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Evaluation of three chemical hybridising agents on two varieties of broad beans (*Vicia faba* L.)

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Abstract

Chemical hybridizing agents (CHAs) offer opportunity to develop hybrids in crops where cytoplasmic, genetic male sterile lines are not available. Keeping this in view, three CHAs namely, benzotriazole, ethrel and surf excel (synthetic detergent) of different concentrations at three stages of development were tested on two genotypes of broad beans (*Vicia faba* L.) ILB 1817 and ILB 4189. The field trials were conducted following randomized block design with three replicates during 2000 and 2001. Ethrel at 0.1, 0.2 and 0.3% gave the best performance in inducing 100% male sterility lasting for 15-25 days in both the varieties. On the other hand, three treatments of benzotriazole at 0.5, 1.0 and 1.5% and three treatments of surf excel at 1.0, 2.0 and 3.0% also induced 100% male sterility lasting for 15-25 and 15-20 days respectively and surf excel proved to be the least phytotoxic in both the genotypes. The variously treated plants exhibiting 100% pollen sterility showed insignificant reduction in the total yield in open pollinated plants.

Key words: *Vicia faba*, Chemical hybridizing agents (CHAs), male sterility, benzotriazole, ethrel, surf excel

Introduction

Chemicals capable of selectively inhibiting pollen development and thus blocking male fertility are known as chemical hybridizing agents (CHAs). These chemicals induce male sterility and have unique potential to provide for the development of hybrids directly out of the elite germplasm without the time and effort required to regressively backcross male sterility genes and fertility restoration systems. The newer generation of chemical hybridizing agents are found to be much useful with increasingly better selectivity and effectiveness [1]. The CHAs facilitate cross breeding in plant species with perfect flowers by selectively sterilizing male sex cells or by interrupting microsporogenesis to prevent self-pollination and to promote fertilization by an outside pollen source. Thus, they offer great opportunities to develop hybrids in rice and wheat [2, 3, 4]. Recently, evaluation of some anilates as the potential chemical hybridizing agents have been successfully done in wheat and chickpea [5, 6]. In recent years some synthetic detergents e.g. Nirma and Surf excel have also been successfully used to induce male sterility in some crops e.g. *Oryza sativa* [7] and *Brassica juncea* [8].

Keeping this in view, during the course of present investigation, the efficacy of three chemicals namely, surf excel, benzotriazole, ethrel have been tested to induce male sterility in broad beans (*Vicia faba* L.) an important protein rich vegetable crop.

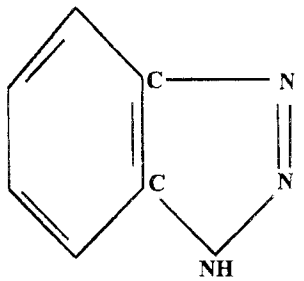
Materials and methods

Present experiment was conducted on *Vicia faba* L. var. ILB 1817 and ILB4189 obtained from the National Bureau Plant Genetic Resources, Pusa Campus, New Delhi during the years 2000 and 2001. The experiment was laid out in a randomized complete block design with three replicates at Experimental Research Station, School of Life Sciences, Dr. B. R. Ambedkar University, Agra. The seeds of both the varieties were alternately sown keeping row to row distance of 30 cm and between plants it was 15 cm. All recommended agronomic practices were followed.

Aqueous solutions of different concentrations of various CHAs namely, surf excel (1.0, 2.0 and 3.0%), benzotriazole (0.5, 1.0 and 1.5%), ethrel (0.1, 0.2 and 0.3%) were prepared in distilled water. The concentrations of various CHAs were finally selected after preliminary experiment conducted earlier. The chemical/ structural formulae and composition of these CHAs are given below:

a. Ethrel or Ethephon (2-Chloroethyl phosphonic acid)

b. Benzotriazole ($C_6H_5N_3$)



c. Surf excel consists of surface-active agents, builders (phosphates) and fillers. In addition, it has additives, e.g. antideposition agents, optical brightners, bluing agent, bleaching agent, foam regulators, organic sequestering agent and enzyme etc. Alkalines, e.g. sodium carbonate (soda ash) and sodium borate are commonly added to neutralize the acid constituents of dirt.

The plants were sprayed with these CHAs at different stages of development. A group of 30 plants of each variety were sprayed only once with each concentration after 21 and 25 days of sowing i.e. a week before floral bud initiation (T_1) in var. ILB 1817 and ILB 4189 respectively. Another 30 plants were sprayed twice i.e. once a week before the floral bud initiation and second 3 days after floral bud initiation (T_2). Another 30 plants were sprayed thrice, i.e. first a week before floral bud initiation, second three days after floral bud initiation and third at the time of anthesis (T_3). Individual plant was sprayed with 20 ml of concerned solution to run off. Each treatment was replicated thrice. A group of 30 plants was sprayed with distilled water to run off to serve as control (T_0).

The flowers of some treated and untreated plants were bagged w'th butter paper bags to check the pollen fertility and seed-set.

Data on the reproductive parameters i.e. days to flowering, pollen sterility, fruits/plant, seeds/fruit, total yield (seed weight)/plant was recorded and statistically analyzed by calculating standard deviation and difference of means using 't' test at $p = 0.05$.

Pollen fertility was tested at regular intervals throughout flowering period with 1% Tetrazolium chloride in 0.15M tris HCl at pH 7.5, FCR test after Shivanna and Rangaswamy [9] and by *in vitro* test after Brewbaker and Kwack [10].

Results and discussion

Days taken to first flowering: All the treatments with different CHAs significantly enhanced the number of days taken to first flowering in both the cultivars as compared to that recorded in control plants (Table 1). The plants treated thrice with 0.1% ethrel (T3) took maximum days (83) for flowering as compared to 67 days taken by control plants. On the other hand, plant sprayed with various concentrations of benzotriazole took 66-68.3 days for first flowering. The plants treated thrice with 3.0% surf excel took 66 days and interestingly plants sprayed with lower concentration of this detergent (1%) took 73-75 days for flowering (Table 1).

Total yield/plant: Data presented in Table 2 clearly shows that various treatments with different concentrations of various CHAs caused significant reduction in total yield (total seed weight)/plant in both the genotypes as compared to that recorded in the control plants and the reduction was directly proportional to the concentration of all the CHAS. The maximum reduction in yield was recorded in plants treated with 0.3% ethrel (4.5 g) and minimum reduction was observed in plants treated once with 1.0% surf excel (25.5 g) as compared to control (28.8 g). The bagged flowers of treated plants failed to show seed-set.

Pollen sterility: Foliar sprays with different concentrations of various CHAs effectively induced pollen sterility ranging between 90.2-100% in both the varieties (Table 3). It was interesting to note that the sterility caused by these treatments lasted for 43 days. All the treatments with ethrel were significantly effective in inducing complete pollen sterility, while treatments with 0.5% (T_3), 1.0% (T_2, T_3) and 1.5% (T_1, T_2, T_3) benzotriazole induced complete pollen sterility. Similarly, the plants treated thrice with 2% and 3% surf excel caused 100% pollen sterility.

From the foregoing observations it is evident that all the treatments with various CHAs at different concentrations are capable of inducing complete pollen sterility in both the varieties of *Vicia faba* and ethrel seems to be the most sterility effective. Rowell and Miller [11, 12] have also induced 100% male sterility in wheat by treatments with 1000-2000 mg^{-1} ethrel at floral stage. Similarly, ethrel has been found to be successful in inducing complete pollen sterility in cotton (Meenu Chaudhary, Unpublished). However, plants sprayed with ethrel caused significant reduction in the number of flowers, fruits, seeds and total yield in both the cultivers of *Vicia faba* as compared to other chemicals.

Similarly, all the treatments with benzotriazole also induced complete pollen sterility in both the varieties of *Vicia faba*. Benzotriazole is a well known inhibitor of microspore development [1]. Graham [13] was able to demonstrate selective induction of male sterility in wheat by sprays with benzotriazole. Complete pollen sterility in *Helianthus annuus* has been successfully induced by treatments with benzotriazole [14]. Similarly, benzoriazole has also been reported to be a potential hybridizing agent for *Brassica juncea* [15]. The plants treated with 0.5% and 1% benzotriazole not only induced 100% pollen sterility but also produced a large number of seeds in treated plants of *Brassica juncea* on open pollination.

Treatments with surf excel induced pollen sterility without significantly changing the yield components in both the varieties of *Vicia faba*. Recently, Surf excel has been used as a potential CHAs in *Brassica juncea* [8]. They have not only induced complete pollen sterility in this important-crop but also successfully obtained

Table 1. Effect of various CHAs on days taken to first flowering in two cultivers of broad beans (*Vicia faba* L.)

Chemicals	Conc. %	Days taken to first flowering					
Varieties		ILB 1817			ILB 4189		
No. of treatments		T_1	T_2	T_3	T_1	T_2	T3
Surf Excel	1.0	73.0*±4.3	73.0*±2.3	75.0*±0.8	72.0*±4.1	74.0*±3.3	73.3*±4.4
	2.0	70.3*±1.2	68.6*±0.4	68.6*±2.8	71.3*±0.9	69.3*±1.6	68.4*±1.6
	3.0	68.6*±3.6	68.3*±1.6	66.0*±1.6	68.5*±3.5	68.2*±1.4	67.1*±1.7
Benzotriazole	0.5	66.0*±1.6	67.3*±1.6	66.3*±4.2	65.6*±1.4	68.2*±1.6	66.3*±4.2
	1.0	66.3*±4.2	66.6*±3.2	67.3*±1.7	65.4*±1.4	67.3*±1.6	66.2*±4.0
	1.5	66.3*±4.1	67.0*±2.1	68.3*±1.6	67.2*±1.7	66.6*±3.2	67.3*±1.6
Ethrel	0.1	76.6*±1.7	79.0*±0.8	80.3*±1.2	75.4*±1.2	76.4*±1.6	79.1*±1.1
	0.2	79.0*±0.8	81.0*±1.4	81.3*±0.9	80.5*±1.3	79.2*±0.8	82.2*±1.5
	0.3	81.3*±0.9	79.0*±0.8	83.3*±1.6	79.9*±0.9	81.2*±1.2	82.3*±1.6
Control			67.0 ±2.9			62.2 ±3.0	

±Standard Deviation; *Significantly different from control at p = 0.05; T_1: Single spray, a week before floral bud initiation; T_2: Double spray, once a week before the floral bud initiation and second 3 days after floral bud initiation; T_3: Triple spray, first a week before the floral bud initiation, second 3 days after floral bud initiation and third at the time of anthesis.

Table 2. Effect of various CHAs on total yield/ plant in two cultivers of borad beans (*Vicia faba* L.)

Chemical	Conc. %	Total yield/plant (g)					
Varieties		ILB 1817			ILB 4189		
No. of treatments		T_1	T_2	T_3	T_1	T_2	T3
Surf Excel	1.0	25.5*±1.6	24.8*±1.3	23.6*±1.5	25.2*±1.6	24.9*±1.3	23.7*±1.5
	2.0	23.1*±2.4	20.8*±2.0	20.5*±1.7	24.5*±2.4	21.1*±2.1	20.8*±1.6
	3.0	18.5*±1.7	16.8±1.9	14.6*±1.8	19.5*±1.8	16.4*±1.8	14.4*±1.7
Benzotriazole	0.5	15.1*±0.8	9.4*±1.1	9.2*±1.2	9.5*±1.3	9.5*±1.3	9.2*±1.3
	1.0	12.2*±1.9	10.4*±1.5	9.9*±1.2	11.5*±1.5	9.6*±1.2	9.4*±1.2
	1.5	10.5*±1.2	9.9*±1.2	9.5*±1.2	10.6*±1.2	9.5*±1.2	9.3*±1.1
Ethrel	0.1	8.6*±1.2	7.0*±1.1	6.9*±1.4	8.6*±1.2	7.1*±1.1	6.8*±1.4
	0.2	6.8*±1.6	6.5*±1.2	6.0*±1.5	6.8*±1.6	6.4*±1.3	6.1*±1.6
	0.3	5.9*±1.5	5.3*±2.0	4.5*±1.9	5.9*±1.5	5.4*±2.1	4.3*±1.9
Control			28.8 ±1.5		26.4 ±2.1		

±Standard Deviation; *Significantly different from control at p = 0.05; T_1: Single spray, a week before floral bud initiation; T_2: Double spray, once a week before the floral bud initiation and second 3 days after floral bud initiation; T_3: Triple spray, first a week before the floral bud initiation, second 3 days after floral bud initiation and third at the time of anthesis.

Table 3. Effect of various CHAs on pollen sterility in two cultivers of broad beans (*Vicia faba* L.)

Chemical	Conc. %	Pollen sterility (%)							
Varieties		ILB 1817				ILB 4189			
No. of treatments		T_0	T_1	T_2	T_3	T_0	T_1	T_2	T3
	1.0.	4.5±1.6	90.2*±1.9	96.9*±1.5	98.0*±1.2	5.4±0.8	91.4*±1.8	97.1*±1.4	98.6*±1.3
Surf Excel	2.0	5.1±1.5	92.6*±0.9	98.5*±1.2	100.0*±0.0	4.6±1.1	92.8*±1.1	98.2*±1.1	100.0*±0.0
	3.0	4.7±1.2	93.4*±2.8	98.9*±1.2	100.0*±0.0	5.4±1.5	94.1*±1.8	97.1*±1.2	100.0*±0.0
Benzotriazole	0.5	3.6±2.1	95.9*±1.4	100.0*±0.0	100.0*±0.0	4.3±1.2	95.3*±1.2	100.0*±0.0	100.0*±0.0
	1.0	4.9±1.2	99.5*±0.0	100.0*±0.0	100.0*±0.0	5.1±1.8	99.1*±1.0	100.0*±0.0	100.0*±0.0
	1.5	4.5±1.9	100.0*±0.0	100.0*±0.0	100.0*±0.0	4.6±1.2	100.0*±0.0	100.0*±0.0	100.0*±0.0
Ethrel	0.1	3.2±1.3	99.9*±1.6	100.0*±0.0	100.0*±0.0	5.4±1.4	100.0*±0.0	100.0*±0.0	100.0*±0.0
	0.2	4.2±1.4	100.0*±0.0	100.0*±0.0	100.0*±0.0	4.8±1.8	100.0*±0.0	100.0*±0.0	100.0*±0.0
	0.3	4.6±1.9	100.0*±0.0	100.0*±0.0	100.0*±0.0	4.7±1.2	100.0*±0.0	100.0*±0.0	100.0*±0.0

± Standard Deviation; *Significantly different from control at p = 0.05; T_0: Control, plants sprayed with distilled water; T_1: Single spray, a week before floral bud initiation; T_2: Double spray, once a week before the floral bud initiation and second 3 days after floral bud initiation; T_3: Triple spray, first a week before the floral bud initiation, second 3 days after floral bud initiation and third at the time of anthesis.

hybrid seeds in young floral buds with increased receptivity of stigma in treated plants. The bagged flowers of plants treated with different concentrations of all the CHAs failed to produce any seeds indicating complete pollen sterility. On the other hand, the open pollinated flowers of treated plants produced seeds.

Thus, it is evident from the present observations that all the three CHAs used are capable of inducing cent percent pollen sterility in broad beans (*Vicia faba*) but the detergent-surf excel seems to be the best in inducing complete pollen sterility without any significant reduction in yield as well as it is less phytotoxic and less expensive.

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