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Thermotolerance in Cotton Roots at Early Stage of Emergence

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Abstract: The objective of the study was to ascertain the effects of high temperature stress on root growth of cotton genotypes viz. Qalandari, MNH-93, Rehmani, 5-12, CIM-70, SLH-41, MS-84, K-68-9, CRIS-6 and CiM-109. The seeds were germinated for 48h at 25 + 0.5°C. After 48 h they were heat stressed for 2 h at 25 (internal control), 30, 35, 37.5, 40, 42.5 and 45°C in a waterbath and re-incubated at 25°C for a further 22 h, making a total germination period of 72 h. The root growth in all cultivars showed a good tolerance upto 35°C. At 45°C the roots of all cultivars were irreversibly damaged. Between 35 and 45°C the cultivars behaved differently, however. Cultivars Qalandari and Rehmani were more heat tolerant, and cv. 5.12 least heat tolerant.

Key words: Cultivars, cotton, terinierature stress, roots

Introduction

The amount of reserve materials present in the seed and the efficiency with which they are mobilized are all important aspects of seedling growth and development in any crop. At the cellular level, the seed vigour and viability depend on the integrity of biochemical processes such as. DNA replication and transcription, protein synthesis, and the functions of subcellular organelles and membrane stability (Bradbeer, 1988; Chachar *et al*., 1999).

The growth and development of the root system are under genetic control, but may be modified extensively by the environment. It has been shown in number of studies that for a variety of species, variability exists in the development of root length and in the initiation and growth of branched roots (McMichael, 1986; Quisenberry *et al*., 1981; McMichael *et al*., 1987). Quisenberry *et al*. (1981) and McMichael (1986) have shown that there were varietal differences in root growth across several environments, such as changes in soil temperature, soil strength, composition of soil atmosphere and soil water content. In general, the growth of roots tends to increase with an increase in soil temperature until the optimum temperature is reached, with a decrease then occurring as temperature rise above the optimum range. Al-Ani and Hay (1983) reported in this respect that root extension rates increased significantly for each 10°C rise in temperature upto 35°C. Krieg and Bartee (1975) reported that mobilisation of the cotton seed reserves was adversely affected by unsuitable temperatures.

It is therefore obligatory to sow the crop at a temperature where nutrients reserves can be properly and efficiently used and where the other biochemical functions of the seedling can take place effectively. The objectives of the present study was to asses the thermotolerance of germination and root radical growth in some cotton genotypes commonly grown in Pakistan.

Materials and Methods

The study was conducted at School of Biological Sciences, University of Wales, Bangor (U.K.). Seeds of cotton cultivars were provided by various cotton research stations/institutes in Pakistan and were stored at 4°C in sealed glass/plastic containers before use.

Delinting of seeds: The seeds were delinted with H_2SO_4, then neutralised by placing them in 1% $NaHCO_3$ solution and finally washed thoroughly with water. They were left standing in distilled water for 1 hour to separate into sinkers and floaters. The sinkers were collected and sterlised in sodium hypochlorite solution (1% available chlorine) for 20 minutes. Finally, the hypochlorite solution was removed by washing the seeds several times in sterile distilled water.

Germination of seeds: All the materials used in the germination experiments were placed under UV light for 30 minutes and all manipulations were done in aseptic conditions in laminar flow cabinet. Batches of 50 delinted, sterilised seeds were placed in 10 rows with 5 seeds in each row on a 23 × 57cm sheet of filter paper (Whatman No.1) which had been moistened with 40 ml sterile distilled water. After seed arrangement, the paper was rolled-up round a wooden stick and placed in a polythene bag. The top of the bag was folded over and tied lightly to allow air exchange into or out of the bag. The seeds were then germinated for 48 h at 25 + 0.5°C in an air-incubator.

Application of heat stress: After 48 h germination, the roots were subjected to heat stress for 2 h. This stress treatment was carried out by putting the plastic bag and its contents in to a waterbath, so that all the seeds were submerged in the water but water was not allowed to enter the bag. These treatments were carriedout at 25 (internal control), 30, 35, 37.5, 40, 42.5 and 45°C. After the stress, the roots were returned to 25°C in the air-incubator for a further 22 h to complete a 72 h germination period. Further controls consisted of seed samples germinated at 25°C in the air-incubator for 48 (control 1) or 72 h (control 2) periods in the air-incubator at 25°C.

Recording and analysis of data: After harvesting, the seedling root length were measured and the resulting data were analysed in 2 ways. Firstly, the means + sd of the roots were calculated. Secondly, the root length values were placed in order from longest to shortest and divided into four quartiles so that the mean length + sd of each quartile could be calculated. Best fit curves were drawn to data points using the computer with the Systat Sygraph DWLS (Distance Weighted Least Squares) soft ware programme.

Results and Discussion

All the seed lots' yielded data with very large standard deviations. It was therefore initially difficult to prove

Table 1: Temperature at which root growth was inhibited by 50% (TIG50) in cotton cultivars

Total roots		First quartile		Second quartile	
Cultivar	TIG50°C	Cultivar	TIG50°C	Cultivar	TIG50°C
Qalandari	41.4	Qalandari	42.0	Rehmani	41.6
Rehmani	41.1	Rehmani	41.8	Qalandari	41.4
CRI5-6	40.9	CIM-109	41.1	CR1S-6	40.9
CIM-109	40.9	CRIS-6	40.9	CIIVI-109	40.7
MS-84	39.6	MNH-93	40.0	MS-84	39.5
K-68-9	39,1	MS-84	39.8	K-68-9	39.5
MNH-93	39.1	K-68-9	39.5	SLH-41	38.6
SLH-41	39.0	CIM-70	39.5	CIM-70	38.6
CIM-70	38.6	SLH-41	38.9	IvINH-93	37.5
5-S-12	38.0	5-12	38.6	S-1Z _ .	37.5

The TIG50 value is the temperature at which root growth is inhibited by 50%

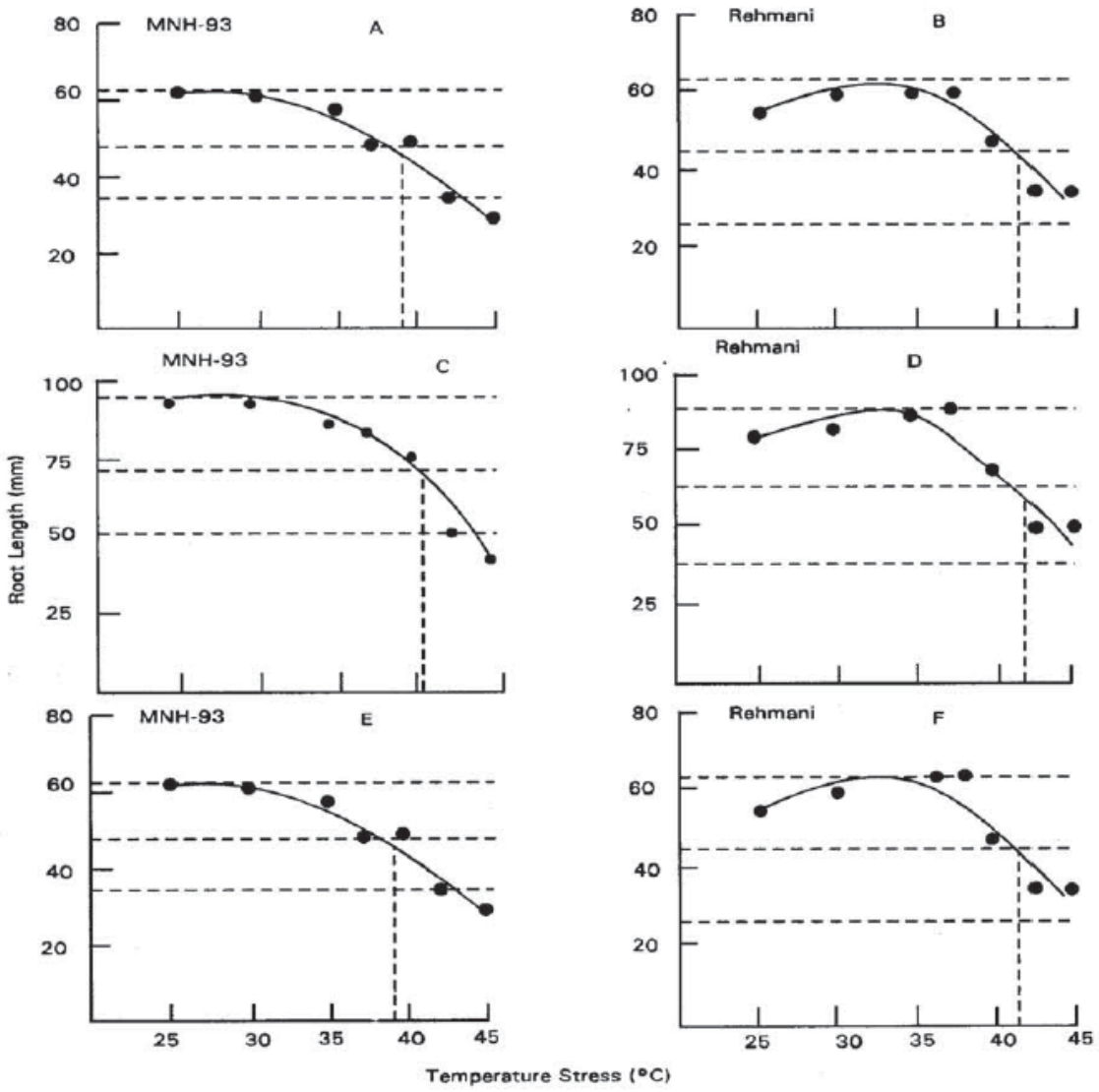

Fig. 1: Effect of heat stress on cotton root length of different cotton cultivars
A, B: Total roots; C, D: First quartile: E, F: Second quartile

differences between the growth rates of the venous cultivars or to demonstrate any cultivar differences with respect to their responses to heat stress. To overcome this problem it was decided to use frequency distribution analysis. As described above, the roots were divided into four quartiles. Only the values for the first and second quartiles (the longest roots) are presented here. The values for third and fourth quartiles showed greater statistical variability and they did riot provide any information which could not be obtained from the first two quartiles, they were therefore discarded.

The root length values of total first and second quartiles after heat stress of cultivars not all are plotted as Fig. 1. In Figure 1 the top and bottom dashed horizontal lines are the root length values obtained after 72 and 48 h respectively incubated in the air-incubator at 25°C. The effect of "heat shock" at 25°C (internal control) were variable, however. In case of some seed hots, there was a large decline in root growth following "heat shock" at 25°C. Examples of this kind of response are cvs. CIM-109, CIM-70, S-12 and SLH-41. There was no such effect with other cultivars however, leg. cv. MNH-93. Heat shock at 30°C or 36°C generally had little effect on root growth, although in some cases (eg. cvs. Rehmani, CRIS-6, Se1-1-41 and K-68-9) it produces a small increase compared with the 25°C control. In some cases, even heat shock at 37.5°C had little effect (cvs. Qalanded, Rehmani and CIM-109O while in other cases (cvs. CIM-70, MNH-93, S-12, SLID-41, ms-84 and K-68-9) some reduction in root growth resulted. Heat shock at higher temperatures produced noticeable inhibition of root growth. In this respect the temperature at which inhibition was first noticed varied from one seed lot to another. For example, cvs. Qalandari, Rehmani, CRIS-6 and CIM-109 appeared to be more heat tolerant than cultivars MNH-93, S-12, SLH-41 and K-68-9. Root growth in all the seed lots was completely stopped following heat shock at 45°C. Indeed, this treatment even caused a small "negative growth" (shortening) in some cases.

Because of unexpected variability in the response of different seed lots to the 25°C "heat shock" treatment, it was difficult to evaluate the effects of higher heat shock temperatures on root growth, In particular, the 25°C line does not appear to be a suitably constant reference points for such evaluations. A decision was therefore taken to dispense with the control 2 and to replace it with a horizontal line passing through the maximum of each curve and a third horizontal line has also been drawn half-way between the new line and control 1 line. Where this line cults the curve, a verticle line has been drawn to the "X" axis. The point where this verticie lines intercept the "X" axis is the temperature at which root growth is inhibited by 50%. This is defined as the TIG-50 values (temperature inhibiting growth to 50%). The TIG-50 values for the whole sample and for the first and second quartiles of each seed lot studied are presented in Table 1. From the data in Table 1 it can be seen that there is about 3°C difference between the TIG-50 values of the most heat tolerant cultivars (Qalandad and Rehmani) and least heat tolerant cultivar (S12). Other cultivars are in the range between 41 and 38.6°C. A comparison of the T1G-50 of the whole sample with the T1G50 of their corresponding first and second quartiles reveals differences. The sample of MNH-93 had the exceptional behavior. In this case ThG-50 for the whole sample was 39°C, while that of the first quartile was 40°C and second quartile was 37.5°C.

It is evident from the result that temperature had a marked effect on root growth. Thus, upto a temperature of 35°C each cultivar showed a increase in root length. Above this temperature, the cultivars responded differently, however. In some cultivars progressively shorter root length (reduced growth rates) were observed above 35°C. The increase in root length was negative fie the root length was actually shortened) when the stress temperature was raised to 45°C. This happened probably due to severe damage and even the death of the cells leading to loss of the turgor and contraction of the tissue, Although there have been few detailed studies of the effect of high temperature on the seedling root growth, the available literature supports our findings. In this regard, Abdelmagid and Osman (1977) reported that the germination and root growth of cotton ceased below or above a temperature range of 15-45°C, While working with different crops (flax, pea, red kidney beans, maize, straw berries, broad beans, rape and oats), Brouwer (1962) determined the TIG-50 values for root growth. He concluded that there was very little difference among the species with respect to the upper temperature limits. However, considerable differences were observed for lower temperature limits.

The cotton cultivars examined in this study behaved very similarly to each other with respect to their root elongation. The increase in root growth observed following stress at 25 to 35°C also supports the finding of Arndt (1945) who reported an optimum daily temperature range from 33 to 36°C for maximum seedling growth in cotton. On the other hand McMichael and Quisenberry (1993) reported that the optimum temperature for cotton root growth was between 28-35°C; temperature below or above this range reduced to the root length of cotton. The findings reported by Arndt (1945), Tharp (1960), Gerard (1971), Wanjura and Buxten (1972) and McMichael and Quisenberry (1993) agree well with our observation that cotton radicle elongation was inhibited above 38°C.

The present results may be compared with those of Murtazi and Portova (1971), Kharlamov and Mousienko (1992) on wheat seedlings. They reported that mitotic cell division was inhibited by temperature stress at 45°C and nucleic acid synthesis was inhibited between 36 and 48°C. Patel and Vora (1987) reported that even a 2 min heat stress at 47.5°C applied to rice seedlings inhibited shoot and root growth, They further stated that the heat stress also altered the percentage water content, free amino acid levels, chlorophyll alb ratio and level of carotenoids.

The exact mechanism(s) of the response of roots to different soil temperatures has not been determined (McMichael and Quisenberry, 1993). Some researchers are of the opinion that changes in protoplasmic resistance can account for the observed responses, particularly the reduction in water uptake (Bowen, 1991). Barlow (1987) indicated that the rate of cell division was reduced at low temperatures and that as the temperature rises the time required for additional cell division is reduced.

Unfavourable temperatures can have profound effects by either slowing or hastening the cell cycle (Francis and Barlow, 1988). Changes in temperature may alter the duration of the cell cycle and consequently the growth of plant organs; growth of roots may then be reduced and less branching can occur. They further showed that the rates of cell division alter with changes of temperature in a tissue-and species-specific manner. The temperature at which the mitotic cycle has minimum duration the optimum temperature) varies in different species, eg., 28°C in *Vicia faba* and 32°C in *Album cepa* (Grif, 1981). The cycle is prolonged at temperature both above and below those optima. Thus, at extreme temperatures, the tight coupling which exists between nuclear division and cell growth may be dissociated (Francis and

Barlow, 1988). This may have been a major reason causing reduced elongation and finally a general mortality of cotton roots at higher temperatures in the present experiments. Elongation rates (Taylor and Ratliff, 1969) the enzymatic activities are reduced by higher than optimum temperatures, while branching may be increased in some cases (Neilson, 1974). Some evidence indicates that there is genetic variability in response to changes in temperature both between and within species. For example, McMichael *et al.* (1987) has shown that there are differences between the responses of seedlings of a number of exotic cottons in terms of primary and lateral root development. Brar *et al.* (1990) showed that the temperature for optimum emergence and growth between a number of forage legumes was significantly different. Heinrich and Neilson (1966) observed differences in root growth between 20 alfalfa varieties in response to different soil temperatures.

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