cultivars of garden beans. He found that the presence of large number of blossoms does not necessarily mean that pod set and yield will be heavy. A negative correlation was found between percentage of

blossom and pod drop and yield/plant. He reported that blossom and pod setting are critical periods affecting yield.

Chemical and growth hormone applications have been tried by many workers to improve pod set. Ketellapper and Bonner (1961, 1963) reported the effect of regular sprays containing mixtures of vitamin B, C, caseinhydrolysate, sucrose or ribosides, on plants grown at different temperatures, including the optimum temperature. He found that application of 10 percent sucrose solution to pea plants grown in artificial light, 23/17°C, caused 56 percent increase in dry weight, which made the dry weight equal to that grown in optimal conditions. Increase in dry weight, up to 40 percent, was obtained from applying vitamin B or ribosides to pea plants grown in a high temperature, 30/23°C. He suggested that partial prevention of the reduction in dry weight caused by unfavorable temperature is possible by application of essential metabolites. Murneek et al. (1944) reported that only under high temperature did the application with alpha-naphthaleneacetamide and beta-naphthoacetic acid increase the yield of snap bean. Allen and Fisher (1943) found a 15 percent increase in wax beans when plants were dusted with naphthaleneacetic acid alone or mixed with insecticide. The same materials were less efficient in spray form. Wittwer and Murneek (1946) reported higher fruit setting and yield obtained by spray or dust application with para-chlorophenoxyacetic acid,

alpha-orthochlorophynoxypropionic acid or 2,4 dichloro-phenoxy acetic acid at the time of blooming. Wester and Jorgenson (1950) reported success of indolbutyric acid and para-chlorophenoxyacetic acid mixed with lanolin and applied to the base of the flower with a dissecting needle. The mixture increased the percent of successful crosses from 18.7 to 28, and caused a significant increase in the average no. of seed/pod from 1.95 to 2.43 in lima bean. Honma and Heeckt (1958) succeeded in obtaining mature seeds with the use of cotton soaked with 500 ppm of N-M-tolylphthalamic and attached to the pedicel in crossing P. coccineus with P. lunatus. When they used a mixture of indolacetic acid and para-chlorophenoxyacetic acid in crosses between P. acutifolius with P. vulgaris, it neither increased the pod set nor delayed the embryo abortion. Al-Yasiri (1964) reported that four applications of naphthaline acetamide and potassium gibberelate to the flower pedicel improved pod set and increased pod growth, but did not delay embryo abortion. The inhibition effect of the bean extract on the embryo growth was also found by Honma (1955).

#### Incompatibility and Male Sterility.

Incompatibility is the failure of the pollen grains to fertilize the ovary of the same flower, or the ovary of another flower from the same variety or species. Self and cross incompatibilities exist in most species and families, from the grasses to the composites. It is believed to

result from the inhibition of pollen tube growth either on the stigma, at, or soon after germination, or in the pistil, usually during the first few hours of pollen tube growth. Among 53 genera that have been studied, inhibition occurs at, or immediately following pollen grain germination. In 17 of the genera cessation of pollen tube growth occurred before penetration of the stigma. In the other 36 genera, pollen germination and stigmatic penetration proceeded normally, but inhibition occurred in the pollen tube during the descent in the style. In some cases the pollen tubes grow as far as the embryo sac (Frey 1967).

Self incompatibility produces a cross pollination mechanism in plants. Some species have more than one incompatibility mechanism. The leguminosae, for example, have both self- and cross-pollinating agricultural species. It is probable that a mutation(s) occurred to wild species giving rise to the development of self-compatible plants. Three types of incompatibility in Phaseolus were discussed by Al-Yasiri (1964): compatible, partially compatible, and incompatible. Cardwell (1961) reported the presence of several mechanical barriers to self-fertility; pollen dehiscence below the stigmatic surface; and the presence of papillar cells on the stigma surface. He argued that these need to be ruptured before pollen tubes can penetrate the stigma. In explaining unilateral incompatibility between P. coccineus with P. vulgaris, Martin and Pandey according to Abdalla and Hermsen (1972) reported the possibility of

disturbed pollen tube growth being involved and a multiple gene system controlling the balance of substances affecting pollen tube growth and stylar inhibition. They mentioned that lines unsuccessful as seed parents would have an abundance of pollen tube inhibiting substances in the style. Additionally, they thought only pollen grains with abundant growth-promoting substance could achieve fertilization, while the lines which are poor as males would have little growth promoting substance in the pollen. Hawkins and Evans (1973) examined the growth of pollen tubes in both P. coccineus and P. vulgaris and found that P. coccineus pollen tubes grow normally in stylar tissue of both P. coccineus with P. vulgaris with no obstacles to self-fertilization. They added that the vascular tissue of the style has considerable nutritional and chemotropic influence upon pollen tube growth. They noticed that the association between the pollen tubes in P. vulgaris and the vascular tissue after the penetration of stigmatic surface was not immediate as in P. coccineus. They observed that the inhibition of the pollen tube in the interspecific cross P. coccineus with P. vulgaris resulted from the slow growth of the pollen tube due to the increase of callose deposit in both pollen tube walls and in the form of callose plug in style tissue. Ballon and York (1959) reported that pollen tubes reached the ovule 6 hours after selfing in Vigna sesquipedalis Fruwirth while in crosses V. sesquipedalis with P. coccineus and V. sesquipedalis with P. vulgaris

pollen tube reached half the length of the style in 6 hours.

Male-sterility has received a great attention from many scientists. In lima beans, Allard (1953), found that male-sterile plants show a seedling abnormality, with a defective terminal bud after the second node. The leaves are much crinkled, thickened, and dark green. He noticed that the anthers do not open during the normal flowering season but there may be up to 50 percent stainable pollen and a few pods set late. Dubey and Singh (1965), Kaul and Singh (1966), Chauhan and Singh (1966), Joppa et al. (1966), Alam and Sandal (1967), Jensen (1969), and Pritchard and Hutton (1972), all reported that the morphological and cytological behavior of the anther was similar in both fertile and sterile plants. The development through meiosis was similar in both except in the case of microspores and tapetum. Those tapetal cells which do not degenerate, but persist, cause a failure in the supply of the substances which are required for the development of microspores, and the result is male-sterility. Putt and Heister (1966), Meyer (1968), and Gill and Anand (1970), reported sterility ranges from partial male-sterility to complete male-sterility. Joppa et al. (1966) reported that tapetal cells in male-sterile wheat formed less starch than in the fertile plants. Kendall et al. (1971) reported that plants with less than 50 percent pollen germination contain half the amount of proline, glycine and alpha-aminobutyric acid of those with more than 50 percent pollen germination. Alam

and Sandal (1969) found a greater amount of protein bonds in fertile plants than in sterile ones, especially in cytochrome oxidase and in total protein. They suggested that heritable abnormal metabolic activity was associated with pollen abortion and subsequent male-sterility in sudan-grass. Putt and Heister (1966) described two kinds of pollen grains produced by male-sterile sunflower plants: no pollen, or pollen of uneven size which are clumped and do not stain with acetocarmine; pollen produced, but smaller than normal, non-staining and not clumped. Miller and Schonhorst (1968) and Kendall et al. (1971) reported the correlation between fertility percentage and pollen tube length, and a significant difference was observed between pollen tube length in the greenhouse versus field grown plants.

Meyer (1969) reported that male-sterility is caused by mutant genes, cytoplasm from other species, environmental stress, or chemical treatment. Brooks and Brooks (1967) observed the effect of environment on male-sterility expression in sorghum. The inheritance of male-sterility has been discussed by many authors. Allard (1953) found that in all commercial varieties of lima beans there is a 0.002 frequency of sterile plants. About 0.1 appeared to be male-sterile. Allard (1953), Putt and Heister (1966), Knight (1969), Meyer (1969), and Briggles (1970), observed that male-sterility is controlled by a single recessive gene in lima beans, sun flowers, crimson clover, cotton and