J. Phytol. Res. 19(2) :191-195, 2006

CHEMICAL INDUCTION OF MALE STERILITY IN *CYAMOPSIS TETRAGONOLOBA* LINN.

NISHA and S.V.S. CHAUHAN

Department of Botany, School of Life Sciences, Dr. B. R. Ambedkar University, Khandari, Agra – 282002, India

Efficacy of three chemical hybridizing agents namely, benzotriazole, maleic hydrazide (MH) and ethrel (ethephon) were tested for inducing pollen sterility and hybrid seed production in *Cyamopsis tetragonoloba* (var. Deshi). Various concentrations and treatments of all the three chemicals induced pollen sterility ranging between 92-100%. Flowering in treated plants was delayed. However, these treatments caused significant reduction in the plant height, number of branches/plant, number of inflorescence/plant ,inflorescence size and total yield/plant.

Keywords: Benzotriazole; *Cyamopsis tetragonoloba;* Ethrel; Maleic hydrazide; Pollen sterility.

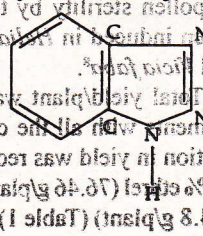
Introduction

Male sterility is failure of production of viable pollen grains and it is of quite common occurrence in higher plants[1]. It has particular importance because of its practical application in the production of hybrid seeds. Since male sterile lines are not always available in various crops, artificial induction of male sterility by the use of chemicals is most convenient way for hybrid seed production. Chemicals capable of selectively inhibiting pollen development and thus blocking male fertility are known as male gametocides or chemical hybridizing agents (CHAs)[2-4]. These chemicals induce male sterility and have unique potential for the development of hybrids directly out of the elite germplasm without taking time and efforts required for regressively back cross male sterility genes and fertility restoring system.

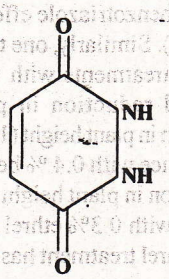
The newer generation of chemical hybridizing agents are found to be much useful with increasing better selectivity and effectiveness[5]. There are at least following four classes of chemical hybridizing agents in the current literature:

1. Plant-growth regulators and substances that disrupt floral development. 2. Metabolic inhibitors. 3. Inhibitors of microspore development. 4. Inhibitors of pollen fertility.

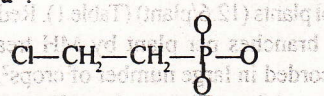
Benzotriazole ($C_6H_5N_3$), a copper chelator which acts as an inhibitor of microspore development has recently been used as a useful CHA for some crops, e.g. *Helianthus annuus*[6], *Brassica juncea*[7], *Vicia faba*[8].



Maleic hydrazide (1,2-dihydro-3,6-pyridazine-dione, $C_4H_4N_2O_2$) is a plant growth regulator which disrupt floral development. Various concentrations of MH have been used to delay flowering and induce pollen sterility in plants but with the reduction in yield. Maleic hydrazide was the first gametocide used to induce male sterility in tobacco[9].



Ethrel or ethephon(2-Chloro-ethyl phosphonic acid) is an ethylene generating synthetic compound which acts as a plant growth regulator. It has the unique property of releasing ethylene in plant tissue. Ethrel is known to induce male sterility in some crops e.g. wheat[10, 11], barley[12], and Okra[13].



In the present study an attempt has been made to find out the efficacy of above mentioned chemical hybridizing agents namely benzotriazole, maleic hydrazide and ethrel on *Cyamopsis tetragonoloba* for obtaining male sterile plants for their possible use in hybrid seed production.

Materials and Methods

The present experiment was conducted on *Cyamopsis tetragonoloba* var. Deshi. The seeds of this variety obtained from National Seed Corporation, Agra were sown

at botanical garden, School of Life Sciences, Dr. B.R. Ambedkar University, Agra. The plants, thus raised were sprayed with aqueous solutions of 0.4, 0.6 and 0.8% (w/v) benzotriazole ($C_6H_5N_3$); 0.3, 0.4, 0.5% (w/v) maleic hydrazide ($C_4H_4N_2O_2$) and 0.1, 0.2 and 0.3% (v/v) ethrel (2-Chloro-ethyl phosphonic acid) at three different stages of development. A group of 90 plants were sprayed a week before the initiation of floral buds at pre-meiotic stage (T_1), while leaving a group of 30 plants after first treatment (T_1), the remaining 60 plants were sprayed again at the time of floral bud initiation stage (T_2) and after leaving a group of thirty plants receiving two sprays (T_2). A group of another 50 plants were sprayed with distilled water (T_0), 20 ml of each solution was sprayed on one plant to run off. Pollen fertility was tested at regular intervals with 1% TTC (Tetrazolium chloride) in 0.15M tris HCl buffer at pH 7.8[14].

Data on plant height, number of branches, leaves, inflorescence and size, days taken to first flowering, and total yield/plant in treated and untreated plants were collected and statistically analyzed.

Results and Discussion

(1) Plant height - Effect of various treatments on plant height is shown in Table 1. Foliar sprays with different concentrations of benzotriazole effectively reduced plant height (Figs.1 & 2). Similarly, one to two treatments with 0.3% and all the treatments with 0.4 and 0.5% maleic hydrazide caused reduction in plant height (Fig.3). Minimum reduction in plant height (105.5 cm) was observed in plants treated once with 0.4 % benzotriazole (Table 1). Maximum reduction in plant height (77 cm) was recorded in plants sprayed with 0.3% ethrel (Fig.4). Reduction in plant height by ethrel treatment has also been reported in *Vicia faba*[8].

(2) Number of branches /plant -The number of branches/ plant decreased with the increase in the concentration and number of treatments of benzotriazole, MH and ethrel. Maximum reduction in number of branches (6/plant) was recorded in plants sprayed twice with 0.3% ethrel as compared to control plants (12.6/plant) (Table 1). Reduction in the number of branches per plant by MH treatment have also been recorded in large number of crops[2].

(3) Number of leaves /plant -The number of leaves/plant decreased with the increase in treatments with benzotriazole, MH and ethrel. The maximum reduction in the number of leaves (32/plant) as recorded in the plants treated twice with 0.3% ethrel as compared to 102.2 leaves/ control plants. Minimum reduction in the number of leaves (69.1/plant) as recorded in the plants treated once with 0.3% maleic hydrazide (Table 1). The leaves treated with benzotriazole were slightly affected as their margins turned white, after 17-18 hours of spray. The leaves got shriveled and also exhibited slight burning (Fig. 5). Reduction in number of leaves/plant was recorded in *Vica faba* plants treated with ethrel[8].

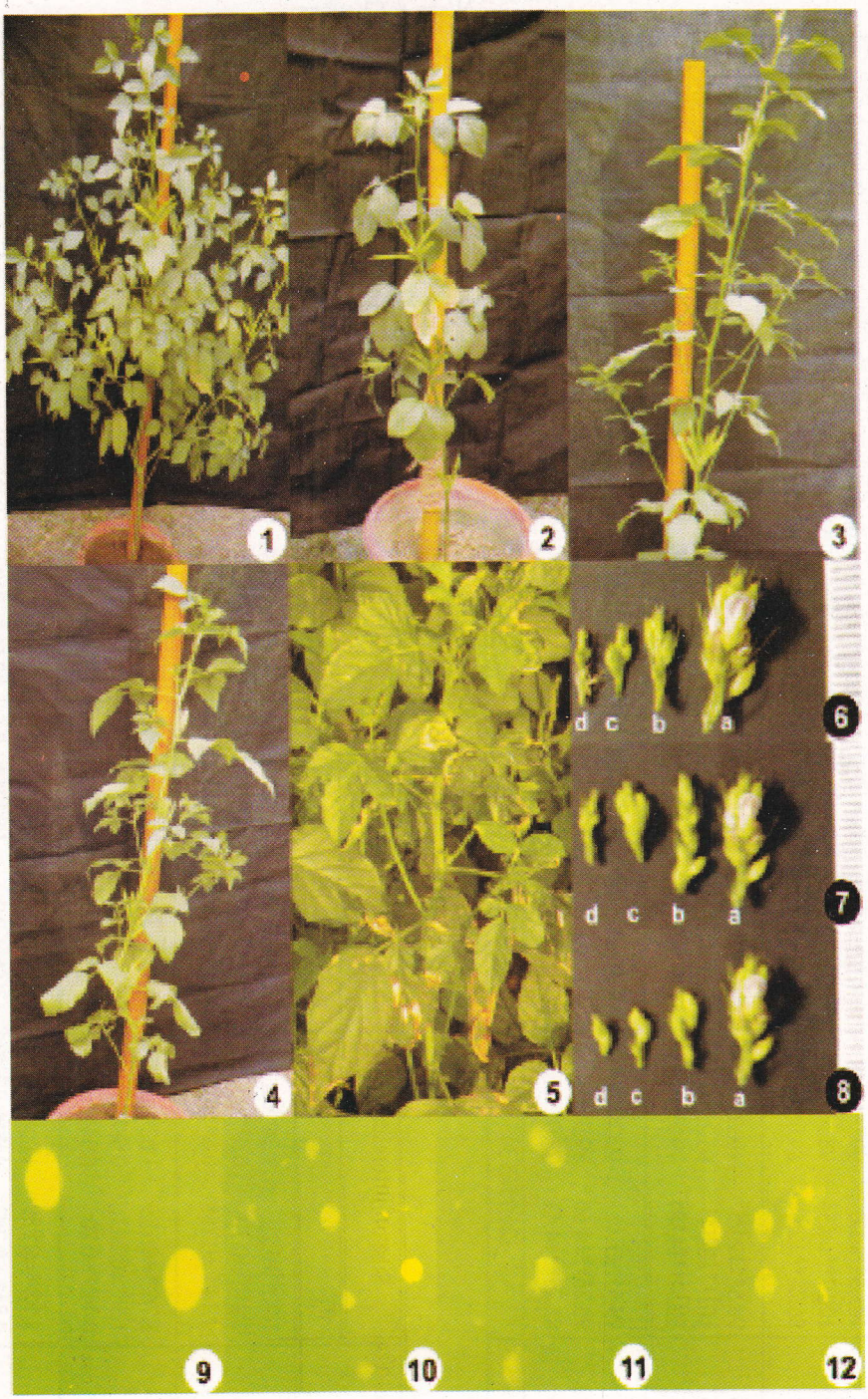
(4) Number of Inflorescence/plant- All the CHAs sprayed caused significant reduction in the number of inflorescence/plant and the reduction was directly proportional to the number of treatments. Maximum reduction in the number of inflorescence (12 inflorescence/ plant) was recorded in the plants treated twice with 0.3% ethrel as compared to untreated plants producing 62.3 inflorescence/plant. Minimum reduction in the number of inflorescence/plant was recorded in plants treated once with 0.4% benzotriazole (Table 1).

(5) Size of inflorescence- The size of inflorescence was significantly reduced by the treatment of all the three chemicals (Figs. 6, 7 & 8). The maximum reduction in size of inflorescence was recorded in the plants treated twice with 0.3% ethrel (Fig. 8). Reduction in flower size in a large number of plants treated with maleic hydrazide has been reported[2]. Minimum reduction in size of inflorescence was recorded in the plants treated twice with 0.3 % maleic hydrazide i.e 1.76 cm as compared to control plants (2.08 cm) (Fig. 7) (Table 1).

(6) Days taken to first flowering - Different concentrations of benzotriazole, MH and ethrel delayed flowering. Plants sprayed with various concentrations of ethrel took more number of days to first flowering (68.2 days) as compared to benzotriazole and MH treated plants. The plants sprayed once with 0.1% ethrel took 68.2 days for flowering as compared to 23 days taken by control plants (Table 1). Delayed flowering by ethrel and benzotriazole has also been observed in *Gossypium hirsutum*[15], *Helianthus annuus*[6] and *Vicia faba*[8]. Delayed flowering by MH has been observed in *Capsicum annuum*, *Datura alba* and *Ranunculus muricatus*[2].

Pollen Fertility - Foliar sprays with different concentrations of benzotriazole effectively induced pollen sterility ranging between 93-100% (Figs. 9 & 10). One to two treatments with 0.3% and all the treatments with 0.4 and 0.5% maleic hydrazide induced complete pollen sterility (Fig.11). On the other hand, all the treatments with various concentrations of 0.2 and 0.3% ethrel induced 100% pollen sterility (Fig.12). The complete pollen sterility has also been induced by treatments with ethrel in *Gossypium hirsutum*[15] and *Ablemoschus esculentus*[16]. Recently, complete pollen sterility by treatments with benzotriazole has been induced in *Helianthus annuus*[6], *Brassica juncea*[7] and *Vicia faba*[8].

Total yield /plant - Total yield/plant was significantly reduced by the treatments with all the chemicals used. The maximum reduction in yield was recorded in plants treated twice with 0.3% ethrel (76.46 g/plant) as compared to control plants (284.8 g/plant) (Table 1). The reduction



Figs. 1-5. Untreated and treated plants of *Cyamopsis tetragonoloba*. **Fig.1.** Untreated plant of *Cyamopsis tetragonoloba*, **Fig. 2.** Plant treated with 0.8% benzotriazole, **Fig. 3.** Plant treated with 0.5% MH, Fig. 4. Plant treated with 0.3% ethrel and Fig. 5. Plant treated with 0.8 % benzotriazole showing burning of leaves, **Figs. 6-8.** Inflorescence of untreated and treated plants. Fig.6. Benzotriazole treated (a: untreated, b: 0.4,c: 0.6 and d: 0.8% treated), Fig. 7. MH treated (a: untreated, b: 0.3%, c: 0.4% and d: 0.5% treated), Fig.8. Ethrel treated (a: Untreated, b: 0.1%, c: 0.2% and d: 0.3% treated), **Figs. 9-12.** Pollen grains (FCR test) of untreated and treated plants.480X, Fig. 9. Viable pollen of untreated plants, Fig. 10. Non-viable pollen of 0.8% benzotriazole treated plants, Fig.11. Non-viable pollen of 0.5% MH, Fig.12. Non-viable pollen of 0. 3% ethrel treated plants.

Table 1. Effect of CHAs on Reproductive Parameters of *Cyamopsis tetragonoloba*.

Chemicals	Conc. (%)	Plant height		No. of branches per plant		Number of leaves per plant		Number of inflorescence per plant	
		T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2
Benzotriazole	0.4	105.5**±3.0	99**±4.58	10.0^{NS}±1.26	9.6*±0.8	66.4**±2.15	60.0**±1.4	58.3^{NS}±1.30	50.2**±0.3
	0.6	100.7**±4.54	98.5**±3.5	9.2**±0.97	8.8**±0.96	57.4**±1.74	51.4**±2.57	45.7**±0.97	32.4**±0.2
	0.8	86.28**±2.69	84.0**±4.002	8.2**±1.6	7.6**±0.48	50.0**±0.63	42**±0.31	30.4**±1.21	29.5**±0.3
MH	0.3	96.5**±2.95	100.25**±1.49	9.6**±0.8	9.1**±0.98	69.1**±2.51	57.1**±0.6	51.9^{NS}±0.1	42.5**±0.2
	0.4	101**±2.54	95.25**±3.83	8.8*±0.96	8.1**±1.01	53.2**±2.57	51.3**±2.57	43.1**±1.26	34.7**±0.60
	0.5	93.51**±4.387	79.75**±4.10	7.2**±0.48	5.3**±0.92	52.3**±0.31	49**±0.2	32.3**±0.19	30.2**±0.42
Ehrel	0.1	99.5**±1.11	92.25**±4.02	8.2**±1.6	8.1**±1.01	52.0**±0.21	50.1**±2.57	22.01**±0.2	20.1**±0.21
	0.2	90.25**±4.60	89.0**±4.12	7.3**±0.91	7.2**±1.0	49.1**±1.26	43.2**±0.26	18.81**±0.31	15.4**±0.12
	0.3	83.25**±4.43	77.0**±3.48	6.5**±0.42	6.0**±0.21	35.1**±1.32	32.0**±0.31	13.41**±0.3	12.0**±0.13
Control		126.75±3.34		12.6±0.8		102.2±1.46		62.3±0.36	

Table 1. Contd.

Chemicals	Conc. (%)	Size of Inflorescence		Days taken to first flowering		Pollen Sterility (%)		Total yield per Plant (gm)	
		T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2
Benzotriazole	0.4	1.72^{NS}±0.21	1.34^{NS}±0.34	32.0**±0.16	36.52**±1.20	93.0**±1.41	95.0**±0.70	256**±1.32	253.6**±0.32
	0.6	1.44^{NS} ±0.10	0.9**±0.268	39.6**±0.46	32.5**±1.62	96.2**±.70	100**±00	250.69**±0.2	232**±1.03
	0.8	1.14^{NS} ±0.268	0.6**±0.141	40.9**±0.46	41.0**±0.32	100**±00	100**±00	222.9**±0.9	179**±0.31
MH	0.3	1.76^{NS} ±0.185	1.02^{NS} ±0.426	34.1** ± 0.9	36.3**±0.46	92.0**±1.14	96.0**±1.14	289.0^{NS} ± 0.16	269.1^{NS}± 1.94
	0.4	1.3^{NS} ±0.282	0.68**±0.305	35.1** ± 0.31	39.1**±0.56	97.0**±1.0	100**±00	239.1** ± 0.4	221** ± 0.16
	0.5	0.44^{NS} ±0.20	0.34**±0.10	39.3** ± 0.61	40.2**±0.61	100**±0.0	100**±00	201.7** ± 1.4	193** ± 0.06
Ehrel	0.1	1.32^{NS}±0.330	1.02^{NS}±0.426	68.2** ± 0.21	62.3**± 0.71	93.0**±1.21	96.0**±7.07	118.8**±0.06	116**±0.40
	0.2	0.8** ± 0.282	0.68**±0.3059	63.4**± 0.9	61.13** ± 0.56	98.0**±.812	100**±0.0	109.0**±0.3	92.2**±1.69
	0.3	0.44**±0.2059	0.34**±0.101	62.30**± 1.6	63.5**± 0.16	100**±00	100**±0.0	82.46**±0.16	76.46**±0.03
Control		2.08±0.30		23.2±035		2.09±0.20		284.8±0.9	

T_1: Single spray, a week before floral bud initiation.
T_2: Double spray, first, a week before floral bud initiation and second, after three days floral bud initiations.
T_0: Simultaneously, a group of plant sprayed by distilled water to serve as a control. ± : Standard deviation, * : Significant at 5% level
** : Significant at 1% level, NS: Not significant.

in total yield is largely because of the reduction in number of fruits/plant. Reduction in the number of fruits and total yield in *Vicia faba* plants treated with ethrel has been reported[8].

Thus, ethrel at higher concentration (0.3%) was found to induce complete pollen sterility in *Cyamopsis tetragonoloba* but associated with significant reduction in yield parameters. It is important to find out the suitable gametocide or chemical hybridizing agent, appropriate concentration, number of treatments and stage at which sprays are to be made which will not only induce complete pollen sterility without significantly reducing yield parameters. From the foregoing observations it is quite clear that single spray of lower concentration of benzotriazole is quite suitable chemical hybridizing agent for this crop.

References

1. Shivanna K R 2003, *Pollen biology and Biotechnology*. Oxford and IBH Publishing Co. Pvt. Ltd. New Delhi. 77-89.
2. Chauhan S V S and Kinoshita T 1982, Chemically induced male sterility in angiosperms a review. *Seiken Ziho.* **30** 54-75.
3. Shivanna K R and Johri B M 1985, *The Angiosperm Pollen Structure and Function.* Wiley Eastern Ltd. New Delhi.
4. Kaul M L H 1988, *Male Sterility in Higher Plants.* Springer Verlag, Berlin, Heidelberg, New York.
5. Cross J W and Schulz P L 1997, Chemical induction of male sterility. In : *Pollen Biology for Crop Production and Improvement* (Eds. Shivanna K R and Sawhney V K) Cambridge University Press, London, pp 218-236.
6. Singh V and Chauhan SVS 2000, Effect of various chemical hybridizing agents on *Helianthus annuus* L. *J. Indian Bot. Soc.* **79** 71-74.
7. Singh V and Chauhan S V S 2001, Benzotriazole-A new chemical hybridizing agents for *Brassica juncea* L. *J. Cytol. Genet.* **2** 81-83.
8. Chauhan S V S and Chauhan Surabhi 2003, Evolution of three chemical hybridizing agents on two varieties of broad beans (*Vicia faba* L.) *Indian J. Genet.* **63 (2)** 128-131.
9. Naylor A W and Davis E A 1950, Maleic hydrazide as a plant growth inhibitor. *Bot. Gaz.* **112** 112-126.
10. Rowell P L and Miller D G 1971, Induction of male sterility in wheat with 2-Chloroethyl phosphonic acid (ethrel). *Crop Sci.* **11** 629-631.
11. Fairey D T and and Stoskopf N C 1975, Effect of granular ethephon on male sterility in wheat. *Crop Sci.* **15** 29-32.
12. Colhoun C W and Steer M A 1983, The cytological effects of the gametocides ethrel and RH-531 on microsporogenesis in barley (*Hordeum vulgare* L.). *Plant Cell Environ.* **6** 21.
13. Chauhan S V S and Agnihotri DK 2006, L M and TEM study in male sterile plants of *Abelmoschus esculentus* L. *Proc. Nat. Acad. Sci.* **76 (I)** 51-55.
14. Shivanna and Rangaswamy 1992, Pollen Biology : *A laboratory Mannual.* Narosa Publishing House, New Delhi.
15. Gupta H K and Chauhan S V S 2005, Efficacy of ethrel and benzotriazole as chemical hybridizing agents in cotton (*Gossypium hirsutum* L.). *J. Cotton Res. Dev.* **19 (2)** 153-156.
16. Agnihotri D K and Chauhan S V S 2005, Ethrel induced male sterility in Okra (*Abelmoschus esculentus* L.). *J. Cytol. Genet.* **6** 75-78.