J. Ind. Bot. Soc. Vol.84 (2005) 14 - 16

FREE PROLINE IN THE ANTHERS OF MALE STERILE LINES OF VARIOUS ORIGINS IN *BRASSICA JUNCEA* (L.) CZERN & COSS.

VANDANA SINGH AND S.V.S.CHAUHAN*

Department of Botany, School of Life Sciences, Dr. B.R. Ambedkar University, Agra-282002

Quantitative analysis of free proline in the anthers of male sterile lines of *Brassica juncea* of various origins (cytoplasmic, chemically induced and aphid infested) and their fertile counter parts was made. The quantity of free proline in the anthers of male fertile plants increased gradually with age and recorded maximum values at engorged pollen stage. On the other hand, in male sterile plants the quantity of proline though increased with age was significantly low as compared to their fertile counter parts.

Key words: *Brassica juncea*, anthers, sterility, proline.

Male sterility, its mode of inheritance, application in hybrid seed production and its mechanism has received considerable attention (Kaul, 1988). Various aspects of male sterility in Brassica including new sources obtained through chemical hybridizing agents have been well documented (Chauhan and Singh, 2002 and Singh and Chauhan, 2003; 2004).Information has been accumulating about high quantity of proline in fertile pollen grains in a wide variety of taxa (Chauhan and Kinoshita, 1993; Singh and Chauhan, 1995). Predominance of free proline in pollen over pistil and its involvement in fundamental reactions of the sexual process is also well known (Britikov et al., 1964 and Tupy, 1964).

Keeping this in view, the present investigation has been carried out to quantify the proline content in the anthers of different male sterile (CMS, chemically induced and aphid infested) and fertile plants of *Brassica juncea* L.

The present work was carried out on male fertile and male sterile plants of various origins in *Brassica juncea*. The seeds of cytoplasmic male sterile lines (Siifolia A & PCMC 10A), maintainer lines (Siifolia B & PCMS 10B) and normal male fertile line (var. Pusa Bold) were obtained from Division of Genetics, IARI, New Delhi were sown in the randomized row design at Botanic garden, School of Life Sciences, Dr. B.R.Ambedkar University, Agra. The distance between row to row was 35 cm and plant to plant was 15 cm.

Foliar sprays of different concentrations of various chemical hybridizing agents (CHAs) were made on male fertile plants to induce male sterility. The plants of *Brassica juncea* var. Pusa bold were sprayed twice. First spray was carried out a week before and second after 3 days of floral bud initiation with aqueous solutions of gibberellic acid 'GA_3' (0.04% w/v), ethrel (0.3 % v/v), surf excel (6 % w/v) and benzotriazole (1.5 % w/v). A group of plants were sprayed only with distilled water to serve as control. The seeds of *B. juncea* var. Pusa bold were sown in the pots inside a glass house and the plants thus raised were inoculated with nymphs of the aphid *Lipaphis erysimi* Kalt. The pollen fertility of the male fertile and sterile plants of various origins was tested at regular intervals with Alexander's stain (1980).

Free proline in the anthers of the normal and male sterile plants was quantitatively measured according to the procedure of Bates et al. (1973). Fresh anthers (200 mg) at different stages of development were homogenized with 5 ml of 3 % sulphosalicylic acid. The homogenate was centrifuged and the supernatant

e-mail : svs250@rediffmail.com
Communicated by : P.M. Swamy

was supplemented with a mixture of 1.25 g ninhydrin, 8ml orthophosphoric acid, 30 ml acetic acid and 12 ml distilled water. The mixture was boiled for an hour and cooled to room temperature. 10 ml toluene was added to the mixture in a separating funnel. Toluene containing the chromophore was separated and allowed to stand till it became clear. Transmittance was read with a colorimeter at 520nm. Standard curve was prepared by using pure proline (BDH).

It is clear from the data presented in Table1 that the amount of free proline decreased with the increase in pollen sterility and the quantity of free proline was lower in the male sterile plants than the male fertile (maintainer and control) plants.

Amount of free proline was significantly lower in cytoplasmic male sterile (Siifolia A and PCMS 10A) plants compared to their fertile maintainer (Siifolia B and PCMS 10B) plants. However, amount of free proline in petaloid anthers of PCMS10A was significantly lower than as compared to that in the anthers of Siifolia A.

It is also evident from the data in Table 1 that the plants sprayed with different chemical hybridizing agents exhibited 100 % pollen sterility (Chauhan and Singh, 2002; Singh and Chauhan, 2003; 2004). It was interesting to note that the anthers of these chemically induced male sterile plants contained lower quantity of free proline than the their fertile counterparts. The reduction in free proline was directly proportional to the decrease in pollen fertility which decreased with the increase in the concentration of different CHAs. The anthers of plants sprayed with different concentrations of gibberellic acid exhibiting 100% pollen sterility contained lowest amount of free proline among all the treated plants. On the other hand, the anthers of benzotriazole treated plants showing 100% pollen sterility exhibited minimum reduction in proline content.

Infestation of aphid (*Lipaphis erysimi* Kalt.) causes great damage to entire plant and floral parts in particular. Such plants exhibited partial to complete pollen sterility, based on the developmental stage at which the plant is infested (Solanki and Chauhan, 1993). The anthers of such plants showed marked reduction in proline content than their control plants and the reduction increased with the intensity of infestation and increase in pollen sterility (Table 1).

Table1. Quantity of free proline in the anthers of male fertile and sterile Brassica juncea plants of various origins at different stages of development (a: pollen mother cell; b: microspore tetrad; c: pollen).

S. N.	Different Male fertile and sterile plants	Pollen Sterility (%)	Proline Content (mg/g fresh wt.)		
			a	b	c
1.	**Cytoplasmic male sterile & male fertile maintainer**				
i.	Siifolia A (CMS)	100 ±0.00	0.540** ±0.075	0.570** ±0.066	0.593** ±0.070
ii.	Siifolia B (MF)	7.9 ±1.69	1.120 ±0.296	1.150 ±0.026	1.350 ±0.126
iii.	PCMS 10 A (CMS)	100 ±0.00	0.150** ±0.046	0.170** ±0.017	0.174** ±0.041
iv.	PCMS 10 B (MF)	9.2 ±1.27	1.080 ±0.048	1.140 ±0.05	1.230 ±0.034
2.	**Chemically induced male sterile plants**				
i.	Gibberellic acid (0.04%)	100 ±0.00	0.188** ±0.019	0.240** ±0.031	0.255** ±0.035
ii.	Ethrel (0.03%)	100 ±0.00	0.360* ±0.030	0.420* ±0.077	0.443* ±0.068
iii.	Surf excel (6%)	100 ±0.00	0.435* ±0.028	0.488** ±0.037	0.510* ±0.030
iv.	Benzotriazole (1.5%)	100 ±0.00	0.563** ±0.033	0.578* ±0.038	0.600** ±0.041
3.	**Aphid infested male sterile plants**				
i.	Infested at pre floral bud initiation	100 ±0.00	0.164* ±0.018	0.201* ±0.024	0.298* ±0.023
4.	Male fertile control plants	8.6 ±2.21	1.020 ±0.222	1.180 ±0.213	1.320 ±0.172

±Standard deviation
*Significantly different from control at P=0.05 using Student's "t" test.
**CMS plants significantly different from their male fertile counterpart of P=0.05 using Student's "t" test.

The results of the present and previous studies clearly indicate that proline, one of the major amino acids of pollen, is closely associated with pollen fertility. It has also been reported by several investigators in large number of cytoplasmic, genic and induced male sterile plants (Kaul, 1988; Chauhan and Kinoshita, 1993; Singh and Chauhan, 1995).

REFERENCES

Alexander M P 1980. A versatile stain for pollen, fungi, yeast and bacteria. *Stain Tech* **55** 13-18.

Bates L S, R P Waldren & I O Teare 1973. Rapid determination of free proline for water stress studies. *Plant and Soil* **19** 205-207.

Britikov E V, N A Mustava, S V Vladimirseeva & M A Protesnko 1964. Proline in the reproductive systems in plants. In: Pollen Physiology and Fertilization.

(Linskens, HF ed.) North Holland Pub Co, Amesterdam pp 77-85.

Chauhan S V S & T Kinoshita 1993. Quantitative estimation of free proline in the anthers of male fertile and sterile lines of varied origins in *Beta vulgaris*. *Proc Nat Acad Sci* **63 (II)** 257-260.

Chauhan S V S & Vandana Singh 2002. Detergent-induced male sterility and bud pollination in *Brassica juncea* L. *Curr Sci* 8 918-920.

Kaul M L H 1988. *Male Sterility in Higher Plants*. Springer-Verlag Berlin.

Singh Jolly & S V S Chauhan 1995. Quantitative analysis of free proline in the anthers and pistils of seasonally transient sterile *Tecoma stans* L. *J Indian Bot Soc* **74** 125-127.

Singh Vandana & S V S Chauhan 2003. Bud pollination and hybrid seed production in detergent-induced male sterile plants of *Brassica juncea*. *Plant Breed* **122** 421-425.

Singh Vandana & S V S Chauhan 2004. Evaluation of three chemical hybridizing agents in *Brassica juncea* L. *Brassica* 6 (3&4) 71-73.

Solanki Seema & S V S Chauhan 1993. Free proline in the anthers of *Brassica juncea* plants under various kinds of stress. *Plant Cell & Incompatibility Newsletter* 25 50-54.

Tupy J 1964. Metabolism of proline in styles and pollen tubes of *Nicotiana alata*. In: *Pollen Physiology and Fertilization*. (Linskens, HF ed.) North Holland Pub Co, Amesterdam pp 86-94.