Indian J. Genet., **63**(1): 85-86 (2003)

Short Communication

Induction of male sterility in wild and related species of sunflower (*Helianthus annuus* L.)

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(Received: June 2001; Revised: January 2003; Accepted: January 2003)

The genus *Helianthus* comprises of about 67 species and many of them have been reported excellent sources of resistance against major sunflower diseases and insect-pests as well as sources of male sterile cytoplasm and fertility restorer genes. The transfer of gene from wild species to cultivated types requires intercrossing between them. Hand emasculation of wild species flowers is very difficult owing to the presence of a large number of very small florets weakly attached to the receptacle. The use of gibberellic acid as a gametocide is routinely used in cultivated types. A similar procedure requires to be evolved to induce male sterility in wild forms to facilitate crossing.

The seeds of two wild related species of sunflower (*Helianthus debalis* and *H. argoplyllus*) were planted on 16th November, 1999 in 6 and 2 row plots, respectively following 60 and 30 cms spacings between and within the rows. At bud initiation stage, separate plants were earmarked for different GA_3 treatments and different buds sizes representing different growth stages were selected. In case of *H. deblis* buds of sizes 0.7, 0.8, 0.9 and 1.0 cm were selected, while in case of *H. argophyllus*, the bud sizes selected were 1.5, 2.0 and 2.5 cm. Care was taken to select only buds which were born on separate branches owing to the poor developments of axillary buds on GA_3 treatments. Four GA_3 solutions of concentration 50, 100, 200 and 500 ppm were prepared by dissolving GA_3 crystals first in a few drops of ethanol and then making up the desired volume of a particular concentration with distilled water. The experiment thus involved 12 treatments (4 concentrations $\times$ 3 days of application) and required 24 buds (12 for each replication) each of size 0.7, 0.8, 0.9 and 1.0 cm. The four GA_3 concentrations thus made a total of four such sets. Under each treatment, 4 buds were sprayed in the morning with GA_3 using a hand sprayer and two of them were covered with butter paper bags to check pollination by foreign pollen while the rest two buds were left unbagged to allow open-pollination to occur. Ten buds of *H. deblis* and five buds of *H. argophyllus* were also tagged and allowed to open-pollinate to record the seed set under normal conditions. Similarly one untreated head from each of the two species was bagged to determine the existence of self-compatibility/ self-incompatibility. At maturity, the number of seeds set in both treated bagged, treated unbagged and untreated unbagged (control) were counted.

The results of the experiment in Table 1 showed that the buds of size 0.7 cm when treated with 50 ppm GA_3 for 5 days recorded an average seed set per head of 3.0 and 12.5 in the bagged and unbagged heads, respectively indicating that the concentration was not potent enough to induce male sterility in *H. deblis*. On the basis of this observation it was assumed that other treatments involving the same dose but buds of large size and fewer days of GA_3 application would not either be able to induce pollen-sterility and were, therefore, abandoned. In case of 100 ppm applied for 1 and 3 days, no induction of pollen-sterility, as indicated by seed setting in the bagged heads, was observed. However, the same dose when applied for 5 days to a bud of size 0.7 cm appeared to be able to induce complete pollen-sterility. The buds of sizes 0.8 and 0.9 cm did not exhibit any male sterility. 200 ppm applied for a single day was not effective in inducing complete male-sterility, however, when applied for 3 days to buds of sizes 0.7 and 0.8 cm was successful in inducing complete pollen-sterility. The effect reduced as the bud size increased to 0.9 and 1.0 cm. When buds of sizes 0.7, 0.8 and 0.9 cm were treated with 200 ppm for 5 days or with 500 ppm for 3 days, induction of complete male-sterility along with appreciable seed set in unbagged heads was observed. 500 ppm GA_3 applied for 5 days resulted in induction of complete male- and female-sterility as evidenced by no seed set in both bagged and unbagged heads. In case of *H. argophyllus* also, 50 ppm treatment for 1 and 3 days had to be abandoned as the 5 days' treatment of the same concentration was ineffective in inducing complete male sterility (parenthesis value). 100 ppm was not effective in inducing complete

Table 1. Seeds per head in bagged and unbagged heads under different GA_3 treatment combinations in wild sunflower

GA_3 concentration	Number of days												Bud size (cm)	
	1 day				3 days				5 days					
	Bagged		Unbagged		Bagged		Unbagged		Bagged		Unbagged			
	A#	B#	A	B	A	B	A	B	A	B	A	B	A	B
50 ppm	-	-	-	-	-	-	-	-	3.0	2.0	12.5	7.0	0.7	1.5
	-	-	-	-	-	-	-	-	-	4.0	-	13.0	0.8	2.0
	-	-	-	-	-	-	-	-	-	11.0	-	25.0	0.9	2.5
	-	-	-	-	-	-	-	-	-	-	-	-	1.0	-
100 ppm	5.5	1.0	15.0	11.0	2.0	0.0	6.5	4.0	0.0	0.0*	1.0	2.7*	0.7	1.5
	8.0	12.0	27.5	27.0	2.5	4.0	10.5	20.0	1.5	0.0*	16.0	13.7*	0.8	2.0
	17.0	10.0	46.0	36.0	9.0	11.0	37.5	28.0	2.0	1.3*	20.0	18.0*	0.9	2.5
	17.5	-	51.5	-	10.0	-	41.0	-	-	-	-	-	1.0	-
200 ppm	2.5	1.0	13.5	11.0	0.0	0.0*	1.5	2.7*	0.0	0.0	0.5	0.0	0.7	1.5
	6.0	6.0	20.5	19.0	0.0	0.0*	5.0	11.3*	0.0	0.0	2.0	0.0	0.8	2.0
	7.0	11.0	29.0	30.0	1.5	0.0*	9.5	15.3*	0.0	-	5.5	-	0.9	2.5
	14.5	-	53.0	-	3.0	-	15.0	-	-	-	-	-	1.0	-
500 ppm	1.0	1.0	6.0	5.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	1.5
	2.5	3.0	7.0	6.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.8	2.0
	2.0	7.0	11.5	13.0	0.0	0.0	3.5	1.0	0.0	-	0.5	-	0.9	2.5
	5.0	-	30.5	-	0.5	-	7.0	-	-	-	-	-	1.0	-

*Avarage of three replications; #A: *Helianthus deblis*; B: *H. argophyllus*

pollen-sterility when applied for 1 and 3 days to buds of sizes 2.0 and 2.5 cm. However, it was effective in inducing male-sterility when a bud of size 1.5 cm was treated. When 100 ppm was applied for 5 days to buds of sizes 1.5 and 2.0 cm, complete male-sterility was observed. Similar results were observed with 200 ppm treatment for 3 days of buds of all the three sizes. 200 ppm for 5 days and 500 ppm for 3 and 5 days induced complete pollen-sterility. No difference was observed in seed set in the unbagged untreated buds (control) and bagged treated buds.

Thus it was evidenced that gibberellic acid at 50 and 100 ppm was not effective in inducing complete pollen-sterility in wild sunflower genotypes, while the same treatments have been reported to induce complete male-sterility in cultivated sunflower [2-3]. Even the two species *H. deblis* and *H. argophyllus* responded differently to different treatments. *H. deblis* gave best results at 200 ppm (for 5 days) and 500 ppm (3 days), while the same treatments induced complete male and female-sterility in *H. argopyllus* suggesting greater sensitiveness of the latin to externally applied GA_3. Schuster [4] has also reported differential response to GA by different genotypes. A progressive decrease in the seed set with increasing GA_3 concentration and a progressive increase with increase in the bud size was also observed. Buds at an early stage of development are likely to be more sensitive to GA_3 compared to when the buds are in the late stages that have developed protective coverings, which reduce their sensitivity. A strong influence of GA_3 on female-sterility was also observed. GA_3 was observed to reduce seed set considerably at all bud stages in all the treatment combinations as evidenced by comparison between seed set in the treated unbagged buds and untreated buds). Female-fertility ranged from 48.4 per cent (200 ppm applied for 1 day to 1 cm bud) in *H. deblis* to 28.5 per cent (100 ppm for 1 day applied to 2.5 cm bud) in *H. argophyllus* to complete male-sterility at higher concentrations applied for more number of days.

Therefore, from the present investigation it was concluded that the applications of 200 ppm for 5 days at bud size of 0.7-0.9 cm and 500 ppm for 3 days at bud size of 0.7-0.9 cm in *H. deblis*, whereas 100 ppm for 5 days at bud size of 1.5-2.0 cm and 200 ppm for 3 days at bud size of 1.5-2.0 cm in *H. argophyllus* sunflower wild species were found effective in inducing complete male-sterility without affecting female-fertility. Further, both the species appeared to be self-compatible, which was indicated by no difference in seed set in unbagged untreated buds (control) and bagged untreated buds. Although the seed set even in the best combinations was low, it should, nevertheless, be of importance to the breeders as it obviates the need adopting tedious emasculation procedure or any other procedure where purity of the hybrid seed is not guaranteed.

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