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CHAPTER 3

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EFFECT OF WATER DEFICIT STRESS ON THE GROWTH, PHYSIOLOGICAL PROCESSES AND LEAF CELL ULTRASTRUCTURE OF SORGHUM

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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 nutrient solution (Nitch solution) with a completely randomised block design with three replications. The three water deficit levels were created by adding 0, 10 and 20% (w/v) PEG 6000 to the nutrient solution. Water deficit, especially severe water deficit (-0.96 MPa), severely affected the growth and physiological processes. Water deficit stress markedly reduced plant height, leaf number, cumulative leaf area, dry matter accumulation (leaf, stalk and total shoot), water use efficiency, root length, and stomatal density. Water deficit stress also increased leaf diffusive resistance. Water deficit stress increased root dry mass. Water stress increased stomatal closure. Stressed plants deposited larger amounts of epicuticular wax on the leaf surface and on the stomatal openings. Water stress significantly reduced the amount of starch grains in the chloroplasts. The seven cultivars displayed distinctive responses 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: epicuticular wax, sorghum, starch, stomata, water deficit stress

Introduction

Water deficit stress is one of the most important environmental stresses affecting agricultural productivity in the semi-arid areas of NE Ethiopia and often results in considerable yield reductions. One of the prevalent features of the climate of the area is the uncertainty of rainfall during the vegetative stage of sorghum. A deficiency of water during any growth stage of sorghum often results in a loss of grain yield (Munamava and Riddoch 2001). Water deficiency reduces plant water potential and thus, impairs many physiological and biochemical functions (Daie 1996).

Among several strategies devised to overcome the problem of drought stress, the selection of crop species or cultivars with drought tolerance traits has been considered an economical and efficient strategy (Ashraf et al. 1992). Existing evidence indicates that genetic variation for drought tolerance in grain sorghum is wide (Blum et al. 1989). Blum et al. (1989) indicated that sorghum genotypes differ for nearly all recognized drought tolerance mechanisms, such as maintenance of high leaf water status, deeper root growth, stomatal sensitivity and epicuticular wax deposition. Genotypic differences in dry matter production and partitioning in response to drought stress have also been reported (Ashraf and Ahmad 1998). This indicates that the opportunity for selecting drought tolerant cultivars is considerable.

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

In NE 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 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 agronomic, physiological and anatomical 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 (Nitch solution), respectively. These water deficit treatments were based on the results of a preliminary trial. The stress period lasted for 12 days. The osmolality of the pure nutrient solution and nutrient solution with PEG 6000 added was determined with a digital Micro Osmometer 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 plants were planted into the trial pots and grown for 24 days under no-stress conditions before water deficit stress treatments were applied for a period of 12 days. Two uniform seedlings per pot were inserted in holes in polystyrene lids fixed on 91 pots containing a nutrient solution at pH (H_2O) 5.4. Plants were grown in growth chambers ($25^0/17^0$ C in 12h day/night cycle, 334-399 μ mol m^{-2} s^{-1} PAR).

Growth measurements

Plants were harvested 51 days after emergence (12 days after the commencement of treatments) at which time leaf, stem (including leaf sheaths) and root dry mass were determined after oven drying at 75^0 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 recorded.

Leaf diffusive resistance (LDR) was determined between 10h00 and 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 adaxial surface of the second and third youngest leaves of each plant. Leaf relative water content of the third and fourth youngest leaves was determined between 10h00 and 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 calculated as the ratio between total dry matter and the corresponding amount of water used.

Scanning (SEM) and Transmission (TEM) electron 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 3 h at 4^0 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 301transmission electron microscope.

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 and Discussion

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 the level of 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. Growth response to stress in terms of plant height was regarded as one component of a multiple selection index for drought tolerance in maize (Fischer et al. 1983).

Table 1

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

	Plant height			Leaf area		
Cultivars	-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
SA1486	117.67efg	105.00hij	94.00jk	100bc	77.9def	50.9gh
SA1488	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 $P \leq 0.05$.

Data on the number of leaves indicate that water stress had significant effects on the production of leaves per plant. Leaf production under severe water deficit stress (-0.96 MPa) was significantly reduced compared to well-watered and mild water deficit (-0.27 MPa) conditions (fig.1). Similar results were reported by Heitholt (1989) in wheat and De Costa et al. (1997) in faba bean who observed reduced leaf number under water deficit stress.

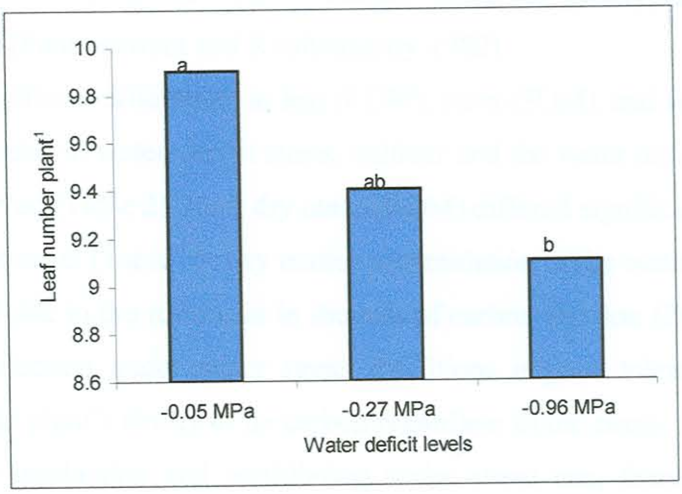

Fig.1 Effect of water deficit stress on leaf number in sorghum. Bars with the same letter do not differ significantly at $P \leq 0.05$.

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 areas of Jigurti, Gambella 1107 and Meko were, however, relatively small. Under severe water deficit conditions, P9403 and SA1486 developed significantly smaller leaf areas.

Leaf area development in the susceptible cultivar SA1486 was significantly reduced by even mild water stress. Leaf area was significantly correlated to leaf number (r=0.48), suggesting lower leaf production as one of the reasons for reduced leaf area development per plant under water stress conditions. The reduction in leaf number implies that leaf surface area per plant available for transpiration and assimilate production will also be reduced, thereby reducing the growth of plants. Rosenthal et al. (1987) also reported reduced leaf area development in sorghum due to fewer leaves produced under water stress conditions. Reduced leaf area development under water deficit conditions could also occur due to inhibition in cell division and enlargement as a result of loss of cell turgor (Lu and Neumann 1998). Reduced leaf area development under water stress conditions will reduce the quantity of solar radiation intercepted by the canopy, thereby reducing dry matter production. Thus, assessment of the ability of a genotype to continue leaf growth or expansion, and maintain a high leaf area, during water deficit stress would help in characterizing the genotypes as drought tolerant or susceptible (Parameswara and Krishnasastry 1982).

Significant differences in leaf (LDM), stem (SDM), and shoot (SHDM) dry matter production due to water deficit stress, cultivar and the water deficit x cultivar interaction were observed (Table 2). Root dry mass (RDM) differed significantly only between water deficit treatments (Table 3). Dry matter accumulation under water stress conditions could be reduced due to the reduction in the rate of carbon fixation (Daie 1996). 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. Genotypic differences in dry matter production and partitioning under stress can, therefore, be used as useful indicators of relative tolerance to water deficit stress (Ashraf and Ahmad 1998). In all cultivars, LDM, SDM and SHDM reductions were markedly higher under severe water deficit than under mild water deficit conditions (table 2). 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 (tables 2 and 3). SHDM was closely correlated to leaf area development (r = 0.93) and root to shoot ratio (r = -0.50) suggesting that the reduction in SHDM under water deficit conditions could be explained by the reduction in leaf area development and increased dry matter partitioning to the roots. Under water stress conditions, owing to low carbon supply, the fixed carbon will be used in osmotic adjustment rather than for growth/storage thus reducing crop growth (Daie 1996).

RDM accumulation was enhanced both by mild and severe water deficit treatments (table 3). In agreement with this observation, Ren et al. (2000) found increased root growth in wheat under PEG-induced drought stress. There is no clear explanation for the increased RDM accumulation under water stress, except the hypothesis by Kramer (1983) that there could be occasional situations where RDM could increase in mildly stressed plants due to more effective osmotic adjustment in roots than in shoots.

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 with Jigurti followed by Gambella 1107 and Meko having the longest, and 76 T1 #23, P9403 and SA1486 having the shortest roots. Root to shoot ratio (RSR) also differed both between water deficit treatments and cultivars (table 3). Water deficit stress enhanced RSR 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).

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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
SA1486	8.25a-d	6.07fg	4.88g	4.41b-f	2.79ghi	2.41hi	12.67a-e	8.86fg	7.29g
SA1488	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.

Table 3

Effect of water deficit stress and cultivar differences on root dry matter (RDM), root length (RL) and root to shoot ratio (RSR) of sorghum cultivars

Water deficit stress levels	RDM (g plant^{-1})	RL (cm plant^{-1})	RSR
-0.05 MPa	5.25b	32.98a	0.37c
-0.27 MPa	7.33a	30.93b	0.55b
-0.96 MPa	6.49a	27.06c	0.66a
Cultivars			
Jigurti	6.28a	35.83a	0.49bc
Gambella 1107	7.58a	32.44b	0.46c
Meko	6.18a	31.63b	0.46bc
76 T1 #23	6.84a	28.86cd	0.52bc
P9403	5.41a	27.89cd	0.59a
SA1486	5.66a	26.28d	0.61a
SA1488	6.57a	28.94bc	0.56ab

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

This indicates that water deficit not only reduces the rate of dry matter production but also affects partitioning. Greater partitioning of dry matter to the root system would enable more thorough exploration of soil water reserves and may, therefore, enhance survival during subsequent drought exposure (Munamava and Riddoch 2001). Surviving a drought may, however, not make cultivars able to perform well under drought condition. The observed increase in RSR under water deficit conditions agrees with the results of Munamava and Riddoch (2001).

Water use (WU) and water use efficiency (WUE)

Differences in WU between water deficit treatments and between cultivars were significant (table 4). The amount of water used by plants under severe water deficit conditions was 34% less than water used by plants under well-watered conditions. Among cultivars, Gambella 1107, Meko and 76 T1 #23 followed by Jigurti used the most water. Differences in WUE were also observed between water deficit treatments and between cultivars (table 4). The results indicate an increase in WUE as the level of water deficit stress was increased. WUE increased by 14 and 24% under mild (-0.27 MPa) and severe (-0.96 MPa) water deficit treatments, respectively. This result is in agreement with the reports of Misra and Chaudhary (1985), who reported increased WUE in sorghum under water deficit conditions. Among cultivars, SA1488 followed by Gambella 1107 were the most efficient in water use, while P9403 was the least efficient cultivar. In terms of WUE Gambella 1107 is a drought tolerant cultivar combining high WUE and superior growth under water deficit condition.

Table 4

Effect of water deficit stress and cultivar differences on water use and water use efficiency

Water deficit stress levels	Water use (kg)	Water use efficiency ($g\ kg^{-1}$)
-0.05 MPa	9.121a	4.270c
-0.27 MPa	8.707a	4.860b
-0.96 MPa	6.031b	5.277a
Cultivars		
Jigurti	8.091bc	4.708bc
Gambella 1107	9.718a	4.977ab
Meko	8.768ab	4.596bc
76 T1 #23	8.492abc	4.632bc
P9403	6.992cd	4.446c
SA1486	6.404d	4.919b
SA1488	6.972cd	5.339a

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

Leaf relative water content (RWC)

No significant differences were observed in RWC of leaves for any of the treatment effects. However, RWC tended to decrease as the level of water deficit increased, although differences were not statistically significant (data not shown). The absence of significant differences in leaf RWC in this study may be explained by the hypothesis suggested by Davis et al. (1994) that roots exposed to water deficit conditions may induce a root hormonal signal to the shoot, thus causing stomatal closure and retardatory growth without any detectable changes in leaf water potential or leaf turgor.

For instance, Cruz de Carvalho et al. (1998) reported stomatal closure in *Phaseolus vulgaris* even before detecting any leaf water deficit.

Leaf diffusive resistance (LDR)

LDR differed between water deficit treatments where marked increases in LDR were observed under severe water deficit condition (fig. 2). The increase in LDR under water deficit conditions could be associated with stomatal closure (fig. 3) arising from non-hydraulic root signals (hormonal, e.g. ABA signals) as suggested by Li et al. (2001). The increased diffusive resistance could also be associated with the wax deposition on the stomatal openings as evidenced by the SEM observations (fig. 5). Differences in cultivars in stomatal resistance in response to water deficit stress were not observed.

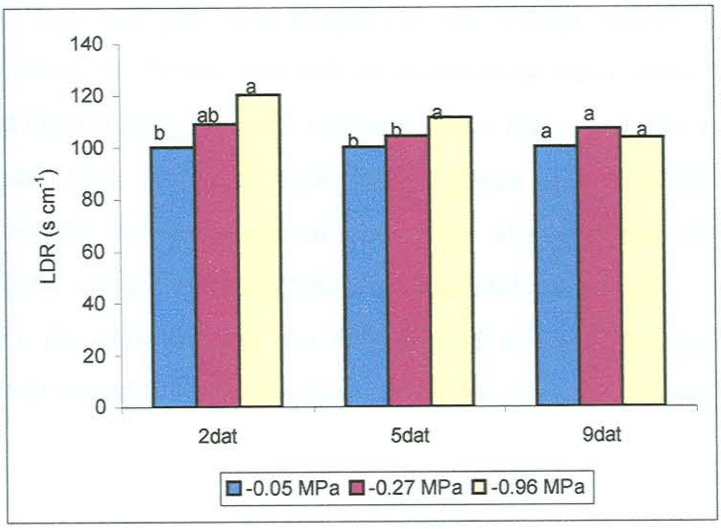

Fig. 2 Effect of water deficit stress on leaf diffusive resistance (LDR) (% of control) of sorghum at 2, 5 and 9 days after application of water stress treatment (dat). Bars with the same letter do not differ significantly at $P \leq 0.05$.

Effect of water stress on leaf cell ultrastructure

Stomatal density and pore size

Differences in stomatal density on the abaxial leaf surface were observed among water deficit treatments. Both mild and severe water deficit treatments significantly reduced stomatal frequency by 18 and 21%, respectively (table 5). This observation is in accordance to the findings of Younis et al. (1993) in *Vicia faba* and Sam et al. (2000) in tomato. It has been indicated that stomatal density and pore size affects diffusion resistance of the epidermis (Muchow and Sinclair 1989). Decreases in stomatal frequency increase drought tolerance through decreasing transpiration (Heichel 1971 as cited by Turner and Begg 1981). Cultivar differences in stomatal density were, however, not observed.

Stomatal pore size (length) on the abaxial surface varied significantly between water stress treatments, but with an inconsistent trend (table 5). 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. Muchow and Sinclair (1989) also reported genotypic variability in sorghum for stomatal pore length. 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.

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Table 5

Effect of water deficit stress and cultivar differences on stomata pore length (μ m) and stomata density (number mm^{-2})

Water deficit levels	Abaxial stomata pore length	Adaxial stomata pore length	Abaxial stomata density
-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 the effect of water stress treatments on the degree of stomatal closure were observed. The micrographs from SEM clearly show the progressive closure of stomata following the level of water stress (fig. 3). Compared to the well-watered condition, stomatal openings under both mild and severe water stress conditions were either completely or partially closed. This effect was also confirmed by the increased stomatal diffusive resistance under water deficit conditions (fig. 2).

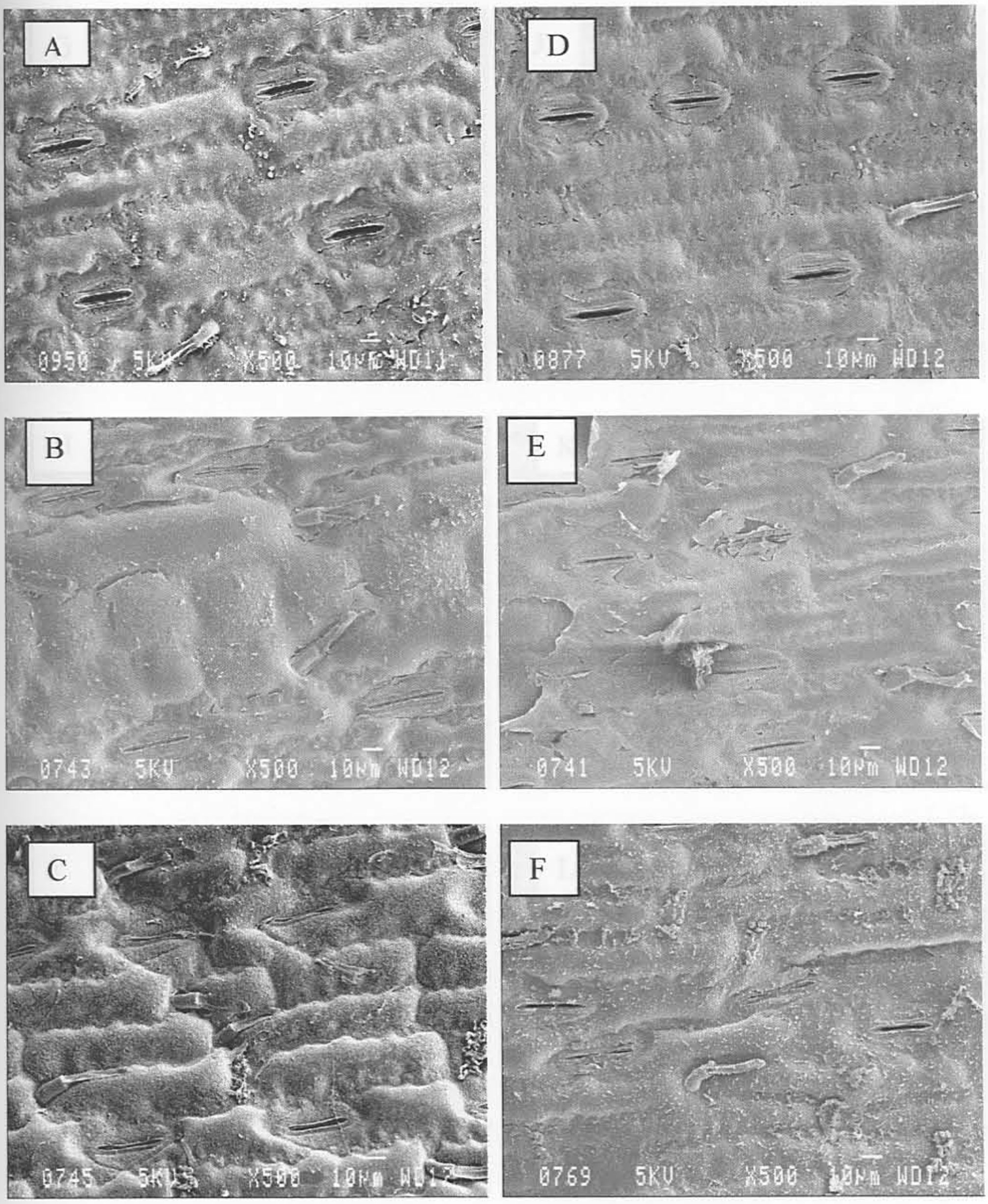

Fig. 3 Scanning electron micrographs of stomata on the adaxial leaf surface of sorghum cultivars (bars 10μm). Note the closure of stomata under water stressed conditions. A, B, C (Jigurti at control, -0.27 MPa, & -0.96 MPa); D, E, F (Meko at control, -0.27 MPa, & -0.96 MPa).

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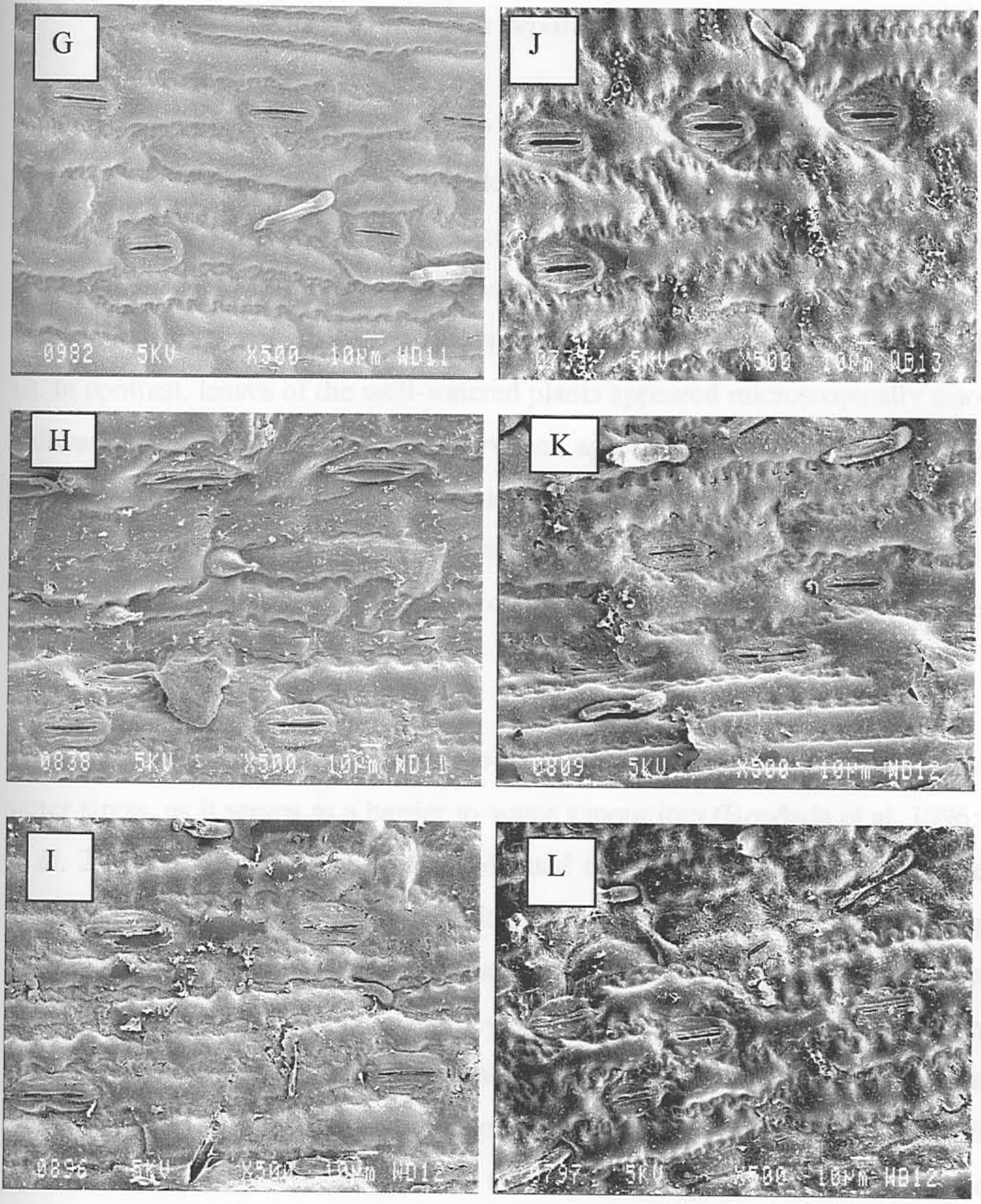

Fig. 3 (Continued). G, H, I (76 T1 #23 at control, -0.27 MPa, & -0.96 MPa); J, K, L (SA1488 at control, -0.27 MPa, & -0.96 MPa).

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. The SEM micrographs reveal that plants grown under well watered and water stressed conditions exhibited different degrees of EW deposition (fig. 4). In all cultivars, leaves from stressed plants showed extensive EW deposition on the adaxial surfaces (fig. 4B, C, E, F, H, I, K, L). In contrast, leaves of the well-watered plants appeared microscopically smooth for all cultivars (fig. 4A, D, G, J). This observation agrees with the findings of Bondada et al. (1996) who reported significant increases in wax concentration in water stressed cotton leaves compared to well-watered plants. Jordan et al. (1983) also found greater EW deposition in water stressed sorghum compared to well-watered plants. Increased wax deposition with drought stress has also been reported in wheat (Johnson et al. 1983) and cotton (Oosterhuis et al. 1991).

The development of EW is known to be advantageous for plants growing under water stress, as it serves as a barrier to water vapour loss (Bondada et al. 1996; Cameron et al. 2002). Cameron et al. (2002) indicated that drought tolerance and increased EW deposition are 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 EW deposition is also associated with reduced cuticular transpiration (Jordan et al. 1983). Blum (1975) as cited by Jordan et al. (1983) also suggested that thick EW layer enhances stomatal control of water loss. Sanchez et al. (2001) indicated that, apart from genetic factors, environmental factors, such as drought, could affect epicuticular wax load, which may explain the observed difference between water stressed and well-watered plants in this study. Thus, the ability to deposit more epicuticular wax could be used as a screening criterion for drought tolerance selection as plants with greater epicuticular wax exhibited higher ability in the retention of tissue water (Jordan et al. 1983).

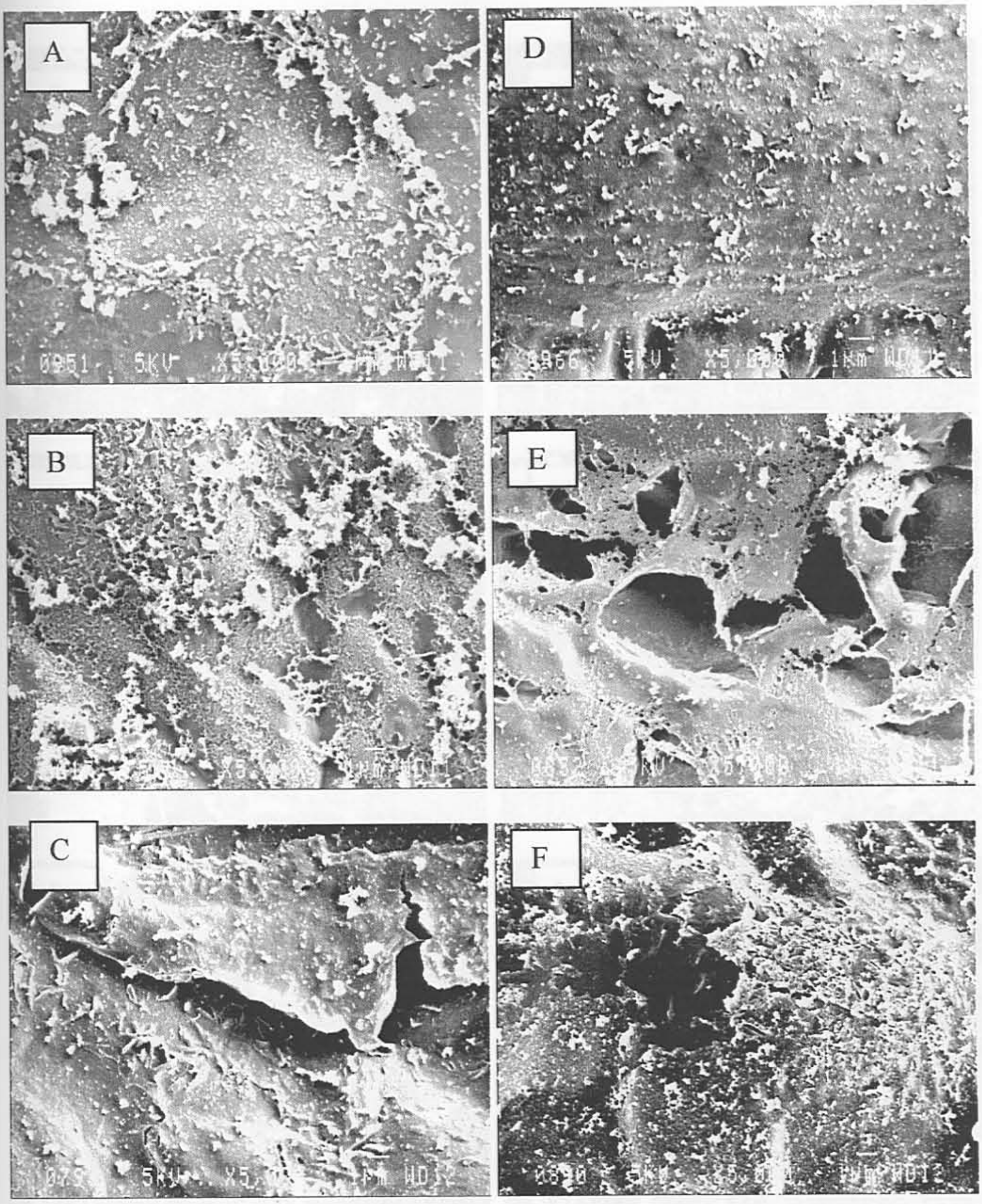

Fig. 4 Scanning electron micrographs showing EW on the adaxial leaf surfaces of sorghum cultivars (bars 1μm). Note the sparse and dense EW deposition on the well watered and water stressed leaves, respectively. A, B, C (Jigurti at control, -0.27 MPa, & -0.96 MPa); D, E, F (Meko at control, -0.27 MPa, & -0.96 MPa).

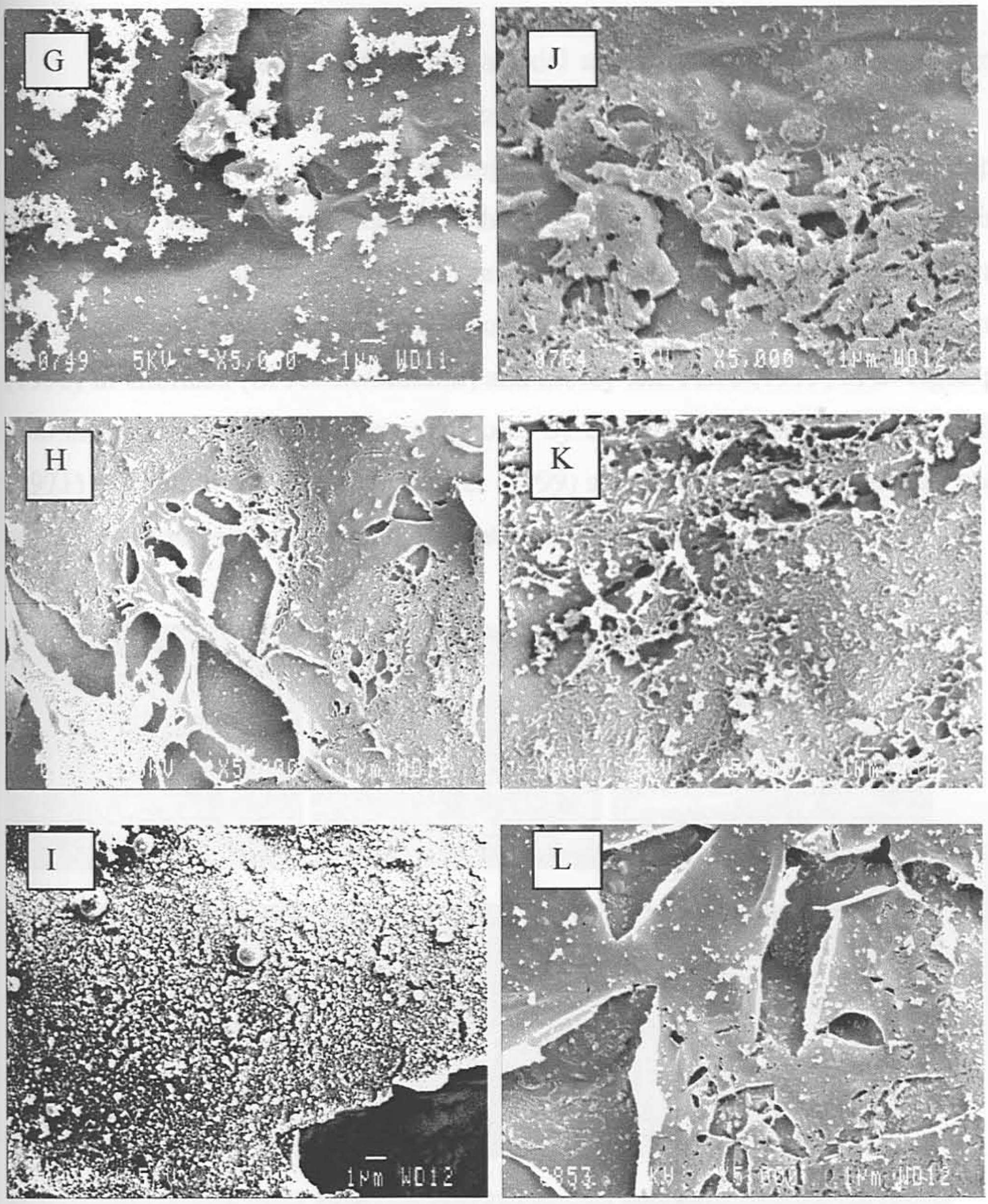

Fig. 4 (Continued). G, H, I (76 T1 #23 at control, -0.27 MPa, & -0.96 MPa); J, K, L (SA1488 at control, -0.27 MPa, & -0.96 MPa).

Epicuticular wax deposition on stomatal apertures

SEM examination of both the adaxial and abaxial leaf surfaces of plants grown under water stress showed partial or complete insulation of stomatal openings with wax deposits (fig. 5). The apparent sealing of the stomatal slit might have impeded the diffusion of water vapour and CO_2 through the stomata. Stomata covered in wax as shown in the bottom panel of fig. 5 are probably dysfunctional. This observation is in agreement with the reports of McWhorter et al. (1990) in sorghum, Ponsamuel et al. (1998) in Gloriosa (*G. rothschildiana*) and Storey and Price (1999) in d' Agen plum fruit who all observed occluded stomatal pores by epicuticular wax deposition. Jeffree et al. (1971) as cited by Jenks and Ashworth (1999) suggested that increased wax occlusion of stomatal openings could increase stomatal resistance to water vapour.

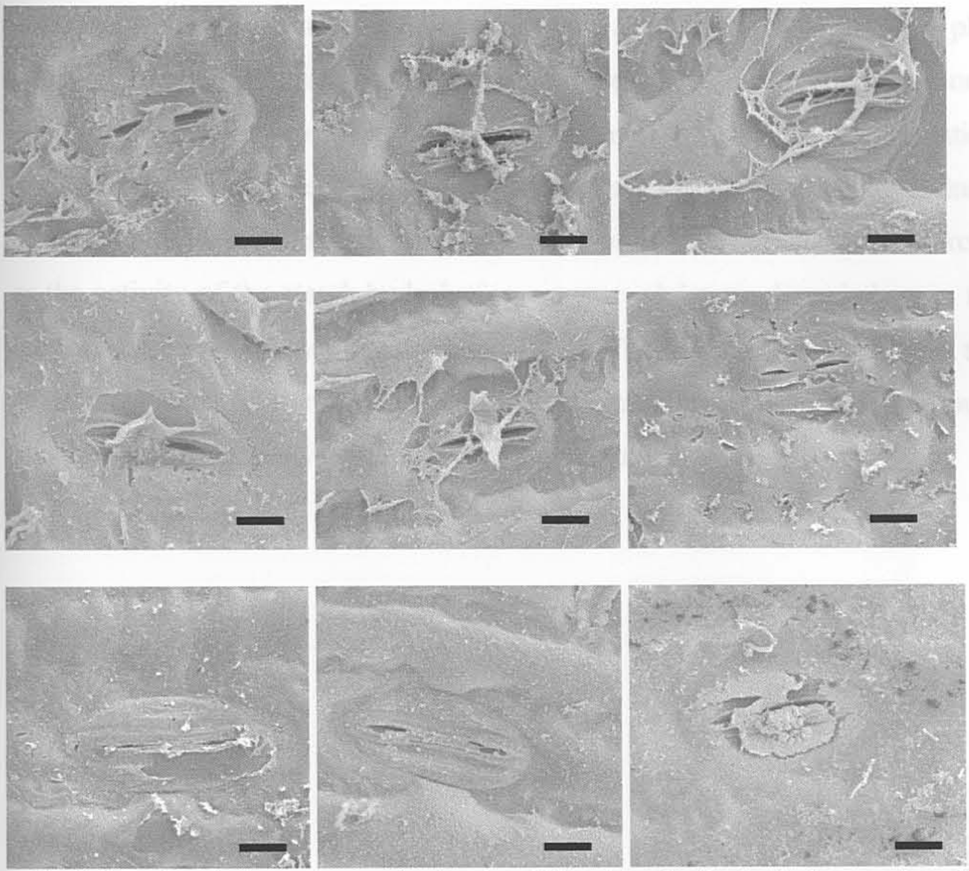

Fig. 5 Scanning electron micrographs showing different levels of epicuticular wax deposition on stomatal openings in 76 T1 #23 under sever water deficit stress (-0.96 MPa) (bars 10µm).

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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. 6). Clear differences in starch deposition between the stressed and unstressed plants were observed in 76 T1 #23 and Meko. In these cultivars plants under severe water deficit (-0.96 MPa) conditions (fig. 6 D and F) showed a marked reduction in the amount of starch grains in the chloroplasts. 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 (fig. 6A and B). SA1488 (tolerant cultivar) deposited more starch grains under water stress compared to the well-watered plants (fig. 6G and H). Giles et al. (1971) also reported a marked reduction in starch deposition in the bundle sheath chloroplasts of stressed sorghum leaves. In the leaf the fixed carbon is temporarily stored in chloroplasts as starch grains during active carbon fixation by photosynthesis (Daie 1996). Considering the low carbon supply under drought conditions, a shift in chemical partitioning of carbon occurs in favor of sucrose accumulation or starch remobilization in the leaf cells of stressed plants (Daie 1996). 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.

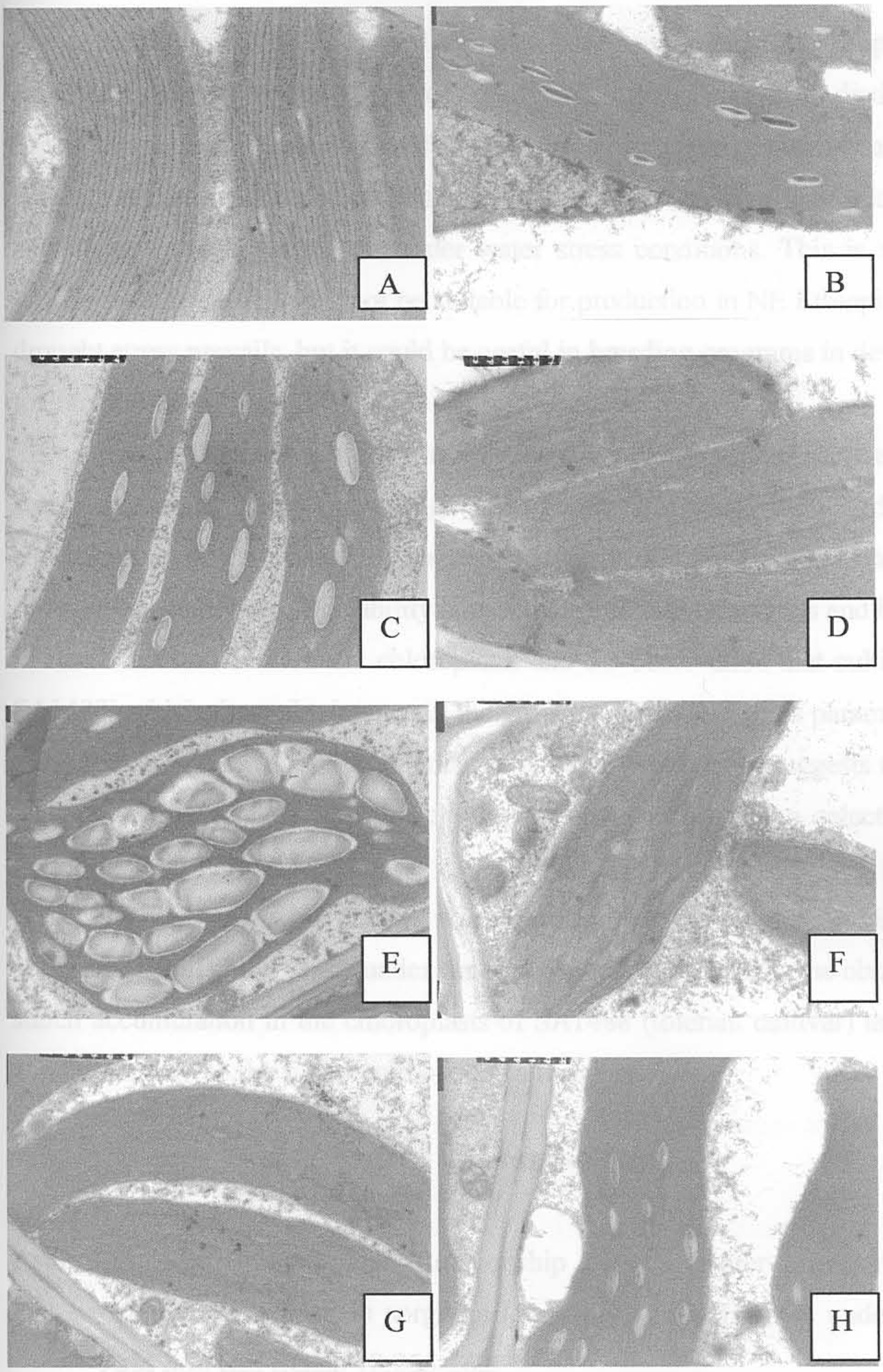

Fig. 6 Starch deposition in unstressed and stressed bundle sheath chloroplasts. A & B = Jigurti unstressed & stressed, C & D = 76 T1 #23 unstressed & stressed, E & F = Meko unstressed & stressed, G & H = SA1488 unstressed & stressed X 13 000.

Conclusion

Water deficit stress caused significant changes in most morphophysiological and anatomical characteristics. The seven cultivars evaluated displayed distinctive responses to water deficit stress for many of the measurements. Jigurti, Gambella 1107 and Meko consistently showed tolerance in most of the parameters. Jigurti showed no reduction in any of the growth parameters under water stress conditions. This is a relatively slow growing genotype and may not be suitable for production in NE Ethiopia where terminal drought stress prevails, but it could be useful in breeding programs in developing drought tolerant cultivars.

The observed variability in epicuticular wax deposition between water deficit stress treatments implies that high epicuticular wax deposition under water deficit conditions could be used as a screening criterion for identifying materials possessing drought tolerance traits. 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.

To our knowledge there is no report in literature indicating an increase in starch accumulation in chloroplasts under water stress conditions. Thus, the observed increase in starch accumulation in the chloroplasts of SA1488 (tolerant cultivar) under water stress conditions may be the first record of such a response.

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