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Monosomics in Common Bean, *Phaseolus vulgaris*

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Summary. Two monosomics of *Phaseolus vulgaris* (2n = 22) were found among selfed progeny of plants treated with colchicine. The monosomic chromosomes involved were identified as chromosomes H and J according to the previously suggested Giemsa karyotype. Both monosomic plants had slower growth rate and smaller size as compared with their respective euploid sibs. However, no apparent morphological characteristics distinguished the two monosomics. The frequencies of transmission through selfing of monosomics H and J were 9% and 10% respectively.

Introduction

Many physiological and morphological characters in common bean, *Phaseolus vulgaris*, have been reported to be conditioned by one or a few genes (Bliss 1971, Coyne and Schuster 1972, Kelley 1971, Moh 1971, Yarnell 1965). However, there is no information concerning the location of gene markers on specific chromosomes. The availability of plants with variations in either chromosome structure (e.g. duplication-deficiency and translocation) or chromosome number (aneuploids) would greatly facilitate the determination of gene-chromosome relationship. Since bean chromosomes are small, structural variations of chromosomes may be difficult to characterize. If individual chromosomes of beans can be distinguished cytologically, the generation of aneuploids for the purpose of gene mapping appears to be more feasible than the synthesis of structural variation. After succeeding in the identification of bean chromosomes (Mok and Mok 1976), our efforts were centered on producing aneuploids through conventional methods of tetraploid-diploid and triploid-diploid matings. In the course of inducing tetraploids with colchicine, unexpectedly, monosomics were found among selfed progeny of treated plants. This paper concerns preliminary studies of two monosomics obtained and discusses implications of their occurrence on the synthesis of aneuploids in *Phaseolus vulgaris*.

Material and Method

Common bean (*Phaseolus vulgaris*, 2n = 2x = 22) cultivars, Bush Blue Lake, Early Harvester, Harvester, and Oregon 1604-E, were plant materials used. Seeds were germinated in vermiculite. Fifty seedlings of each variety were transplanted to soil when the first true leaves emerged. All plants were grown in the greenhouse at temperatures of 24°C (day) to 18°C (night) and with 15 hours of light. Three days after transplanting, shoot tips of seedlings were treated with 0.1% colchicine for six hours. The colchicine was applied with cotton balls (3 cm in diamter) soaked with the chemical. The treatment was repeated the following day. Selfed seeds (S_1) were collected from treated plants.

Root tips for chromosome counting, were pretreated with 0.1% hydroxyquinoline for four hours and then fixed in ethanol-glacial acetic acid (3:1) for 24 hours. The somatic chromosome number of individual S_1 seedlings was determined by making aceto-carmine squashes of root tips. A modified Giemsa technique (Mok et al. 1974, Mok and Mok 1976) was used to identify the monosomic chromosomes.

Flower buds, for the examination of microsporogenesis, were fixed in a solution consisting of equal volumes of ethanol-glacial acetic acid (3:1) and 45% iron-acetate for 24 hours. Anthers were squashed and stained with aceto-carmine.

Results

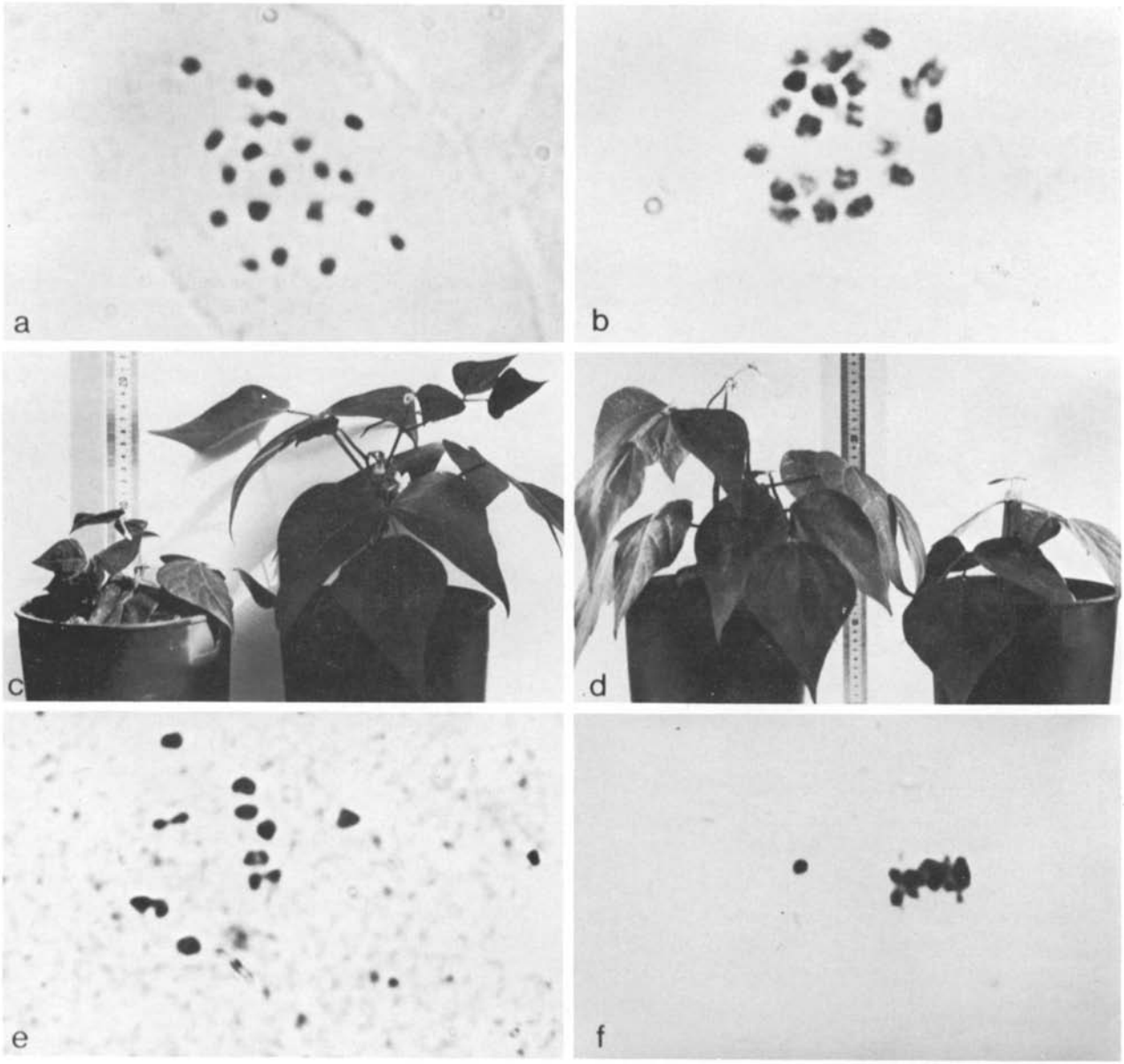
A total of 122 selfed seeds were obtained from plants treated with colchicine, and 95 seedlings survived. The somatic chromosome number of each plant was determined (Table 1). Two monosomics (2n = 21) and three tetraploids (2n = 44) were found. The designations C-8-3 (Fig. 1a) and D-2-2 (Fig. 1b) were given to the monosomic plants derived from Early Harvester and Bush Blue Lake respectively.

A common characteristic of C-8-3 and D-2-2 was the slower rate of growth and later development as compared with euploids. The mature plants were also

Table 1. Somatic chromosome number of selfed progeny obtained from four cultivars treated with colchicine

Cultivars	Number of seedlings obtained	Number of seedlings with the indicated chromosome number		
		21	22	44
Bush Blue Lake	28	1	26	1
Early Harvester	29	0	29	0
Harvester	24	1	23	0
OR 1604-E	14	0	12	2

considerably smaller than normal. Figures 1c and 1d illustrate the differences in plant height, at 29 days after germination, of C-8-3 and D-2-2 as compared with their respective diploid sibs. The first trifoliate leaves in both monosomics were atypical. A pair of small tendril-like leaves replaced the central leaflet of the first trifoliate in D-2-2. In C-8-3 two of the three trifoliate leaves were fused. However, all other trifoliate leaves that developed subsequently were normal.



Figs. 1. a to f, Somatic cells, morphology and meiotic cells of monosomics. (a) Somatic metaphase of C-8-3 with 21 chromosomes (× 1300). (b) Somatic metaphase of D-2-2 with 21 chromosomes (× 3600). (c) Monosomic C-8-3 (left) as compared with its euploid sib, 29 days after germination. (d) Monosomic D-2-2 (right) as compared with its euploid sib, 29 days after germination. (e) PMC of C-8-3 at Metaphase I, with 10 bivalents and 1 univalent. (f) PMC of D-2-2 at Metaphase I, with univalent

Table 2. Percent* of pollen mother cells of monosomics with, (1) lagging chromosomes at Anaphase I, (2) tripolar separation and micronuclei at Anaphase II, and (3) tri- and multi-nucleate cells at tetrad stage

Monosomic	Ana. I	Ana. II	Tetrad Stage		
	with lagging chromosome	tripolar separation	with micro-nuclei	tri-nucleate cell	multinucleate cell
C-8-3	10	4	7	6	8
D-2-2	16	7	11	10	14

* At least 250 cells were examined at each stage in each monosomic

The monosomic chromosomes in C-8-3 and D-2-2 were identified as chromosomes H and J, according to our previously suggested Giemsa karyotype (Mok and Mok 1976).

The results of cytological examination of microsporogenesis in monosomic plants are summarized in Table 2. Metaphase I of pollen mother cellls (PMCs) of monosomic plants had 10 bivalents and one univalent (Figs. 1e and 1f). At Anaphase I, a lagging chromosome was found in 10 % and 16 % of PMCs of C-8-3 and D-2-2 respectively. At Anaphase II, tripolar configurations and micronuclei were observed, and at tetrad stage, trinucleate and multi-nucleate cells were found. Pollen sterility as determined by iodine potassium iodide (I_2KI) was 14 % in C-8-3 and 30 % in D-2-2.

Practical considerations precluded the determination of male and female transmission frequencies of monosomic chromosomes by crossing monosomic plants with diploids. As flowers of *Phaseolus vulgaris* are cleistogamous, selfed seeds are easily obtained. In order to obtain the maximum number of seeds, monosomics were allowed to set selfed seeds.

The chromosome numbers of selfed progeny obtained from C-8-3 and D-2-2 were determined (Table 3). Three of the 34 selfed seedlings derived from C-8-3 had 21 chromosomes. The monosomic chromosome was identified as chromosome H in each case. Two monosomic plants were found among 20 selfed seedlings of D-2-2. The monosomic chromosome in each plant was identified as chromosome J. Again, these progeny monosomic grew more slowly than their euploid sibs, but the first trifoliate leaves were normal. As no univalent shift has occurred, the variant leaf types observed in the two original monosomics do not appear to be associated with the monosomic conditions.

Discussion

As far as we know, this is the first reported case of aneuploids in *Phaseolus vulgaris*. The occurrence of these monosomics and the transmission of gametes with 10 chromosomes through selfing suggest the possibility of generating and maintaining other aneuploids in beans. However, the chromosomes involved in these monosomics, H and J, are short chromosomes; their respective monosomic condition in the genome may not have drastic effects on fertility and viability of the plant. The lack of correlation between morphological characteristics and monosomy, except for the smaller plant size and slower growth rate, suggests that visual selection of aneuploids associated with specific chromosomes in beans may be difficult. Similar observations were reported in cross-pollinated species such as *Solanum* (Hermsen et al. 1970, Kessel and Rowe 1974) and self-pollinated species such as *Glycine max* (Palmer 1974, 1976). However, the possibility of unique morphological traits relating to other monosomics or trisomics, as reported in *Datura* (Blakeslee and Avery 1919), *Lycopersicum* (Rick and Barton 1954) and *Sorghum* (Schertz 1966) can not be excluded at present.

Table 3. Somatic chromosome number of selfed progeny derived from two monosomics

Monosomics	Number of progeny with the indicated chromosome number		Frequency of monosomics
	21	22	
C-8-3	3	31	9 %
D-2-2	2	18	10 %

The frequency of monosomics recovered from selfing for chromosomes H and J were 9% and 10% respectively. We were unable to study the male and female transmission rate of 10-chromosome gametes by crossing with normal diploids due to the relatively few flowers of the original monosomics. However, five aborted embryos of C-8-3 were examined cytologically and one had 20 chromosomes in somatic cells of the young root. This observation may be indicative of the viability of microspores with 10 chromosomes.

Aneuploids may arise in various ways (Burnham 1962), such as unequal distribution of chromosomes during meiosis of polyploids, and abnormal disjunction at meiosis of desynaptic mutants. The exact mechanism of the origin of the monosomics described here is not known. Their occurrence appears to have been caused by colchicine treatment, since all selfed progeny obtained from untreated plants were normal euploids. Extensive investigations of the effects of colchicine on the stability of chromosome number in *Sorghum* have been reported (Sanders and Franzke 1964, 1976). Chromosome substitution resulting from the doubling of chromosome number and subsequent reduction has been generally accepted as the basis for the occurrence of complex *Sorghum* mutants. It is tempting to speculate that similar events may have occurred in beans, as tetraploids were induced by colchicine and the monosomic may have resulted from subsequent chromosome reduction. However, the proposed explanation for the induced mutants in *Sorghum* appears to be dependent on the polyploid nature of the 20-chromosome *Sorghum* and reduction of polyploidy as a general phenomenon rather than an exceptional event. There is no evidence suggesting similar conditions occur in *Phaseolus vulgaris*. Another possible explanation for the occurrence of aneuploids is the induction of chromosome instability by colchicine. Somatic instability induced by wide crosses (Nielsen and Nath 1961) and low temperature (Huskins 1948, Huskins and Cheng 1950) have been reported, and spontaneous occurrence of variable chromosome number in vegetatively propagated grasses has been observed (Nielsen 1966). However, in *Nicotiana*, although incomplete polyploids were reported to be associated with colchicine treatment (Lefevre and Heslot 1953), no reduction of the diploid chromosome number was observed. Further studies are needed in order to ascertain the mechanism involved in the origin of the bean monosomics.

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