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Screening of Cotton Genotypes for Heat Tolerance via *In vitro* Gametophytic Selection Technique

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Abstract: According to some estimates, cotton plant sheds about 65 to 70% of its fruiting points. Major percent of shedding in the form of squares, flowers and small bolls, however was attributable to high temperatures. The temperatures above 35°C have been observed rendering pollen grains inviable, thus unfertilized flowers wither without forming bolls. Conventional methods of selecting heat tolerant cotton genotypes become ineffective due to confounding effects of disease, insect-pest, drought, micro and macro-environment of plant, nutrition and water stresses and others. In that situation, *in vitro* gametophytic selection in the controlled laboratory conditions is rather a reliable and effective technique that takes care-of all the confounding factors mentioned as above. Four cotton genotypes, all *hirsutum* types (CRIS-121, CRIS-134, CRIS-168 and NIAB-78) were screened for heat tolerance via *in vitro* male gametophytic selection technique. The percent of pollen germination and the pollen tube length were considered as selection criteria. Cotton genotypes did not differ in both germination percent and pollen tube length at control temperature of 33°C, however, they differed at 36 and 39°C. At 36 and 39°C, varieties CRIS-134 and CRIS-121 were more heat tolerant than CRIS-168 and NIAB-78 as both the varieties gave higher germination percent and longer pollen tubes. At highest temperature of 39°C, CRIS-134 and CRIS-121 gave average of 50 and 45% pollen germination and 19.0 and 13.0 millimeter tube length respectively. The control NIAB-78 however, gave significantly lower germination percent (15%) and smaller (10.0mm) pollen tubes at the same temperature.

Key words: Heat tolerance, *in vitro* gametophytic selection, Cotton

Introduction

Cotton is fairly heat tolerant as compared to may C_3 plants, even then excessively high temperatures (above 36°C) cause severe square, flower and small fruit shedding. Consequently, substantial loss occurs in cotton yield. Researchers have observed that high temperatures prevent the production of viable pollen grains, thus unfertilized ovary withers in the form of flower shedding.

If it is known that sufficient number of genes expressing in the sporophytic stage of plant life cycle are also expressed at gametophytic level (in pollen grains), then it is possible that different levels of heat stresses could be imposed to pollen grains and the genotypes that produce viable pollens at high temperature are expected to be heat tolerant at plant level also. Significant research findings support this possibility. Tanksley *et al.* (1981) found that some genes were expressed in both sporophytic and gametophytic cycles of plant. They observed that 18 of 30 isozymes, isolated in the sporophytes, also expressed in the gametophytes, and 18 of 19 pollen isozymes were found in one or more stages of sporophytic development. In terms of percent gene overlap, it is by now well established that, on an average, 60% of structural genes express in both the phases of plant life cycles (Tanksley *et al.*, 1981). Zamir *et al.* (1981) demonstrated that pollens from a wild, cold-tolerant species of tomato *Lycopersican hirsutum* L. had higher germination and fertility and were selectively more functional at low temperatures than pollen from non-selected cultivars. They subsequently succeeded in transferring genetic cold-tolerance from wild species' gametophytes. Benjamin nd Barrow (1988) reported that cotton cultivars showing heat tolerance in the field generally expressed higher fertility after heat treatment than pollens from heat sensitive cotton cultivars. They successfully transferred heat tolerance in the progeny of 7456 *G.barbadense* (heat tolerant) and Paymaster 404 (heat sensitive pollen parent). The objective of this study was to screen cotton genotypes at gametophytic (pollen grain) level by treating them artificially with variable heat temperatures. It is expected that genotypes giving high percent of *in vitro* pollen germination and produce comparatively longer pollen tubes at relatively high temperatures be considered heat tolerant genotypes. These criteria for selection against salt tolerance in oil seed *Brassica* have already successfully been used by Tyagi and Rangaswamy (1993).

Materials and Methods

Four high yielding genotypes, CRIS-121, CRIS-134, CRIS-168 and NIAB-78 as a control were grown in the field during 1999 so as to test their heat tolerance via *in vitro* male gametophytic selection technique in the laboratory. In our conditions, cotton crop sown in the month of May flowers in July, however, peak flowering occurs in August and September. The temperatures in these two months, on an average, range from 37.3 to 40.8°C (average of three years, 1997 to 1999) where pollen grains become completely sterile at 40°C. The varieties tested are early maturing and set bottom fruit which suggested that they can be fairly heat tolerant to high temperatures. Yet, above varieties shed lot of flowers in the hot months as mentioned earlier.

We are therefore interested to determine their real genetic tolerance via *in vitro* studies on pollen viability at controlled temperatures. Thus other factors such as insect-pest and disease attack, irrigation and nutrition stresses and others that also contribute to flower shedding are selectively eliminated. The flowers of each variety were placed day before anthesis in incubator at one temperature treatment at a time with 70% humidity. Three heat temperatures, 33, 36 and 39°C were applied to the flowers of each variety. The 33°C was considered as control temperature in our conditions.

The medium used was prepared with 300mg $[CaNO_3]_2$, 140mg $MgSO_4.4H_2O$, 50mg H_3BO_3 and 40% sucrose, dissolved in 100ml of distilled water (Barrow, 1980). Five Petri plates of each variety for each heat treatment were prepared by placing wet filter papers in the bottom of Petri

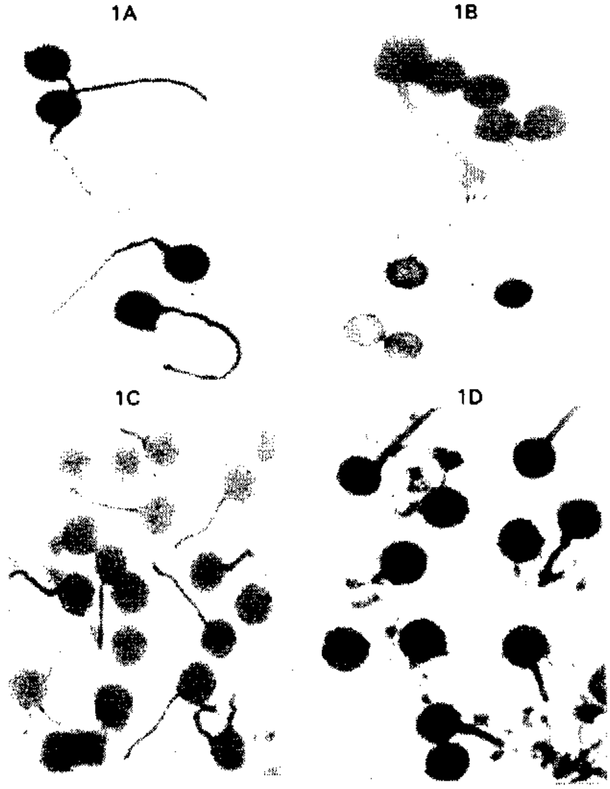

Fig. 1A: Pollen tubes grown at control temperature, 1B: Pollen tubes of CRIS-134, IC: Pollen tubes of CRIS-121, ID: Pollen tubes of NIAB-78 at 39°C

Table 1: *In-vitro* pollen germination percent and pollen tube length as affected by various temperatures

Genotypes	Pollen germination %			Pollen tube length (mm)		
	33°C (control)	36°C	39°C	33°C (control)	36°C	39°C
CRIS-134	98.0	75.0	50.0	20.0	18.0	15.0
CRIS-121	90.0	70.0	45.0	19.0	15.0	13.0
CRIS-168	85.0	65.0	30.0	18.5	13.5	11.0
NIAB-78	82.0	63.0	15.0	18.0	13.0	10.0
S.E.	ns	5.0	8.5	ns	2.0	1.2

The values were considered significantly different from each other when the differences were as great or greater than their respective standard errors.

plates. One or two drops of medium were placed on the microscope glass slides in circular form. Single flower with about 500 to 2000 pollen grains were shed onto each glass slide, thus in total 20 glass slides were cultured for each heat treatment. After inoculating the glass slides, they were placed in Petri plates covered half a way with their tops so as to assure enough air flow in the inoculum. After 3 hours of incubation, the observation on percent of pollen grain germination was taken on five random microscopic fields from each glass slide and scored for germination. Only those pollen grains with a pollen tube that measured at least to diameter of grain size were considered as germinated grains. For determining the average tube length, 50 to 60 pollen tubes that were intact with the pollen grain and having symmetrical tube-walls were measured, thus pollen bursts were selectively eliminated from germinated pollen grain counts. In our study, the percent of pollen germination and the tube length were considered as selection criteria for heat tolerance as done by Tyagi and Rangswamy (1993) for salt tolerance in oil seed *Brassic*

Results and Discussion

The pollen grains of cotton are vulnerable to temperature above 35°C. Thus high temperatures cause fruit sheddir either by preventing the production of viable pollen grair or causing abscission of squares, flowers and even sma bolls. In conventional methods, breeding cotton for he tolerance is, to select the cotton genotypes that set botto and earlier fruiting, sympodial branches towards botto nodes, sympodial node number bearing Ist effective bc and etc.

Researchers have also noted that plant population, mic and macro-environments of plants and many other facto render these criteria of selecting heat tolerant genotyp invalid in field conditions. Other factors such as spac light, water and nutrition stresses, insect-pest and disea attack, all also contribute to square, flower and small fru shedding. Thus these factors create confounding effects f cotton breeders to select with confidence the heat tolera cotton plants.

Unconventional and rather a new technique called *in vit* gametophytic selection for heat tolerance is more reliab and free of above mentioned perplex effects. He temperatures, 33, 36 and 39°C were given to the flowe before anthesis and the pollen grains of these flowers we grown in Taylor's (1972) medium modified by Baloch *et a* (2000). The genotypes that gave higher germinatic percent and longer pollen tube length were considered heat tolerant genotypes.

The results presented in Table 1 and Fig.1A suggested th at control temperature of 33°C, the genotypes did n differ in pollen germination percent and tube lengt however, 36 and 39°C temperatures significantly affecte the germination percent and tube length where CRIS-13 gave 50% pollen germination with tube length up 15.0mm at 39°C (Table 1 and Fig.1B). The next in the ra was CRIS-121 that gave 45% pollen germination wi maximum tube length of 13.0mm at the same temperatu (Table 1 and Fig.1C). Variety NIAB-78 as a contr however, gave only 20% pollen germination with maximu tube length of 10.0mm (Table 1 and Fig.1D).

These results suggested that CRIS-134 and CRIS-121 we first and second heat tolerant genotypes which can set t fruits even in the hot months of July, August a September. These results further reveal that these varieti can be a desirable parents to be used in hybridizatic program for breeding heat tolerant cotton varietie Benjamin *et al.* (1988) have successfully transferred he tolerance from 7456 *G. barbadense* as donor parent to t heat sensitive cultivar Paymaster 404 by screening c pollen basis.

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