Pakistan Journal of Biological Sciences
Screening of Cotton Genotypes for Heat Tolerance via
In vitro Gametophytic Selection Technique

Dr. Mohammad Jurial Baloch¹, Abdul Rahim Lakhoo¹,
Rahmatullah Rind² and Hidayatullah Bhutto¹
1Central Research Cotton Institute, Sakrand, Sindh, Pakistan
Department of Microbiology, Sindh, Agriculture University, Tandojam, Pakistan

Abstract: According to some estimates, cotton plant sheds about 65 to 70% of its fruiting points. Major percent of
sheding 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 NIA-B-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 NIA-B-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 18.0 and 13.0 millimeter tube length respectively. The control
NIA-B-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 many C₃ 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.

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 Lycopersicon 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 NIA-B-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 [Ca(NO₃)₂],
140mg MgSO₄·7H₂O, 50mg H₂BO₃ 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
Rangswamy (1993) for salt tolerance in oil seed Brassica

Results and Discussion

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

Researchers have also noted that plant population, micro- and macro-environments of plants and many other factors render these criteria of selecting heat tolerant genotypes invalid in field conditions. Other factors such as spacing, light, water and nitrogen stresses, insect-pest and diseases attack, all also contribute to square, flower and small fruit shedding. Thus these factors create confounding effects for cotton breeders to select with confidence the heat tolerant cotton plants.

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

The results presented in Table 1 and Fig.1A suggested that at control temperature of 33°C, the genotypes did not differ in pollen germination percent and tube length. However, 36 and 39°C temperatures significantly affected the germination percent and tube length where CRIS-134 gave 50% pollen germination with tube length up to 15.0mm at 39°C (Table 1 and Fig.1B). The next in the rank was CRIS-121 that gave 45% pollen germination with maximum tube length of 13.0mm at the same temperature (Table 1 and Fig.1C). Variety NIAB-78 as a control, however, only 20% pollen germination with maximum tube length of 10.0mm (Table 1 and Fig.1D).

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

References


