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Cell Membrane Thermostability as a Measure of Heat-Tolerance in Cotton

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High termperature stress is a major constraint to increased productivity of cotton in Pakistan. A technique based on physiological characteristics is therefore required to screen the cultivars for heat-tolerance. The membrane thermostability test conducted on fully grown young leaves of cotton is an appropriate technique for screening breeding material for heat-tolerance. Genotypes tested showed wide variations in adaptation to high temperature stress and could be broadly grouped into three categories on the basis of cell injury level. Genotypes showing high membrane thermostability gave higher seed cotton yield.

Key words: Cotton, Heat tolerance, Cell membrane thermostability.

Introduction

Heat stress has been recognized as the main environmental limitation to cotton production in Pakistan. Excessive temperatures (42-44°C day temperatures and 28-31°C night temperatures) cause heavy shedding of young flower buds and bolls. It is only after mid-August that effective period of boll setting starts in cotton. Irrespective of planting dates and cotton cultivars, about 2/3 of total boll load is set during the month of September (Taha *et al* 1981; Malik 1991). Increase in cotton productivity potential is possible by lengthening effective bolling period. This could be achieved by selecting heat-tolerant cultivars. A wide diversity in heat tolerance has been reported among cultivars belonging to *G. hirsutum* species.

At present, selection for heat-tolerance is carried out on the basis of early fruit set and yield. This approach is empirical and does not take into account any chemical or physiological characteristics that are indicative of heat-tolerance.Several physiological tests have been developed recently to screen the breeding material for adaptation to high temperature (Sullivan 1972; Sullivan *et al* 1977; Sullivan and Ross 1979; Bjorkman *et al* 1980; Raison *et al* 1980). Among these, one component of temperature tolerance that has received more emphasis is cellular membrane thermostability. In this test, the degree of membrane stability to temperature stress is evaluated by measuring leakage of ions from leaf discs. This test is based on the observation that when leaf tissue is injured by exposures to high temperatures, cellular membrane permeability is increased and electrolytes diffuse out of the cells. If the tissue is subsequently bathed in deionized water for specified period after the application of heat stress, the amount of electrolyte leakage can be evaluated by electrical conductance measurements. Since the amount of electrolyte leakage is the function of membrane permeability (which in turn is a function of the degree of injury induced by the elevated temperature), the membrane thermostability of different genotypes can be assessed in terms of electrolyte conductance. This technique has been used successfully to identify genetic variations in heat tolerance in different crop species. Genetic variations determined by this procedure in sorghum related to differences in plant photosynthesis and yield performance (Sullivan 1972, Sullivan *et al* 1977; Sullivan and Ross 1979).Thermostability technique has also been successfully employed in evaluating temperature tolerance of soybean by Martineau *et al* (1979) and in wheat by Shanahan *et al* (1990).

The objective of the present study was to conduct thermostability test in several strains of cotton and relate it to yield performance under field conditions.

Materials and Methods

Plant Materials. Advanced strains as well as standard varieties of cotton were planted at experimental farm of Central Cotton Research Institute, Multan during the years 1984-90. The genotypes screened each year were different depending on the availability of new strains. The crop was planted in mid-April, so that its reproductive phase coincided

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with the hottest period of the season. During early reproductive phase the crop experienced 42-44°C day temperatures and 28-31°C night temperatures during the years of experimentation. Cotton strains were planted in a randomized complete block design and had four repeats. The plot size in each replication was 8 m wide and 15 m long having plant configuration of 75x30 cm for each strain. Seed cotton was hand picked in each plot at 150 days from planting and total yield was calculated on area basis.

Membrane Thermostability. Leaf discs were collected in the month of July at 10.00 hours from plots of each cotton strain repeatwise for labortory evaluation of heat tolerance, using a procedure similar to that described by Sullivan (1972) for sorghum and Martineau *et al* (1979) for soybean. Samples for assay consisted of 20 leaf discs of 1 cm diameter cut from a group of 20 fully expanded young leaves using specially constructed leaf disc punch. Each assay was replicated 3 times, i.e. 60 leaf discs from each cotton strain divided into 3 samples containing 20 discs in each assay sample. Leaf material was rinsed in tap water and then again washed thrice with deionized water spanning over a period of 1.5 h at room temperature to remove possible surface contaminants and solutes from the cut surface of the discs. After the final wash, the tubes were drained, retaining 2 ml of water to prevent dessication of tissues during heat treatment. The tubes for heat treatment were covered with plastic wrap and incubated for one hour in a controlled temperature water bath already maintained at 50°C. On the other hand, test tubes containing discs for control treatment were kept in incubator maintained at 25°C for the same period, and thus did not suffer from cold stress. After the treatment period, 40 ml of deionized water was added to both control and treated test tubes. The tubes were periodically shaken to mix the contents and held for 18 to 24 hours at ambient temperature to allow diffusion of electrolytes from the leaf discs. Electrical conductivity of the efflux solution was measured with conductivity meter. After measurement, the heat treated samples were placed in an autoclave at 0.10 MPa pressure for 15 min to completely kill leaf tissues and release all of the electrolytes. Subsequently, test tubes were cooled to 25°C, the contents were mixed and final conductance measurements were made. Cellular damage due to the 50°C heat treatment was calculated from conductivities as follows:

$$\% \text{ Cellular damage} = \left[\frac{(\text{Heated control})}{(\text{Autoclaved control})} \right] \times 100$$

The absence of cellular damage expressed as (100-% cellular damage) was taken as a measure of heat tolerance (Quisenberry *et al* 1981).

Results and Discussion

There were significant differences in cellular damage among cotton strains tested each year. The cellular damage ranged between 60 to 90% for 41 strains tested over seven years. Strains could be broadly placed into three groups (1) low heat tolerance, 80-90% cell injury (2) medium heat-tolerance, 70-80% cell injury and (3) high heat tolerance, 60-70% cell injury (Table 1). It may be stated that day and night temperatures recorded during the experimentation were normally distributed and temperature data did not show skew in the distribution patttern. Hence, the differences shown in Table 1 can only be ascribed to varieties rather than differences among seasons. Genetic differences in cellular damage has been reported in other crop species such as sorghum (Sullivan and Ross 1979), soybean (Martineau *et al* 1979) and wheat (Shanahan *et al* 1990).

Cotton strains differed in seed cotton yield and differences in yield were related to cell injury level (Table 2, Fig 1). The association between cell injury level and seed cotton yield was negative and linear. Cotton strains showing less yield under a thermally stressed field environment always showed higher cell injury in heat treatment. Differences in correlation co-efficient values among years may be ascribed to difficulty of obtaining precise control of the variables involved in the technique, i.e. cellular membranes tend to acclimate

Table1

Cell injury (%) as determined by the membrane thermostability test on fully expanded leaf tissues in different cotton genotypes planted at Multan, Pakistan

Heat-tolerance (in order of damage)		
Low heat-tolerance	Medium heat-tolerance	High heat-tolerance
Cell injury % = 80-90	Cell injury % = 70-80	Cell injury % = 60-70
CIM-83	NIAB-82	SLH-117
SLH-41	S-2	CIM-243
CIM-84	CIM-360	CRIS-9
CIM-173	1717	BH-36
B-557	S-12	644/84
272/79	CIM-140	CIM-240
MS-2	CIM-109	CRIS-6
CIM-90	RH-114	CIM-70
DNH-25	FH-367	CIM-196
BH-4	TH-3/83	NH-26
Rehmani	Cyto-9/85	CIM-251
MNH-93	H-1	CIM-241
	H-2	CIM-200
		NIAB-78
		727/84

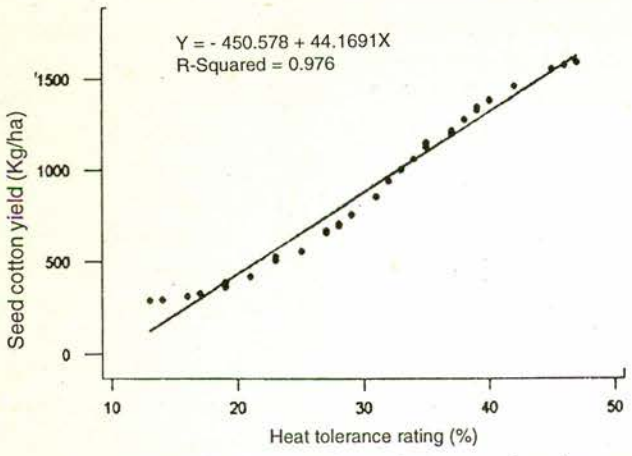

Fig 1. Relationships between heat tolerance and seed cotton yield.

Table 2

Correlation co-efficient between cell injury (%) and seed cotton yield (kg ha^{-1})

Year	Correlation co-efficient (r)	Equation
1990-91	-0.71	1532.96-18.19 x
1989-90	-0.86	1259.19-11.69 x
1988-89	-0.57	4899.91-44.76 x
1987-88	-0.58	8234.9-61.33 x
1986-87	-0.70	2568.89-21.98 x
1985-86	-0.44	1945.00-13.66 x
1984-85	-0.66	2371.12-19.68 x

or harden in response to the ambient field temperature and variation in seed cotton yield due to pest damage. These results are similar to those reported by Sullivan and Ross (1979) for sorghum and Blum and Ebercon (1981) for wheat. It is concluded from these experiments that membrane thermostability test can be useful procedure for selecting cotton strains tolerant to higher temperature stresses. The increase in membrane thermostability as recorded for various genotypes in these experiments may be ascribed to synthesis of heat shock proteins produced in response to high temperatures as suggested by Key *et al* (1981). The relationship between heat tolerance level and seed cotton yield illustrated in Fig. 1 demonstrates the importance of heat tolerance in raising seed cotton yield. Cotton breeders in Pakistan have been endeavouring to breed heat tolerant cotton varieties and manifestation of certain genotypes to be heat tolerant in the present experiment may be ascribed to their efforts in this direction.

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