CHAPTER 7

MORPHOLOGICAL STUDY OF THE LEAVES OF FIVE SUB-TROPICAL PERENNIAL GRASS SPECIES

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Abstract

The effect of four levels of water availability on the leaf morphology of five subtropical perennial grasses (*Cenchrus ciliaris,* a *Cynodon* hybrid*, Digitaria eriantha subsp. eriantha, Panicum maximum* and *Pennisetum clandestinum*) was evaluated in a small plot trial under a rain shelter at the University of Pretoria. The four treatments were: soil profile brought to 25 (W1), 50 (W2), 75 (W3) and 100% (W4 - the control) of field capacity on a weekly basis.

C. ciliaris and the *Cynodon* hybrid tended to have a large number of trichomes on the leaf surfaces, while the stomata were well protected from the environment by the veins. This may explain the adaptation of these species to drought conditions. In contrast *P. clandestinum* leaves had few trichomes and the stomata were much more exposed, which may result in poor water use efficiency and in-ability to grow under drought conditions. *P. maximum* also has the characteristics which should

have made it very productive and adaptive to drought conditions, but it did not fare very well under such conditions. *D. eriantha* had more stomata on the abaxial than adaxial leaf surface and the epidermis was covered by large wax crystals, it was however not enough to ensure the yields and water use efficiency of *C. ciliaris* and the *Cynodon* hybrid, although it was better than both *P. maximum* and *P. clandestinum*.

The availability of water and species interaction was significant ($P \leq 0.05$) with *C. ciliaris*, the *Cynodon* hybrid and *P. clandestinum* tending to have more stomata with more water when looking at the adaxial leaf surface, while *C. ciliaris*, the *Cynodon* hybrid and *D. eriantha* also exhibited this tendency on the abaxial surface of leaves. No tendencies were observed for the other species. Overall, it may be concluded that water had very little effect on the number of stomata when compared with species differences, which had a much stronger effect.

Keywords

Cenchrus ciliaris, Cynodon hybrid*, Digitaria eriantha* subsp. *eriantha, Panicum maximum, Pennisetum* clandestinum**,** trichome, stomata, epidermis, wax crystals

7.1 Introduction

In a series of papers by Marais *et al.* (unpublished), it had become apparent that certain grass species were better adapted to drought conditions than others. The reasons for this may be genetic and the genetic make-up of a plant is reflected in its morphology. Of particular importance are the stomata and cuticle layers on the leaf surface, which could affect water loss and thus photosynthesis. Many authors have found not only morphological but also anatomical changes in water stressed plants (Sullivan, 1972; Bleckmann *et al.*, 1980; Traore *et al.*, 1989). According to them, these changes could be linked to better water use and productivity under drought conditions.

In this paper the leaf surface morphology of the five grass species under non water limiting conditions are described, as different levels of water did not have an effect on the morphology of the species. The effect of water availability on the number of stomata was also evaluated.

7.2 Materials and Methods

Five subtropical perennial grasses were established under an automatic rain shelter on the Hatfield Experimental Farm, of the University of Pretoria in Pretoria, (25˚45'S, 28˚16'E), South Africa, during December 1995. The trial ended in June 1998. The five grasses were *Cenchrus ciliaris* cv. Molopo (Blue buffel-grass), a *Cynodon* hybrid cv. K11 (Coach-grass), *Digitaria eriantha subsp. eriantha* cv. Irene (Smuts finger-grass), *Panicum maximum* cv. Gatton (Guinea grass) and *Pennisetum clandestinum* cv. Whittet (Kikuyu grass).

The soil at the site is a Shorrock series of the Hutton form (MacVicar *et al.*, 1991) with 30% clay in the top soil. The A-horizon of the soil is uniform to a depth of 1.2 m, before reaching the B-horizon, which contains coarse gravel. The experimental plots were 2.5 x 2.0 m in size and separated by asbestos plates to a depth of 1.2 m. During June 1995, seeds of *C. ciliaris*, *P. maximum*, *D. eriantha* and *P. clandestinum* were sown in seedling trays and kept in a greenhouse until December 1995. *C. ciliaris, P. maximum* and *D. eriantha,* which are tufted or bunch grasses, were established at a rate of 300 000 plants ha^{-1}, while the creeping grasses (the *Cynodon* hybrid & *P. clandestinum*) were established at 160 000 plants ha^{-1}. The *Cynodon* hybrid was established using vegetative material collected on the experimental farm. The initial germination rate of *D. eriantha* was less than adequate and additional seedlings had to be propagated. This delayed transplanting of this species from trays to the field site by ten weeks compared to the other species.

A neutron probe access tube was located in the centre of each plot. Neutron probe counts in all the plots were taken at nine depth increments, each of 200 mm, on a weekly basis. These counts, which are related to the volumetric water content, were then incorporated into a calibration equation to determine the water deficit for each layer. Just before the onset of each growing season, the soil profiles of all the plots were brought to field capacity. Only then were the plants subjected to four levels of water availability

The water availability levels used were:

W1 - apply 25% of the amount given to W4.

W2 - apply 50% of the amount given to W4

W3 - apply 75% of the amount given to W4

W4 - control, the soil profiles were brought to field capacity on a weekly basis

Water was applied by means of flood irrigation and the amounts of water applied were monitored using water flow meters.

During the establishment season (1995/96), the grasses were not subjected to differential irrigation treatments to ensure a good establishment as it has been found that some of these grasses only start to produce optimally during the second or third year . To ensure a fair comparison of the species, treatments were thus only imposed in the second (1996/97) and third seasons (1997/98).

According to soil analyses, the pH(H_2O) of the experimental soil was neutral. The phosphorus (Bray II) and potassium (Ammonium acetate extractable cations) status in the top soil (30 mg kg^{-1} P; 108 mg kg^{-1} K) was much higher than that of the subsoil (8 mg kg^{-1} P; 67 mg kg^{-1} K). To achieve a non-limiting soil phosphorus and potassium status of 40 mg kg^{-1} P and 150 mg kg^{-1} K, which would ensure that these nutrients were not limiting, annual applications of these nutrients were necessary. As the plots were not grazed, but harvested as hay, N, P and K were lost from the soil and the fertilizer regime was designed to replace these losses. At planting (1995/96 season), the plots received 75 kg N ha^{-1}, 40 kg P ha^{-1} and 200 kg K ha^{-1}. Nitrogen and potassium were also applied to all plots as top dressings during the summer growing season, resulting in a total of 450 kg N ha^{-1} a^{-1}, 40 kg P ha^{-1} a^{-1} and 400 kg K ha^{-1} a^{-1}. In the subsequent seasons (1996/97 and 1997/98), nitrogen and potassium were applied to all plots as top dressings at rates of 225, 338, 394 and 450 kg N ha^{-1} a^{-1} and 200, 300, 350 and 400 kg K ha^{-1} a^{-1} for the W1, W2, W3 and W4 water availability levels respectively. The fertilizers used were limestone ammonium nitrate (LAN) (28.0% N), superphosphate (8.3% P) and potassium chloride (KCl) (50.0% K).

The grasses were harvested at the 10% flowering stage, except for *P. clandestinum*, which was not allowed to grow taller than 40 cm. This resulted in an average of three to four cuts during each season. *C. ciliaris, D. eriantha* and *P. maximum* (tufted grasses) were cut to a height of 10 cm while the *Cynodon* hybrid and *P. clandestinum* (creeping grasses) were cut to 5 cm. A sample plot of one square metre, in the middle of each plot, was harvested, after which the rest of the plot was also cut to the same height. The sample plots were permanently marked to ensure that the samples were taken from the same area at each harvest. The material was dried to constant mass for 48 hours at 65˚C.

During the last harvest in 1998, leaf samples were collected for use in a microscopy study. The oldest, actively growing, leaves were sampled as younger leaves can have a higher stomata density due to cells which have not yet fully expanded (Shearman & Beard, 1972). A sub-sample from the middle of the leaf blade was taken to describe the leaf surface morphology of the five grass species and for evaluating the effect of water availability on stomata number and appearance. The sub-samples were always taken from the middle of the leaf blade , for all the treatment combinations, as it has been found that the morphology may differ on different parts of the same leaf blade (Pazourek, 1969; Shearman & Beard, 1972). A JEOL, JSM840 scanning microscope was used to study the leaf surfaces. Before the leaf samples could, however, be placed into the scanning microscope, they had to be prepared according to the method set out by Hayat (1981). During this preparation, the leaf materials were fixed and dried under controlled conditions before being placed on a button and coated with a thin layer of gold.

A fully randomized block design with three replications was used. The statistical analysis was done using the Statistical Analysis System (SAS, 1996). Tukey's least significant difference at the 5% level of probability was used to determine significant differences between treatment means. Relevant statistical analysis data is presented in the Appendix (Tables A7.1 - A7.4).

7.3 Results

7.3.1 Terminology

Before the presentation of results, it is meaning-full to define the terminology used in this paper.

The leaf can be divided into two zones. The costal zone above the veins and the intercostal zone between veins (Metcalfe, 1960). Two types of epidermis cells are present namely long and short cells, where long cells are horizontally elongated and vertically narrow, while short cells are almost the same size horizontally and vertically.

Short cells often contain silica and/or cork bodies (Metcalfe, 1960; Prat, 1967; Dahlgren & Clifford, 1982; Fahn, 1982) and can be exodermic, bearing salient prolongations *it est* hooks, spikes, hairs, setae, spines or papillae (Prat, 1967). The epidermis layer of some species may have no short cells (Prat, 1967). In a surface view the silica cells can have a circular, elliptic, dumb-bell, acutely angled, cross shaped, crenate or saddle shape (Figure 7.1).

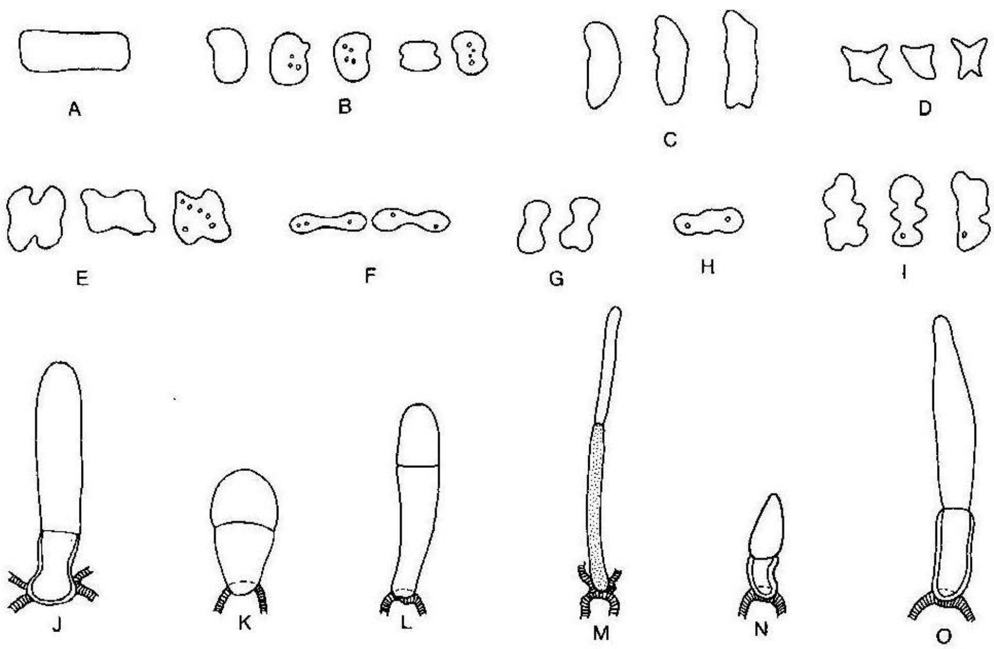

Figure 7.1 Illustration of silica bodies and (A - I) and microhairs (J - O) generally found on the epidermis of grasses.

Silica bodies: A - rectangular, B - saddle shaped, C - large narrow, D - acutely angled, E - cross shaped, F - dumbbell shaped, G - transversely dumbbell shaped, H - crenate, I - transversely crenate.

Microhairs: J - L with inflated distal cell, M - O with non-inflated distal cell. (Dahlgren *et al.*, 1985)

The waxy layer above the epidermis of the leaf forms part of the cuticle (Coombe & Bell, 1965). The wax can be in the form of granules, rods or scales and restricts the movement of water into or out of the leaf. Epicuticular wax structures can have different patterns and differ between species, age of leaf, etc. (Traore, *et al.*, 1989; Webb & Almeida, 1990; Balok & St. Hilaire, 2002).

The stomata are situated in the epidermis layer of the leaf and consist of two guard cells and an opening (cleft or pore) between them (Coombe & Bell, 1965; Fahn, 1982). Stomata, together with subsidiary or accessory cells which border the guard cells, are called the stomatal apparatus or the stomatal complex (Paliwal, 1969). The guard cells can be flush with the interstomatal cells or overlap them (Watson & Johnston, 1978).

In the grasses, the stomata are most often found in intercostal zones. In dry conditions they might also be restricted to the sides or bases of furrows and grooves on the leaf surface (Metcalfe, 1960). Sometimes the grooves can be protected by prickles, or stomata can be covered by overlaying papillae. The stomata are usually in well defined horizontal bands, but Shearman and Beard (1972) noted that the stomata were in parallel rows on the adaxial side of *Agrostis* species, while scattered throughout the abaxial surface.

The guard cells may be level, sunken or raised relative to the other epidermal cells. The guard cells, of members of the Poaceae, are usually dumb-bell shaped in comparison to the kidney shaped guard cells of most other plants. Stomata are distributed more or less equal distances from each other and are specific to the species and leaf side. Different shapes of subsidiary cells are to be found and can differ not only between species but even on a single leaf blade. In the current study the stomata were not classified according to the shape of the subsidiary cells, since it can be so variable.

The number of stomata, per square millimetre of leaf surface, differs among plants (24 for *Avena sativa* and *Triticum sativum* to 1 198 for *Quercus lyrata*), between the adaxial and abaxial side of the leaf (Gray, 1881; Coombe & Bell, 1965; Martin & Juniper, 1970; Fahn, 1982, Gardner *et al.*, 1990; Balok & St Hilaire, 2002) as well as between cultivars of the same species (Shearman & Beard, 1972; Joubert, 1981; Traore, *et al.*, 1989). External factors such as light (Pazourek, 1970; Onwueme & Johnston, 2000), temperature (Bleckmann *et al.*, 1980), water (Shearman & Beard, 1972; Dreyer & Human, 1974; Joubert, 1981; Balok & St. Hilaire, 2002), cultivation practices (Teare *et al.*, 1971; Dreyer & Human, 1974) etc., can also have an influence on the number of stomata per unit area.

Trichomes are classified as all the uni- and multicellular appendages of the epidermis (Fahn, 1982). Trichomes are widely used to distinguish between plant families and genera. There are several shapes of trichomes and in grasses they are classified as macro-hairs, micro-hairs, prickle hairs and papillae (Metcalfe, 1960). A trichome consists of a foot (the part inserted in the epidermis) and body (the part projecting from the epidermis).

Macro-hairs are usually unicellular and can easily be seen with the naked eye. When the foot of a macro hair is surrounded by epidermal cells which are large and inflated and raised above the surface level it is called a cushion hair. Macro-hairs can often be confused with prickles. Macro-hairs are to be found in the intercostal zones or are common over the veins or at the leaf margins. Intercostal macro-hairs are often cushion hairs or have a sunken base/foot.

Micro-hairs are almost always two celled. The distal cell is usually thin walled and can be damaged or destroyed easily, while the basal cell is more durable due to a thicker cell wall. It is also believed that the distal cell could be filled with material that is secreted and therefore looks damaged or is totally absent. The micro-hairs can not be seen with the naked eye. The micro-hairs occur in the stomatal bands, or in the intercostal zones between the stomatal bands and veins.

Prickle hairs are robust, sharply but shortly pointed structures with swollen bases. Associated with short cells, they arise directly from the epidermis and the swollen bases form an integral part of the epidermis. The sharp point is usually directed towards the apex of the leaf, but exceptions can occur. They have thick lignified walls. Two sizes of prickle hairs, namely large size or prickles and small size or hooks, can be recognized. The latter have a more rounded base which can best be seen when studied from above. None, one or both types can be found on the leaves. The prickles/hooks can be found above or between veins with prickles above the veins and hooks between them. Angular, strongly pointed prickles are common at leaf margins.

Papillae are variously shaped protrusions from the outer walls of epidermal cells. They are dome shaped structures over veins but are found most often in the intercostal zone. There can be one or more papillae per long cell. The papillae of the epidermis next to a stomata, often overarch to protect the stomatal pores.

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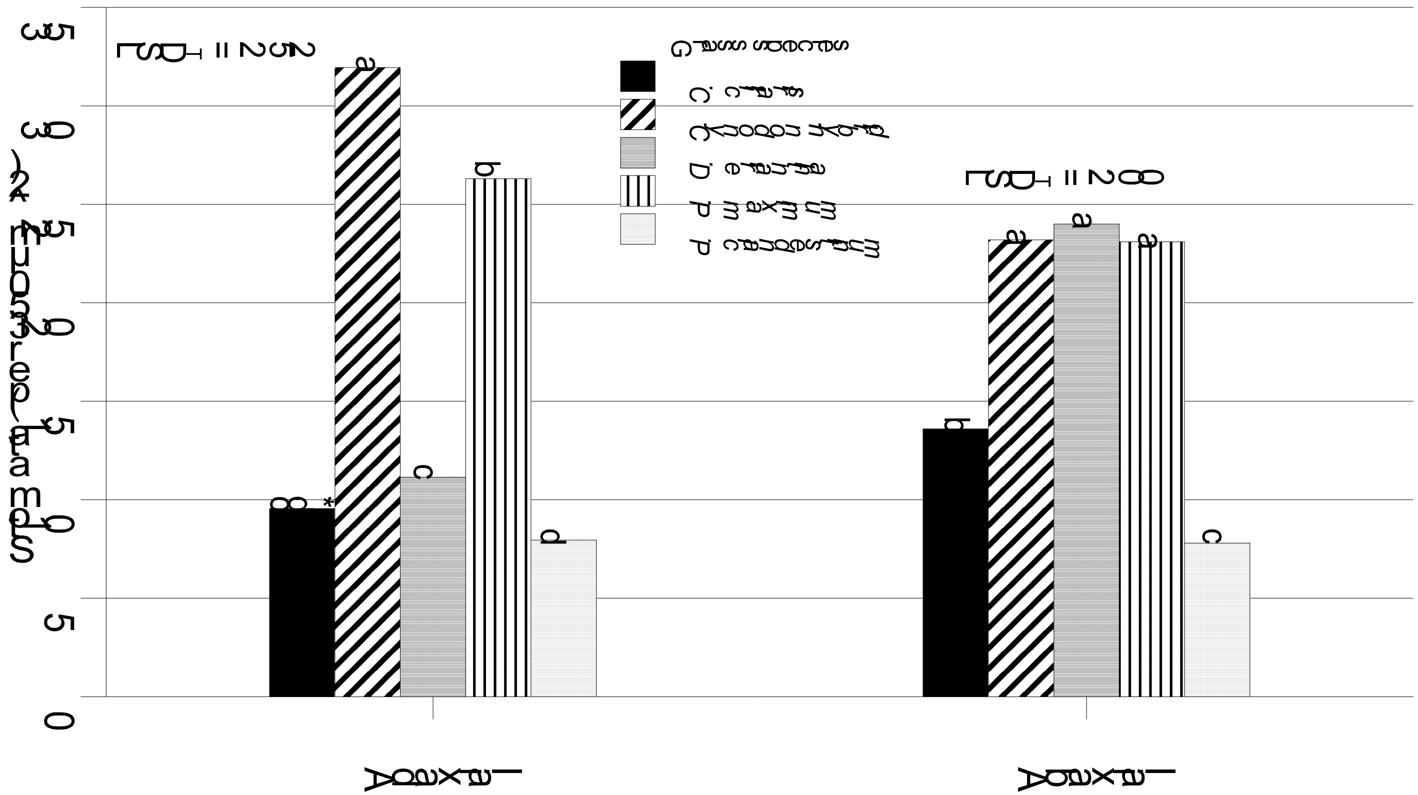
7.3.7 Number of stomata

On average, the *Cynodon* hybrid and *P. maximum* tended to have the highest number of stomata in total (adaxial and abaxial), followed by *D. eriantha* and *C. ciliaris* with *P. clandestinum* having the least number of stomata (Figure 7.42). When the adaxial and abaxial sides of the leaves of the different species are compared, the *Cynodon* hybrid tended to have significantly ($P \leq 0.05$) the highest number of stomata on the adaxial surface. *P. maximum* had the second highest number, followed by *D. eriantha*. *D. eriantha*, however, had 15 to 20 stomata per unit area less than *P. maximum* and the *Cynodon* hybrid. There was not much of a difference in number of stomata on the adaxial surface between *C. ciliaris*, *D. eriantha* and *P. clandestinum*, but *P. clandestinum* still had the lowest number and had significantly ($P \leq 0.05$) less than *D. eriantha* (Figure 7.42).

On the abaxial surface, *D. eriantha* tended to have the highest number of stomata, but it did not differ significantly ($P \geq 0.05$) from either the *Cynodon* hybrid or *P. maximum*. *C. ciliaris* had about 10 stomata per unit area less than this group, with *P. clandestinum* having an even lower number of stomata, and significantly ($P \leq 0.05$) so (Figure 7.42). Despite the low number of stomata in *P. clandestinum*, this species had the biggest stomata (36 - 41 µm) followed by *C. ciliaris* (30 - 32 µm), and *D. eriantha* (25 - 27 µm). The two species with the largest number of stomata, the *Cynodon* hybrid and *P. maximum* had correspondingly the smallest stomata, of 17 - 19 µm and 17 - 24 µm respectively.



*Species with the same letter do not differ significantly from each other (P < 0.05)

Figure 7.24 Number of stomata on the adaxial and abaxial leaf surface of five grass species without water stress

There was no significant ($P \geq 0.05$) main effect of water availability on the number of stomata on the adaxial surfaces of the leaves of the five grass species (Table 7.1). The only significant ($P \leq 0.05$) differences were recorded between the abaxial leaf surfaces of W2 and W4 plants (Table 7.1), with W4 plants having significantly ($P \leq 0.05$) more stomata than the W2 plants.

Table 7.1 Effect of the availability of water on the average number of stomata on the adaxial and abaxial leaf surfaces of five grass species.

	W1*	W2	W3	W4
Adaxial surface	17 a[#]	18 a	17 a	19 a
Abaxial surface	19 ab	17 b	18 ab	20 a

* W1 - severe water limiting conditions, W4 - control.

[#] Values in a row with the same alphabetical letter, do not differ significantly from each other ($P \leq 0.05$).

There was, however, a significant ($P \leq 0.05$) species x water availability interaction (Figures 43 and 44). While *C. ciliaris*, the *Cynodon* hybrid and *P. clandestinum* tended to have an increased number of stomata on the adaxial leaf surfaces with an increase in the amount of water available (Figure 43), there was no clear tendency for *D. eriantha* and *P. maximum*.

On the abaxial leaf surfaces, *C. ciliaris*, the *Cynodon* hybrid and *D. eriantha* tended to have a higher number of stomata with an increase in the amount of water available (Figure 44). For *P. maximum* and *P. clandestinum* there was no clear tendency on the abaxial leaf surface.

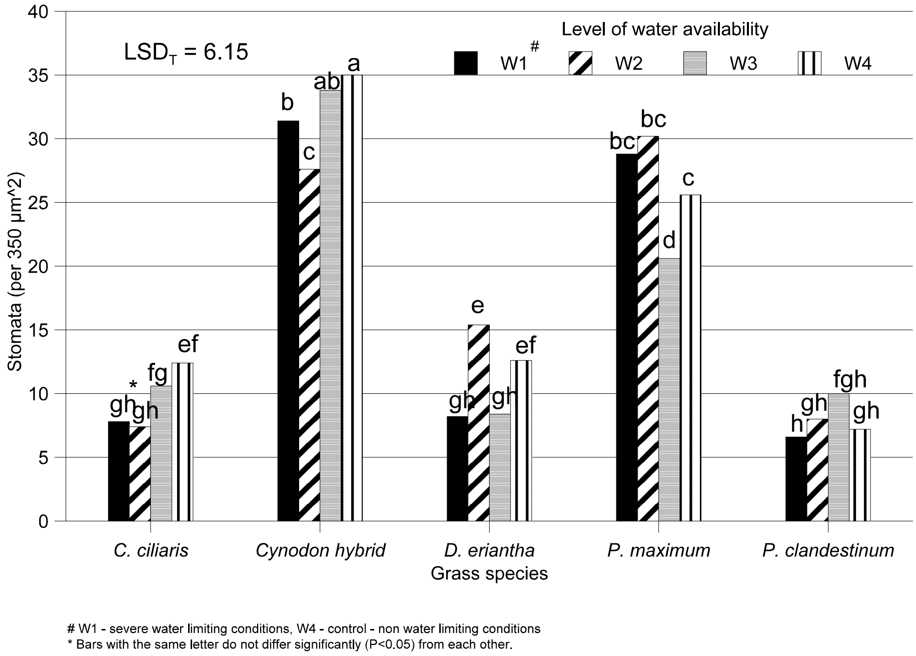


Figure 7.43 Number of stomata on the adaxial leaf surface of five grass species as influenced by water availability.

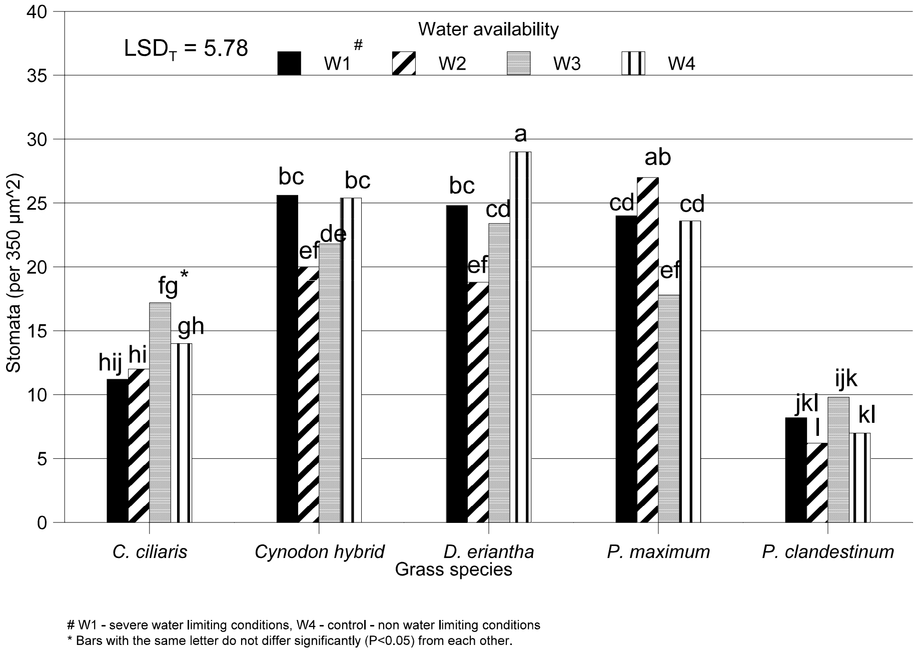


Figure 7.44 Number of stomata on the abaxial leaf surface of five grass species as influenced by water availability.

7.4 Discussion and Conclusions

From the leaf surface descriptions of the grasses the following important features, in terms of adaptations that may reduce water loss, were noted:

Both leaf surfaces (adaxial and abaxial) of *C. ciliaris*, the *Cynodon* hybrid and *D. eriantha* and the adaxial leaf surface of *P. maximum* tended to have a wax deposit. In the case of the *Cynodon* hybrid and *D. eriantha* it even covered the stomatal openings. The wax layer did then not only restrict water loss from the epidermal cells, but also from the main structures for water loss, namely the stomata. This could explain the good water use of the *Cynodon* hybrid.

In the *Cynodon* hybrid some of the papillae sculpted over an adjacent stoma, further restricting excessive water loss from the stomatal aperture. In *D. eriantha* (especially abaxial) and *C. ciliaris* (adaxial and more so abaxial) the stomata are located in deep grooves between the veins, or tucked away below the veins to create the same restriction barrier for water loss as the papillae in the *Cynodon* hybrid. The stomata of *P. maximum* and *P. clandestinum* were not as protected as in the previous three grass species. The stomata were, however, a little lower than the surrounding epidermis cells, which could also be seen as a type of protection. This was, unfortunately not enough, especially in the case of *P. maximum*, and resulted in disappointing yields and water use efficiency values.

Another strategy to combat excessive water loss from the leaves, is to reduce the number, and/or size, of the stomatal aperture on the leaf as a whole or on one side of the leaf. On the adaxial leaf surfaces the *Cynodon* hybrid had significantly ($P \leq 0.05$) the highest number of stoma, followed by *P. maximum*. The wax layer and protecting papillae might explain why the *Cynodon* hybrid had better yields and water use efficiency values than *P. maximum* despite this high potential water loss due to the number of stomata. The same holds true for the lower side where there was no significant ($P \geq 0.05$) difference in the number of stoma for the *Cynodon* hybrid, *D. eriantha* and *P. maximum*. Here not only the *Cynodon* hybrid, but also *D. eriantha* had extra protection against water loss, which *P. maximum* did not have.

P. clandestinum had significantly ($P \leq 0.05$) the lowest number of stomata on both leaf surfaces, followed by *C. ciliaris*. This might be the way, or additional way for *C. ciliaris*, in which these two species combat excessive water loss, despite the bigger size of the individual stoma.

Plant species adapted to dry conditions often have fewer stoma on the adaxial than abaxial side of the leaf. If that holds true, it can explain why it is reflected in *C. ciliaris* and *D. eriantha*. The *Cynodon* hybrid and *P. maximum* had more stomata on the adaxial than abaxial leaf surface and can explain why the stomata are that much smaller and are adapted in other ways to combat water loss. *P. clandestinum* had almost the same number of stomata on either side of the leaf.

Shearman & Beard (1972), Van de Roovaart & Fuller (1935) and others noted less stomata per area for well watered than for water stressed grass plants. This can be explained by the larger size of the epidermis cells under optimal conditions resulting in a greater distance between stomata and a lower stomatal frequency as compared to plants growing under adverse conditions. This was not the case in this trial. Some of the grasses (*C. ciliaris*, the *Cynodon* hybrid and *P. clandestinum* adaxial and *C. ciliaris*, the *Cynodon* hybrid and *D. eriantha* abaxial) tended to have less stomata with less water available. The other species had no clear tendency.

Although the number of trichomes per leaf area was not determined, *P. clandestinum* clearly had the least trichomes. The *Cynodon* hybrid and *P. maximum* leaves were densely covered with macrohairs, while *C. ciliaris* and *D. eriantha* were covered by prickles and hooks. Traore, *et al.*, (1989) concluded that plants with a higher trichome density are better adapted to stressful environments than their hairless counterparts. Trichomes increase the leaf boundary layer resistance to air flow and increase radiation reflectance. As with stomata number, the number of trichomes per leaf, and between species (Balok & St. Hilaire, 2002) and cultivars can also differ. The low number of trichomes might explain why *P. clandestinum* did not do as well under drought conditions as the other grass species, but cannot fully explain the poor performance of *P. maximum*.

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7.3.2 Cenchrus ciliaris

There were no significant differences in the leaf surface appearance at the different levels of water availability, and it was, therefore, decided only to describe the leaf surfaces of the control (W4) plants. Photos of the leaves as affected by the other levels of water availability are included in the appendix (Figures A7.1 - A7.5). There were, however, differences between species.

The adaxial leaf surface of *C. ciliaris* consists of well defined costal (over vein) and intercostal (between veins) zones (Figures 7.2 (x50)and 7.3 (x300)). At the summit of the costal zone a single row of prickles can be found with two rows of prickles at the lower edges of the veins. Numerous hooks can be found in the intercostal zone. Stomata can also be found in the same area as the hooks, and sometimes the hooks and stomata alternate with each other. At the summit of the veins, silica bodies in the shape of dumbbell, cross shaped or nodular forms can be found alongside and between the prickles. Micro-hairs with two distinct cells are situated closely beside the vein. The distal cells of the micro-hairs have collapsed as has been found in many grass species (Metcalfe, 1960). No macro-hairs were present.

In a closer view (x2500) (Figure 7.4) one can clearly see wax deposits on the surface in the form of rods. In some instances the wax crystals have merged to form a solid structure. In this view one can also clearly see the dumbbell shaped guard cells with two subsidiaries, one at each side of the guard cells of the stoma. The leaf edge consists of a single row of prickles (Figure 7.5). The points of these prickles are rather blunt in comparison to those on the abaxial side of the leaf (Figure 7.9).

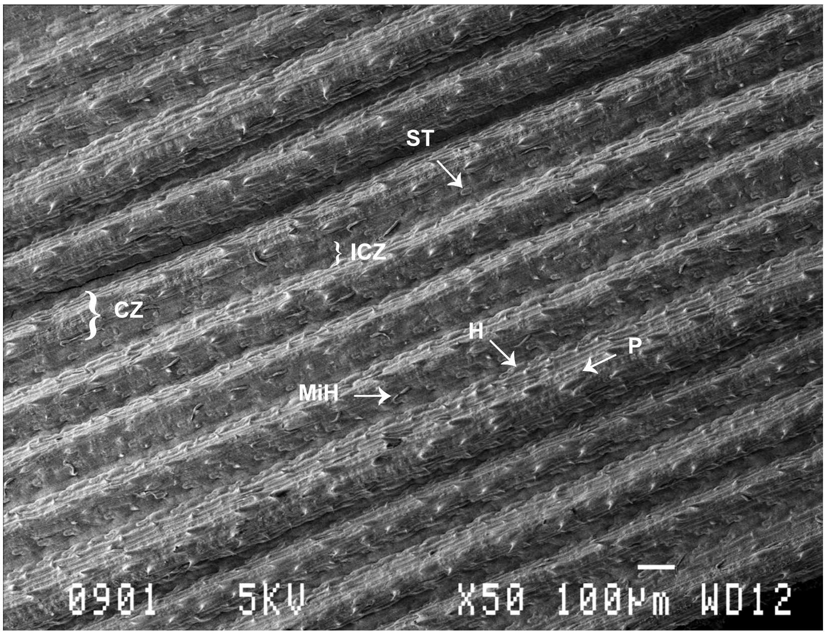

Figure 7.2 Surface of the adaxial side of a *C. ciliaris* leaf grown without any water stress (W4). MiH -Micro-hair, CZ - Costal zone, ST - Stoma, H - Hook, ICZ -Intercostal zone, P -Prickle.

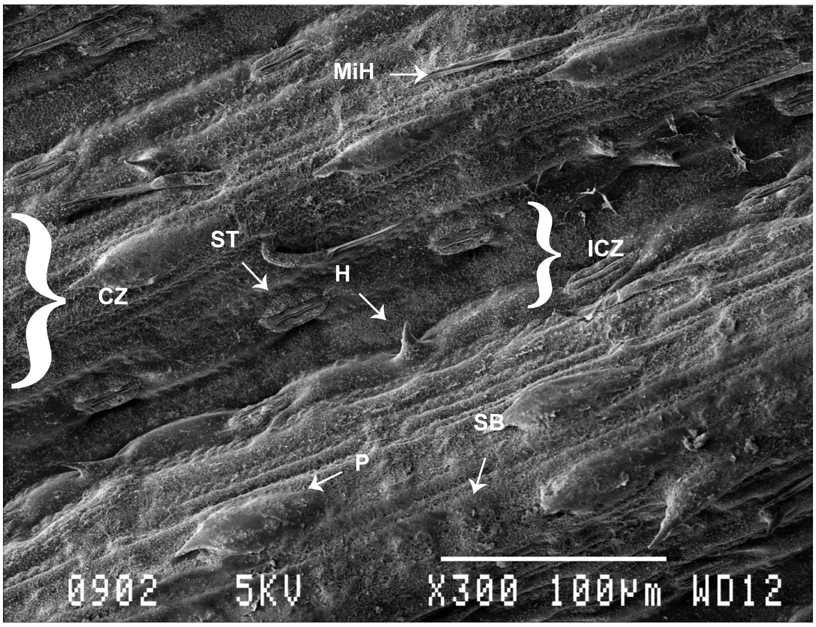

Figure 7.3 Surface of the adaxial side of a *C. ciliaris* leaf grown without any water stress (W4). MiH - Micro-hair, CZ - Costal zone, ST - Stoma, H - Hook, ICZ -Intercostal zone, P -Prickle, SB - Silica body.

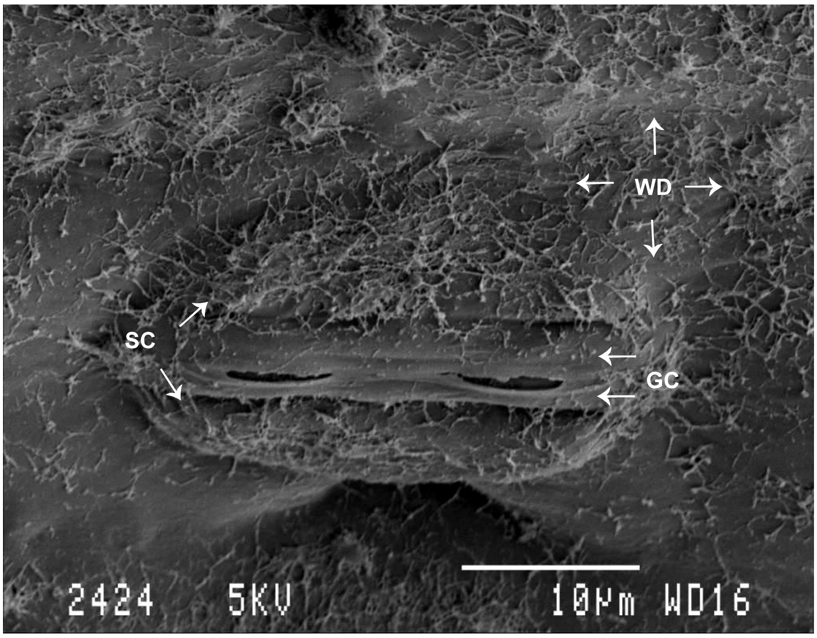

Figure 7.4 A stoma found on the adaxial side of a *C. ciliaris* leaf grown without any water stress (W4) (x2500). GC - Guard cell, SC - Subsidiary cell, WD - Wax deposit.

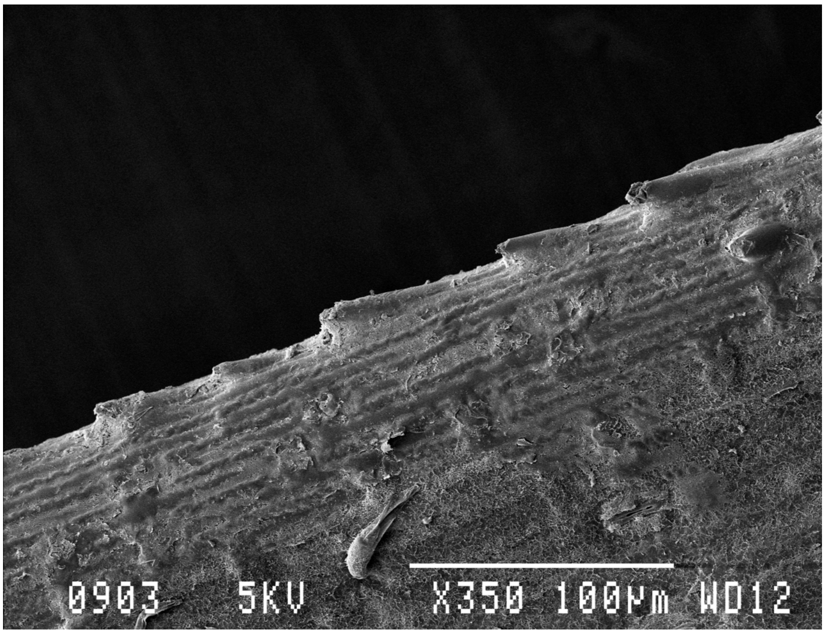

Figure 7.5 Leaf edge of the adaxial side of a *C. ciliaris* leaf grown without any water stress (W4).

On the abaxial side of the leaf surface of *C. ciliaris*, the costal and intercostal zones are much more pronounced (Figures 7.6 and 7.7). The micro-hairs are closely situated beside the veins with prickles at the summit and edges of the vein and numerous hooks between the veins. Once again no macro-hairs were found. The silica bodies are similar to those found on the adaxial leaf surface. The stomata are to be found in the valley between the veins and are similar to the ones found on the adaxial side (Figure 7.8). The leaf edge consists out of three rows of pointed prickles (Figure 7.9).

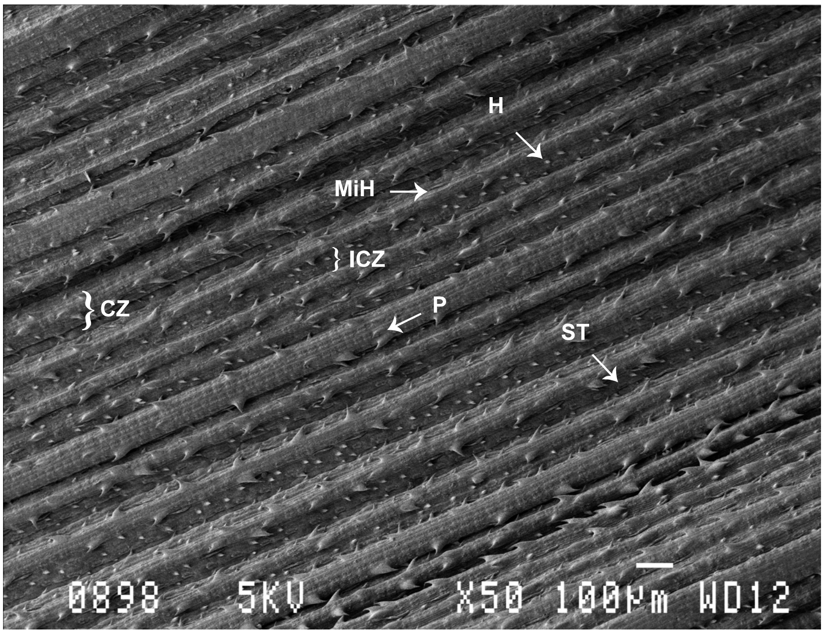

Figure 7.6 Abaxial Surface of a *C. ciliaris* leaf grown without any water stress (W4). MiH - Micro-hair, CZ - Costal zone, ST - Stoma, H - Hook, ICZ - Intercostal zone, P - Prickle.

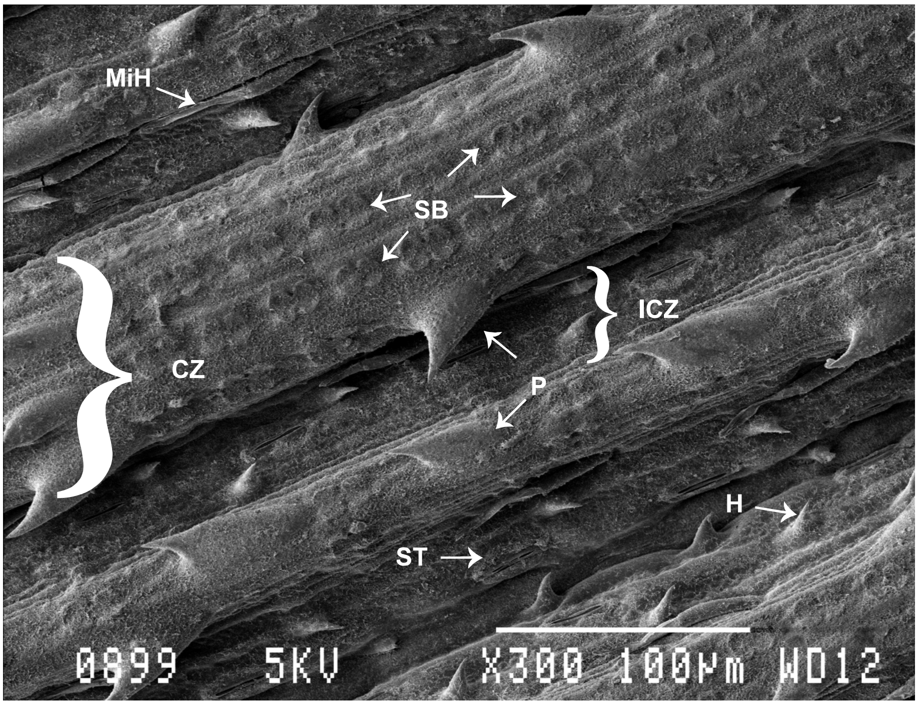

Figure 7.7 Abaxial surface of a *C. ciliaris* leaf grown without any water stress (W4). MiH - Micro-hair, CZ - Costal zone, ST -Stoma, H - Hook, ICZ - Intercostal zone, P - Prickle, SB - Silica body.

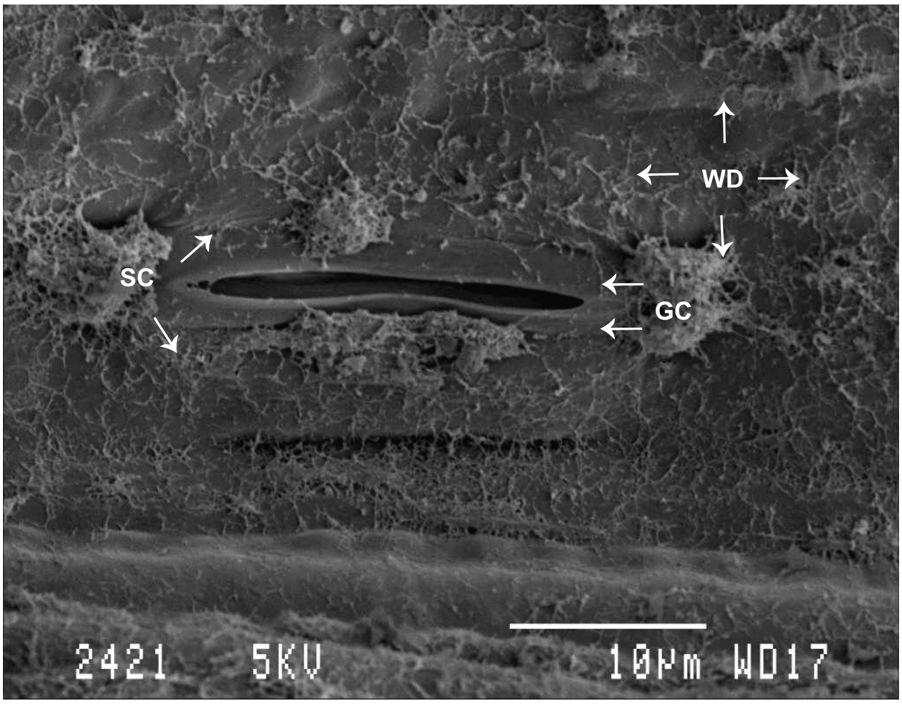

Figure 7.8 A stoma found on the abaxial side of a *C. ciliaris* leaf grown without any water stress (W4) (x2500). GC - Guard cell, SC - Subsidiary cell, WD - Wax deposit.

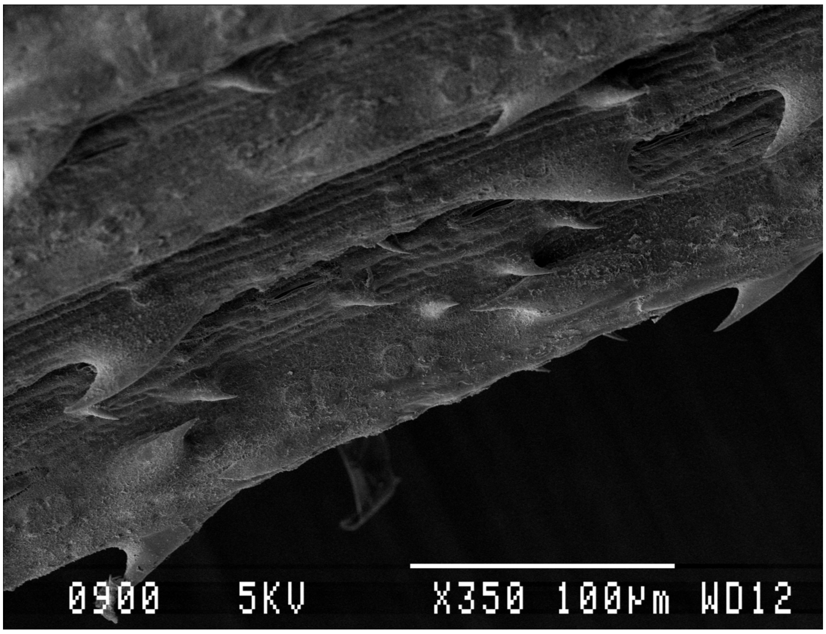

Figure 7.9 Leaf edge of the abaxial side of a *C. ciliaris* leaf grown without any water stress (W4).

7.3.3 Cynodon hybrid

The adaxial side of the *Cynodon* hybrid leaf has fairly long and numerous macro-hairs with sunken bases (foots) in the intercostal zone and prickles spread throughout the surface (Figure 7.10). Micro-hairs with inflated distal cells, sunken into pits can be found in the intercostal zone. The prickles are found in rows, but are further apart than those of *C. ciliaris*, with some of the prickles appearing in pairs. The costal and intercostal zones are not as pronounced (Figure 7.11) as found for *C. ciliaris* (Figure 7.3). In this closer view (Figure 7.11) numerous nodule like papillae can also be observed.

As with the leaf surface of *C. ciliaris*, the leaf surfaces of the *Cynodon* hybrid leaves are covered by a rod like wax layer. The rods sometimes merge to form a solid structure which protrudes over or even covers some of the stomata (Figures 7.12). The stomata are flush with, or sometimes lower than, the surrounding epidermis cells and papillae sculpting over a stoma will sometimes be found (Figures 7.11 and 7.12). The leaf edge consists out of a single row of sharp pointed prickles (Figure 7.13).

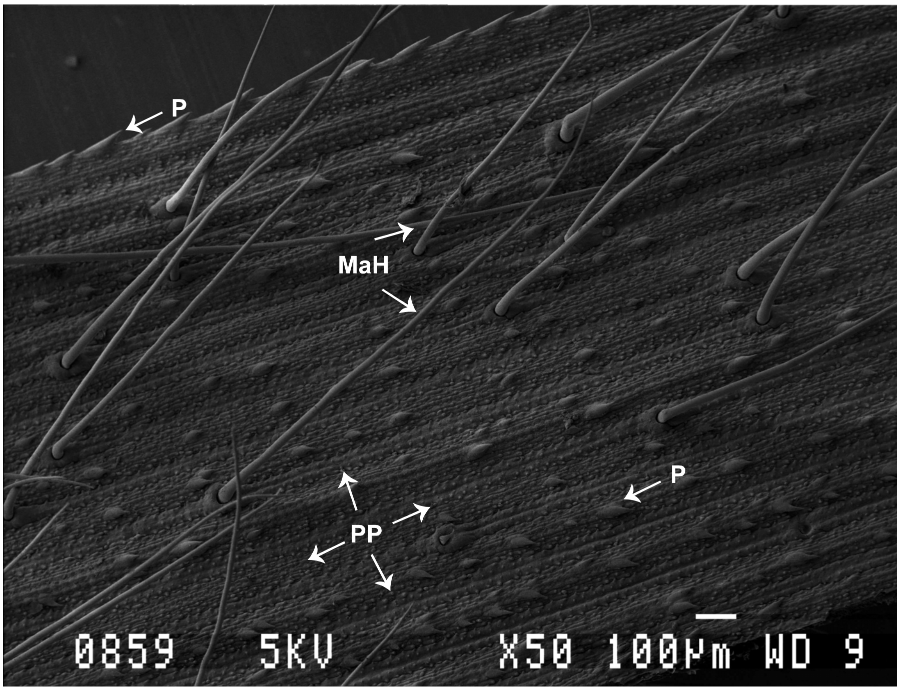

Figure 7.10 Adaxial surface of a *Cynodon* hybrid leaf grown without any water stress (W4). MaH - Macro-hair, P - Prickle. PP - Papillae.

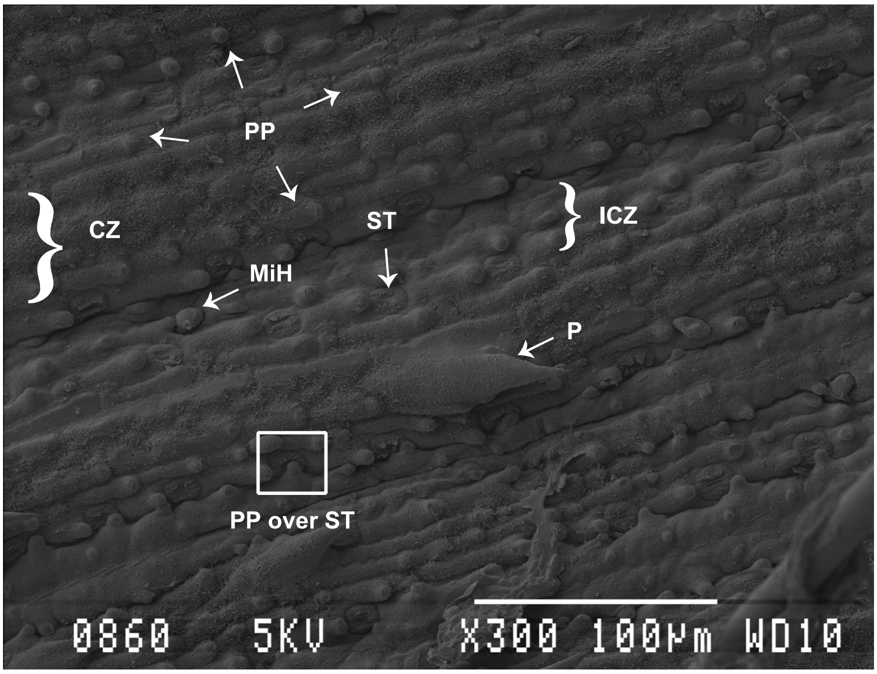

Figure 7.11 Adaxial surface of a *Cynodon* hybrid leaf grown without any water stress (W4). CZ - costal zone, ICZ - Intercostal zone, ST - Stoma, PP - Papillae, P -Prickle, MiH - Micro-hair, PP over ST - Papillae overlying a stoma.

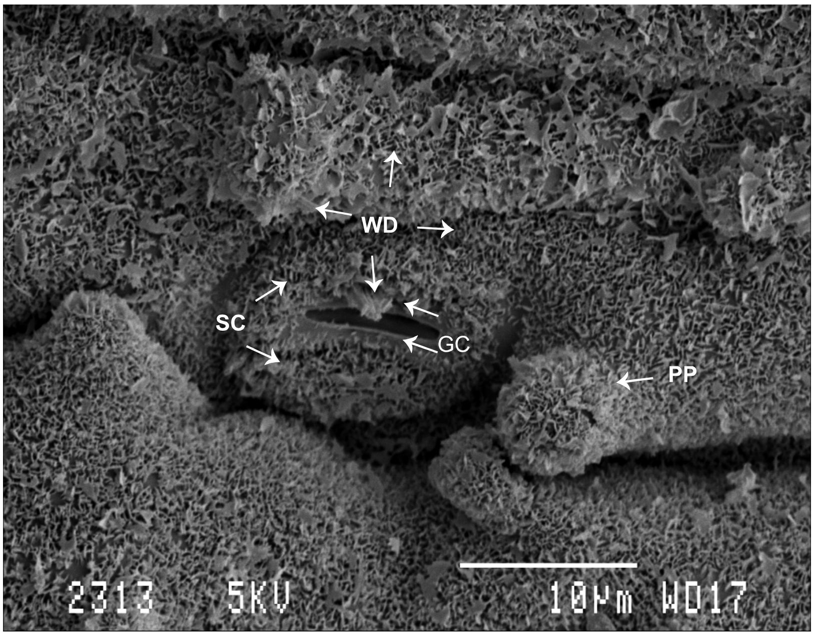

Figure 7.12 A stoma found on the adaxial side of a *Cynodon* hybrid leaf grown without any water stress (W4) (x2500). GC - Guard cell, SC - Subsidiary cell, WD - Wax deposit, PP - Papillae.

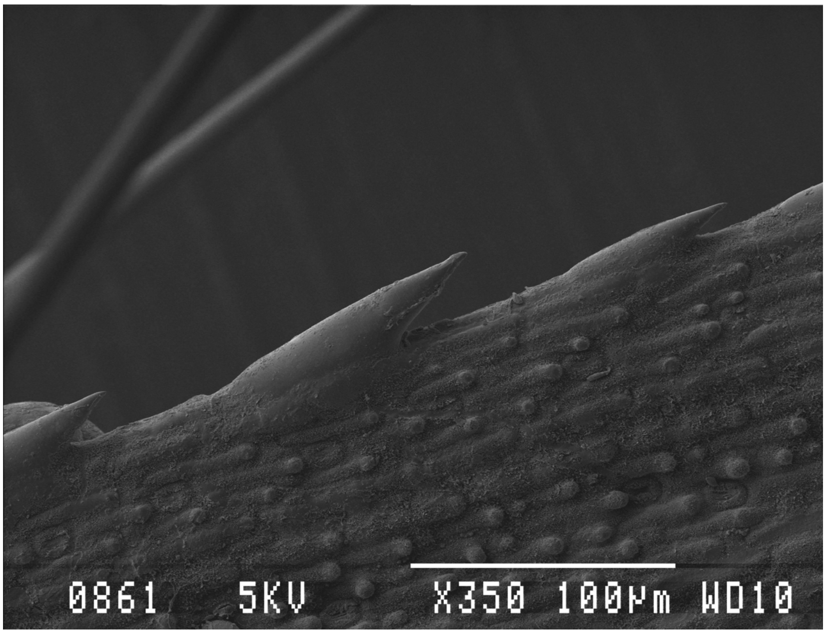

Figure 7.13 Leaf edge of the adaxial side of a *Cynodon* hybrid leaf grown without any water stress (W4).

Compared to the adaxial side (Figure 7.10) of a *Cynodon* hybrid leaf, the macro-hairs are more numerous on the abaxial side (Figure 7.14), but no prickles were observed at this magnification (x50). The macro-hairs on the abaxial side (Figure 7.14) are also shorter than those on the adaxial side (Figure 7.10), but are further the same. At higher magnification (x300) (Figure 7.15), still no prickles were observed, but micro-hairs of the same sort found on die adaxial side were observed. The leaf surface was again covered by a wax deposit with some of the wax crystals protruding over the stomatal openings (Figure 7.16). Some of the papillae adjacent to a stoma, also protrude over the stoma (Figure 7.16), as was found on the adaxial side of the *Cynodon* hybrid leaf. A single row of pointed prickles were to be found on the leaf edge (Figure 7.17).

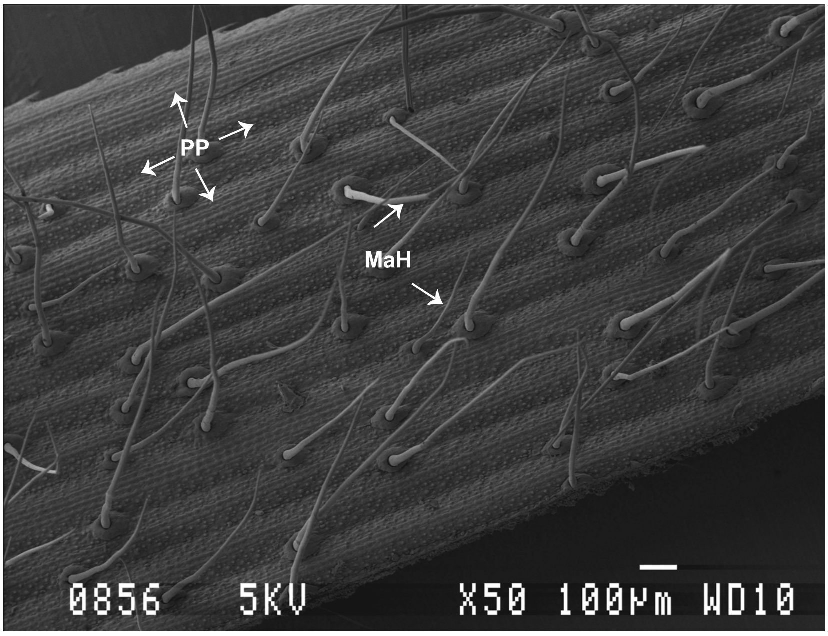

Figure 7.14 Abaxial surface of a *Cynodon* hybrid leaf grown without any water stress (W4). MaH - Macro-hair, PP - Papillae.

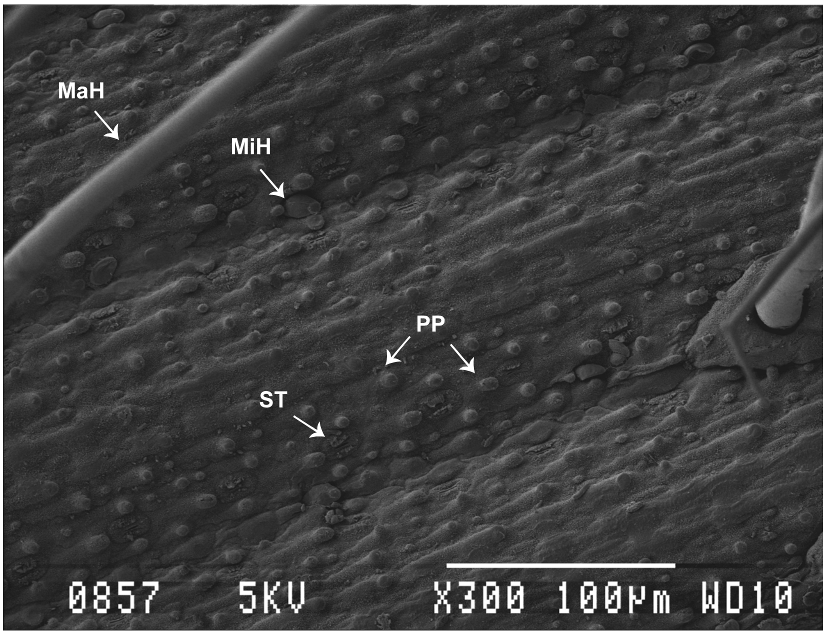

Figure 7.15 Abaxial surface of a *Cynodon* hybrid leaf grown without any water stress (W4). MaH - Macro-hair, MiH - Micro-hair, ST - Stoma, PP - Papillae.

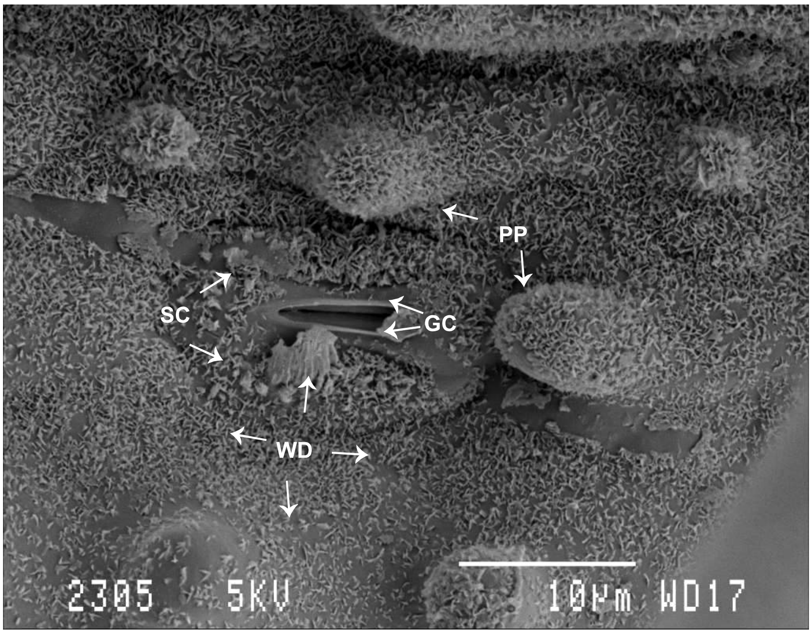

Figure 7.16 A stoma found on the abaxial side of a *Cynodon* hybrid leaf grown without any water stress (W4) (x2500). GC - Guard cell, SC - Subsidiary cell, WD - Wax deposit, PP - Papillae.

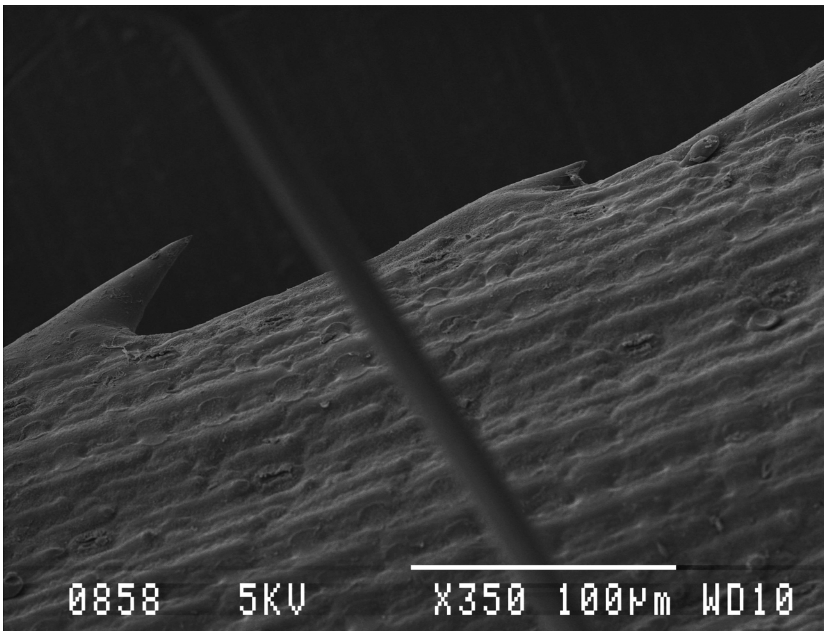

Figure 7.17 Leaf edge of the abaxial side of a *Cynodon* hybrid leaf grown without any water stress (W4).

7.3.4 Digitaria eriantha subsp. *eriantha*

Well defined costal and intercostal zones can be observed on the adaxial surface of a *D. eriantha* leaf (Figures 7.18 and 7.19). There are only micro-hairs (Figure 7.19), as is the case with *C. ciliaris* (Figure 7.3). The micro-hairs are in two adjacent rows parallel to the vein with prickles (sometimes paired) over the veins (Figure 7.19). A row of hooks can be found in approximately the middle of the intercostal zone (Figure 7.19). Silica bodies with cross or butterfly shapes were found over the veins. The stomata borders both sides of a vein (Figure 7.19) and are covered with wax crystals (Figure 7.20), making it difficult to identify the guard and subsidiary cells. There are prickles present on the leaf edges (Figure 7.21).

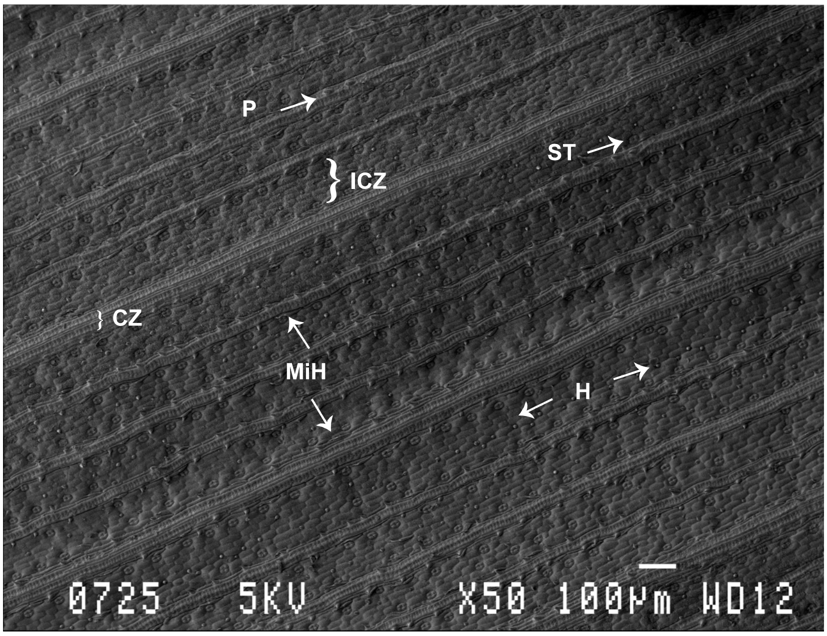

Figure 7.18 Adaxial surface of a *D. eriantha* leaf grown without any water stress (W4). CZ - Costal zone, ICZ - Intercostal zone, MiH - Micro-hair, P - Prickle. H -Hook, ST - Stoma.

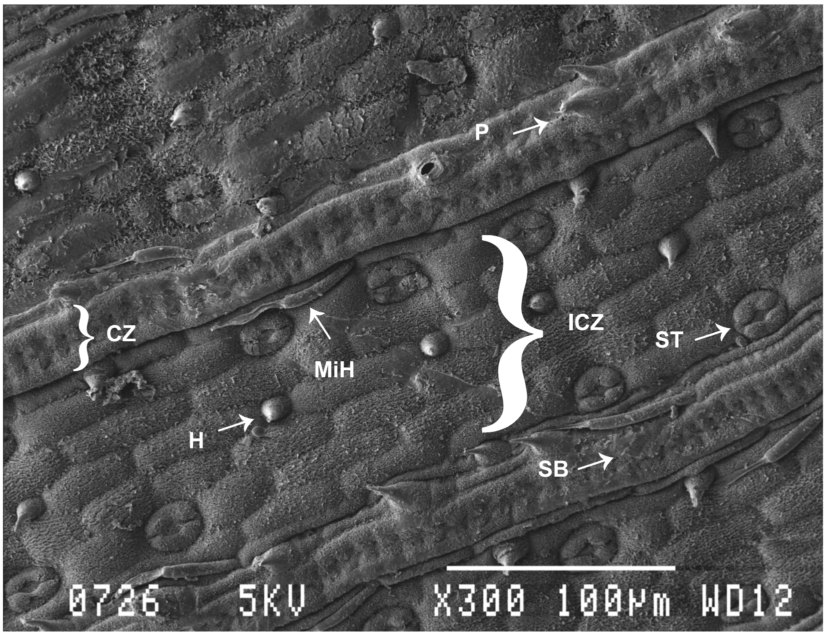

Figure 7.19 Adaxial surface of a *D. eriantha* leaf grown without any water stress (W4). CZ - Costal zone, ICZ - Intercostal zone, MiH - Micro-hair, P - Prickle, H - Hook, ST - Stoma, SB - Silica bodies.

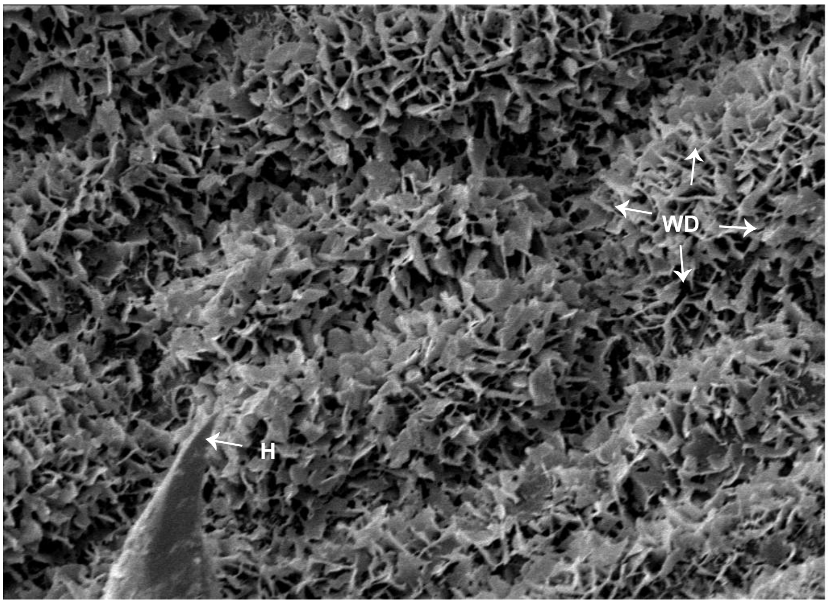

Figure 7.20 A stoma found on the adaxial side of a *D. eriantha* leaf grown without any water stress (W4) (x2500). WD - Wax deposit, H -Hook.

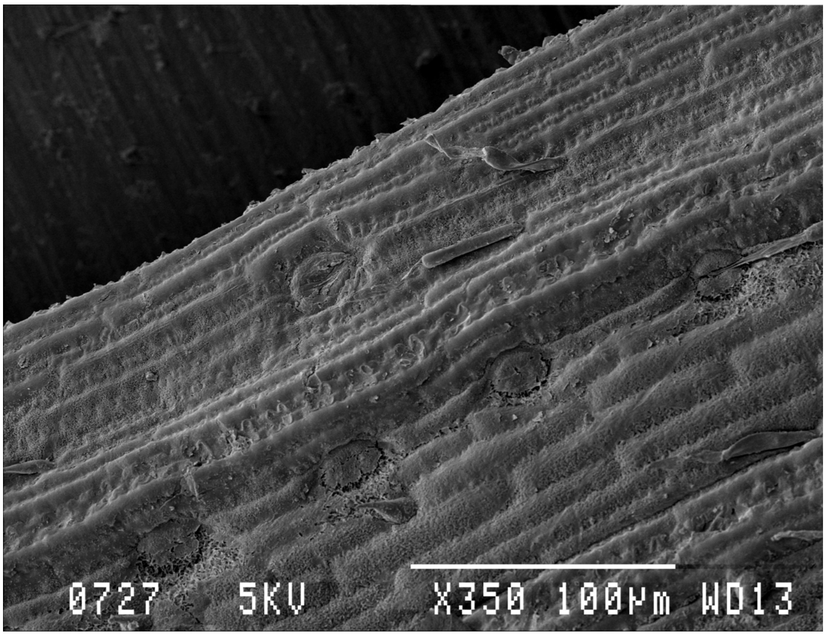

Figure 7.21 Leaf edge of the adaxial side of a *D. eriantha* leaf grown without any water stress (W4).

Costal and intercostal zones are also clearly visible on the abaxial side of the *D. eriantha* leaf (Figures 7.22 and 7.23). The same type of micro-hairs, although fewer, were found on the abaxial (Figures 7.22 and 7.23), than on the adaxial (Figures 7.18 and 7.19), side of the leaf. The abaxial leaf surface was also covered with prickles (costal zone) and hooks (intercostal zone) (Figure 7.23). As on the adaxial side, silica bodies of the same shape can be found on the abaxial side of the leaf. The stomata are to be found in the intercostal zone (Figure 7.23) and are covered by wax crystals (Figure 7.24). Some of the stomata are overshadowed by the adjacent veins (Figure 7.23). A three rowed prickle covered leaf edge can be observed on the abaxial side of the leaves (Figure 7.25).

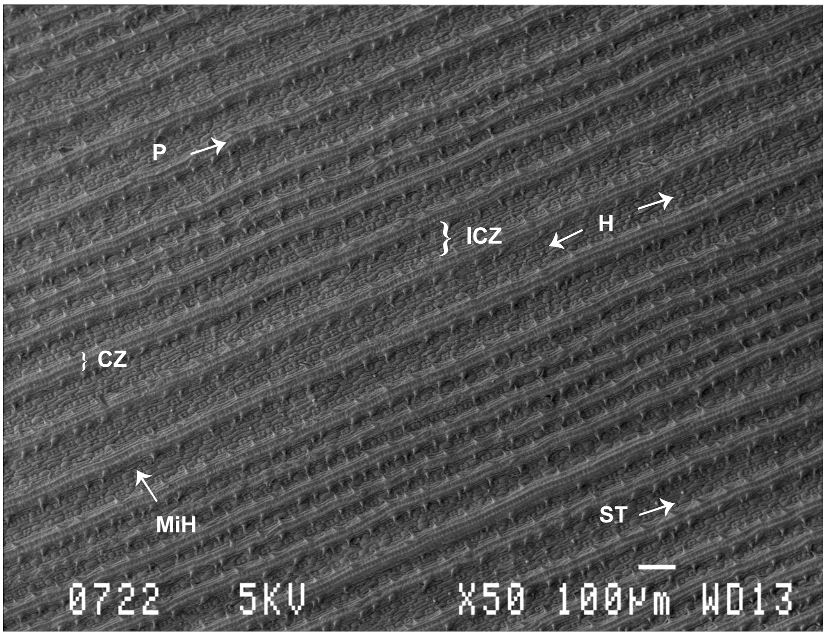

Figure 7.22 Abaxial surface of a *D. eriantha* leaf grown without any water stress (W4). CZ - Costal zone, ICZ - Intercostal zone, MiH - Micro-hair, P - Prickle. H -Hook, ST - Stoma.

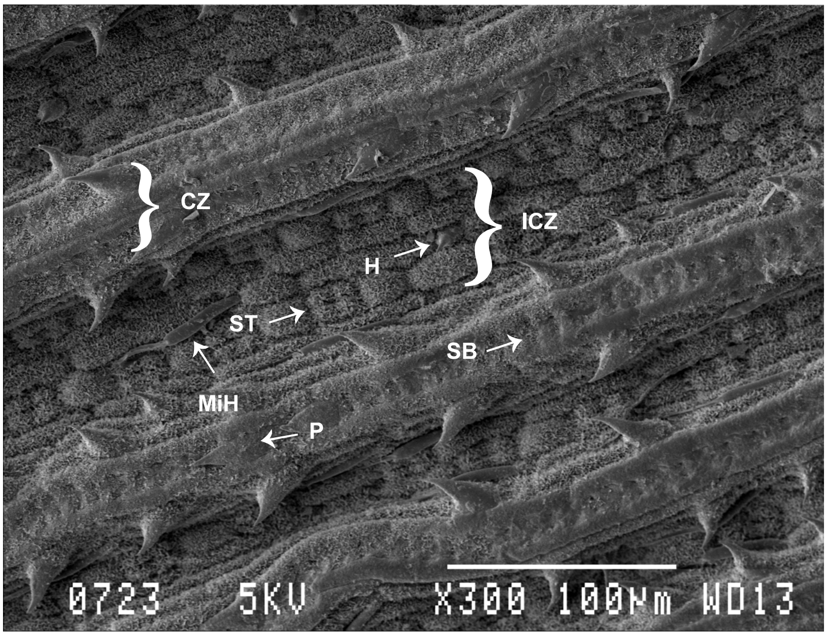

Figure 7.23 Abaxial surface of a *D. eriantha* leaf grown without any water stress (W4). CZ - Costal zone, ICZ - Intercostal zone, MiH - Micro-hair, P - Prickle, H - Hook, ST - Stoma, SB - Silica bodies.

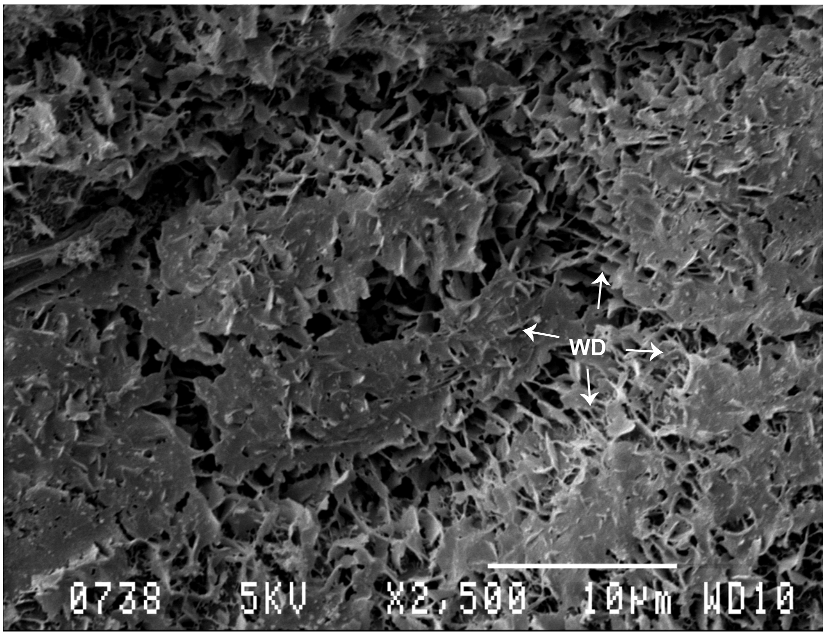

Figure 7.24 A stoma found on the abaxial side of a *D. eriantha* leaf grown without any water stress (W4) (x2500). WD - Wax deposit.

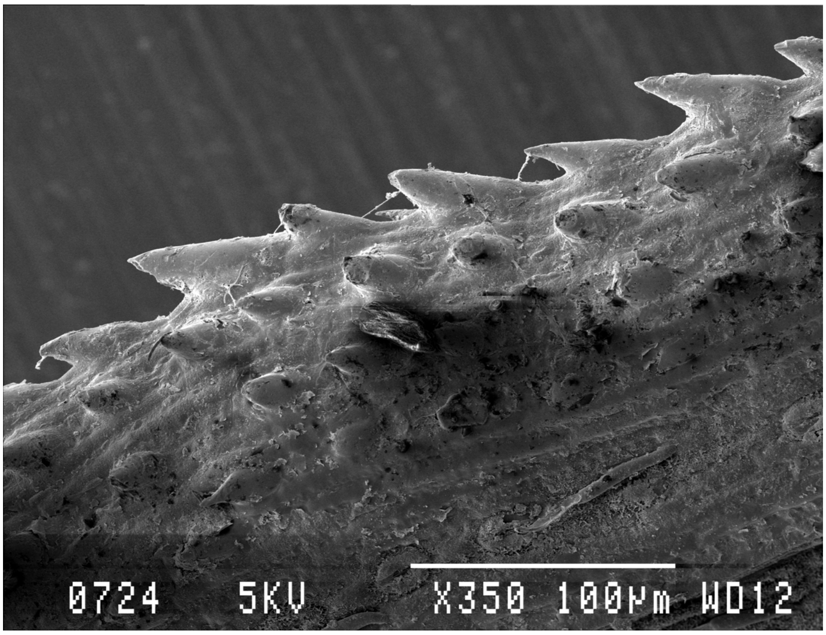

Figure 7.25 Leaf edge of the abaxial side of a *D. eriantha* leaf grown without any water stress (W4).

7.3.5 Panicum maximum

Numerous prickles and short macro-hairs (Figure 7.26) are to be found in the intercostal zone on the adaxial side of a *P. maximum* leaf. Micro-hairs, similar to the ones found on *C. ciliaris* (Figure 7.3) and *D. eriantha* (Figure 7.19), are also present, but are less abundant (Figure 7.27). Costal and intercostal zones can be identified, but are less pronounced than those in *D. eriantha* and *C. ciliaris*. Silica bodies, in the form of dumbbells or nodules, are associated with the veins (Figure 7.27). As in *D. eriantha*, the stomata border the veins (Figure 7.27) and are seldom, if ever, found in the middle of the intercostal zone. The leaf surface is once again covered by a wax deposit (Figure 7.28), but it does not cover the guard cells as was the case for *D. eriantha*. Two to three rows of prickles can be found on the leaf edge (Figure 7.29).

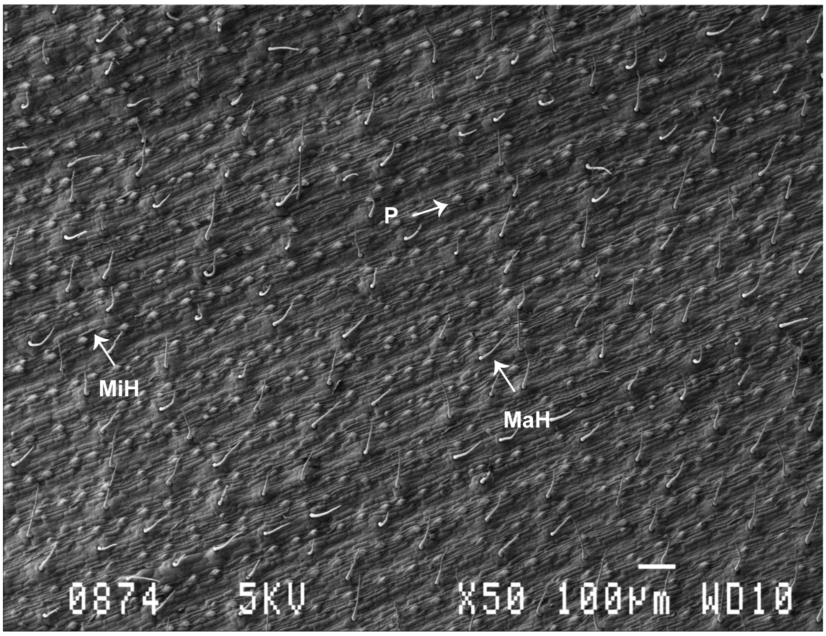

Figure 7.26 Adaxial surface of a *P. maximum* leaf grown without any water stress (W4). MaH - Macro-hair, MiH - Micro-hair, P - Prickle.

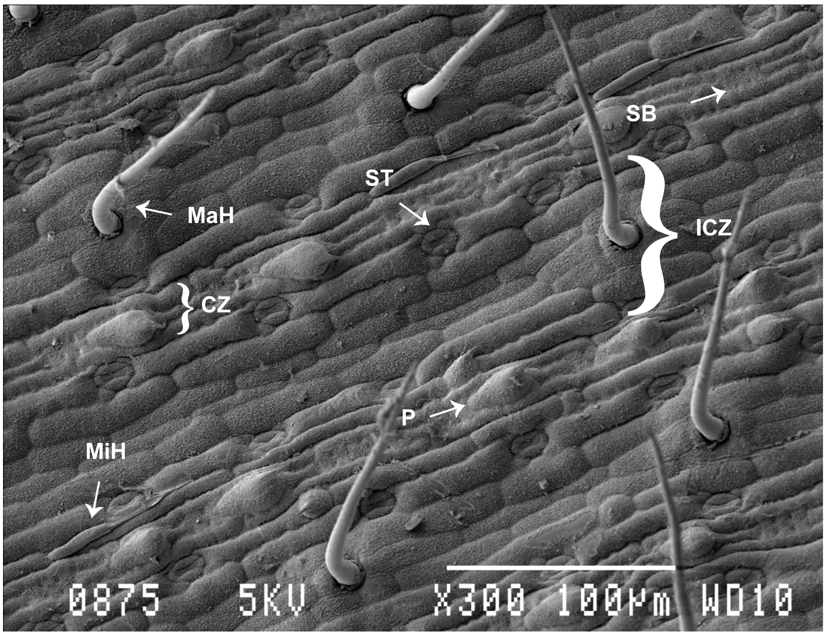

Figure 7.27 Adaxial surface of a *P. maximum* leaf grown without any water stress (W4). CZ - Costal zone, ICZ - Intercostal zone, MaH - Macro-hair, MiH - Micro-hair, P - Prickle, ST - Stoma, SB - Silica body.

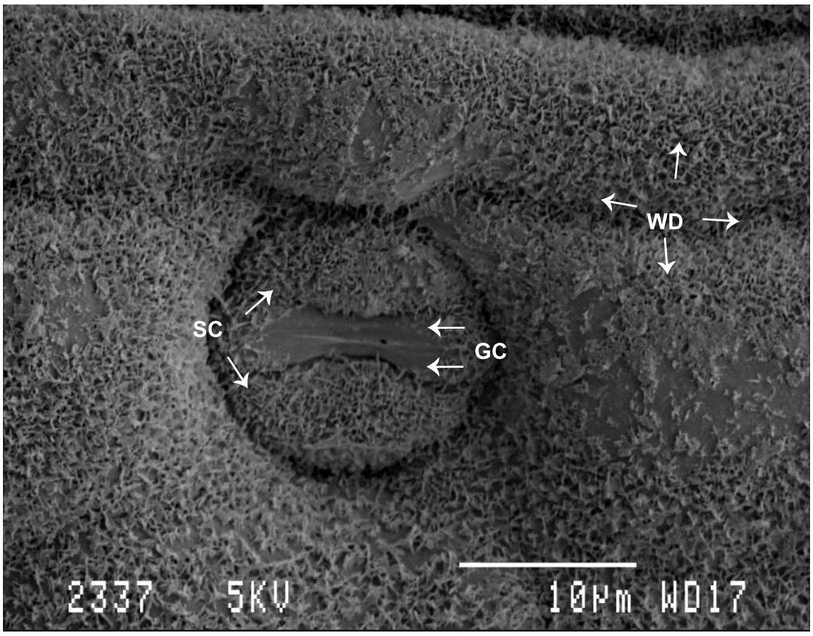

Figure 7.28 A stoma found on the adaxial side of a *P. maximum* leaf grown without any water stress (W4) (x2500). WD - Wax deposit, SC - Subsidiary cell, GC -Guard cell.

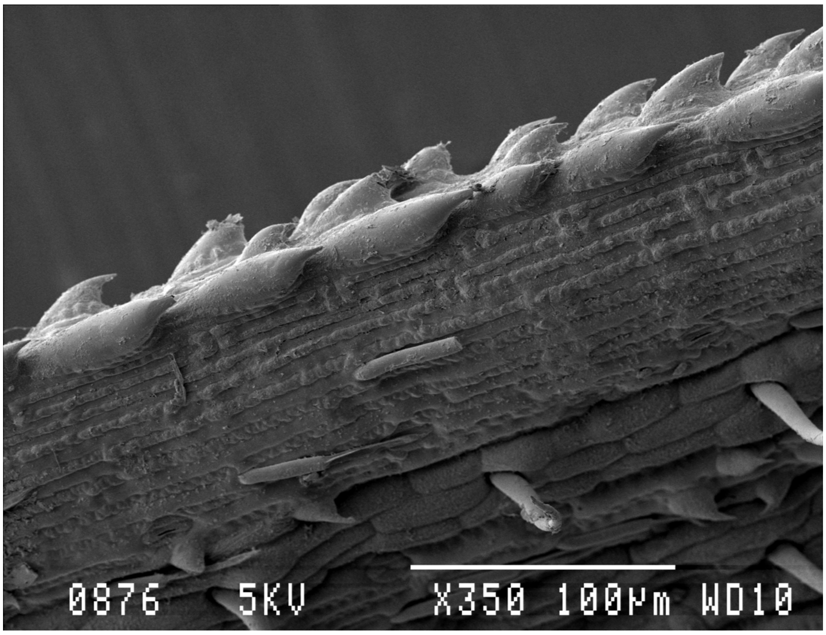

Figure 7.29 Leaf edge of the adaxial side of a *P. maximum* leaf grown without any water stress (W4).

The abaxial (Figure 7.30) leaf surface has the same components as the adaxial side, but the position of some of these components differ between the two surfaces. The difference being that the prickles can be found on the border of the costal and intercostal zones on the abaxial surface (Figure 7.31), while it is in the middle of the costal zone on the adaxial surface (Figure 7.27). The macro-hairs are in straight rows in the intercostal zone (Figure 7.31). The micro-hairs are in the same row, adjacent to the macro-hairs. The surface is smoother and the costal silica bodies are clearly dumbbell to saddle shaped. The stomata are found close to a row of macro- and micro-hairs, or prickles, and little wax deposit is evident (Figure 7.32). Prickles can be found in two to three rows on the leaf edge (Figure 7.33).

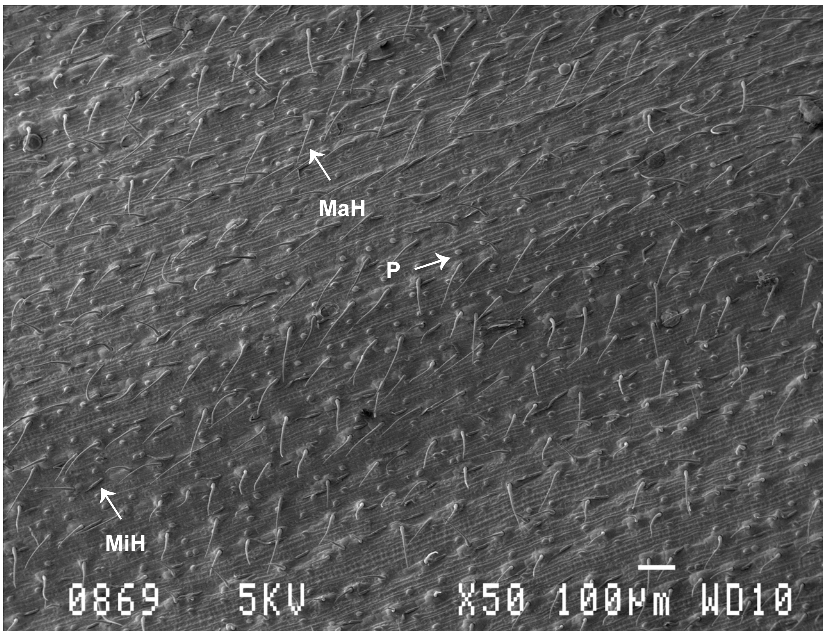

Figure 7.30 Abaxial surface of a *P. maximum* leaf grown without any water stress (W4). MaH - Macro-hair, MiH - Micro-hair, P - Prickle.

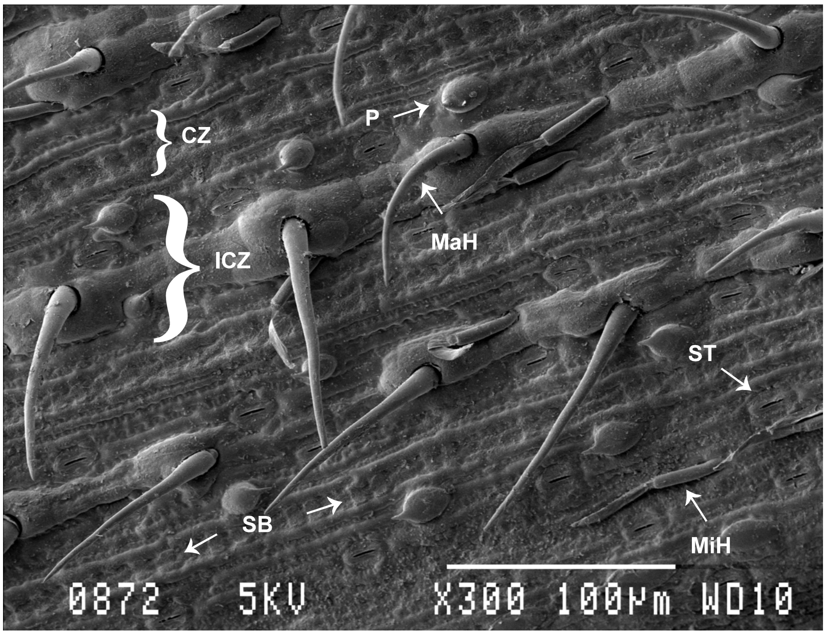

Figure 7.31 Abaxial surface of a *P. maximum* leaf grown without any water stress (W4). CZ - Costal zone, ICZ - Intercostal zone, MaH - Macro-hair, MiH - Micro-hair, P - Prickle, ST - Stoma, SB - Silica body.

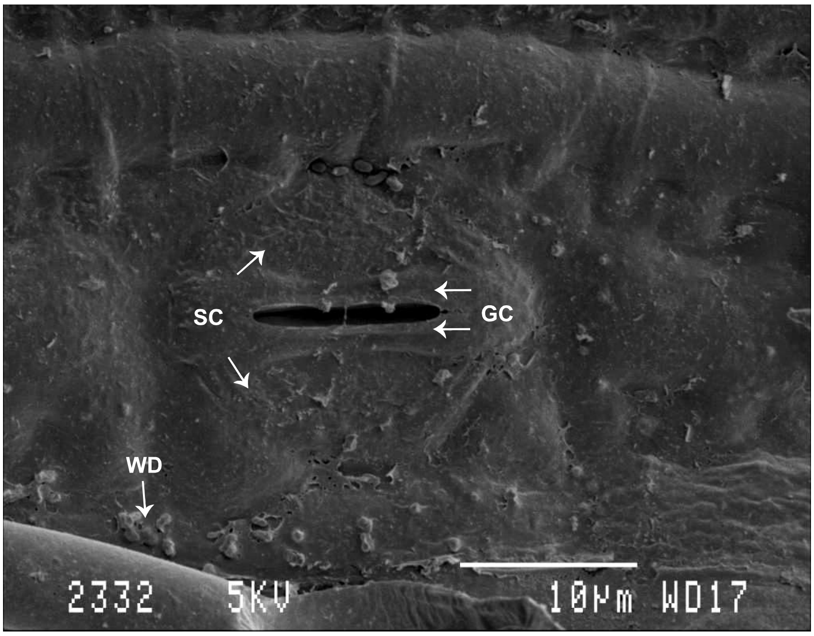

Figure 7.32 A stoma found on the abaxial side of a *P. maximum* leaf grown without any water stress (W4) (x2500). WD - Wax deposit, SC - Subsidiary cell, GC -Guard cell.

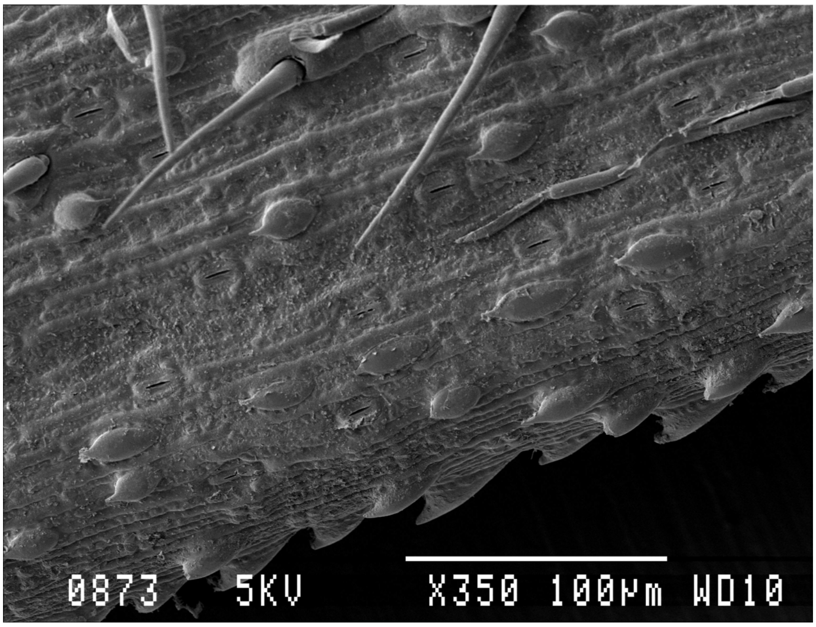

Figure 7.33 Leaf edge of the abaxial side of a *P. maximum* leaf grown without any water stress (W4).

7.3.6 Pennisetum clandestinum

The adaxial leaf surface of a *P. clandestinum* plant had infrequent macro- and micro-hairs (Figure 7.34). The micro-hairs differed somewhat from the previous four grass species, in that the distal cell had not collapsed (Figure 7.35), and more closely relates to the prickles of the other species. Silica bodies in the shape of dumbbells and prickles are to be found. The stomata are clearly visible and not covered with wax crystals (Figure 7.36), while a single row of prickles exists on the leaf edge (Figure 7.37).

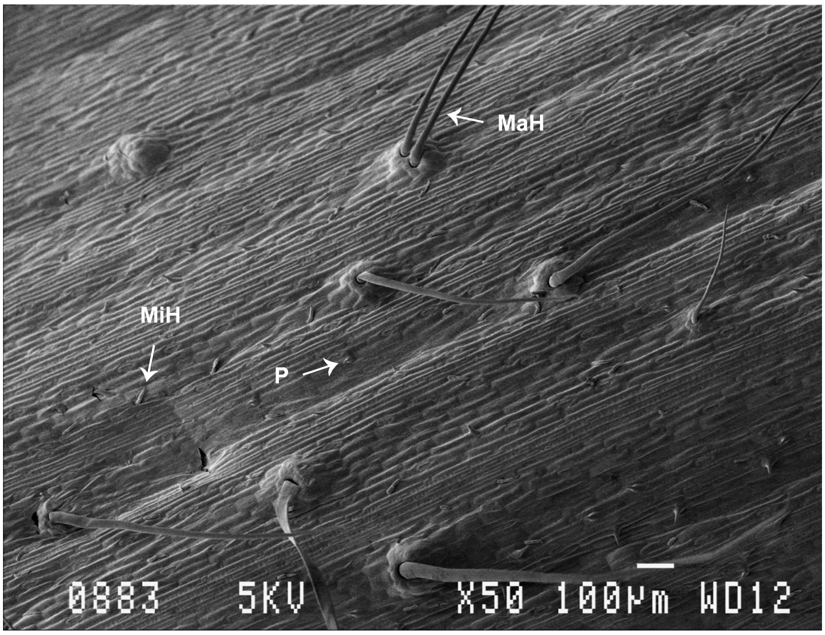

Figure 7.34 Adaxial surface of a *P. clandestinum* leaf grown without any water stress (W4). MaH - Macro-hair, MiH - Micro-hair, P - Prickle.

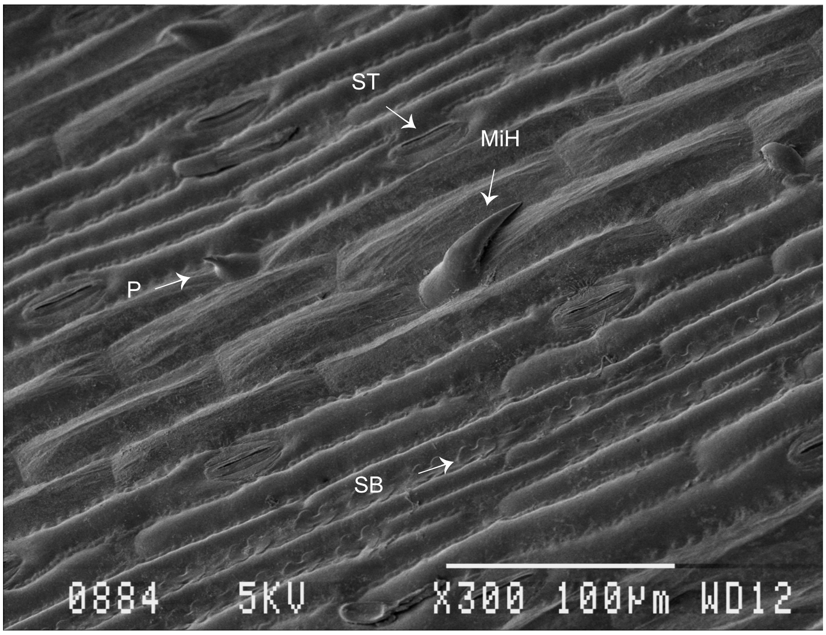

Figure 7.35 Adaxial surface of a *P. clandestinum* leaf grown without any water stress (W4). MiH - Micro-hair, P - Prickle, ST - Stoma, SB - Silica body.

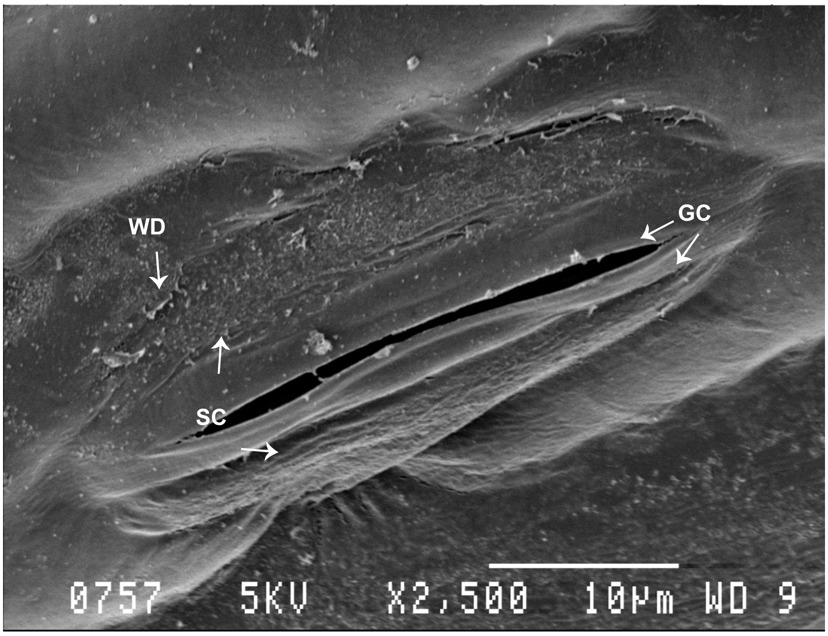

Figure 7.36 A stoma found on the adaxial side of a *P. clandestinum* leaf grown without any water stress (W4) (x2500). WD - Wax deposit, SC - Subsidiary cell, GC -Guard cell.

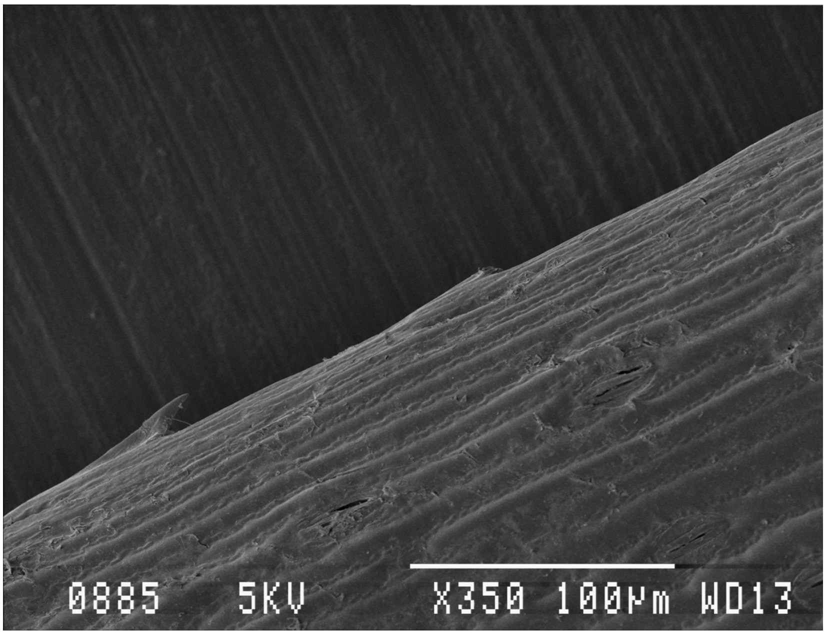

Figure 7.37 Leaf edge of the adaxial side of a *P. clandestinum* leaf grown without any water stress (W4).

Infrequent macro- and micro-hairs are found on the abaxial leaf surface of *P. clandestinum* (Figure 7.38). The long cells are not as angular (Figure 7.39) as on the adaxial side of the leaf. The leaf surface is smooth without any prickles, while the stomata are clearly visible (Figure 7.40). The leaf edge consists out of a single row of prickles (Figure 7.41).

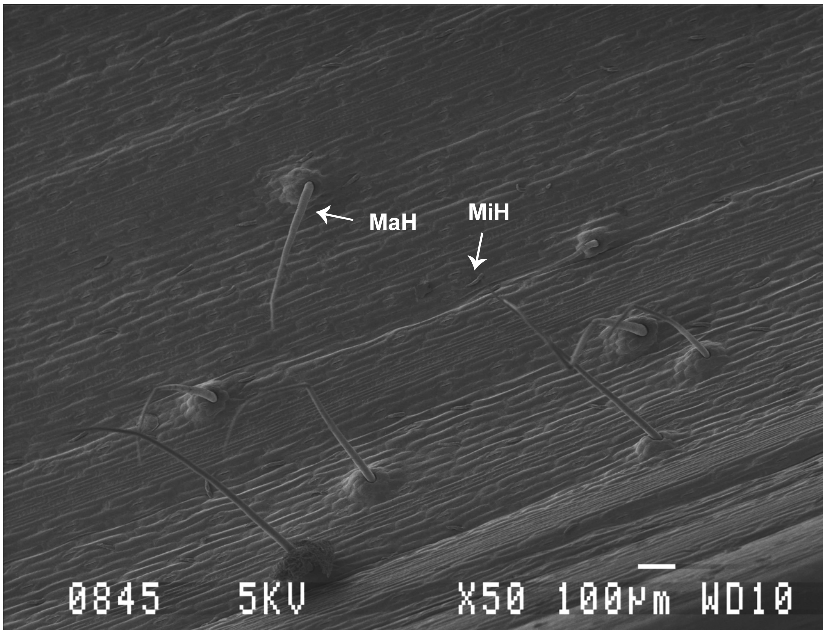

Figure 7.38 Abaxial surface of a *P. clandestinum* leaf grown without any water stress (W4). MaH - Macro-hair, MiH - Micro-hair.

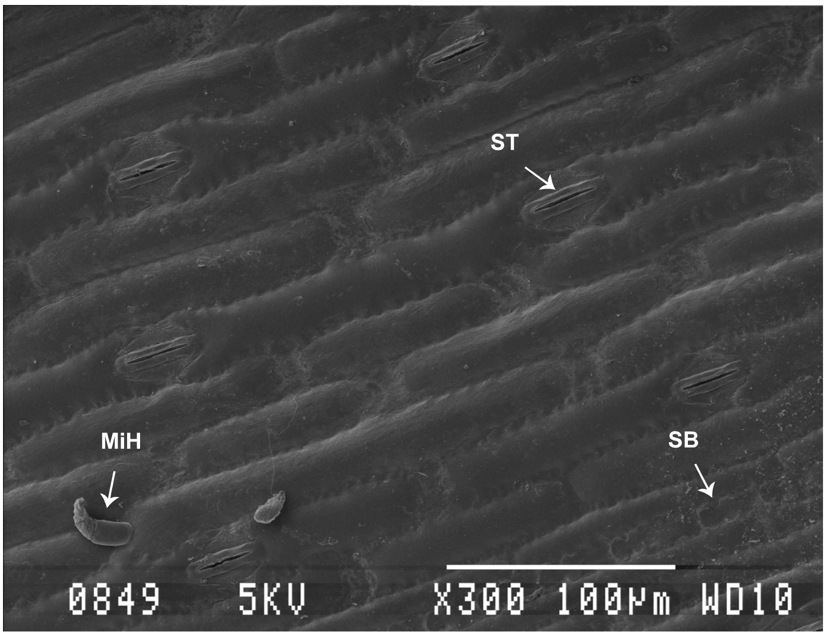

Figure 7.39 Abaxial surface of a *P. clandestinum* leaf grown without any water stress (W4). MiH - Micro-hair, ST - Stoma, SB - Silica body.

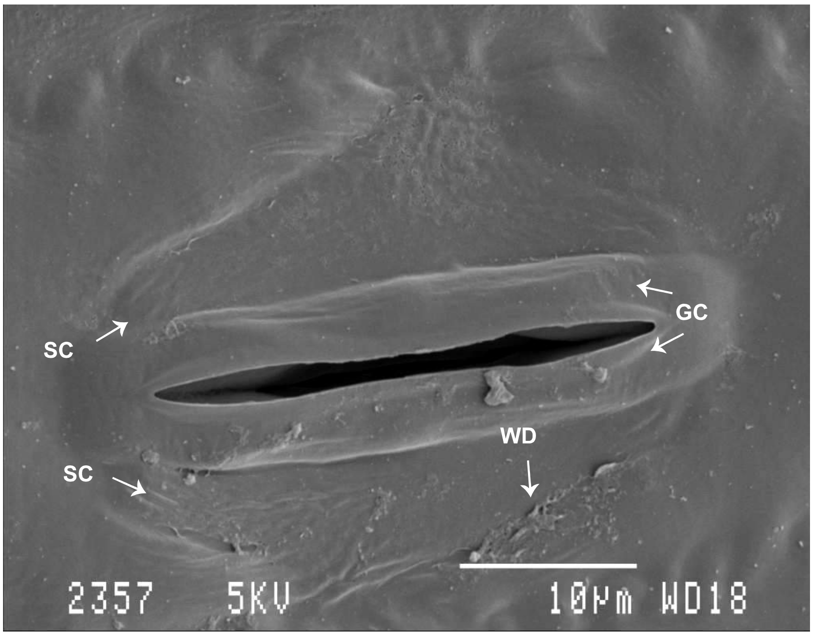

Figure 7.40 A stoma found on the abaxial side of a *P. clandestinum* leaf grown without any water stress (W4) (x2500). WD - Wax deposit, SC - Subsidiary cell, GC -Guard cell.

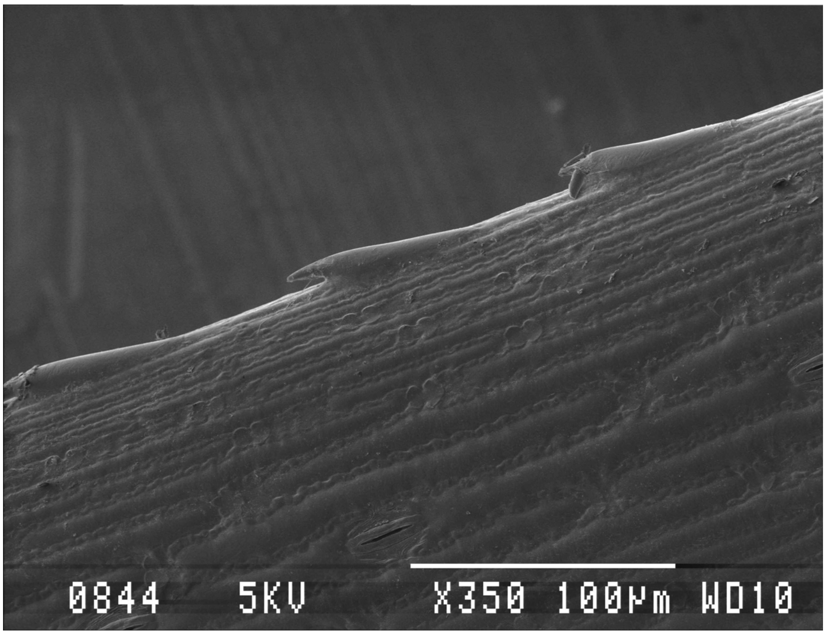

Figure 7.41 Leaf edge of the abaxial side of a *P. clandestinum* leaf grown without any water stress (W4).