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Meiosis and Fertility of Interspecific Hybrids Between *Phaseolus vulgaris* L. and *P. acutifolius* A. Gray*

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Summary. Meiosis and fertility of interspecific hybrids obtained from reciprocal crosses between *Phaseolus vulgaris* and *P. acutifolius* were examined. Bivalents as well as univalents were found at Metaphase I. The majority of the microsporocytes had four or more univalents and the average was 6.3 univalents per cell. The average number of lagging chromosomes at Anaphase I was 2.3 per cell and the most frequent chromosome distribution at late Anaphase I was 10-12. The lower than expected number of lagging chromosomes as compared with the number of univalents at Metaphase I suggests the possible occurrence of precocious separation of bivalents. The male fertility as measured by pollen stainability was 17%, however, the frequency of pollen germination in selfing was 3.5%. Upon selfing of the interspecific hybrids, no dividing embryos were found even though 7 and 26% of the ovules were fertilized at 12 hours and four days after pollination. In backcrosses to *P. vulgaris* (male), 6 and 20% of the ovules were fertilized and 0 and 4% of the ovules contained dividing embryos at the same sampling times. When *P. acutifolius* was the male parent, respective values were 8 and 31% for fertilization and 0 and 13% for ovules with dividing embryos. The frequencies of backcross embryos recovered at 14-26 days were in agreement with the frequencies of dividing embryos at four days. The ability to obtain backcross plantlets demonstrates the feasibility to further utilize interspecific hybrids for the improvement of *P. vulgaris*.

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Introduction

The primary objective of generating interspecific hybrids between *P. vulgaris* and *P. acutifolius* is to facilitate gene transfer between the two species. This approach would promote the utilization of *P. acutifolius* germplasm for the improvement of *P. vulgaris*. Among the desirable traits of *P. acutifolius* are resistance to common blight (*Xanthomonas phaseoli*) and bean golden mosaic virus (Coyne and Schuster 1973, Yoshii et al. 1978, CIAT, 1977), and tolerance to drought and high temperature (Chavan et al. 1956).

Interspecific hybrids between *P. vulgaris* and *P. acutifolius* were first reported by Honma (1955, 1956). Subsequently, it has been shown that fertilization and division of the embryo and endosperm occur regularly in reciprocal crosses involving these two species (Rabakoarihanta et al. 1979). The hybrid embryos are characterized by the uneven growth of the two cotyledons (Mok et al. 1978). However, the development of the reciprocal hybrid embryos ceases at the cotyledon stage (with the final size of 3 to 4 mm) at approximately 17 to 21 days after pollination. Culturing of immature embryos on artificial medium is required to recover hybrid plantlets (Honma 1955, Mok et al. 1978). As an exception to these observations, the formation of mature hybrid seeds between two particular genetic lines was reported (Smartt 1970).

The efficient utilization of interspecific hybrids for gene transfer is dependent on sufficient fertility of these hybrids. Previous studies on interspecific hybridization of *Phaseolus* conducted in our laboratories (Mok et al. 1978; Rabakoarihanta et al. 1979) have established satisfactory procedures to recover hybrid plants between *P. vulgaris*

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and *P. acutifolius*. The availability of sufficient numbers of hybrids provides the opportunity to identify screening parameters for fertility and to determine genetic factors which may influence hybrid fertility. This paper describes results of our first attempt, namely the examination of meiosis and the assessment of fertility in reciprocal hybrids involving two parental genotypes.

Materials and Methods

Phaseolus vulgaris L. (2n=22) cv. Gallatin 50 (G 50) and *P. acutifolius* A. Gray (2n=22) P. I. 321637 (AC 2) were used as the parental genotypes. Flowers were emasculated one day before opening and pollinated with appropriate pollen. Embryo culture techniques and the medium composition were the same as previously described (Mok et al. 1978), with the exception that 0.125 uM N^6-benzyladenine (bzl^6 Ade) was added to the medium. The inclusion of this cytokinin in the medium was found to increase the growth rate of the plantlets. Embryos were grown on this initial medium under constant light (8000 lux) for approximately three weeks. They were then transferred to medium without bzl^6 Ade and with only one half (15 g/l) of the original amount of sucrose. Six week old plantlets were transferred to medium containing only one-quarter (7.5 g/l) of the sucrose. At this time the photoperiod was reduced to 12 hours. The temperature during embryo culture was 27° C. Sufficiently large plantlets were grown in hydroponic culture and maintained in the growth chambers. Large mouth jars containing 500ml of one-quarter strength mineral salts as described by Murashige and Skoog (1962) were used and proper aeration was provided. On the average, hybrids were grown in hydroponic cultures for one month and then transplanted to Jiffy Mix Plus (obtained from George Ball Pacific Company, CA). Hybrid plants were grown in the growth chamber at the temperature regime of 22° C/20° C (day/night) with 12 hours of artificial lighting.

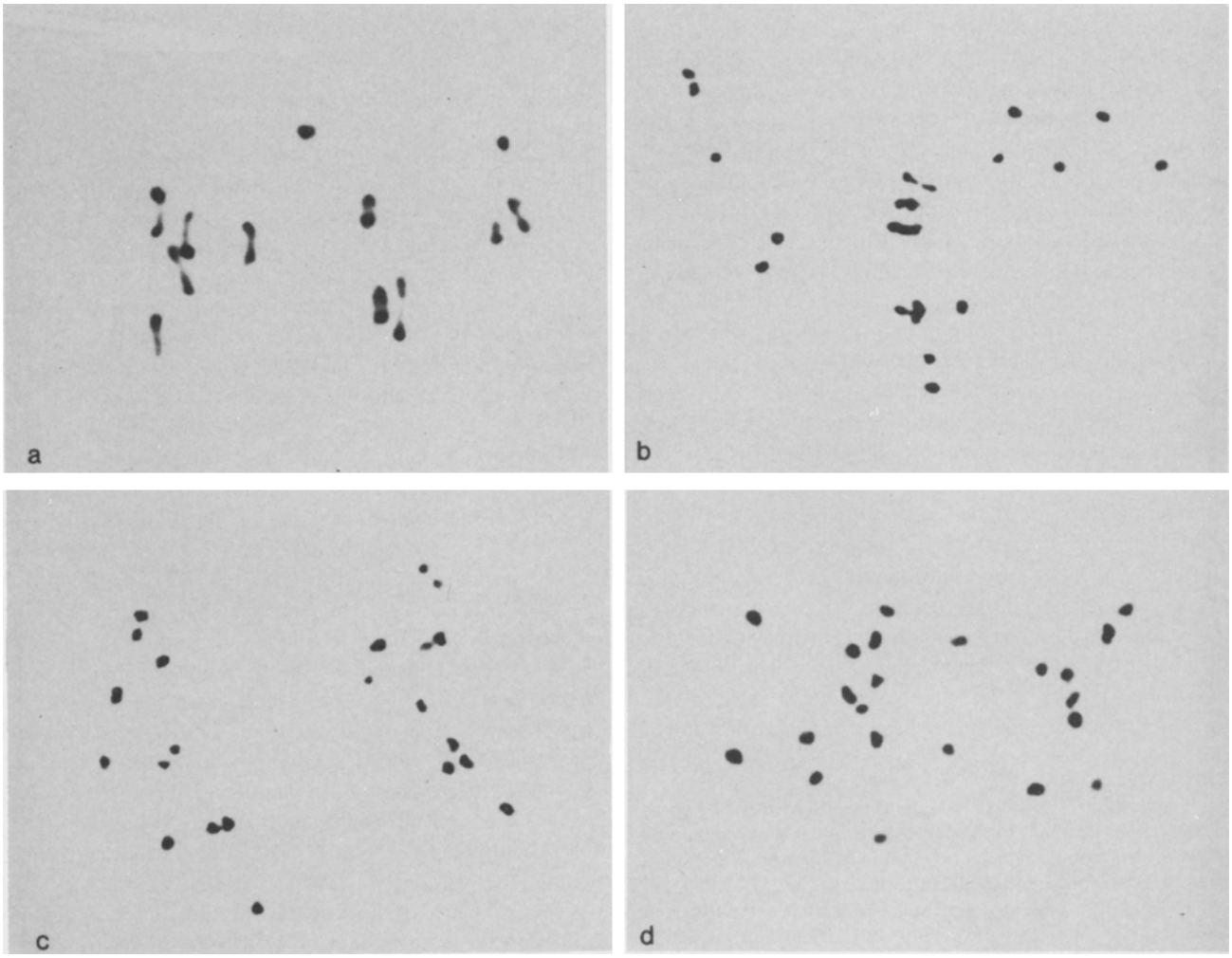

Fig. 1a-d. Meiosis of interspecific hybrids of *P. vulgaris* and *P. acutifolius*. a Metaphase I showing 10 bivalents and 2 univalents; b Metaphase I showing 4 bivalents and 14 univalents; c Anaphase I with 11-11 disjunction; d Anaphase I with 8-11 disjunction and with 3 chromosomes remaining in the center

Microsporogenesis was examined in two hybrids each of the reciprocal crosses between G 50 and AC 2. Flower buds were fixed in a solution containing three parts of 95% ethanol and one part of glacial acetic acid (volume to volume) with saturated iron acetate added (5 ml/100 ml of fixative). Anthers were dissected and microsporocytes were stained with 45% aceto-carmine. Particular attention was directed at key stages of meiosis. The male fertility was estimated by pollen stainability (with aceto-carmine) and in vivo germination rate at 12 and 24 hours on stigmas of hybrids by self pollination. The frequencies of fertilized ovules and ovules containing dividing embryos were determined at different time intervals after pollination in selfing and backcrosses to G 50 and AC 2.

Embryos obtained from backcrosses were dissected from 14 to 26 day old pods and were cultured on embryo culture medium. The frequency of immature embryos was recorded. The procedures for recovering backcross progeny were the same as those employed to obtain the initial F_1 hybrids.

Results

Microsporogenesis of reciprocal hybrids between *P. vulgaris* (cv. G 50) and *P. acutifolius* (AC 2) was examined. Chromosome pairing at Metaphase I was analyzed (Fig. 1a and b) and the frequencies of cells with univalents are presented in Table 1. The majority of the cells had four or more univalents and the average numbers of univalent per microsporocyte in AC 2 × G 50 and G 50 × AC 2 were 6.0 and 6.6 respectively. Since there was no reciprocal cross difference, results of further meiotic analyses of hybrids were combined.

Analysis of Anaphase I consisted of two parts; the number of cells with lagging chromosomes were recorded, and the numbers of chromosomes at opposite poles (Fig. 1c and d) after disjunction were determined (Table 2). The majority of the microsporocytes (54%) had two lagging chromosomes and the average number of laggards per cell was 2.3. Despite the observation that all cells at early Anaphase I had one or more chromosomes lagging, the frequency of cells with 11 chromosomes at each pole at late Anaphase was 17%. The remaining cells at late Anaphase I had unequal number of chromosomes and the most frequent disjunction was 10 and 12.

The male fertility of the interspecific hybrids was estimated by pollen stainability and by the germination rate on the stigmas of the hybrids (in selfing). The frequency of stainable pollen of reciprocal hybrids was 17% (over 1,000 pollen grains counted). The pollen germination rates (over 500 grains examined per each of the five samples) were 0.5 and 3.5% respectively at 12 and 24 hours after pollination. No difference in stainability or germination rate was detected between reciprocal hybrids.

The frequencies of fertilized ovules and ovules with dividing embryos in selfing and backcrosses are presented in Table 3. Crosses were classified according to the source of the pollen since there was no difference between reciprocal hybrids (AC 2 × G 50 and G 50 × AC 2) serving as female parents. At 12 hours after pollination, fertilization had occurred in 7% of the ovules. In backcrosses with pollen of G 50 and AC 2, fertilization was completed in 6 and 8% of the ovules respectively. The frequencies of fertilized ovules increased at later time intervals until four days after pollination. At this last sampling time, fertilization was completed in 26% of the ovules in selfing, and 20 and 31% of the ovules in backcrosses to G 50 and AC 2 respectively.

The frequencies of ovules containing dividing embryos were much lower than the frequencies of fertilized ovules

Table 1. Chromosome pairing at Metaphase I of interspecific hybrids between *P. vulgaris* (G 50) and *P. acutifolius* (AC 2)

	No. and percent of cells			
No. of univalents	G 50 × AC 2		AC 2 × G 50	
0	1	1.1	3	2.7
2	6	6.7	5	4.5
4	14	15.6	21	18.9
6	35	38.9	49	44.1
8	17	18.9	25	22.5
10	11	12.2	7	6.3
12	5	5.6	1	0.9
14	1	1.1	0	0
Total no. of cells examined	90		111	
Average no. of univalent per cell	6.6		6.0	

Table 2. Anaphase I disjunction of chromosomes of interspecific hybrids of *P. vulgaris* (G 50) and *P. acutifolius* (AC 2)

Early Anaphase I			Late Anaphase I		
No. of lagging chromosomes	No. and percent of cells		Chromosome distribution at Ana. I poles	No. and percent of cells	
0	0	0	11-11	7	17
1	8	21	10-12	18	45
2	21	54	9-13	12	30
3	6	15	8-14	3	8
4	2	5	7-15	0	0
5	2	5	6-16	0	0
Total no. of cells examined	39			40	
Average no. of lagging chromosomes per cell	2.3		Average distribution of chromosomes at Ana. I poles	9.7-12.3	

Table 3. Frequencies (in percent) of (a) fertilized ovules and (b) ovules with dividing embryos, in selfing and backcrosses of interspecific hybrids to *P. vulgaris* (G 50) and *P. acutifolius* (AC 2) at different time intervals after pollination

Cross		Time intervals after pollination				
		12 hours	24 hours	2 days	3 days	4 days
(G 50-AC 2) self	a)	7 (94)[a]	15 (144)	21 (57)	21 (66)	26 (89)
	b)	0	0	0	0	0
(G 50-AC 2) x G 50	a)	6 (61)	11 (46)	19 (54)	20 (60)	20 (64)
	b)	0	3	4	4	4
(G 50-AC 2) x AC 2	a)	8 (65)	17 (58)	19 (88)	25 (84)	31 (70)
	b)	0	4	7	13	13

[a] Number of ovules examined at each sampling time

at all the time intervals examined (Table 3). No dividing embryos were found in selfing of interspecific hybrids. When backcrossed to G 50, 3% of the ovules at 24 hours contained dividing embryos. The frequency increased only slightly in later sampling times and then remained rather constant at 4%. In backcrosses to AC 2, the frequency of dividing embryos at 24 hours after pollination was 4%. Substantial increase was observed in later sampling times; at the fourth day, 13% of the ovules contained dividing embryos.

Pods resulting from backcrosses which were retained on the plants for a period of 14 to 26 days, were collected. Immature embryos (with sizes ranging from 0.15 to 1.5 mm) were excised and cultured. A higher frequency of embryos was recovered from backcrosses to AC 2 (14%) than from backcrosses to G 50 (4%) (Table 4).

Discussion

As expected in most diploid interspecific hybrids, bivalents and univalents were observed at Metaphase I (Fig. 1a, b and Table 1). The number of univalents ranged from 0 to 14 with the average number of approximately 6 per microsporocyte. These observations suggest that there is reasonable homology between seven to eight pairs of chromosomes. Based on the assumption that all univalents at Metaphase I resulted from a lack of synapsis, the number of lagging chromosomes at Anaphase I should closely correlate with the number of univalents. Further more, the unequal distribution of chromosomes to Anaphase I poles should also reflect the number of univalents present at Metaphase I. According to this assumption, the majority of the Anaphase I cells should have at least six or more lagging chromosomes, and the failure of Anaphase I disjunction should have resulted in the majority of the late Anaphase I cells having normal distributions of only seven or eight pairs of chromosomes. However, the actual observations did not agree with this expectation. Most of the cells (75%) at Anaphase I were found to have two or less lagging chromosomes and the chromosome distribution at late Anaphase I was mainly 11 to 11 (17%) or 10-12 (45%). One of the possible explanations of higher than expected frequency of equal distribution of chromosomes may be that some of the univalents observed at Metaphase I were the result of precocious separation of loosely paired bivalents. Although these chromosomes appeared to be univalents, they were capable of co-orientation and normal disjunction. Indeed, close examination of cells at Metaphase I (Figs. 1a and 1b) revealed proximity of 'pairs' of univalents which may lend some support to the suggested explanation.

The determination of the actual fertility of the interspecific hybrid is more complicated. Pollen stainability was apparently an overestimate of the male fertility since 17% of the pollen was stainable but only 3.5% of the pollen germinated at 24 hours after pollination. Whether all pollen contained viable gametes is not certain since no dividing embryos were found in selfing of the interspecific hybrids. A definite assessment of the female fertility is not feasible either, since the completion of fertilization

Table 4. Number and frequency (in percent) of viable backcross embryos obtained at 14 to 26 days after pollination

Cross	No. of ovules	No. and percent of embryos	
(G 50-AC 2) x G 50	90	4	4
(G 50-AC 2) x AC 2	129	18	14

does not indicate that the female gametes involved are functional and the frequency of embryos capable of dividing was, for large part, influenced by the male parent. Thus, the measurement of the fertility of the interspecific hybrids would be somewhat arbitrary. With regard to the utilization of interspecific hybrids, however, the number of viable embryos in relation to the total number of ovules is the measurement of practical value. In predicting the potential of hybrids, the frequencies of dividing embryos may be the useful criterion, as they corresponded quite well with the frequencies of viable embryos in both selfing and backcrosses.

The difference in the developmental potentials of backcross embryos obtained from crossing to G 50 and AC 2 constitutes an interesting phenomenon. As the contribution of the female gametes of the interspecific hybrids should be the same, the much higher survival rate of backcross embryos of AC 2 suggests the possibility that the relative dosages of genetic material of the two species may be involved in the regulation of embryo development.

The genetic implications with respect to promoting gene transfer between *P. vulgaris* and *P. acutifolius* are encouraging. Although the frequency of backcross progeny obtained was low, they nevertheless demonstrated that further utilization of interspecific hybrids between these two species is possible. As the hybrids used in this study were derived from only two randomly chosen parents, it is conceivable that by screening large number of genotypes, interspecific hybrids with higher fertility can be obtained. Based on the frequency of bivalents at Metaphase I, it is speculated that crossovers can occur on at least seven or eight pairs of chromosomes. Whether these chromosomes regulate genetic traits of agronomic interests remains to be tested.

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