ETHREL INDUCED CHANGES IN FLORAL MORPHOLOGY, YIELD, POLLEN STERILITY AND ANTHER DEVELOPMENT IN TOBACCO (*NICOTIANA TABACUM* L.)

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The effect of foliar sprays of ethrel or ethephon (2-chloroethyl phosphonic acid) on floral morphology, pollen fertility and yield in *Nicotiana tabacum* L. var. NP 70 (Solanaceae) was studied. Treatments with ethrel caused delayed flowering, reduction in number of fruits, fruit size and other yield components. However, it was interesting to note that due to elongation of style, stigma protrudes out of the floral buds of plants sprayed twice or thrice with 0.3% ethrel, and in a limited number of floral buds, the stigma became four lobed. The plants treated thrice with 0.2 and 0.3% ethrel, exhibited complete pollen sterility but this was associated with reduction in yield. The tapetal cells in the anther of treated plants remained intact till anthesis. However, their organelles were in degenerated form. The pollen mother cells underwent normal meiotic division producing microspore tetrads. The microspores released from the common callose wall, were converted into sterile pollen grains of abnormal shape and size and consisted of degenerated protoplast. The exine and intine was thick. At the time of anthesis, the pollen grains degenerated followed by the degeneration of tapetal cells.

Keywords: Anther development; Ethrel; *Nicotiana tabacum*; Pollen sterility.

Introduction

During the past five decades, extensive work has been carried out on chemical induction of male sterility in angiosperms[1-3]. Naylor[4] was the first to induce male sterility in tobacco by sprays with maleic hydrazide. Similarly, Jos and Singh[5] have studied the gametocidal effect of maleic hydrazide (MH), FW-450, 2,4-D and Embutox-4 on two varieties of tobacco. They found that MH in lower concentrations caused 80% pollen sterility. Recently, new generation of chemical hybridizing agents have been used for the induction of male sterility in different crops[6]. Ethrel or ethephon (2-chloroethyl phosphonic acid), an inhibitor of microspore development has been demonstrated as selective inducers of male sterility in different crops e. g. *Cannabis sativa*[7]; *Ipomoea nil*[8]; *Vicia faba*[9]; *Abelmoschus esculentus*[10]; *Gossypium hirsutum*[11] and *Cicer arietinum, Lens culinaris, Lycopersicon esculentum, Nicotiana tabacum*[12]. The present work was undertaken to find out the efficacy of ethrel as an effective chemical hybridizing agent in tobacco (*Nicotiana tabacum* L.)

Material and Methods

The present experiment was conducted on *Nicotiana tabacum* var. NP 70. The seeds of this variety obtained from National Seed Corporation, Agra were sown at Botanic Garden, School of Life Sciences, Dr. B. R. Ambedkar University, Agra during 2004-2005. The experiments were laid out in a randomized row design with five replicates. The distance between row to row was 75 cm and plant to plant it was 45 cm. The plants were sprayed with aqueous solutions of 0.1, 0.2 and 0.3% (v/v) ethrel at three different developmental stages. A drop of liquid soap (Ezeey) was added in each solution to serve as surfactant. A group of ninety plants were sprayed one week before the initiation of first floral buds (T$_1$), the remaining sixty plants were sprayed again at the time of floral bud initiation (T$_2$) and ,remaining thirty plants were sprayed third time at the time of anthesis (T$_3$). A group of ninety plants grown in between treated plants were sprayed with distilled water containing a drop of surfactant to serve as control. 15 ml of each concentration was sprayed on one to run off.

Data on days taken to first flowering, floral morphology, pollen fertility and anther development and total yield component were collected from the treated and untreated plants. Pollen fertility was tested at regular intervals with 1% TTC (Tetrazolium chloride) in 0.15 M Tris-HCL buffer at 7.8 pH.

Light microscopic studied, floral buds of both treated and untreated plants were fixed in formalin-acetic-
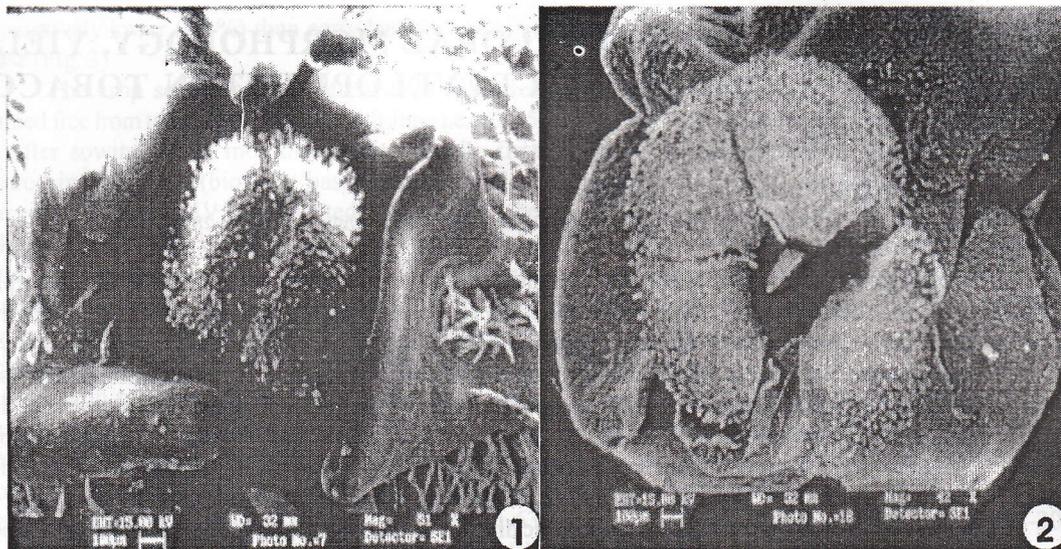
Figs.1-2. Scanning electron microscopic (SEM) photographs of stigma in control and ethrel treated plants of *Nicotiana tabacum* L.
Fig.1. Bilobed stigma of control plant.; **Fig.2.** Stigma of ethrel treated plant. Note stigma became four lobed.

alcohal. These were dehydrated, cleared and embedded in paraffin by customary method. The sections were cut at 8-12 μm and stained with Delaffiled haemotoxylin.

For Transmission electron microscopic studied, anthers at various developmental stages were fixed in 3% glutaraldehyde in 0.1 M PO4 buffer at pH6.8. Post fixation was done in 1% osmic acid. Samples were dehydrated in an ethylene oxide series and embedded in spurr's low viscosity embedded media. Ultra thin were stained uranyl acetate lead citrate and observed under electron microscope at All India Institute of Medical Sciences, New Delhi.

For scanning electron microscopic (SEM) studies, the floral buds were fixed in 2.5% glutaraldehyde in phosphate buffer (pH 7.4) at 4°C and dehydrated through aqueous acetone series. These were dried in HCP-2 Hitachi critical point dryer using CO_2. These floral buds were coated with gold in a SCD 020 sputter-coating unit, mounted on stubs and observed and photographed in a LEO EM-SEM at All India Institute of Medical Sciences, New Delhi.

Results and Discussion

(1) Days taken for first flowering and floral morphology- Flowering in control plants was initiated 67 days after sowing but it was delayed in all the treated plants (Table 1). In plants treated thrice with 0.1, 0.2 and 0.3% ethrel, the flowering was initiated after 75, 78 and 80 days respectively. Thus, maximum delay in flowering was recorded in plants treated thrice (T_3) with all the concentrations of ethrel.

The size of flowers and their parts was reduced in variously treated plants and the reduction was directly proportional to the concentrations and number of treatments. However, it was interesting to note that in plants treated thrice (T_3) with 0.3% ethrel, the receptive stigma protruded out of young floral buds. Another interesting feature recorded was that the stigma of flowers of plants treated thrice with 0.3% ethrel became four lobed (Fig.2) as compared to bilobed stigma of control plants (Fig. 1).

(2) Pollen sterility and anther development- Foliar applications of different concentrations of ethrel effectively induced pollen sterility ranging between 95-100% (Table 1). Two or three sprays with 0.2 and 0.3% ethrel brought about complete pollen sterility. The bagged flowers of treated plants failed to show any seed-set. Thus, conforming induction of complete pollen sterility by various treatments.

The anther wall development in *Nicotiana tabacum* is of dicotyledonous type[13] and in both sterile and fertile anthers, at sporogenous tissue stage anther wall consisted of an epidermis, 2-3 layers of endothecium, a middle layer and a glandular tapetum. The behaviour of these layers until the beginning of meiosis was more or less similar in both sterile and fertile (control) plants. The cells in middle layer in the anthers of control plants

Table 1. Effect of different concentrations and number of treatments of ethrel on various reporductive parameters in *Nicotiana tabacum* L. NP 70.

Chemical	Conc. (%)	Days taken to first flowering			Pollen sterility (%)			Number of fruits/plant			Fruit size* (cm^2)			Total yield/ plant		
Number of Treatments		T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3
Ethrel	0.1	73.0	74.4	75.0	95.0	97.9	100	257.2	250.0	248.8	1.4	1.3	1.3	20.9	19.9	17.4
	0.2	74.2	77.0	78.0	97.0	100	100	239.8	237.2	230.0	1.3	1.2	1.2	18.5	16.0	15.5
	0.3	75.0	78.0	80.0	97.9	100	100	237.2	233.8	226.4	1.2	1.1	1.1	18.0	16.9	15.5
Control			67.0			1.72			262.8			1.52			23.0	
CD at 5% level			1.5			2.1			3.8			1.8			2.9	

*: Fruit diameter

T_1 : Single spray before floral bud initiation

T_2 : Double sprays, first before floral bud initiation, second 2-3 days after floral bud initiation

T_3 : Three sprays, first before floral bud initiation, second 2-3 days after floral bud initiation and third at the time of anthesis

degenerated at early vacuolated pollen grain stages, while in sterile anthers, the cells in this layer persisted until maturity. The endothecial cells in fertile anthers elongated tangentially but with the commencement of tapetal degeneration, they started elongating radially. On complete degeneration of tapetum, cells in endothecial layer attained maximum radial enlargement and characteristic fibrous thickening appeared on their radial walls. However, the endothecial cells in sterile anthers elongated in tangential direction with age and characteristic fibrous bands on their radial walls failed to appear. At late vacuolated pollen grain stage, the tapetal cytoplasm more or less disappeared leaving a thin plasmalemma on which a large number of ubisch bodies were observed. On the other hand, the tapetal cells in the anthers of ethrel treated plants exhibited complete pollen sterility, started elongating radially with the onset of meiosis in pollen mother cells. Tapetal cells were poorly stained cytoplasm but highly vacuolated. The pro-orbicules formation was also reduced but sporopollenin synthesis was not much influenced. The enlargement of tapetal cells continued even after the formation of microspore tetrads and the release of microspores from the callose wall. Tapetal cells of sterile anthers consisted of large vacuoles and mitochondria, plastids, ER, ribosomes and dictyosomes that were in degenerated form. At the time of anthesis, tapetal cells degenerated followed by the degeneration of pollen grains.

The pollen mother cells in both the fertile and sterile anthers underwent normal meiotic division and both the divisions took place in quick succession to convert it into a tetrad of microspores, each of which is surrounded by a callose wall. Later, a prime exine appeared between the callose wall and microspore stage. The microspores cytoplasm contained plastids, ER, mitochondria and several small vacuoles. On released from the callose wall, each microspore developed into a pollen grain with a well-differentiated exine and intine. The pollen grains were spherical and tricolpate. The sterile pollen grains of treated plants were of various shape and size. It was interesting to note that mature pollen grains ethrel treated plants consisted of a large vacuole and degenerated cell organelles. Their exine and intine was highly thick (Fig. 3-14).

(3) Number of fruits, their size and total yield/plant-There was a reduction in number of fruits, their size and total yield in the treated plants. The reduction was directly proportional to number of treatments as well as concentrations (Table 1). The maximum reduction in the number of fruits (226.4), fruit size (1.1 cm) and total yield (15.0 g) has also been recorded in plants treated thrice

with 0.3% ethrel as compared to 262.8 fruits, 1.52 cm fruit size and 23.0 g/control plants respectively. The total yield decreases due to the reduction of fruits and their size.

It is evident from the foregoing observations that various treatments with ethrel induced pollen sterility ranging between 95-100%, although associated with reduction in yield component. Thus, the plants sprayed only once at pre-floral bud initiation (T$_1$) exhibited 95% pollen sterility associated with minimum yield loss.

Present studies on anther development in ethrel treated plants clearly indicated that pollen abortion is associated with abnormal tapetal behaviour similar to a large number of cytoplasmic, genic as well as chemically induced male sterile plants [1,2,14]. The control of tapetum on endothecial development was also shown in the anthers of induced male sterile plants as shown earlier by Chauhan[15,16].

Colhoun and Steer[17] have studied the effect of ethrel on microsporogenesis in barley and observed abortion of sporogenous cells in plants sprayed at both pre-and post-meiotic stages of microsporogenesis. According to them, the cytological effects of the gametocides are similar to those induced by male sterile genes in a variety of plants. Keyes and Sorrells[18] have also induced pollen sterility in various genotypes of wheat by treatments with ethrel. According to them, the consistent liner relationship between the number of *Rht* alleles and sensitivity of ethylene-induced male sterility suggests that gibberellic acid and its recognition may exert a stabilizing effect in pollen development in the presence or stress or an ethylene shock. Chauhan and Chauhan[9] have also successfully induced complete pollen sterility lasting for 20-25 days in broad beans (*Vicia faba*) by treatments with 0.1, 0.2 and 0.3% ethrel, but this was associated with significant reduction in yield. Recently, Chauhan and Singh[19] and Singh and Chauhan[20] have observed protruding stigma in young buds of detergent induced male sterile plants of *Brassica juncea*. Su et al[21]. have recorded eight male-sterile lines of *B. juncea* with fertile ones. Although all the lines were of different origin, but they exhibited protruded and receptive stigmas, associated with stamen degeneration and pollen abortion. According to them, plants, plants with a protruding may be classed as male sterile. Bud pollination is one of the most successful techniques for overcoming self-incompatibility. It has been achieved in several crops including *Nicotiana*[22].

Nicotiana species are well known for their response to yield to insect pollination. It is known to be directly proportional to the extent of self-sterility[23]. The
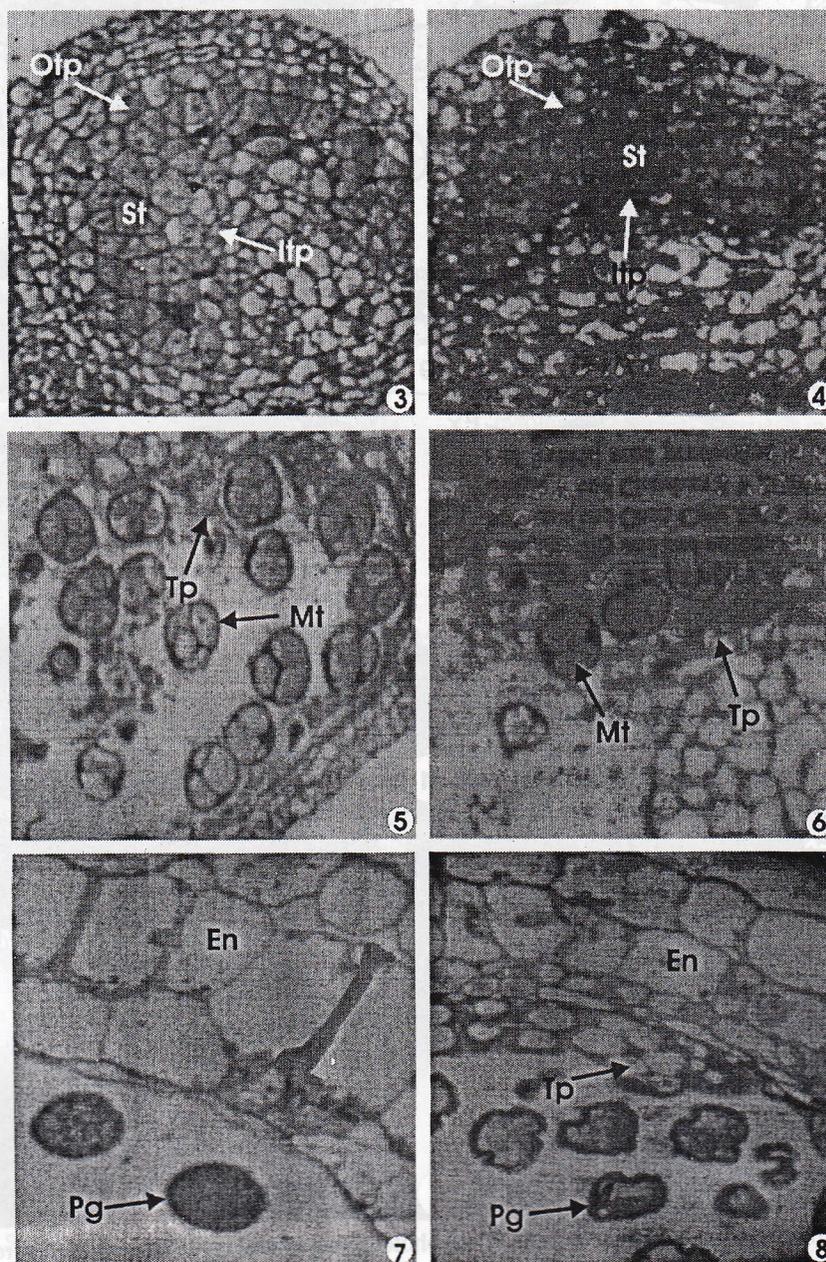
Figs. 3-8. Light microscopic (LM) photographs of anthers of control and ethrel treated plants of *Nicotiana tabacum* L. **Fig. 3.** LM photograph of anther at sporogenous tissue (St) stage of control plant showing well developed epidermis, endothecium, middle layer and tapetum (Tp) 430X.; **Fig. 4.** LM photograph at sporogenous tissue (St) stage of treated plant. Note the presence of vacuolated and poorly stained cytoplasm 430X.; **Fig. 5.** LM photograph of anther at microspore tetrad stage (Mt) of control plant. Note tapetum (Tp) degeneration started at this stage 620X.; **Fig. 6.** LM photograph at microspore tetrad stage (Mt) of treated plant. Note tapetum remained intact 620X.; **Fig. 7.** LM photograph of anther at pollen grain stage (Pg) of control plant. Note the presence of fibrous bands (Fb) in the endothecial cells (En) and completely degenerated tapetum 680X.; **Fig. 8** LM photograph at pollen grain (Pg) of treated plant. Note lacking of fibrous band (Fb) in endothecium and vacuolated tapetal cells (Tp) also enlarge radially and remain intact 620X.
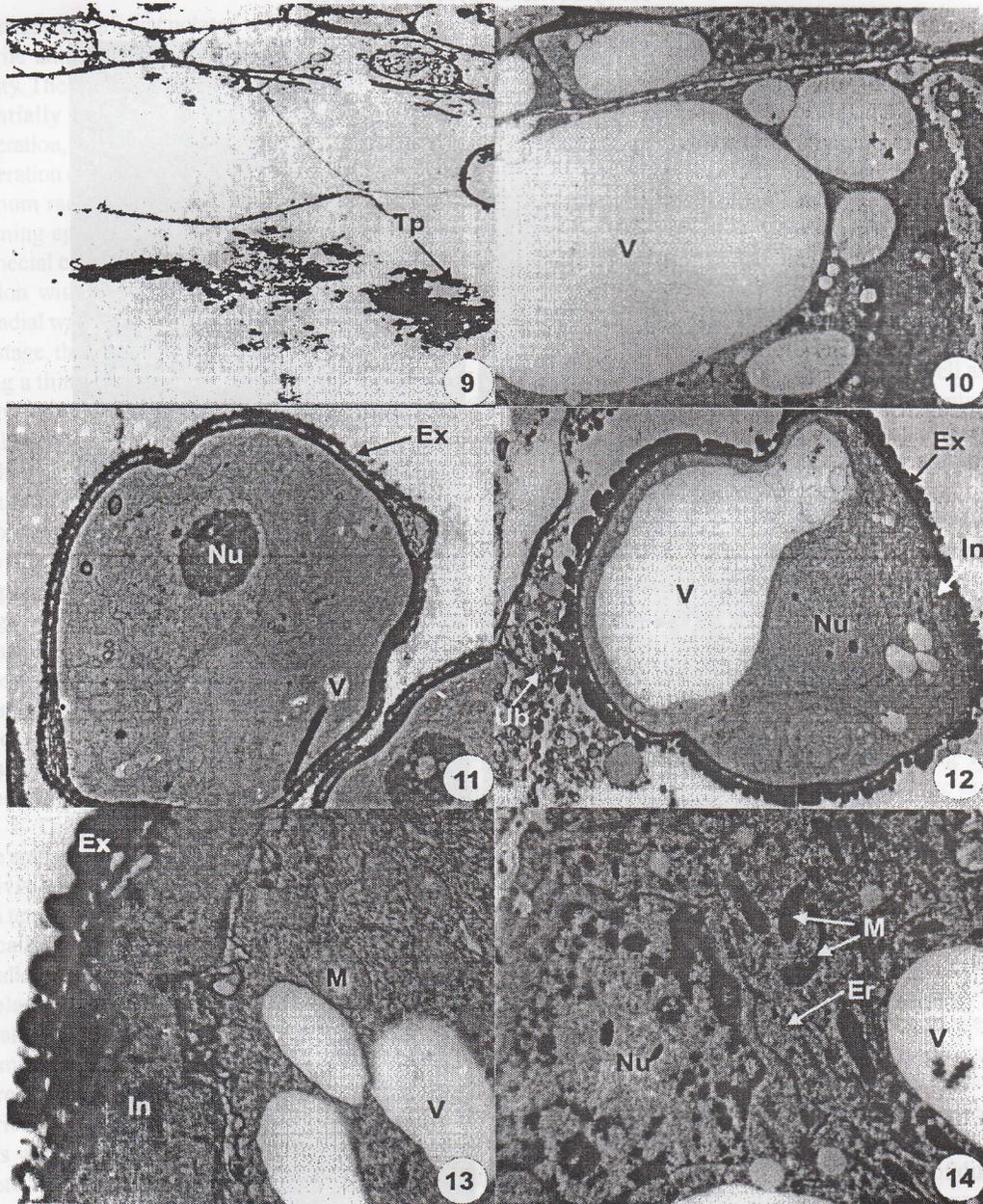
Figs.9-14. Transmission Electron Microscopic (TEM) photographs of anthers of control and ethrel treated plants of *Nicotiana tabacum* L.

Fig.9. TEM photograph of anther at pollen grain stage (Pg). Note completely degeneration of tapetum (Tp) 1100X; **Fig.10.** TEM photograph of a tapetal cell (Tp) at sterile pollen grain stage (Pg). Note highly vacuolated (V) and degenerated cell organelles 2100X; **Fig.11.** Mature pollen grain (Pg) stage of control plant. Note presence of well developed exine (Ex), nucleus (Nu) and few number of vacuoles (V) 2100X; **Fig.12.** TEM photograph of anther at mature pollen grain (Pg) stage. Note thick exine (Ex) and intine (In) and large size of vacuoles (V) at two cell stage of pollen grain 2100X; **Fig.13.** Magnified view of Fig.12 showed a part of pollen grain (Pg). Note thick exine (Ex), intine (In), few vacuoles (V) and degenerated mitochondria (M) 2800X; **Fig.14.** Magnified view of tapetal cell (Tp) Fig.10 showed degenerated cell organelles including plastids (P), endoplasmic reticulum (Er), nucleus (Nu) and vacuoles.4800X.

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buds of treated plants with protruding stigma are quite capable of cross- pollination at this stage and can avoid self-pollination due to pollen sterility.

Thus, on the basis of the results of the present investigation and those of others mentioned above it may be suggested that treatment with lower concentrations of ethrel (0.1% or 0.2%) at pre-meiotic stage will be able to induced pollen sterility to significant extent without much reduction in yield and such male sterile plants can be used for hybrid seed production on commercial basis in this important cash crop.

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