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SHORT COMMUNICATION

SALICYLIC ACID AS A SELECTIVE GAMETOCIDE IN A THERMOSENSITIVE GENIC MALE STERILE RICE

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Thermosensitive genic male sterility (TGMS) is a genic male sterility expression regulated by certain temperature conditions. This male sterility system is considered more efficient than CMS (cytoplasmic male sterility) system for hybrid rice breeding because CMS lines require specific maintainer and restorer line. So an attempt was made to identify a suitable chemical that can induce complete sterility without affecting the female organs in a stable TGMS line, TS 29 even when the temperature drops below the critical sterility temperature. Salicylic acid at 800 ppm at third and fifth stages of panicle development was found to effect near complete pollen sterility in TS 29 without affecting the female fertility. The fluorescent microscopic studies showed that the pollen from the restorer line (MDU 5) reached the ovule of TS 29, and the histological studies also showed the entry of the pollen tube into the ovule of TS29 proving the selective sterility of the chemical.

Key words : **Female fertility, pollen germination, rice, salicylic acid, TGMS**

The cytoplasmic male sterility system, is presently the most widely used technology for producing F_1 rice hybrids. Although effective, yet it is cumbersome, because CMS lines require specific maintainer and restorer line. Recently, thermosensitive genic male sterility, has been identified to develop rice hybrids in China.

Chemical emasculators, despite many studies, have been least used in practical hybrid breeding programme. If a potential chemical is found and its application is optimised to realise desired level of selective male sterility, several of the advantages of chemical hybridising agent can be made use of for a successful two line breeding (Dotlacil and Apltauerova 1978).Chemical induction of male sterility, leaving the female organs fertile, by a simple chemical treatment would be of great importance for the hybrid seed production in TGMS lines, even when the temperature fluctuates or drops below critical sterility temperature (Ilyas Ahmed 1996).

An experiment was conducted to study the effect of various plant growth regulators and chemicals in inducing sterility at Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore during two seasons, Rabi 2001-2002 and Kharif 2002 (Partial sterility phase of TS 29 line). The stable line namely, TS 29 was used in this experiment. Pollen sterility before anthesis was estimated by I-KI (Iodine-Potassium Iodide) staining.The experiment was carried out in an RBD with 5 treatments and 4 replications. T_{1-} Control; $T_{2\,-}$ Salicylic acid – 800 ppm; $T_{3\,-}$ Salicylic acid – 1000 ppm; $T_{4\,-}$ Ethrel – 800 ppm ; $T_{5\,-}$ Maleic hydrazide – 2000 ppm sprayed at StageIII and Stage V of panicle development.

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Per cent pollen sterility was observed before anthesis. Pollens were stained with I-KI dye, and observed under microscope. The pollen grains that were stained fully were considered as fertile, while unstained, shrivelled and empty pollen grains were considered as sterile. After confirmation of 100 per cent pollen sterility, induced by salicylic acid, the spikelets of TS 29 line were opened by clipping method and dusted with pollen grains collected from the pollinator (MDU 5) and covered with white crossing covers. Then, the ovaries collected from TS 29 line after four and eight hours were fixed in alcohol: acetic acid mixture in the ratio of 3:1 for 24 hours. After that, the ovaries were washed with distilled water for 3 to 5 times. NaOH of 8N was added to it and kept for four hours. Again three to five washings with distilled water were carried out. Aniline blue (0.1%) dissolved in 1 N K_3PO_4 was added and kept in dark overnight. Then, ovaries were mounted in 50 per cent glycerol and observed under fluorescence (Sitch 1990).Further confirmation of female fertility was done by taking microtome sections of the bottom of the ovary by adopting the method of Johansen (1940).

Table 1. Effect of spraying certain chemicals and growth regulators during panicle development on pollen sterility (%) and crossed seed set percentage of TS 29

Treatments	Pollen sterility (%)		
	Season I	Season II	Pooled mean
Control	57.22	17.04	37.13
Salicylic acid (800 ppm)	99.60	98.13	98.87
Salicylic acid (1000 ppm)	99.95	98.78	99.36
Ethrel (800 ppm)	75.88	88.50	82.19
Maleic hydrazide (2000 ppm)	81.91	80.71	81.31
Mean	82.91	76.63	
CD (P=0.05)	1.698	2.498	
For pooled analysis CD (P=0.05)	S	0.804	
	T	1.431	
	S x T	2.023	

During season I, pollen sterility percentage ranged from 57.22 in control to 99.95 in 1000 ppm salicylic acid. But in season II, only 17.04 per cent pollen sterility was recorded in control, and the maximum sterility of 98.78 per cent was also recorded in salicylic acid (1000 ppm) treatment. In both the seasons, the differences among treatments were statistically significant. But, the values recorded in salicylic acid at 800 and 1000 ppm treatments, in both the seasons, were at par with each other and hence, it can be construed that 800 ppm concentration of salicylic acid is optimum for obtaining almost complete pollen sterility**.** Restorer lines were also raised along with TS 29 to study the percentage of crossed seed set as an indirect way of knowing the female fertility. In both the seasons, maximum percentage of crossed seed set was observed in salicylic acid at 800 ppm in both rope pulling and clipping methods of crossing. Although salicylic acid 1000 ppm also recorded comparable results, the lower dose of 800 ppm could be adopted for getting higher crossed seed set (Table 1). Ali (1996) emphasized the need for obtaining stable male sterility by a simple chemical treatment without any accompanying adverse effect on female fertility in two line breeding using TGMS system. Salicylic acid seemed to be the appropriate chemical for achieving this goal. The effectiveness of salicylic acid in achieving near complete male sterility without affecting the female fertility in TGMS lines was also reported by Senthil (2001).

Since salicylic acid @800 ppm was very effective in bringing out 100 per cent pollen sterility, the lines treated with salicylic acid @800 ppm were tested for female fertility using fluorescent microscopy and microtomy studies. Fluorescent microscopy study showed that the pollen germination occurred after the pollen of the restorer line MDU 5 reached the stigmatic hairs of TS 29 line treated with salicylic acid (Fig 1a) The pollen tube then elongated(Fig 1b) and passed through the centre hole of the stigmatic hair(Fig 1c). In the stigmatic and stylar tissues, the pollen tube passed through the transmitting tissue located in the space between the centre area and the vascular bundle. Finally, the pollen tube reached the bottom micropyle end of the ovary(Fig 1d), proving the selective sterility of the chemical showing the fertilized ovule (Fig 1e). Lasa and Bosemark (1993) reported that a gametocide could emasculate the plants

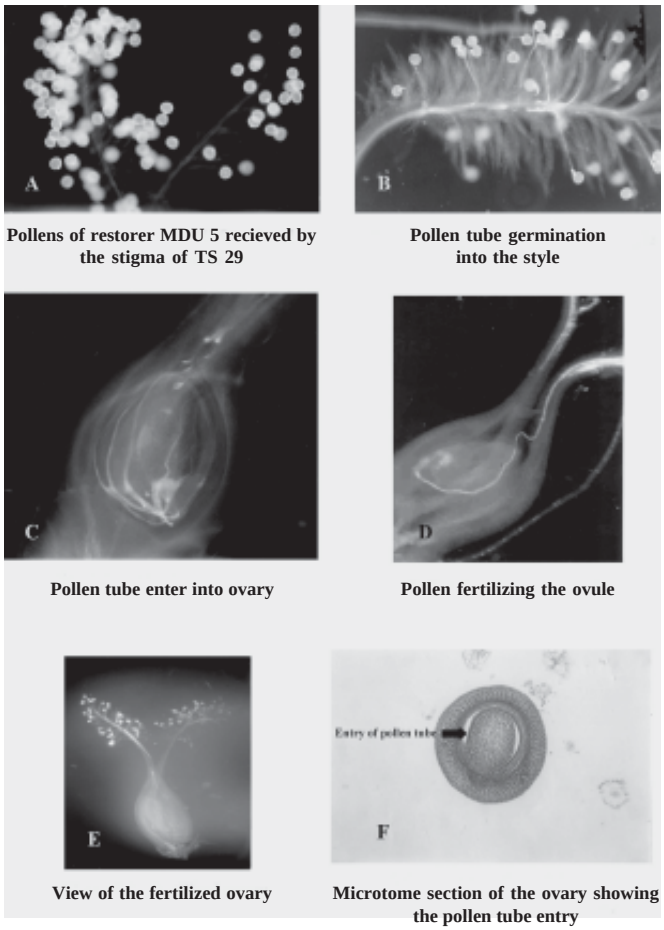

Fig. 1. Fluorescence microscope study revealing female fertility of salicylic acid treated TS 29 line, 1a. pollens of restorer MDU 5 received by the stigma of TS 29, 1b. pollen tube germination into the style, 1c. pollen tube entry into the ovary, 1d. pollen fertilizing the ovule, 1e. view of the fertilized ovary, 1f. microtome section of the ovary showing the pollen tube entry.

resulting in male sterility while maintaining normal female fertility. The microtome sections of the bottom of the ovary showed a narrow space between the outer wall of the ovule and the ovary wall. This space showed where the pollen tube passed through the micropyle, confirming the female fertility of the chemically induced TS 29 line (Fig 1f). Hattori and Wada (1996) observed conditions of pollen tube elongation on the stigmatic hair and pollen tube entry into the ovary.

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