**Cytological Behavior of an F_1 Species Cross
(*Phaseolus lunatus* L. var. Fordhook $\times$ *Phaseolus*
polystachyus L.)¹**

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The genus *Phaseolus* comprises 470 species which grow mostly in warm and temperate regions (Small 1933). The genus is of considerable economic importance. The various species and varieties furnish a large variety of beans for human food. Some of the species are of ornamental value.

Cytological investigations concerning the genus have been combined to 17 species (Darlington and Ammal 1945, and Darlington and Wylie 1956). In this investigation no work has been initiated along this line, because of the small chromosomes and the difficulty in standardizing the staining technique. The present paper concerns the cytology of the F_1 cross (*Phaseolus lunatus* L. var. Fordhook $\times$ *P. polystachyus* L.) under Utah environmental conditions.

Materials and methods

P. lunatus L. and *P. polystachyus* L. have epigeal and hypogeal germination, respectively (Fig. 1). The cross was made in Florida, to introduce the character of hypogeal germination from *P. polystachyus* L. to *P. lunatus* L., to help solve the emergence problem in lima beans. A few F_1 beans were brought to Utah and were grown in the greenhouse. The germination behavior of an F_1 plant is shown in Fig. 2, where about 50 per cent of the hypogeal character was attained by the hybrid, which was intermediate between both the parents. Plants flowered profusely and a few pods were formed but dropped at the later stages of development. As a result of this, no mature seeds were obtained for F_1 plants by manual self pollination.

In Florida, under greenhouse conditions, open pollinated (presumably selfed) F_1 material has produced two F_2 individuals but, in view of the extremely profuse flower production of the F_1 hybrid, fertility can be expressed as less than one one-seeded pod per 1000 flowers, or less than 0.1%. Seed

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production is therefore comparatively rare but not impossible. This is important because it may be the means of overcoming the barrier between the two species.

Figs. 1-5. 1, *Phaseolus lunatus* L. with epigeal germination. 2, *Phaseolus polystachyus* L. with hypogeal germination. 3-5, the germination behavior of an F_1 species cross (*P. lunatus* L. var. Fordhook $\times$ *P. polystachyus* L.) with 50 percent of the hypogeal character.

Meiosis was studied on plants growing in the greenhouse to find out if chromosomal aberrations were responsible for a part of the sterility shown by the plants. The flower buds were fixed in propiono-alcohol made up in the following proportion: 9 parts absolute alcohol, 2 parts propionic acid,

and 1 part propionic acid saturated with ferric acetate. The fixed flower buds were stored for about five days at 6°C. The stain used was propionocarmine, prepared by boiling 0.5 grams of carmine in 100 ml. of 45% propionic acid and filtering after cooling. This stain worked satisfactorily with *Phaseolus* material. Earlier trials with aceto-carmine, aceto-orcein, and several other stains with several modifications did not give satisfactory staining. Passable results were obtained with aceto-orcein, but aceto-carmine did not stain fixed chromosomes of this material.

To make the slides permanent, the slide and the coverslip were separated in a mixture of acetic acid and absolute alcohol in equal parts and were carried through two changes of absolute alcohol. The coverslips and slides were recombined by mounting in Diaphane.

To estimate the pollen fertility, dehiscent anthers were lightly tapped on a drop of mixture of aceto-carmine and glycerine 1:1, on a slide. For each slide a minimum of ten microscopic fields was chosen at random and the grains were counted in fertility and sterility classes.

Initial routine observations were made with a Spencer research microscope with objectives $\times 10$, $\times 43$ dry and $\times 98$ oil emersion and an eye piece $\times 10$.

Results

Both parents (*P. lunatus* L. var. Fordhook, and *P. polystachyus* L.) showed a normal meiotic behavior without any irregularity. The pairing of the chromosomes was normal and eleven bivalents were seen in diakinesis and metaphase I (Fig. 6). This was followed by a regular anaphase I with eleven chromosomes going to either pole (Fig. 7). The second meiotic stage also showed no abnormality from its usual course. The pollen size was uniform; almost all of the grains were capable of germination.

Although a few pollen mother cells from the hybrid showed normal meiosis, most of them exhibited abundant irregularities. Pollen mother cells with normal and abnormal meiosis were found within the same flowering bud. In a majority of the pollen mother cells, 6 bivalents and ten univalents were present at metaphase I (Fig. 8). The occurrence of ten univalent may be due to the failure in their pairing during prophase I. Early disjunction of a bivalent at metaphase I would also lead to a similar situation. In most of the cases some of the chromosomes remained away from the equatorial plate (Fig. 9). In some pollen mother cells three bivalents, twelve univalents, and one quadrivalent were also noticed (Fig. 10).

It is rare that chromosomes at metaphase I form a mass in which individual bivalents cannot be deciphered. Johnsson (1944) reported a similar situation in *Alpecurus myosuroides*. Such an irregularity has usually been attributed to disturbed nucleic acid metabolism (Darlington 1943). Abnormalities at anaphase I are illustrated in Figs. 8 to 11. A pollen mother cell with

10 chromosome distribution, with one laggard chromosome and one bivalent at one pole, was also noticed (Fig. 9). An anaphasic bridge with 8 and 14 distribution of chromosomes was also commonly observed (Fig. 13). One bivalent and one univalent laggards, one bivalent laggard, and two bivalent

Figs. 6-14. 6, metaphase I. 7, anaphase I. 8-10, metaphase I. 11-14, anaphase I.

laggards, were commonly observed (Figs. 15, 16, and 17). In addition to the above mentioned abnormalities at anaphase I, cells with 5/17, 6/16,

Figs. 15-18. 15-17, anaphase I showing bivalents and univalents laggards. 18, micronuclei.

and 7/15 distribution of chromosomes at this stage were also observed. Six microspores of varying sizes, with four large nuclei and one and two micronuclei inside 4 spores are shown in Fig. 18. Cells with four large nuclei and three micronuclei were commonly found. As a result of the variation in the size and number of nuclei, the pollen formed is extremely variable in size (Fig. 19). The pollen grains

are still in the pollen mother cell in Fig. 19, and most of the small grains are behind the large ones. High sterility and variation in size of the pollen grains are shown in Fig. 20. In this plate only one pollen grain is apparently fertile. This is the large, darkly stained body located in the center

Figs. 19-20. 19, cells showing more than 4 microspores. 20, pollen grains showing sterility in F₁ species cross (*P. lunatus* L. var. Foordhook $\times$ *P. polystachyus* L.)

of the plate. Such apparently fertile pollen grains were rarely found. Meiotic irregularities in general seem to be responsible for the high pollen sterility observed in these plants.

Discussion

The haploid and diploid chromosome numbers for *Phaseolus lunatus* L. and *Phaseolus polystachyus* L. were determined as eleven and twenty-two, respectively, which confirmed the findings of Darlington and Ammal (1945) and Darlington and Wylie (1956).

The meiotic behavior of chromosomes was found to be normal in the material fixed from parent plants. A number of meiotic abnormalities were, however, recorded in the material fixed from hybrid plants under the same environmental conditions and method of preparation of slides, as all these factors were kept constant for both parental as well as hybrid plants. The fact that the abnormalities were not recorded in parental plants rules out the possibility that the irregularities may be due to other factors than the cytologic or genic causes. The cause of the meiotic irregularities in the hybrids may thus be attributed to differences in genetic composition (Grun 1952) or the size of the chromosomes of the two genomes. The present investigation has thus clearly shown the importance of genetic make up in relation to the meiotic behavior of hybrids in genus *Phaseolus*.

Summary

The paper describes the cytological behavior of F_1 cross (*Phaseolus lunatus* L. var. Fordhook $\times$ *Phaseolus polystachyus* L.) under Utah environmental conditions. The germination behavior of parental and hybrid plants was studied. The flower buds were fixed in propiono-alcohol and were smeared in propiono-carmin.

The meiotic behavior of chromosomes, as seen from the dividing pollen mother cells, was normal in parent species. A number of meiotic irregularities such as non-pairing of chromosomes, early disjunction, the presence of bridges and laggards with unequal distribution of chromosomes at anaphase, and polyspory were recorded from hybrid plants. The meiotic irregularities in general seem to be responsible for high pollen sterility in F_1 hybrid plants.

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