Indian J. Genet., **59**(1): 53-58 (1999)

FOREIGN POLLEN TUBE GROWTH IN MAIZE AFTER CHEMICAL TREATMENTS

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(Received : August 4, 1998; accepted : January 24, 1999)

ABSTRACT

In maize × sorghum and maize × pearl millet crosses, pollen germination and entry of pollen tube in maize silk were normal. The foreign pollen tube grew only to a few millimeter in length and did not reach embryo sac to allow fertilization. To overcome this barrier, various treatments of chemical viz., 2, 4-D, GA_3 and salicylic acid were tried. Growth hormones mostly had favourable effect on pollen tube growth, whereas, the immunosuppressant had inhibitory effect over the control. The treatment of 2,4-D @ 100 ppm was found to be the best, which improved the foreign pollen tube growth significantly over the control. The sorghum pollen tube growth was better than that of pearl millet. Although certain chemical concentrations improved the tube growth of sorghum and pearl millet, it was not sufficient for the foreign pollen tubes to reach maize embryo sac.

Key Words: *Zea mays*, pollen tube growth, maize silk

The use of wide crosses (both interspecific and integeneric) in plant breeding is well established. Apart from acting as a source of useful agronomic traits, wide crosses also help in producing haploids in large numbers. No report is available yet for the production of maize haploids from wide crosses. Certain attempts were made earlier to hybridize maize with sorghum and pearl millet, none of these were successful, due to certain incompatibility barrier [1]. To overcome such barriers, various growth hormones and immunosuppressants were used [2-5]. To study the barrier between maize and sorghum or pearl millet (as pollen parents) and to test the effect of various chemicals on foreign pollen tube growth, this experiment was conducted and the results are presented here.

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MATERIALS AND METHODS

Two genotypes of maize (CM 111-inbred line and Paras - single cross hybrid) and one genotype each of sorghum (PC 121) and pearl millet (Pusa 322) were used. Crosses were made in the field grown plants during *kharif*, 1994 and 1995. Proper care was taken to avoid contamination from stray pollen.

The chemical treatments included spraying growth hormones viz., 2,4-D or GA_3, both at a concentration of 75 or 100 ppm, or the immunosuppressant (salicylic acid) at a concentration of 0.01 or 0.02 M, sprayed either one day before or one day after pollination with foreign pollen.

In case of spraying one day before pollination (DBP), the silks were cut close to the tip of the ear on previous afternoon and covered again with silk bags. The next morning, 5 ml of the solution was uniformly sprayed on the silk with a hand sprayer and covered immediately with silk bags. Different colored tags were used for different treatments. Pollinations were made 24 h later with freshly collected pollen of either sorghum or pearl millet using camel hair brush. In case of pollination day after (DAP), the ears were pollinated first, tagged accordingly and chemical spray was given the next day on the silk and silk bag cover was secured. The pollinated silks were collected for fixation after 48 h, fixed in alcohol : acetic acid mixture (3:1, V/V), and stored at 4-10°C for 24 h. The silks were then transferred to 70% ethyl alcohol and stored in a refrigerator till further use.

The pistils were stained with 0.01% aniline blue [6] and observed under Nikon Microphot-FX microscope with fluorescence attachment, illuminated with 200 W high pressure mercury lamp. The observations were taken with B (380-490 nm) and/or BG 38 (650 nm) excitation filters in combination with BA 520 or BA 530 barrier filters. The pollen tube length was measured in the silks from three different ears pollinated at more or less identical growth stage and each ear was considered as a replication. In each replication, 10 pistils were measured for pollen tube length. Apart from the treatments, a control was included in each cross without any chemical treatment. The data along with control were analyzed cross-wise, following a completely randomized design analysis [7] to study the effect of various chemicals on foreign pollen tube growth.

RESULTS AND DISCUSSION

The pollen pistil interactions were studied during the first year (*kharif*, 1994) to establish the incompatibility barrier between maize and sorghum or pearl millet

(pollen parent). Fluorescent microscopic study revealed that both sorghum and pearl millet pollen grains germinated freely throughout maize silk either on trichomes or on the silk epidermis directly. Once germinated, most of the foreign pollen grains propelled their tubes into the silk. However, in an early study [1] it was observed that only a small proportion of the tubes were able to enter through the cuticle of the papillate shaft cells.

Once the pollen tubes entered the silk, they grew through intercellular space of cortex to reach the transmitting tract. Only a few pollen tubes were directed towards transmitting tract while others simply grew without any defined direction.

Several abnormalities were also noticed in the foreign pollen tubes viz. branching, zig-zagging, callose plugging, enlarging of tube tip and growth in opposite direction. Similar observations were reported earlier also [1, 8-9].

The foreign pollen tubes grew only few millimeter in length even after 48 h of pollination either in cortex or in transmitting tract. It clearly indicated that the limited growth of foreign pollen tube in maize silk is the main reason for the failure of fertilization between these parental species. To overcome pre- fertilization barrier, different chemical treatments (growth hormones and immunosuppressants) were tried.

Foreign pollen germination and its entry into the maize silk were similar in both treated and untreated silks. However, different chemicals had noticeable effect on the extent of the sorghum and pearl millet pollen grains growth in maize silk.

The mean pollen tube growth in length (mm) of PC 121 and Pusa 322 pollens on CM 111 and Paras after treatment with various chemicals along with the control are given in Table 1. Analysis of variance (Table 2) showed significant difference between various treatments. In all the cross combinations, 2, 4-D @ 100 ppm was the best treatment and it improved foreign pollen tube growth significantly over the control. In general, the growth hormones had favourable effect on tube growth. Stimulatory effect of growth hormones on pollen tube growth and embryo development have been reported widely in a range of wide crosses [10]. The hormones also prolong the receptivity of stigma and prevent early abscission of pollinated flowers. In contrast to the growth hormones, salicyclic acid treatment had unfavourable effect on pollen tube growth of both pearl millet and sorghum. The earlier reports also supports inhibition of pollen germination and pollen tube growth by post pollination application of immunosuppressants in *Oryza* spp. [11]. Although there are reports on use of immunosuppressants to overcome the physiological barriers to hybrid embryo development, results are not very conclusive [10]. In the present study only one type of immunosuppressant was used, however, some others like Epsilon - Amino Caproic Acid (EACA) may be tried to see the effects.

Table 1. Effect of various chemical treatments on pollen tube length (mm) of pearl millet and sorghum on maize silk

Maize Genotypes	Treatment	× PC 121 (Sorghum)		× Pusa 322 (Pearl millet)	
		DBP	DAP	DBP	DAP
CM 111	Control	7.28	7.28	4.98	4.98
	2, 4-D 75 ppm	6.73	6.52	4.36	4.30
	2, 4-D 100 ppm	7.35	**8.31**	5.21	**6.02**
	GA_3 75 ppm	6.82	7.47	5.51	4.82
	GA_3 100 ppm	6.93	6.85	4.23	4.19
	SA 0.01 M	7.51	6.95	4.21	4.38
	SA 0.02 M	6.36	6.43	3.99	4.24
PARAS	Control	7.51	7.51	5.65	5.65
	2, 4-D 75 ppm	6.99	6.73	5.12	4.98
	2, 4-D 100 ppm	7.69	**7.87**	**6.72**	5.52
	GA_3 75 ppm	7.21	7.05	5.49	5.94
	GA_3 100 ppm	7.72	7.36	6.55	4.51
	SA 0.01 M	7.77	7.41	5.57	4.73
	SA 0.02 M	6.59	6.32	4.91	4.82

Bold figures indicate the best treatment within each cross

DBP: One day before pollination; DAP: One day after pollination; SA: Salicylic acid

When we compare the two genotypes of maize for tube growth of foreign pollen, Paras - hybrid allowed more growth than CM 111-inbred. It may be inherent in the genotype itself as the hybrid allowed more pollen tube growth in its control and has extended stigma receptivity with delayed abscission of silk.

The 2,4-D at 100 ppm improved tube growth over the control but the treatments at lower concentration had inhibitory effect on the tube growth. So an optimum dose of the chemical has to be found out to get improved growth. The effect of spraying time, in general, had more favourable effect on tube growth when sprayed before pollination in comparision to after pollination. It is consistent with the observation of Sitch and Romero [11] in rice pollen germination and tube growth following post-pollination application of GA_3.

Table 2. Analysis of variance for effect of various chemicals on pollen tube growth of pearl millet and sorghum pollen on maize silk

Source	d.f.	CM 11 × PC 121	Paras × PC 121	CM 11 × Pusa 322	Paras × Pusa 322
Treatment	13	0.87**	0.71**	1.13**	1.38**
Error	26	0.13	0.16	0.02	0.12
SE(d)		0.30	0.33	0.12	0.29
CD (5%)		0.61	0.67	0.24	0.59

If we compare the two pollen parents for the extent of tube growth, sorghum pollen tube growth was longer than the pearl millet. This may probably be due to their differential taxonomic distance from the maize parent, sorghum being closer to maize than pearl millet.

From the above, it is clear that the effect of various factors following treatment with different chemicals are specific, either favourably or unfavourably, on foreign pollen tube growth and differ between crosses.

Though various chemical treatments had specific effect on the extent of pollen tube growth of either sorghum or pearl millet, their pollen germination, tube entry into the silk, pathway in the silk and the abnormalities exhibited by them were more or less similar to the control. Also, the favourable effect of these chemical treatments were not sufficient for the foreign pollen tube to reach maize embryosac to effect fertilization.

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