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Induction of Semisterility Mutations in Common Bean, *Phaseolus vulgaris* L.[1]

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Abstract. **Bean pollen treated with 2, 4, 8, and 16 kR of gamma irradiation was used to produce M_1 seed. The M_1 plants were screened for semisterility of the pollen. The inheritance of the semisterility mutations was studied in crosses to a tester stock with recessive marker genes and in M_2 and M_3 progeny tests. The 4, 8, and 16 kR treatments produced nearly 100% lethal mutations while 2 kR produced 70% lethality. The semisterility factors behaved genetically like dominant alleles in F_1 test cross progeny but segregated again for a ratio of 1 semisterile to 1 fertile plant in the F_2, indicating that the mutants are probably chromosome aberrations and not genic mutations. They may in fact be chromosome translocation heterozygotes. The semisterility factors produced 40 to 60 % pollen abortion and a significant reduction in seed set per pod. Semisterility factors were found in about 5% of the M_1 plants tested.**

There are no known reports concerning work to induce chromosome translocations in common bean. Translocations have numerous uses in research applications (4), including providing a source of trisomic progeny and facilitating the task of mapping genes.

One method of inducing translocations is to treat pollen with ionizing radiation and make pollinations onto untreated plants to reproduce the translocations. Research reports on the effects of pollen irradiation have been reviewed extensively by Brewbaker and Emery (3). Stadler and Sprague (7) treated maize pollen with X-rays and made direct cytological examination of 100 unselected plants of the F_1 progeny produced by pollinations with the treated pollen. They found that 44% of the plants of the X-ray progeny had translocations. Anderson (1) treated maize seeds with 14 kR of X-rays and screened for chromosome aberrations by searching for M_1 plants with tassel sectors showing abnormal pollen. After test-crossing with pollen from the selected plants and planting 10 seeds per test cross, only 6% of the test progeny segregated for semisterility.

In maize translocation heterozygotes, the average frequency of pollen abortion is typically 50% and seed set is reduced to about 50% of normal (4). In tomato pollen, abortion in translocation heterozygotes averages about 30%, ranging from 27% to 32% (2).

The long term objective of the work reported below is to develop an efficient technique for inducing and detecting translocations in bean. The specific objective of this research was to test the effectiveness of treating bean pollen with gamma irradiation to produce semisterility mutations, which are assumed to indicate the presence of a translocation heterozygote in most cases.

Materials and Methods

A bean breeding line, 7-1404, was selected for this project because it has good adaptation and a broad range of disease resistance. This line is a black-seeded, half-runner type with resistance to most of the root rot and foliar fungus diseases observed at Gainesville, Fla.

Newly opened flowers were harvested each day between 0800 and 0830 HR, and these intact flowers were irradiated with a Cesium-137 source (Gammator Model M) producing about 2,000 roentgens/min. Irradiated pollen from these flowers was then used to pollinate emasculated buds on 7-1404 plants, using the stylar "brush" to transfer the adhering pollen to the emasculated bud.

Flowers were irradiated with 2, 4, 8, or 16 kR. About 140 to 160 pollinations were made for each irradiation treatment, using greenhouse plants in November 1978. An additional 120 pollinations were made 3 months later with flowers exposed to 2 kR of gamma irradiation.

Abortion frequency of flower buds pollinated with irradiated pollen was determined. The pods produced by successful pollinations were scored for seed set and seed abortion. Seed produced by crosses with pollen exposed to 2 kR were planted in 2 greenhouse plantings, 1 in December 1978 (group 1) and the other in September 1979 (group 2). These M_1 plants were tested for male semisterility by making stained pollen counts. Acetocarmine dye was used to stain normal pollen; aborted pollen was distinguished by its failure to stain (5).

The M_2 progenies from the semisterile M_1 plants in group 1 were field-planted in April 1979. Forty seeds from each semisterile M_1 were planted, and buds from each surviving plant were examined for male semisterility by the stained pollen count technique.

All M_1 plants in group 2 that exhibited pollen semisterility were crossed as male parent to a tester line, 9-408. This line has 2 recessive markers, reclining foliage and white flowers. Test cross progenies of 10 to 20 plants were either greeenhouse-planted in December 1979 or field-planted in March 1980., All test cross progeny were screened for male semisterility by mak-

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ing stained pollen counts. Three of the F_1 test cross progenies that segregated for semisterility in the greenhouse were tested in the F_2 generation in the field by planting 35 seeds from each of 2 semisterile F_1 plants.

Semisterile M_1 plants in group 2 were scored for missing seeds in their pods. Those M_1 plants with reduced seed set were further investigated in small M_2 progenies planted in the greenhouse. Eight or more plants of each M_2 were scored for missing seed. All the pods on each M_2 plant were assayed to estimate the average seeds per pod for that plant.

Results and Discussion

Seed set and embyro abortion in the pods of M_0 plants. The results of pollination with irradiated pollen are presented in Table 1. Note that 7-1404 produced an average seed set per pod of 6.2 by natural self-pollination. The pod and seed set resulting from the 3 highest treatment levels of gamma irradiation are strikingly similar. One would expect decreasing seed set with increasing levels of radiation, unless the frequency of lethal mutations is overwhelming. Our interpretation is that treatments in the range of 16 to 4 kR produce at least 1 lethal mutation in nearly every pollen cell. All the seeds obtained from these treatments are probably the results of self-fertilization due to imperfect emasculation. If this is true, then about 10% of the emasculations left a few untreated pollen grains on the stigma.

All pods resulting from pollination with irradiated pollen were scored for the frequency of seed set and seed abortion. Pods produced from the 2 kR treatment had much higher frequencies of abortion in the later stages of seed development. These aborted seeds usually had full color development (black) and looked like collapsed seed coats. In the higher irradiation treatments, ovule development rarely proceeded far enough to develop seed coat color. In other words, seeds in pods from the higher treatments were usually either fully developed seeds or had aborted at very early stages of embryo development. Seeds that had aborted at intermediate stages of development were seldom seen.

An average of 5.7 seeds per pod was produced by pollinating emasculated buds with untreated pollen (Table 1). Multiplying 5.7 times the 65 pods obtained from the 2 kR treatment, we obtain 371, the expected number of mature seeds if the pollen were untreated. A minimum estimate of the frequency of lethal mutations is 1-(106/371) = 0.71. The second group of pollinations, using pollen treated with 2 kR gamma irradiation, gave 116 pods containing 163 seeds. The estimated minimum lethal mutation rate was 1-(163/661) = 0.75.

Seventy-two (53%) of the 2 kR pollinations resulted in abortion, usually within 1 or 2 days from pollination (Table 1). The random variation in the lethal mutation frequency easily accounts for this abortion rate, considering that the pods which did set had only 1.6 seeds on the average. Also, consider that 12% of the natural self-pollinations aborted, and 31% of the pollinations using untreated pollen on emasculation buds resulted in early abortion (Table 1).

A 4 kR treatment for the pollen appears to be excessive. The 2 kR treatment is quite satisfactory in our view. A lethal mutation frequency over 80% would require an unacceptable burden of hand-pollination work. A lethal mutation rate much below 70% would yield more seed per pollination, but it would also increase the size of the M_1 population that must be screened to obtain a given number of translocations. It would be necessary to use pollen irradiation treatments below 2 kR to further evaluate the possible trade-offs.

Screening for semisterility in M_1 and derived generations. Normal pollen abortion rates are usually less than 10% under greenhouse conditions. Pollen abortion rates between 10 and 20% are too low to be easily distinguished from normal on a routine basis. In group 1 and 2, about 17 to 20% of the M_1 plants had pollen abortion rates over 20% (Table 2). Those plants with over 20% pollen abortion we classify as semisterile.

The M_2 progenies (40 plants each) from group 1 semisterile plants were field-planted and screened for semisterility. The data did not show 2 completely separate modes of pollen abortion rates, one normal range and another higher range, for any of the M_2 progenies. The frequency distributions were continuous and without distinct modes. Adverse weather and field conditions may have produced greater variability in abortion rates and prevented a clear 1:1 segregation. Subsequent greenhouse screening of small M_3 progenies from selected M_2 parents identifield 4 lines that segregated clearly for semisterile progeny.

A more precise method was used to screen group 2 M_1 plants. All semisterile group 2 M_1 plants were crossed to 9-408. The recessive marker characters in 9-408 permitted us to be sure that each F_1 plant was a true cross. Of the 27 semisterile M_1 plants test-crossed to 9-408, 8 gave F_1 test cross progenies that segregated for semisterility in ratios approximating a ratio of 1 semisterile to 1 fertile plant. Semisterile plants from 3 of these testcross progenies were selfed, and the F_2 plants were tested for the segregation ratio of fertile to semisterile plants (Table 3). All 3 test cross progenies fit a 1:1 ratio satisfactorily.

The expression of semisterility in F_1 test cross progeny indicates that the mutation in the M_1 pollen parent is heritable and behaves like a dominant genetic allele. If the semisterile F_1 plants

Table 1. Number of pollinations, pods set, embryo abortions, and mature seeds in group I normal plants pollinated with irradiated and unirradiated pollen.

Pollination results	Pollen treatments				Untreated pollen	
	16 kR	8 kR	4 kR	2 kR	Emasculated buds	Natural selfs
No. pollinations	162	158	144	137	98	115
No. pods set[z]	15	15	15	65	68	101
No. aborted embryos[y]	72	71	77	317	---[w]	---[w]
Total seeds matured[x]	24	22	20	106	390	625
Avg seed/pod	1.6	1.5	1.3	1.6	5.7	6.2

[z]Pods which aborted at any stage were not included in these figures.
[y]Seed abortion after visible degrees of development; mutations lethal at previsible developmental stages not counted.
[x]Only viable seeds were counted, including some poorly developed.
[w]No data was taken on embryo abortion in this class.

Table 2. The frequency distribution of percentage of pollen abortion in M_1 plants derived from pollination of untreated plants, using pollen exposed to 2 kR of gamma irradiation.

M_1 group	Frequency distribution: Upper class limits of pollen abortion 10 %	20 %	30 %	40 %	50 %	60 %	70 %	80 %	90 %	Total
1	76	5	1	10	1	2	1	2	3	101
2	122	7	5	5	10	3	4			156
Total										257

Table 3. Segregation ratios of fertile to semisterile plants in the F_2 generation derived from semisterile F_1 plants in test cross progenies.

M_1 plant code	F_2 segregation[z]		χ^2 (1:1)	P
	Fertile	Semisterile		
II-3	28	22	0.72	.50–.25
II-70	31	27	0.276	.75–.50
II-130	30	27	0.158	.75–.50

[z]Pooled progeny from 2 semisterile plants from the same F_1 test cross progeny.

were heterozygous for a dominant allele, they would segregate in a ratio of 3 semisterile to 1 fertile in the F_2. The 3 M_1 mutants tested segregated 1:1 in the F_2, indicating a chromosomal aberration rather than a genic mutation.

A similar dominant semisterility factor was reported by Mutschler and Bliss (6). Plants carrying this factor had exactly 50% pollen abortion. Progeny produced by self-pollination segregated in the ratio of 1 semisterile to 1 fertile plant. They attributed the semisterility to translocation heterozygosity. Similarly, we infer that our semisterility factors may be chromosome translocations.

Our semisterile factors differ from the one studied by Mutschler and Bliss (6) in 2 important features. The average pollen abortion rates of some of the semisterile lines differ significantly from an expected value of 50% (Table 4). The rate of pollen abortion in translocation heterozygotes is a function of the frequencies of 4 principal orientations of a ring of 4 chromosomes at metaphase I (4). A few consistent deviations from 50% pollen abortion are known in maize and other species and have been discussed by Burnham (4). The semisterile factor of Mutscheler and Bliss (6) did not reduce seed set per pod, but our semisterility factors consistently reduced seed set per pod in semisterile M_2 progeny (Table 5). It is well-known that translocation heterozygotes result in duplication and deficiencies of chromosome segments, which produces an ovule abortion frequency similar to the pollen abortion frequency (4). Hence, semisterile bean

Table 4. Mean pollen abortion rates in M_2 plants from semisterile M_1 parents and *t*-tests of the deviation of the observed means from a hypothetical 50% abortion rate.

Semisterile M_1 plants		Pollen abortion in semisterile M_2 segregates					
Code	% pollen abortion	No. of plants	No. cells counted	Avg abortion[z] (%)	s^2	Estimated t'	Significant t', 5% level
I-52	38	3	1203	33	12.3	4.75	4.30
I-85	36	5	4233	45	41.2	0.81	NS
I-97	35	3	2088	49	19.0	0.23	NS
II-3	63	7	3921	60	16.9	2.53	2.45
II-6	28	5	1090	34	3.8	7.12	2.78
II-14	38	6	1613	52	25.5	0.35	NS
II-70	45	9	3322	44	20.0	1.34	NS
II-109	43	5	1154	55	66.7	0.64	NS
II-121	57	5	4136	56	34.8	1.08	NS
II-122	34	11	7588	52	32.4	0.22	NS

[z]The *n* values used to calculate the average percentage of abortion, s^2, and t' for each mutant were the numbers of plants in column 3.
[NS]Not significant.

Table 5. Comparison of the mean number of seeds/pod in selfed populations segregating for fertile and semisterile M_2 plants from the same semisterile M_1 parent.

M_1 code number	Mean seeds/pod ± SD in M_2 plants[z]				Percent reduction in seed set	Estimated t'	Significant t', 5% level
	No. plants	Fertile	No. plants	Semisterile			
20 k-B	5	5.1 ± 0.38	4	2.8 ± 0.38	45	17.22	2.36
0-350	4	5.7 ± 0.38	4	3.5 ± 0.23	39	16.98	2.45
I-52	5	5.5 ± 0.92	3	2.8 ± 0.34	49	9.13	2.45
I-85	3	6.2 ± 0.50	5	4.7 ± 0.51	24	7.10	2.45
I-97	5	6.2 ± 0.71	3	5.0 ± 0.18	19	5.55	2.45
II-3	2	4.3 ± 0.26	3	2.9 ± 0.64	33	3.69	3.18
II-14	2	6.1 ± 0.11	7	3.3 ± 0.75	46	12.13	2.36
II-70	4	5.7 ± 0.41	3	3.8 ± 0.08	33	13.09	2.57
II-109	4	6.0 ± 0.39	5	3.2 ± 0.22	47	23.87	2.36
II-122	21	6.2 ± 0.57	11	3.5 ± 0.29	44	62.07	2.04

[z]The *n* values used to calculate the SD of mean seed set and the t' for each mutant were the numbers of plants in columns 2 and 4.

plants resulting from heterozygosity for a chromosome translocation would be expected to have reduced seed set per pod.

The efficiency of the pollen treatment technique. Treatment of pollen with 2 kR of gamma irradiation yielded about 5% semisteriles 8 successes among 156 group 2 M_1 plants). The selection for semisterility in group 1 M_2 progenies was not precise enough to include those results in the computation of efficiency. If a recessive genic male sterility were used to eliminate the need for emasculation in the M_0 and tester stock, the pollen treatment approach would be much less laborious. We have considered the alternative method of inducing translocations, by irradiating dry seed and searching M_2 populations for plants showing reduced seed set per pod. Searching M_2 populations for translocations has not been feasible at Gainesville for both climatic and practical considerations. The rainy season begins at the time of pod maturity, making rapid harvest of all bean plots and selections an urgent consideration, thus preempting the time needed to search for translocations.

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Regulation of Growth and Flowering in *Chrysanthemum* x *superbum* Bergmans[1]

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Abstract. **Seedlings of *Chrysanthemum* x *superbum* shasta daisy varied greatly in their requirements for flowering. Some vegetatively propagated clones of selected seedlings required cold in order to flower, some required long photoperiods, some required both, and some required neither. Flowering bedding plants were produced from seed in 6–8 months.**

Most of the material cultivated as *C. maximum* Ramond is probably *C.* x *superbum* Bergmans, a presumed hybrid between *C. lacustre* and *C. maximum* (1). Unfortunately, most references and seed companies continue to list cultivated types as *C. maximum*.

Extensive information exists on initiation of *C.* x *morifolium* types but is not extendable to *C.* x *superbum* types (7). Flower initiation of shasta daisy cultivars 'Esther Read' and 'T. E. Killian' depends on long photoperiods (2). 'Esther Read' flowers at photoperiods greater than 13 hr, while 'T. E. Killian' flowers at photoperiods greater than 15 hr. A 15°C greenhouse temperature was used in determining the above requirement, but recorded temperatures within the plots averaged 2° to 3° cooler during December through February. Extension of light beyond the critical photoperiod increased flower numbers, length, weight, and diameter for 'Esther Read'. Under short photoperiods, stems grew horizontally some distance from the main stem before bending upwards. Basal leaves showed a progressive increase in length, width, and weight as photoperiod increased (2). Field-grown shastas illuminated 4 hr in the middle of each night flowered considerably earlier than those exposed to the natural photoperiod (3). Laurie and Poesch (5) also reported earlier flowering when plants in the greenhouse were given supplementary illumination from 1800 to 2200 HR in midwinter.

The objective of the present study was to explore the environmental requirements for flowering in shasta daisy and to determine the most rapid means to obtain flowering plants.

Materials and Methods

This study was limited to the 'G. Marconi' cultivar of shasta daisy. Seeds were sown in a peat-lite mixture and were germinated under intermittent mist. Germination required 14–17 days. Seedlings were transplanted about 5 weeks after sowing into 10-cm clay pots in a 1 soil:2 sphagnum peat moss:2 perlite (by volume) mixture. The mix was amended with 0.9 kg of treble superphosphate, 0.6 kg of potassium nitrate, 0.6 kg of magnesium sulfate, and 5.34 kg of agricultural limestone per m^3 of mix. Some seedlings were later cloned by vegetative division. Each division contained at least 1 crown and was planted into a 10-cm clay pot using the same growing mix. Plants were fertilized at each watering with 200 mg/liter each of nitrogen and potassium. A 10-hr photoperiod was used to establish and grow all plants. Overhead 60-watt incandescent lamps supplied about 1.4 ± 0.4 μ E m^{-2} sec^{-1} light from 1500 to 1800 HR. Black cloth covered the plants from 1600 to 0800 HR daily. A

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