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Changes in Growth and Leaf Water Status of Sugarcane (*Saccharum officinarum*) During Heat Stress and Recovery

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ABSTRACT

A pot experiment was conducted to assess the changes in growth water content of control, heat stressed (42 ± 2°C) and recovered plants of heat-tolerant (CP-4333) and heat-sensitive (HSF-240) sugarcane varieties at formative (30 days), grand growth (150 days) and maturity (250 days after sprouting) stage of growth. One set of plants at each stage was kept in a growth room at 27 ± 2°C (control), two sets were shifted at 42 ± 2°C (heat stress) in a growth room and one of the heat stressed set shifted back to 27 ± 2°C for recovery. Measurements from all treatments were made at 24, 48 and 72 h. Under heat stress CP-4333 rolled the leaves more quickly than HSF-240. There was a reduction in the fresh weight but no change in the dry weight and leaf area, leading to a reduction in shoot fresh-to-dry weight ratio and leaf water potential. Upon recovery, both varieties de-rolled leaves, regained fresh weight and improved leaf water potential, but these changes were readily noted in CP-4333. Results suggested that improved heat tolerance in sugarcane was accompanied with curtailed water loss by leaf rolling and quicker reversal of these effects during recovery.

Key Words: Heat tolerance; Leaf water potential; Leaf rolling; Recovery

INTRODUCTION

Heat stress is defined as a rise in temperature for a time sufficient to cause irreversible damage to plant functions and is therefore a limiting factor for growth. According to Mark and Davidho (1991) heat stress is a rise in temperature transiently, usually about 10 to 15°C above that required for normal plant growth. Optimal crop growth requires a non-limiting supply of water, nutrients and radiations, while under high temperature the demand for growth resources increases due to increased metabolic rate, development and evapo-transpiration (Rawson, 1992). When growth resources are limited by heat stress the size of plant organs such as leaves, tillers and spikes are reduced (Wahid *et al*., 2007). Under heat stress leaf size and leaf extension rate, show tight correlation between photosynthesis and growth (Karim *et al*., 2000). Shah and Paulsen (2003) reported that high temperature hastened a decline in photosynthesis and leaf area, decreased shoot and grain biomass, weight and sugar contents of kernels.

Heat stress perturbs the cell metabolism. Since heat stress is a dehydrative force, such perturbations may arise due to hampered cell water balance with decreased water uptake by the root and excessive loss from leaves (Machado & Paulsen, 2001). Specific effects of heat stress include hampered leaf water potential (Wahid & Close, 2007), increased fluidity of membrane lipids (Xu *et al*., 2006) and production of reactive oxygen species (Wahid *et al*., 2007). These processes lead to altered growth patterns (Young *et al*., 2004; Porter, 2005).

Sugarcane (*Saccharum officinarum* L.) is a multipurpose commercial crop. It is a prime source of sugar production and cultivated on approximately 0.99 Mha of irrigated land in Pakistan (Anonymous, 2006). Increasing ambient temperature in sugarcane growing tracts is a great threat, which warrants understanding of detailed physiological and growth responses in sugarcane. It is surmised that heat tolerance in sugarcane is associated to specific changes, which reduce the dehydration effect of heat stress. The objective of this study was to determine the short term changes in growth, water status of two differentially heat tolerant sugarcane varieties at three growth stages under heat stress and during recovery.

MATERIALS AND METHODS

Experimental details and plant growth conditions. This study was performed using two sugarcane varieties CP-4333 (moderately heat tolerant) and HSF-240 (heat-sensitive). Nodal setts were sown in 12 kg capacity soil containing

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loam soil and kept in a net house. During the experimental period, the plants were regularly supplemented with half strength nutrient solution (Hoagland & Arnon, 1950). Experimental design was completely randomized with three replications.

The effect of heat stress was determined at formative, grand growth and maturity stages at 30, 150 and 250 days after seedling emergence respectively. Before the start of heat stress treatment at each growth stage, the pots were acclimated to growth room condition by shifting at 27/23 ± 2°C (day/night) for three days. For heat stress treatment, the plants were shifted in a growth room, where lights were supplemented using white fluorescent tube lights and mercury lamps hanging with the ceiling and walls. Photosynthetically active radiation (PAR) at leaf surface in the growth room ranged from 650-700 µmol m^{-2} s^{-1}. For heat stress the day temperature was 42 ± 2°C, while night temperature was 34 ± 2°C. The temperature was raised to 42°C in about 5 h. Fans were used to circulate air in the growth room. Relative humidity ranged between 55-60%. For recovery experiments, one set of plants was shifted to 27 ± 2°C growth room. Harvesting for control, heat stress or recovery treatments was made at 24, 48 and 72 h. All experiments were laid out in completely randomized design with three replications.

Growth and water status determinations. Leaf rolling time under heat stress was visually noted in both the varieties. Leaf area per shoot was determined of intact growing plants as leaf length × leaf width × 0.68 (correction factor). Leaf water potential was determined using pressure chamber (Arimed Pressure Bomb, Germany) from second fully expanded leaf. Fresh weight of shoot was determined immediately after harvesting, while dry weight taken after drying the shoots in an oven for a week. The leaf fresh-to-dry weight ratio was derived by dividing fresh weight with dry weight.

Fig. 1. Changes in shoot fresh (A) and dry (B) weight of two differentially heat tolerant sugarcane varieties under heat stress and during recovery at three growth stages. In this and subsequent figures, H, harvests; T, temperatures and V, varieties

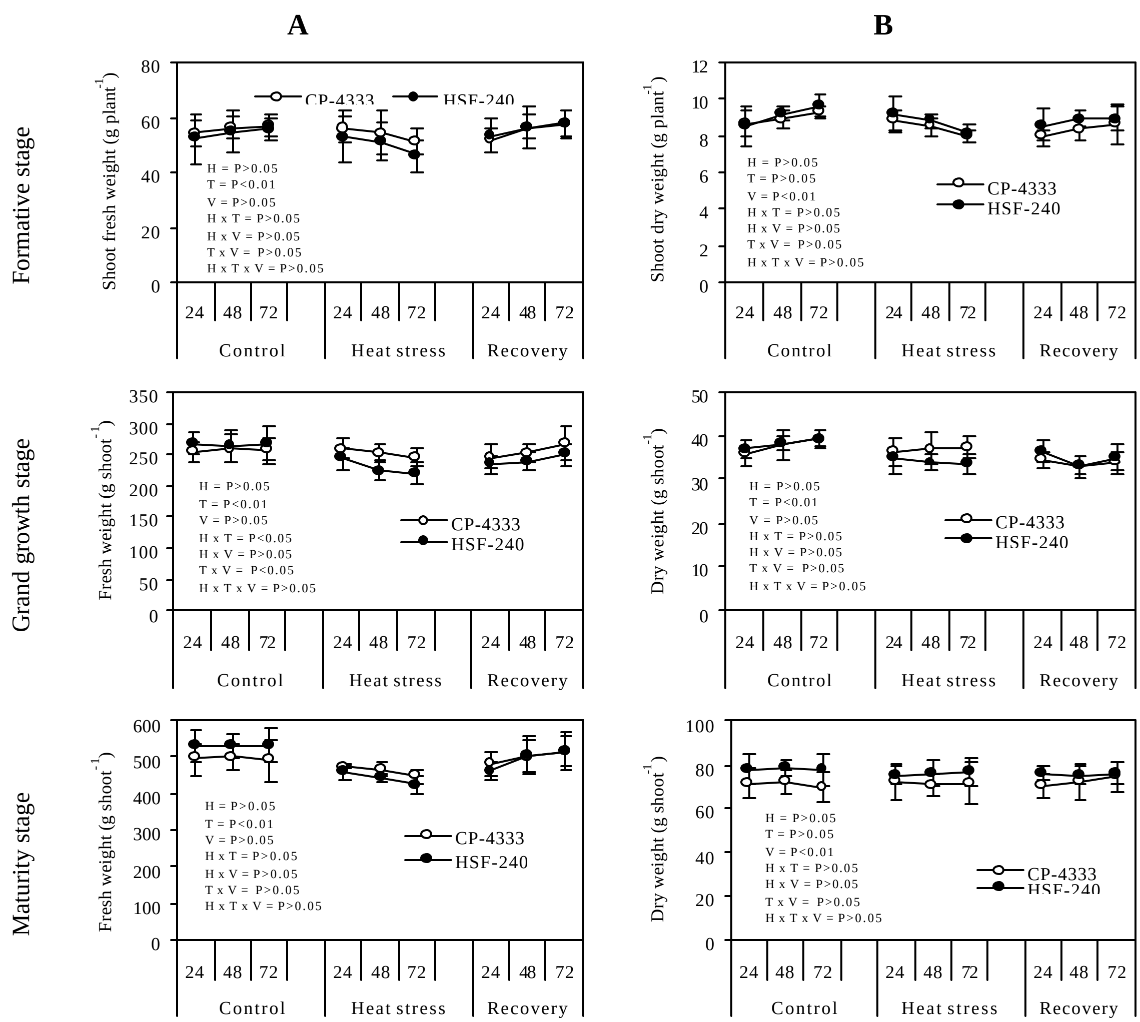
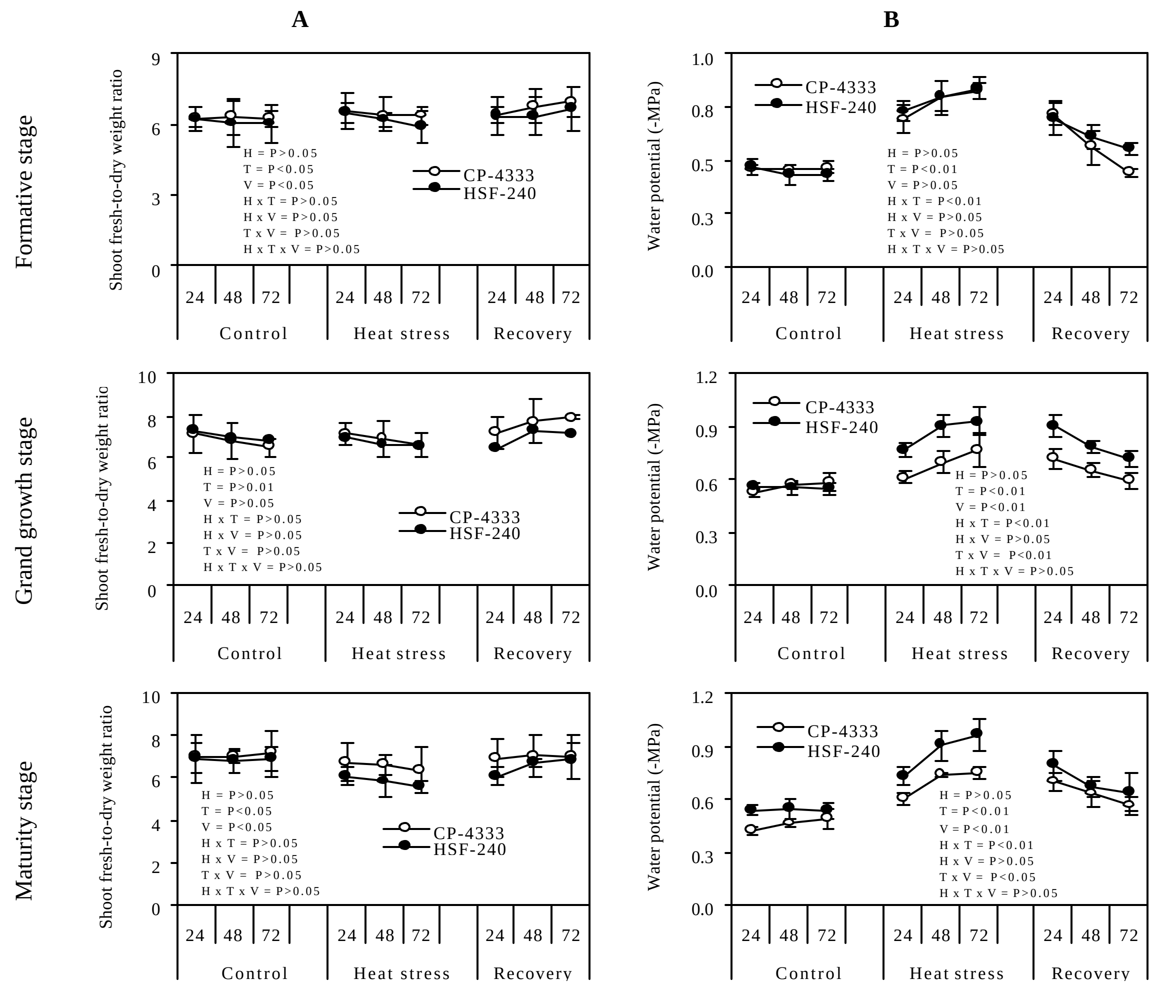


Fig. 2. Changes in Changes in shoot fresh-to-dry weight ratio (A) and leaf water potential (B) of two differentially heat tolerant sugarcane varieties under heat stress and during recovery at three growth stages



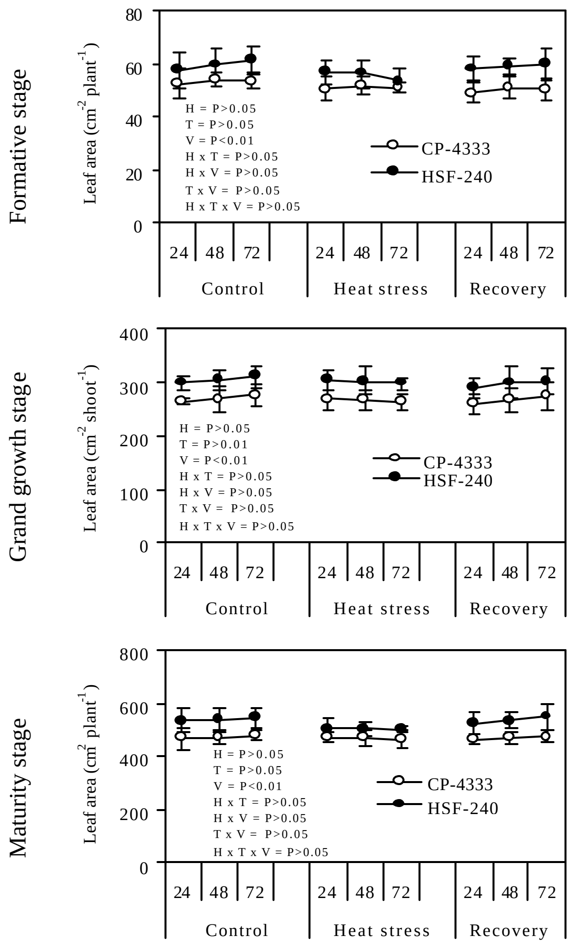
Statistical analysis. Variance analysis of data to find meaningful differences among the various factors was performed using COSTAT computer software.

RESULTS

With a rise in temperature at all stages, both the varieties indicated leaf rolling, being relatively quicker in CP-4333, which took place more readily at formative stage compared to grand growth and maturity stages. At formative stage, shoot fresh weight was significantly less due to heat stress (P<0.01) although there was no differences (P>0.05) between varieties and harvests together with a non-significant (P>0.05) interaction of these factors. Under control conditions, CP-4333 indicated higher fresh weight, but decline in this parameter under heat stress was evident in HSF-240. Recovery from heat stress at formative stage was faster in CP-4333 (Fig. 1a). At grand growth stage, there was no (P>0.05) difference between the varieties and stages but temperature treatments showed significant differences (P<0.01) for shoot fresh weight. Among the various interactions, harvest x days and varieties x treatments interaction were significant (P<0.05). Under heat stress, HSF-240 depicted more decline in shoot fresh weight while upon recovery, CP-4333 recovered more readily than HSF-240 (Fig. 1a). Towards maturity, all the factors and their interactions were non-significant (P>0.05) except temperature which showed significant differences (P<0.01) for this attribute. Under control conditions, although CP-4333 indicated lesser shoot fresh weight, its reduction due to heat stress was slightly greater in HSF-240. However, recovery from heat stress was almost similar in both the varieties (Fig. 1a). At each stage, under heat stress or recovery, the dry weight did not differ appreciably in the varieties over the experimental period under heat stress or recovery (Fig. 1b).

Data revealed that at formative stage varieties and temperature treatments indicated significant (P<0.01) difference for shoot fresh-to-dry weight ratio. However, there was no interaction (P>0.05) of these factors. CP-4333 and HSF-240 had similar value of this attribute under control condition, although it was greatly reduced in HSF-

Fig. 3. Changes in leaf area per shoot of two differentially heat tolerant sugarcane varieties under heat stress and during recovery at three growth stages



240 under high temperature stress. CP-4333 indicated an earlier recovery from heat stress than HSF-240. At grand growth stage, the differences in none of the factors or their interactions were significant. Nevertheless, CP-4333 indicated an earlier and rapid recovery from heat stress than HSF-240. At maturity, only varieties and temperature treatments showed significant differences (P<0.05), while the harvest days or interaction interactions of these factors were not significant (P>0.05) for this parameter. At this stage, under control condition, no great difference was noted in both the varieties. However, under heat stress, this ratio was more reduced in HSF-240, while CP-4333 indicated an earlier and rapid recovery than CP-4333 (Fig. 2a).

At formative stage, temperature treatments indicated significant (P<0.01) difference in leaf water potential (?$_{w}$) together with only significant (P<0.01) interaction of harvests and temperature treatments. Under control or heat stress conditions, CP-4333 and HSF-240 behaved similarly for changes in leaf ?$_{w}$, while CP-4333 indicated a quicker recovery (Fig. 2b). At grand growth stage, temperature treatments and varieties indicated significant (P<0.01) difference, with a significant harvests x temperature treatments and temperature treatments x varieties interactions (P<0.01). Under control condition, leaf ?$_{w}$ was similar in both the varieties, but heat stress lowered the ?$_{w}$ greatly in HSF-240 than CP-4333. Recovery from heat stress was quicker in CP-4333 than HSF-240 (Fig. 2b). At maturity too, data revealed indicated significant (P<0.01) difference in temperature treatments and varieties, with a significant harvests x temperature treatments and temperature treatments x varieties interactions (P<0.01). At 27^{o}C, leaf ?$_{w}$ was relatively lower in CP-4333, but under heat stress, HSF-240 showed a greater reduction than CP-4333. Recovery from heat stress was quicker and better in CP-4333 than HSF-240 (Fig. 2a, b).

Changes in leaf area per plant were similar at all growth stages, although growth stage related increase in this variable was noted under all growth condition in both the varieties. At all stages, only varieties indicated significant (P<0.01) differences in this attribute, while other factors and interactions of these factors were not significant (P>0.05). At all these stages, HSF-240 exhibited a greater leaf area per shoot under control, stress or recovery treatments. However, difference was noted in the stress responsiveness of both the varieties, where HSF-240 tended to maintain greater leaf area under stress and an early recovery from stress (Fig. 3).

DISCUSSION

Crop plants including sugarcane show sensitivity to increased temperature at all the growth stages and aerial parts are mainly affected (Ebrahim *et al*., 1998; Bonnett *et al*., 2006; Wahid *et al*., 2007). Exposure to heat stress caused the leaves of both the varieties to roll at formative (~25%), grand growth (~20%) and maturity stages (~18%) and then de-roll upon recovery with the action of bulliform cells in the lower epidermis of leaf, although this tendency was quicker in CP-4333 (tolerant variety). This revealed that leaf rolling is an important adaptation to curtail water loss under dehydrating forces like heat stress. In view of this finding, further study was conducted on the determination of leaf water status at three growth stages.

Like most mesophytic species, sugarcane also shows sensitivity to supra-optimal growth temperature despite the fact that it has relatively higher optimum temperature compared to C_3 species (Wahid, 2004). Plants subjected to heat stress initially indicated a severe disturbance in leaf water status (Hall, 1992). Determinations made in this study on two differentially heat responsive varieties revealed that although heat stress had little effect on the growth attributes, a decrease in fresh weight (Fig. 1a) was greater than dry weight (Fig. 1b), leading to a reduction in the shoot fresh-to-dry weight ratio (Fig. 2a). Moreover, a depression in the leaf water potential, which also indicated the perturbed water status of shoot (Fig. 2b) as dehydrative effect of heat stress due to excessive evapo-transpiration. Like dry weight, leaf area remained almost unchanged at all stages (Fig. 3), although intrinsic difference in the varieties in the leaf area production was evident. Recovery from stress treatments indicated that all these attributes tended to improve nearly approaching the controls over the experimental period, although this recovery was more prompt in CP-4333. These data suggested that the overall growth of both the sugarcane varieties was not reduced appreciably. Such a response can be attributed to the fact that sugarcane is a long duration crop and remarkable reductions in the overall growth and biomass yield are seldom evident over short periods of time, although such responses are evident under longer heat stress periods (Bonnett *et al*., 2006). Nevertheless, short-term responses are mainly evident on physiological phenomena.

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