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EFFECT OF WATER DEFICIT STRESS ON THE PHYSIOLOGY, GROWTH AND LEAF CELL ULTRASTRUCTURE OF SORGHUM (*SORGHUM BICOLOR* (L.) MOENCH)

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ABSTRACT: An experiment was conducted in growth chambers to study the response of sorghum cultivars to water deficit stress. Seven sorghum cultivars were evaluated under three water deficit levels [control (-0.05 MPa), mild stress (-0.27 MPa), and severe stress (-0.96 MPa)] in a hydroponic culture in a completely randomised block design replicated three times. The three water deficit levels were created by adding 0, 10 and 20% (w/v) polyethylene glycol 6000 (PEG 6000) to the nutrient solution. The results indicated that water deficit, especially severe water deficit (-0.96 MPa), severely affected the growth and physiology of sorghum. Water deficit stress reduced plant height, leaf area, dry matter accumulation, water use efficiency, root length, and stomatal density. Water deficit stress increased stomatal closure and thus leaf diffusive resistance. Stressed plants deposited larger amounts of epicuticular wax on leaf surfaces and stomatal openings. Water stress significantly reduced the amount of starch grains in the chloroplasts. Sorghum cultivars displayed distinctive response to water deficit stress for many of the parameters. Jigurti, Gambella 1107 and Meko grew better than P9403 and SA1486 under water deficit conditions. Jigurti showed no significant reduction in any of the growth parameters even under severe water deficit (-0.96 MPa) conditions.

Keywords/phrases: Epicuticular wax; Leaf diffusive resistance; Leaf relative water content; Starch; Stomata; Water use efficiency.

INTRODUCTION

Water deficit stress is one of the most important environmental stresses affecting agricultural productivity in the semi-arid areas of north eastern Ethiopia and often results in considerable yield reductions. One of the prevalent features of the climate in this part of the country is the uncertainty of rainfall at the vegetative stage of sorghum. It is indicated that deficiency of water during any growth stage of sorghum often results in a loss of grain yield (Munamava and Riddoch, 2001). Daie (1996) indicated that water

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deficiency reduces plant water potential and thus, impairs many physiological and biochemical functions.

Among several strategies devised to reduce the drought stress effects, the selection of crop cultivars with drought resistance traits has been considered an economical and efficient strategy. Existing evidence indicates that genetic variation for drought resistance in grain sorghum is wide (Blum *et al.*, 1989). Blum *et al.* (1989) indicated that sorghum genotypes differ for nearly all recognized drought resistance mechanisms, such as maintenance of high leaf water status, deeper root growth, stomatal sensitivity and in epicuticular wax deposition. Genotypic differences in dry matter production and partitioning in response to drought stress has also been reported (Ashraf and Ahmad, 1998). This indicates that the opportunity for selecting for drought tolerant cultivars is considerable.

Most of the techniques for estimating drought responses have indicated that drought responses at early vegetative stages were reasonably well correlated with drought response of mature plants (Wright and Jordan, 1970). Thus, it appears that identifying drought response at an early vegetative stage can give reasonable estimates of the response of mature plants.

In northeastern Ethiopia, drought escaping sorghum cultivars have been bred and released for production considering only drought escaping traits and yield performance. However, unpredictable and intermittent periods of water deficit often occur also during the vegetative stages. Under such conditions, the use of varieties which combine drought tolerance and drought escape strategies should be advantageous. However, the agronomic and physiological attributes of these cultivars in response to water deficit at the vegetative stage have never been studied. Knowledge of the drought tolerance attributes of the cultivars should provide a good foundation for more efficient water management and for exploiting genetic variability. The objective of this investigation was to study the effect of water stress on the growth of sorghum and to examine variability in the response of sorghum cultivars to water deficit in the early vegetative stages.

MATERIALS AND METHODS

This experiment was conducted in growth chambers at the University of Pretoria. Five sorghum cultivars from Ethiopia (Jigurti, Gambella 1107, Meko, 76 T1 #23 and P9403) and two from South Africa (SA1486 and SA1488) were evaluated at three water deficit levels [control (-0.05 MPa), mild stress (-0.27 MPa), and severe stress (-0.96 MPa)]. The three water stress treatments were created by adding 0, 10 and 20% (w/v) PEG 6000 to the nutrient solution, respectively. These water deficit treatments were based on the results from a preliminary trial. In the preliminary observation trial with ranges of stress treatments, plants which received stress treatment beyond -0.96 MPa suffered from huge growth depression and plants which received stress treatments below -0.27 MPa did not show any stress symptoms. The stress period lasted for 12 days. The osmolarity of the pure nutrient solution and nutrient solution with PEG added was determined with a digital Micro-Osmometer (Wescor, Logman, UT, USA) and converted from mOsmol kg^{-1} to MPa using the Van't Hoff equation. The experiment was designed as a completely randomised block with three replicates. Fifteen-day-old seedlings, grown in a greenhouse, were used as planting material. Two uniform seedlings per pot were inserted in holes in polystyrene lids fixed on 9 litre pots containing a nutrient solution at pH (H_2O) 5.4. Plants were grown in growth chambers (25°C /17°C in 12h day $night^{-1}$ cycle, 334-399 μ mol m^{-2} s^{-1} PAR). Seedlings were subjected to the water deficit stress treatments for 12 days 39 days after emergence.

Growth measurements

Plants were harvested 51 days after emergence (12 days after the commencement of treatments) at which time leaf, stem (with leaf sheaths) and root dry matter yields were determined after oven drying at 75°C to constant weight. Leaf area (LA) at harvest was measured with a LI-3100 leaf area meter (LI-COR, Inc., Lincoln, NE, USA). Root length was estimated by measuring the longest root. Plant height to the tip of the longest leaf was measured at harvest.

Physiological measurements

Leaf diffusive resistance (LDR) was determined between 10h00 to 13h00 using a LI-1600 steady state porometer (LI-COR, Inc., Lincoln, NE, USA) on three occasions during the drought cycle. Measurements were made on the mid-portion of the upper surface of the second and third youngest leaves of each plant. Leaf relative water content (RWC) of the third and fourth youngest leaves was determined between 10h00 to 13h00 on days 1, 4 and 9 after treatments commenced, using five leaf discs (each 0.65 cm^2). RWC was determined using the method of Nepomuceno *et al*. (1998). Water use was calculated from the difference in water supplied and water left at harvest. Water use efficiency (WUE) was estimated as the ratio between total dry matter and the corresponding amount of water used.

Scanning (SEM) and transmission (TEM) election microscopy

After eight days of treatment application, leaf samples from three plants of the cultivars Jigurti, Meko, 76 T1 #23 and SA 1488 were taken from the third youngest leaf. Specimens of ca. 10 mm^2 size for SEM and ca. 2 mm^2 size for TEM were fixed in 2.5% glutaraldehyde in a 0.1 M phosphate buffer (pH 7.4) for 3h at 4°C. Specimens were post-fixed in 1% osmium tetraoxide, rinsed in buffer and dehydrated in a graded series of ethanol spending 15 minutes in each one of the series and subsequently critical point dried in liquid CO_2. Specimens for SEM observation were mounted on aluminium stubs, coated with gold and viewed with a JEOL JSM-840 scanning electron microscope (JEOL, Tokyo) at 5 kV.

Stomatal density was counted for three randomly selected fields per leaf sample. Stomata dimensions were measured for three randomly selected fields, each with four stomata. The dimensions of 12 stomata per leaf sample were measured with an Image Tool (version 2.00) computer program. Epicuticular wax deposition on the adaxial leaf surfaces was also examined. Specimens for TEM observation were embedded in Quetol 651resin. A Reichert ultracut E microtome was used to cut thin cross-sections (0.1 µm) with a diamond knife. Sections were double-stained with uranyl acetate and lead citrate. Electron micrographs were obtained with a Philips EM 301 transmission electron microscope.

Statistical analysis

Analysis of variance for the measured parameters was performed using the SAS statistical program (SAS V8.2, SAS Institute Inc., Cary, NC, USA). Whenever treatment differences were found to be significant, based on the results of *F*-test, critical differences were calculated at 5% level of probability using the least significant difference (LSD) technique.

RESULTS

Results of the ANOVA revealed that highly significant differences existed between both water deficit stress treatments and cultivars for most parameters. Significant cultivar x stress interactions existed only for plant height, leaf area and for leaf, stem and shoot dry mass.

Plant growth

Water deficit stress adversely affected plant growth in most of the cultivars. Plant height was markedly reduced by water deficit stress (Table 1). Compared to the well-watered plants, plant height under severe water stress was significantly reduced in all cultivars, except in Jigurti. The reduction in plant height was more pronounced in P9403 and SA1486 with 15 and 34% reduction in P9403 and 11 and 20% reduction in SA1486 under mild and severe water stress conditions, respectively. In terms of plant height, Jigurti, Gambella 1107, Meko, 76 T1 #23 and SA1488 showed better tolerance to water deficit. The response of Jigurti is interesting in that its shoot height was enhanced by mild water stress and the reduction under severe water stress was not significant.

Table 1 Effect of water deficit stress on plant height (cm) and leaf area (cm^2 $plant^{-1}$, % of control) of sorghum cultivars.

Cultivars	Plant height			Leaf area		
	-0.05 MPa	-0.27 MPa	-0.96 MPa	-0.05 MPa	-0.27 MPa	-0.96 MPa
Jigurti	126.67b-e*	134.67bc	126.33cde	100bc	131.8a	96.5bcd
Gambella 1107	147.00a	138.00ab	122.33def	100bc	87.6cd	66.6efg
Meko	133.67bcd	137.50abc	116.33e-h	100bc	116.1ab	77.0def
76 T1 #23	122.67def	121.33ef	108.67ghi	100bc	92.6cd	61.7fg
P9403	126.33cde	107.67ghi	83.00k	100bc	82.5cde	38.8h
SA 1486	117.67efg	105.00hij	94.00jk	100bc	77.9def	50.9gh
SA 1488	117.50e-h	113.67f-i	104.00ij	100bc	98.6bc	59.5fg

*Means in rows and column for each parameter followed by the same letter do not differ significantly at $p \leq 0.05$.

Significant differences in leaf area development were observed between water deficit treatments, between cultivars and their interaction effects. In all cultivars, except Jigurti, leaf area development was significantly impaired by the severe water deficit treatment (Table 1). The reductions in leaf area of Jigurti, Gambella 1107 and Meko were, however, relatively small. Under severe water deficit conditions, P9403 and SA1486 developed significantly smaller leaf area. Leaf area development in the susceptible cultivar SA1486 was significantly reduced by even mild water stress.

Significant differences in leaf (LDM), stem (SDM), and shoot (SHDM) dry matter production due to water deficit stress treatments, cultivars and the water deficit x cultivar interaction were observed (Table 2). In all cultivars, LDM, SDM and SHDM reductions were markedly higher under severe water deficit than under mild water deficit conditions. Dry matter reduction under mild water deficit conditions was significant only for the susceptible cultivar SA1486. Under severe water deficit conditions, although LDM, SDM and SHDM tend to decline in all cultivars, except Jigurti, significant reductions were observed in 76 T1 #23 (except for LDM), P9403, SA1486, and SA1488 with 54, 41 and 34% reduction in LDM, 40, 69, 45 and 38% reduction in SDM and 33, 60, 42 and 35% reduction in SHDM. Dry matter accumulation in Jigurti, Gambella 1107 and Meko was relatively less affected.

Table 2 Effect of water deficit stress on leaf, stem and shoot dry matter (g plant^{-1}) of sorghum cultivars.

Cultivars	Leaf dry matter			Stem dry matter			Shoot dry matter		
	-0.05 MPa	-0.27 MPa	-0.96 MPa	-0.05 MPa	-0.27 MPa	-0.96 MPa	-0.05 MPa	-0.27 MPa	-0.96 MPa
Jigurti	6.45efg*	9.07abc	7.59b-f	3.95d-h	5.92a	4.91a-d	10.40efg	14.98ab	12.49b-e
Gambella 1107	11.91ab	10.66ab	8.30abc	7.45a	6.91a	5.10a-d	19.36ab	17.56ab	13.40a-d
Meko	8.02a-e	9.91abc	6.83d-g	5.63ab	7.14a	4.62b-e	13.65a-d	17.05a	11.44c-f
76 T1 #23	10.12abc	9.45abc	7.27c-f	5.91ab	5.50a-e	3.52e-i	16.02ab	14.95abc	10.79d-g
P9403	8.10a-d	7.29cdef	3.73g	5.23abc	4.07c-g	1.64i	13.33a-d	11.36c-f	5.37g
SA 1486	8.25a-d	6.07fg	4.88g	4.41b-f	2.79ghi	2.41hi	12.67a-e	8.86fg	7.29g
SA 1488	9.530abc	8.88abc	6.33fg	4.93a-e	4.50b-f	3.08f-i	14.46abc	13.38a-d	9.41fg

*Means in rows and column for each parameter followed by the same letter do not differ significantly at p≤0.05.

Root length was impaired by the water stress treatments, with the highest reduction observed under severe water stress (Table 3). Root length also varied between cultivars where Jigurti followed by Gambella 1107 and Meko having the longest, and 76 T1 #23, P9403 and SA1486 having the shortest roots (Table 3). Root to shoot ratio also differed between water deficit treatments and between cultivars (Table 3). Water deficit stress enhanced root to shoot ratio with increases of 33 and 44% under mild and severe water deficit treatments, respectively, indicating more dry matter partitioning into the roots relative to the shoot. Cultivars P9403, SA1486 and SA1488 appeared to partition a greater proportion of assimilate into their root systems (Table 3).

Table 3 Effect of water deficit stress and cultivar differences on root length, root to shoot ratio, water use and water use efficiency.

Comparisons	Root length (cm plant^{-1})	Root to shoot ratio	Water use (kg)	Water use efficiency (g kg^{-1})
Water deficit stress levels				
-0.05 MPa	32.98a*	0.37c	9.121a	4.270c
-0.27 MPa	30.93b	0.55b	8.707a	4.860b
-0.96 MPa	27.06c	0.66a	6.031b	5.277a
Cultivars				
Jigurti	35.83a	0.49bc	8.091bc	4.708bc
Gambella 1107	32.44b	0.46c	9.718a	4.977ab
Meko	31.63b	0.46bc	8.768ab	4.596bc
76 T1 #23	28.86cd	0.52bc	8.492abc	4.632bc
P9403	27.89cd	0.59a	6.992cd	4.446c
SA 1486	26.28d	0.61a	6.404d	4.919b
SA 1488	28.94bc	0.56ab	6.972cd	5.339a

*Means within a column for each comparison followed by the same letter do not differ significantly at p≤0.05.

Water use and water use efficiency

Differences in water use between water deficit treatments and between cultivars were significant (Table 3). The amount of water used by plants under severe water deficit condition was 34% less than water used by plants under well-watered condition. Among cultivars, Gambella 1107, Meko and

76 T1 #23 followed by Jigurti used the highest amount of water (Table 3). Differences in water use efficiency were also observed between water deficit treatments and between cultivars (Table 3). The results indicated an increase in water use efficiency as the level of water deficit stress was increased. Water use efficiency increased by 14 and 24% under mild (-0.27 MPa) and severe (-0.96 MPa) water deficit treatments, respectively. Among cultivars, SA1488 followed by Gambella 1107 were the most efficient in water use, while P9403 was the least efficient cultivar (Table 3). In terms of water use efficiency, Gambella 1107 is a drought-tolerant cultivar combining high water use efficiency and superior growth under water deficit condition.

Leaf relative water content and leaf diffusive resistance

No significant differences were observed in relative water content of leaves for any of the treatment effects. However, leaf relative water content tended to decrease as the level of water deficit increased, although differences were not statistically significant (data not shown).

Leaf diffusive resistance differed between water deficit treatments where marked increases in leaf diffusive resistance were observed under severe water deficit condition (Fig. 1). Differences in cultivars in leaf diffusive resistance in response to water deficit stress were not observed.

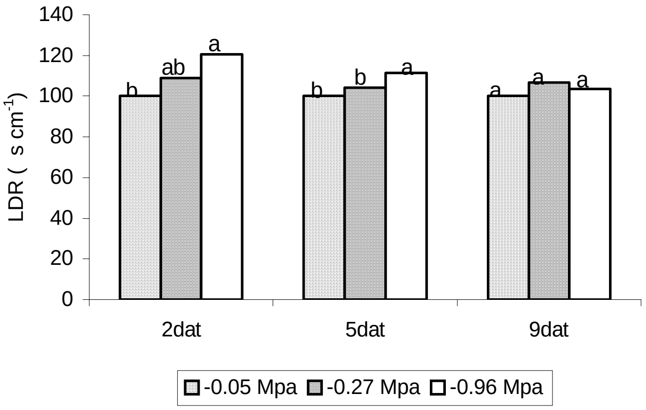

Fig. 1. Effect of water deficit stress on leaf diffusive resistance (LDR) (% of control) of sorghum at 2, 5 and 9 days after treatment (dat) application. Bars followed by the same letter do not differ significantly at P≤0.05.

Effect of water stress on leaf cells ultrastructure

Stomatal density and pore size

Differences in stomatal density on the lower leaf surface were observed among water deficit treatments where both mild and severe water deficit treatments significantly reduced stomatal frequency by 18 and 21%, respectively (Table 4). Cultivar differences in stomatal density were, however, not observed. Stomatal pore size (length) on the lower surface varied significantly between water stress treatments, but with an inconsistent trend (Table 4). Stomatal pore length also varied significantly between cultivars where Meko and 76 T1#23 had significantly larger stomatal pore sizes on both leaf surfaces and SA1488 (a tolerant cultivar) had significantly smaller stomatal pore size (Table 4).

Table 4 Effect of water deficit stress and cultivar differences on stomata pore length and stomata density.

Comparison	Lower leaf surface stomata pore length (μ m)	Uppper leaf surface stomata pore length (μ m)	Lower leaf surface stomata density (number mm^{-2})
Water deficit levels			
-0.05 MPa	25.74b*	26.22a	123.78a
-0.27 MPa	28.32a	28.08a	101.31b
-0.96 MPa	22.75ab	27.28a	102.05b
Cultivars			
Jigurti	26.82b	26.32ab	102.66a
Meko	28.49ab	29.40a	101.68a
76 T1#23	29.50a	28.65a	108.07a
SA1488	24.26c	24.41b	123.78a

*Means within a column for each comparison followed by the same letter do not differ significantly at $p \leq 0.05$.

Stomatal closure

Differences between water stress treatments were observed on the degree of stomatal closure. The micrographs from SEM in Fig. 2 clearly show the progressive closure of stomata following the level of water stress. Compared to the well-watered condition (Figs. 2 A and D), stomatal openings under both mild (Figs. 2 B and E) and severe water stress (Figs. 2 C and F) conditions were either completely or partially closed. This effect was also confirmed by the increased stomatal diffusive resistance under water deficit conditions (Fig. 1). Clear differences between cultivars were not observed, thus representative electron micrographs only from two cultivars are presented (Fig. 2).

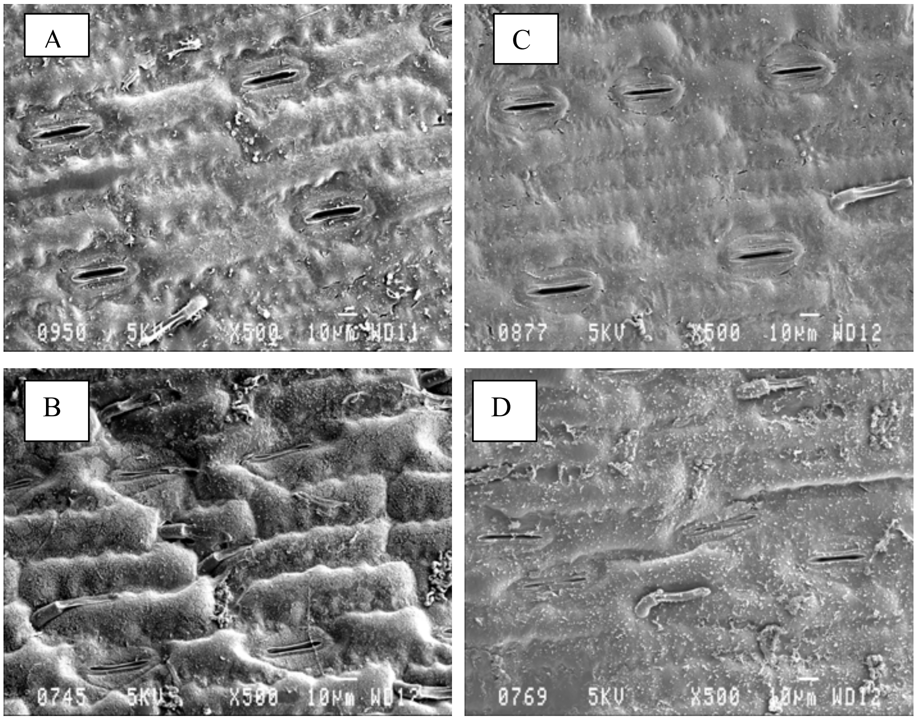

Fig. 2. Scanning electron micrographs of stomata on the upper leaf surface of sorghum cultivars (bars 10 µm). Note the closure of stomata under water stressed conditions. A and B (Jigurti at control and -0.96 MPa); C and D (Meko at control and -0.96 MPa).

Epicuticular wax deposition on leaf surfaces

Considerable differences were observed between water stress treatments in relation to epicuticular wax (EW) deposition on sorghum leaf surfaces (Fig. 3). Epicuticular wax deposition in response to water stress tend to be similar between cultivars, thus representative electron micrographs only from two cultivars are illustrated (Fig. 3). The micrographs from SEM reveal that plants grown under well-watered and water stressed conditions exhibited different degrees of EW deposition (Fig. 3).

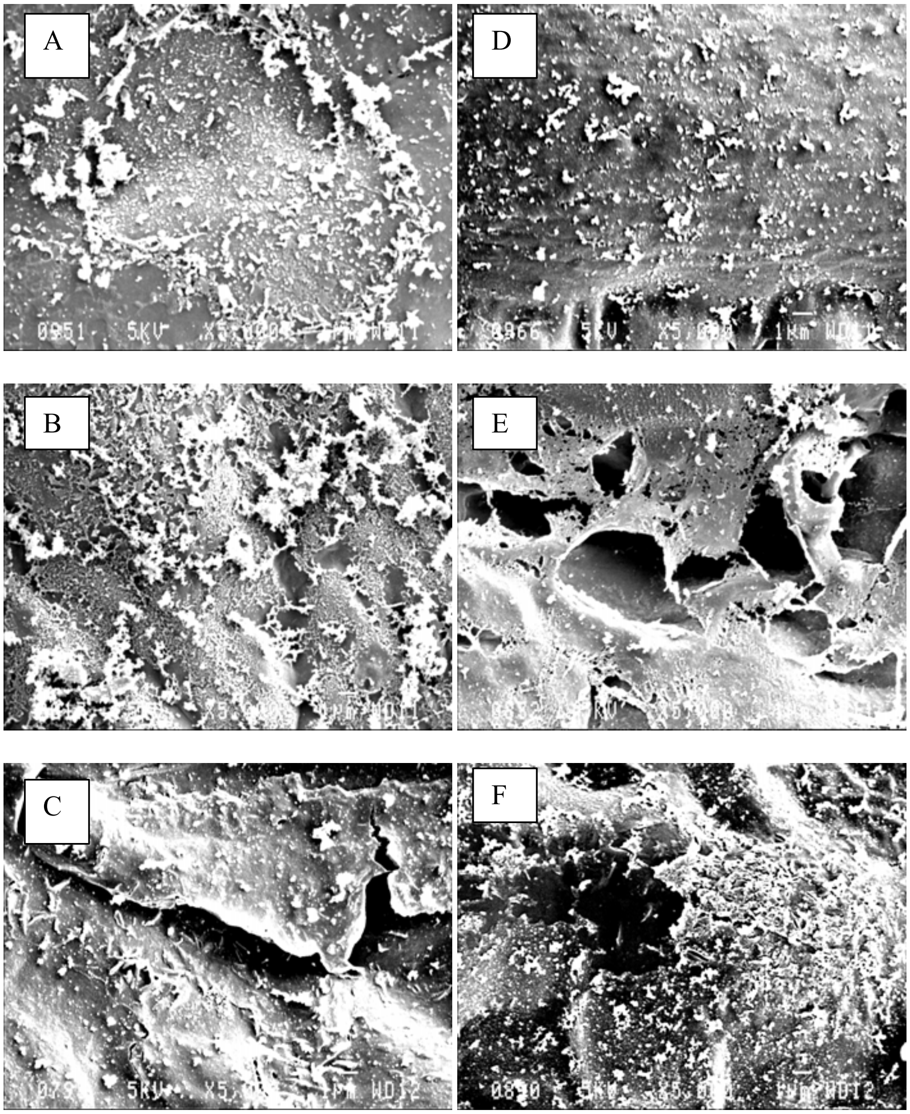

Fig. 3. Scanning electron micrographs showing epicuticular wax deposition on the upper leaf surfaces of sorghum cultivars (bars 1µm). Note the sparse and dense EW deposition on the well-watered and water stressed leaves, respectively. A, B, C (Jigurti at control, -0.27 MPa, and -0.96 MPa); D, E, F (Meko at control, -0.27 MPa, and -0.96 MPa).

In all cultivars, leaves from stressed plants showed extensive EW deposition on the upper surfaces (Figs. 3 B, C, E, F). In contrast, leaves of the well-watered plants appeared microscopically smooth for all cultivars (Figs. 3 A, D).

Starch deposition in chloroplasts

Variability in the amount of starch grains deposited in the bundle sheath chloroplasts was observed between water stress treatments and between cultivars (Fig. 4). Plants under severe water deficit (-0.96 MPa) conditions showed a marked reduction in the amount of starch grains in the chloroplasts. This effect was more noticeable in 76 T1 #23 (Figs. 4 C and D) and Meko (Figs. 4 E and F). Cultivars also tended to differ in terms of starch deposition in the chloroplasts. Clear differences between the stressed and unstressed plants were not observed in Jigurti (Figs. 4 A and B). SA1488 (tolerant cultivar) deposited more starch grains under water stress compared to the well-watered plants (Figs. 4 G and H).

DISCUSSION

Generally water deficit stress adversely affected the growth and development of all sorghum cultivars. Water deficit stress has significantly reduced plant height of all cultivars. According to Kramer (1983), the alteration of plant height under water deficit stress could be due to reduced cell division and elongation resulting from loss of cell turgour. Among cultivars, Jigurti, Gambella 1107, Meko, 76 T1 #23 and SA1488 showed the least plant height reduction.

Similar to the results of Lu and Neumann (1998), reduction in cumulative leaf area under water deficit stress could presumably be due to inhibition in cell division and enlargement. Cultivars also differed in leaf area development in response to water deficit stress. Reduction in leaf area was small in Jigurti, Gambella 1107 and Meko. Thus, larger leaf area development under water deficit stress could be an important trait in screening genotypes for drought tolerance.

Water deficit stress has reduced dry matter (leaf, stem, total shoot) production in all cultivars. It is hypothesized that the reduction in biomass accumulation could be associated with the reduction in leaf area development, which in turn might have decreased radiation interception, radiation use efficiency and then photosynthesis. Because leaf area is the main site of assimilate production, its reduction due to water stress might have strongly affected dry matter production.

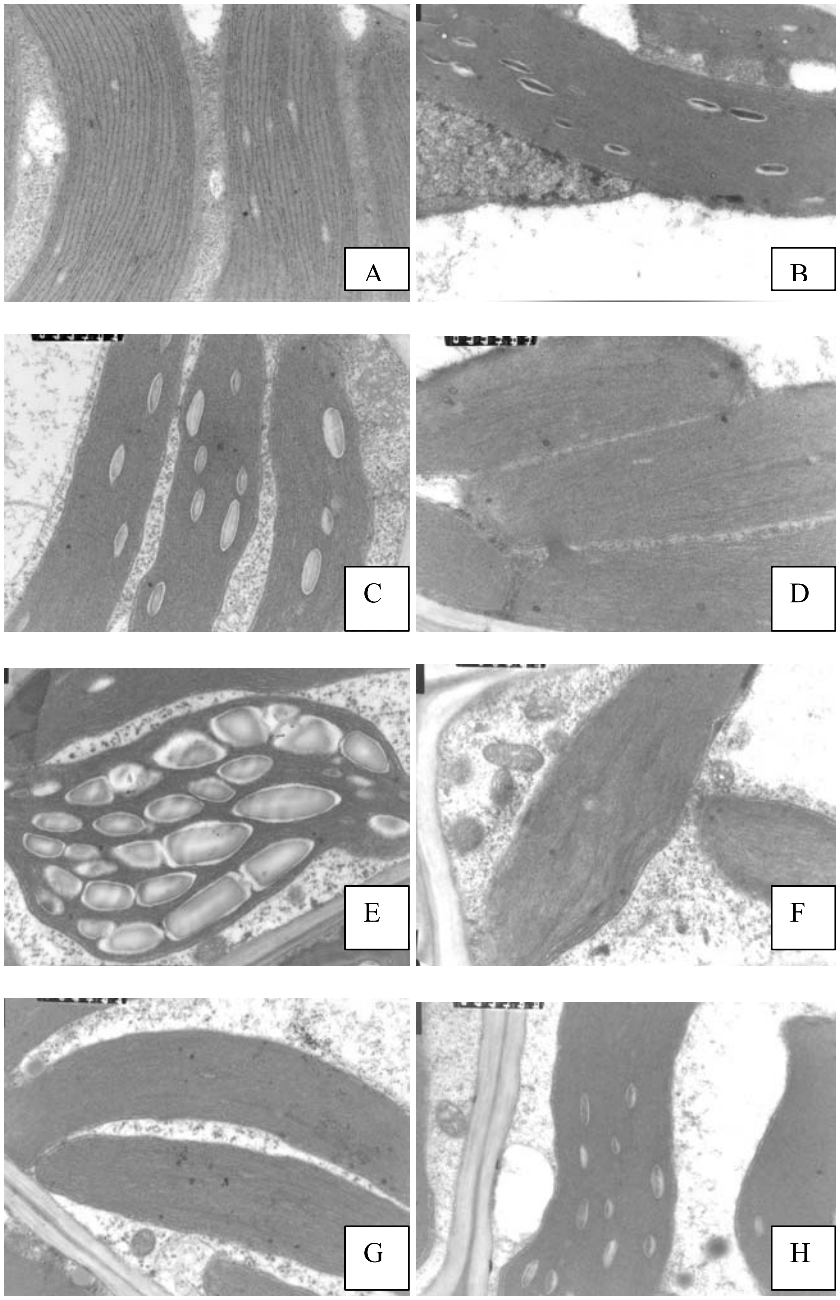

Fig. 4. Starch deposition in unstressed and stressed sorghum plant bundle sheath chloroplasts. A and B = Jigurti unstressed and stressed, C and D = 76 T1 #23 unstressed and stressed, E and F = Meko unstressed and stressed, G and H = SA1488 unstressed and stressed (X 13 000).

Dry matter accumulation under water stress conditions could also be reduced due to the reduction in the rate of carbon fixation as a result of stomatal closure. It was shown in this study that sorghum closed its stomata almost completely under water deficit stress (Figs. 3 and 5). As indicated by Nwalozie and Annerose (1996), although stomatal closure under water deficit stress was an important drought avoidance mechanism, it can happen at the expense of carbon fixation and dry matter production. Thus, effective dry matter production under water stress conditions implies tolerance to the stress as it indicates the plant's ability to fix carbon regardless of the stress. Among the cultivars, dry matter accumulation was least affected in Jigurti, Gambella 1107 and Meko. Genotypic differences in dry matter production and partitioning under stress can, therefore, be used as useful indicators of relative tolerance to water deficit stress.

High root to shoot ratio, together with deep roots, are important mechanisms for surviving drought in many species (Munamava and Riddoch, 2001). In the present study, it was observed that root length was negatively affected by water deficit stress, presumably due to the retardation effect of the stress on cell division and enlargement. Alteration of dry matter partitioning in favor of the roots is one of the recognized effects of water stress on plants. Preferential allocation of dry matter to the roots at the expense of shoot growth is a classic drought avoidance mechanism. It reflects high water absorption capacity, which would enhance survival during subsequent drought exposures. Cultivars differed in root to shoot ratio with P9403, SA1486 and SA1488 maintaining high root to shoot ratio to survive the effect of water deficit. The observed increase in RSR under water deficit conditions agrees with the results of Munamava and Riddoch (2001).

It is an established fact that stomata play an integral part in the process of CO_2 fixation and in controlling water vapor loss. Reduction in stomatal opening would therefore affect photosynthesis and reduce dry matter production. Stomatal response to water deficit stress as determined by measuring leaf diffusive resistance and scanning electron micrographs revealed closure of stomata which in turn indicated reduction in CO_2 fixation and photosynthesis. Stomata covered in wax as shown in the bottom panel of Fig. 4 were probably dysfunctional. The insulation of stomatal apertures by epicuticular wax deposition, observed in this experiment, confirms other findings (McWhorter *et al*., 1990).

Reduction in stomatal density in plants grown under water deficit stress was observed. This is in accordance to the findings of Younis *et al*. (1993) in

Vicia faba and Sam *et al*. (2000) in tomato. According to Muchow and Sinclair (1989), stomatal density and pore size affect diffusion resistance of the epidermis. According to Fitter and Hay (1987), the resistance to the diffusion of water molecules offered by the stomata is inversely proportional to the diameter of the stomatal aperture. Thus, cultivars with smaller stomatal pores may adapt to water deficit stress conditions better. Muchow and Sinclair (1989) on the other hand reported that stomatal pore length differed between sorghum genotypes, indicating the possibility of selecting cultivars with smaller stomatal pore length. In this study, SA1488 (a drought tolerant cultivar) had smaller stomatal pore size.

Sorghum plants grown under water deficit stress conditions deposited larger amounts of epicuticular wax on leaf surfaces (Fig. 3). Epicuticular wax deposition has been shown to serve as a barrier to water vapor loss, thus enhancing plant survival in stressful environments (Cameron *et al*., 2002). Cameron *et al*. (2002) indicated that drought tolerance and increased epicuticular wax deposition were positively associated traits. The mechanism of reduction in transpiration is supposed to be that wax filaments lower the net radiation by increasing reflectance and thickening the boundary layer, thereby increasing the diffusive resistance to gas and water vapour exchange (Jenks and Ashworth, 1999). The thick epicuticular wax deposition is also associated with reduced cuticular transpiration (Jordan *et al*., 1983). Thus, epicuticular wax deposition could be used as a screening criterion for drought tolerance selection as plants with greater epicuticular wax deposition exhibited higher ability in the retention of tissue water (Jordan *et al*., 1983). Increased epicuticular wax deposition in plants grown under water deficit stress was reported by Oosterhuis *et al*. (1991) in cotton, Jordan *et al*. (1983) in sorghum and Johnson *et al*. (1983) in wheat.

Variability in the amount of starch grains deposited in the bundle sheath chloroplasts was observed between water stress treatments and between cultivars (Fig. 5). Plants under severe water deficit (-0.96 MPa) conditions showed a marked reduction in the amount of starch grains in the chloroplasts. Marked reduction in starch deposition in the bundle sheath chloroplasts of stressed sorghum leaves was also reported by Giles *et al*. (1976). Daie (1996) indicated that due to the low carbon supply under drought conditions, a shift in chemical partitioning of carbon occurred in favour of sucrose accumulation or starch remobilization in the leaf cells of stressed plants. Moreover, due to a decline in newly fixed carbon, sucrose accumulation could have resulted from starch breakdown as the activity of the starch hydrolyzing enzyme, alpha-amylase, is known to increase in leaves of drought stressed plants (Daie, 1996). These justifications suggest that the small amount, or lack, of starch deposition in the stressed sensitive cultivars could be due to less carbon fixation and/or breakdown of starch into sucrose. Variability between water stress treatments and between cultivars in starch accumulation in the chloroplasts and the observation that cultivars (Jigurti and SA1488) which showed tolerance in the growth and physiological parameters accumulate more starch in the chloroplasts under water stress conditions, suggests that selection for high starch accumulation in the chloroplasts can be used as a selection criterion for drought tolerance in sorghum.

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