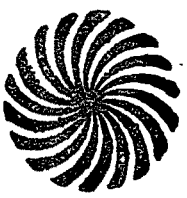
Chapter IV



Drought Resistance
[Responses of Proso Millet (Panicum miliaceum L.) to WaterStress]

1. INTRODUCTION :

One of the basic ingredients of plant production is the availability of soil water. Water is a well known limiting factor in plant growth and numerous studies have been done to understand its mode of action. Water is a major constituent of a tissue, a reagent in photosynthetic reactions and hydrolytic processes, the solvant for and mode of translocation for metabolities and minerals within plants and is essential for cell enlargement and growth. It is self evident that water deficits cause a general reduction in the size of most plants. Water dificit occur in the plant whenever transpiration exceeds water absorption, or may be due to excessive water loss or reduced absorption or both.

According to Kanitkar (1960) seventy seven (77) million acres of land in India can be considered as definitely liable to drought. These are primarly millet growing areas. These areas are mostly dominated by sorghum, pennisetum and other minor millets. Thus in dry land agriculture, a greater understanding of crop water deficits and their influence on growth and development, metabolism and yield is essential.

Stanhill (1957) found that in 66 out of 80 papers dealing with crop response to different soil moisture regimes water shortage was related to a depression in plant growth and in most cases to a reduction in yield. There has also a lot of

work done on the effect of water stress, on plant morphology, physiology and biochemistry (for reviews Maximov, 1929; Crafts, 1968; Parker, 1968; Todd, 1972; Hsiao, 1973).

The integrity of specific protein water structure and the entire cytoplasm is essential for the continuance of most physiological process at maximum rates. Most process are probably not unduly suppressed by the degree of stress which exists diurnally in well watered plants, but as soil water stress increases, key process will become progressively inactivated. Although any factor which affects cell metabolism must affect cell enlargement and plant growth, some effect of water deficits on plants appear to be more directly mediated by turgor pressure. The guard cell turgor directly regulates stomatal aperture which ultimately influences both transpiration and photosynthesis. Complete or partial closure can reduce both process and so ultimately reduce growth.

According to Hsiao (1973) the water deficit may be expected to have following physical and chemical effects : 1) The chemical potential or activity of Cellular water is reduced. 2) Turgor pressure decreases in the cell. 3) Small molecules and macromolecules become more concentrated as cell volume is reduced by water loss. 4) Macromolecules may be affected through water or through modification of the structure of adjacent water.

HOW WATER STRESS AFFECTS YIELD :

Water stress reduces plant yield by considerably supressing photosynthesis and to some extent, mineral absorption. In addition, water shortage promotes an irreversible loss in dry matter and assimulating surface by accelerating leaf senescence and death. Two main modes of action of water deficit on photosynthesis can be recognized. In the first place, complete or partial stomatal closure and reduced rate of CO_2 exchange can influence the supply of CO_2 which was observed in wheat and millet by Slatyer (1973). Second there is a direct effect of water deficit on the biochemical processes involved in photosynthesis. The photosynthetic rate is considerably lowered by water deficit (Jones, 1973; Lawlor, 1976). A suppression in chloroplast Hill activity in both severely and moderately desiccation leaf tissue has been observed by Boyer and Bowen, (1970). Keck and Boyer (1974) found that the activity of the photosystem II was affected more than that of the photosystem I and suggested that electron transport was inhibited during early desiccation. While photophosphorylation was affected at more severe water stress. A prerequisite of high yield is a high production of total dry matter (Yoshida, 1972). There is well established experimental evidence that water deficit causes reduction in leaf area. Leaf production and laminar expansion found in tobacco (Hopkinson, 1968), Sunflower (Mare and Palmer, 1976) and field beans (Karamanos, 1978). According to Wardlaw (1969), Hsiao

~~and Acevedo~~ et al (1976) water shortage affects yield mainly by reducing the assimilating area.

Leaf shedding constitutes both a loss in dry matter and reduction in the assimilatory area. Premature leaf shedding occurs due to water stress. Usually shaded leaves are the first to die under waters stress in beans (Karamonos, 1978) and in cereals (Boyer and Mc Pherson, 1976). Reduction in respiration due to hydration of tissue and inhibition of respiratory enzyme under water deficit observed by Todd (1972).

As soil water content is one of the important factors with respect nutrient availability, drought also exert a great influence on ion uptake and translocation (Samuels, 1972; Singh + Raghuvanshi et al., 1979). It is now clearly established that water stress injury has a metabolic base that is concerned with damage to the protein synthesizing mechanism (Stewart + Hungate et al., 1966; Huffaker et al., 1970). Further water stress is accompanied by definite changes in the level of free amino acids and amides (Singh et al., 1973). Water deficit leads to release or activation of degradative enzymes (Genkel et al., 1967). Water stress exerts a profound effect on harmonal distribution, particularly on the content of cytokinins and abscisic acid (El Beltagy and Hall, 1974). Lastly the translocation of photosynthetic assimilates is suppressed by water stress (Crafts, 1968). All the above metabolic disturbances

finally lead to a retardation of plant growth and a considerable reduction in overall yield.

The stage of growth at which water stress occurs can exert an important influence on the final yield of some crop plants. Particularly in annual cereals. Denmead and Shaw (1960) found that a reduction in yield about 50% was caused by water stress at the silking stage in corn. For wheat and cotton, somewhat similar results have been observed. However, each crop has a different period when it exhibits pronounced sensitivity to stress.

There is a great variation in drought resistance capacity among various crop species. The crops like Sorghum, Wheat, Chick-pea, Safflower and millet are well known for their resistance nature, while rice tomato and other vegetable crops are prone to water stress Proso millet is generally cultivated on lands where rainfall is scanty and only marginal irrigation facilities are available for poor farmers. It is evident from literature that not much work has been done on drought resistance mechanism in proso millet. However, some attempts have been made in the last few years on other species of genus. Panicum (Ramati et al., 1979; Wilson et al., 1980; Ludlow, et al 1980). In the present investigation, therefore, an attempt has been made to study the mechanism of drought resistance in common millet (P.miliaceum) which is generally regarded as hardy cereal.

2. MATERIAL AND METHODS :

Seeds of a local strain of common millet were sown in earthenware pots in the month of September. The plants were equally watered and given equal doses of fertilizers. After six weeks, water was withheld from various pots so that at the time of harvest there were pots receiving no water for 4, 8, 12 and 16 days. The plants which received regular water supply (control) and which were exposed to water stress were harvested seperately and were randomly sampled. The methods for estimation of organic and inorganic constituents were essentially the same as described earlier in Chapter-II.

The method for stomatal studies was that of Stoddard (1965). The widths of stomatal apertures were estimated under the microscope on films of clear nail polish. In this method nail polish was applied to the middle of the lower as well as upper epidermis of the leaf. To avoid errors, maximum care was taken to select 3rd leaf of plant from each group of plants like control, 4, 8, 12 and 16 days water stress.

The films were made at 11.0 A.M. Measurements of stomatal apertures, maximum width and length of Stomatal apparatus were made under precalibrated microscope. Two impressions were taken each time and about 10 stomata were studied at different positions on the film from each impression.

FIG.4·1 EFFECT OF WATER STRESS ON GROWTH OF *P. miliaceum*.

1. CONTROL
2. 4-DAYS STRESS
3. 8-DAYS STRESS
4. 12-DAYS STRESS
5. 16-DAYS STRESS

3. RESULT AND DISCUSSION :

A. Organic Constituents :

i) Soil moisture and Leaf Water potential :

The representative values of soil moisture % and water potential in prosomillet leaves during water stress have been recorded in Table 4.1 and illustrated schematically in Fig.4.2.

It is evident that progressive decline in the soil moisture content as the soil dries due to evoporation and transpiration by plants. It result in progressive decline in leaf water potential also. When the soil is sufficiently moist (26% moisture). The leaf water potential is the lowest (-3.92 bars). However, when the plants are exposed to 16 days stress i.e. when soil is dry (only 6.5% moisture) the leaf water potential increases considerably to the order -66.38 bars to maintain the water flow at the desired level. At this stage the plants were wilted considerably . This might be resulting due to loss of water due to transpiration and finally at this point partial stomatal closure may retard the demand for water absorption. However, by the 16 days the leaf moisture % has also fallen to the level as low as 23%. Root and stem moisture level also falls down from 84.52% and 83.12% in control to 62.80% and 53.30% respectively. It is then impossible for the leaf water potential to recover. The plant

is permantly wilted and recovery may be possible only with soil water recharge.

Water flow through the soil plant system tends to occur along the gradient of decreasing water potential. This means that plant water potential has to be lower than soil water potential inorder to accomplish the flow of water. The removal of water due to transpiration reduce the water potential of Xylem. Thus a potential gradient will exist between leaf and root Xylem to transfer water from the root system to the leaves in the plant. Gardner (1960) and Cowan (1965) reported that the rate of water flow toward the root surface is controlled by the hydraulic conductivities of the soil and the only water available is that occuring within a few centimeters of the root. Kilie, (1973) reported that the rate of water uptake reaches to a limiting value as the potential gradient increases between soil and root xylem. Therefore, an equilibrium condition may exist between total transpiration and root surface with a minimum root potential and optimum water uptake under a normal environments.

ii) Moisture percentage :

The reduction in moisture percentage is essentially the first detectable change caused by water deficits. Therefore, the 16 days stressed common millet leaves retain only about 23% moisture. It was evident that at this stage the leaves were almost dry and rolled. The roots and stem retain

about 50-60% moisture (Table 4.1 and Fig.4.2). Water deficits cause dehydration of protoplasm (Levitt, 1956) and this results in loss of turgor. May and Milthorpe (1962) reported that growth is reduced by a decrease in relative turgidity to below 90% . According to Levitt (1972) a sufficiently severe dehydration leads to a pronounced decrease in respiration rate but this decrease is usually found only after degree of dehydration is severe enough to cause.

Iljin (1923) reported that the first effect of water reduction in leaves is a partial or complete stomatal closure. Such a closure shows decreased movement of CO_2 in the assimulating leaves, reducing the photosynthetic rate two to ten times according to amount of water removal and the sensitivity of the plant. Such a water shortage in leaves not only reduces photosynthetic rate but also retards translocation. However, Levitt (1972) suggested that metabolic disturbances which are not severe enough to injure by themselves may nevertheless amplify the other effects of the dehydration strain and therefore, the injury. It is obvious that there are correlations between drought hardiness and water retention of leaves or other plant parts when the later rate exposed to desiccation (Bayles et al., 1937). In proso millet water retention capacity is considerably lost only due to severe water stress (16 days). However, the root and stem of this plant show a good water

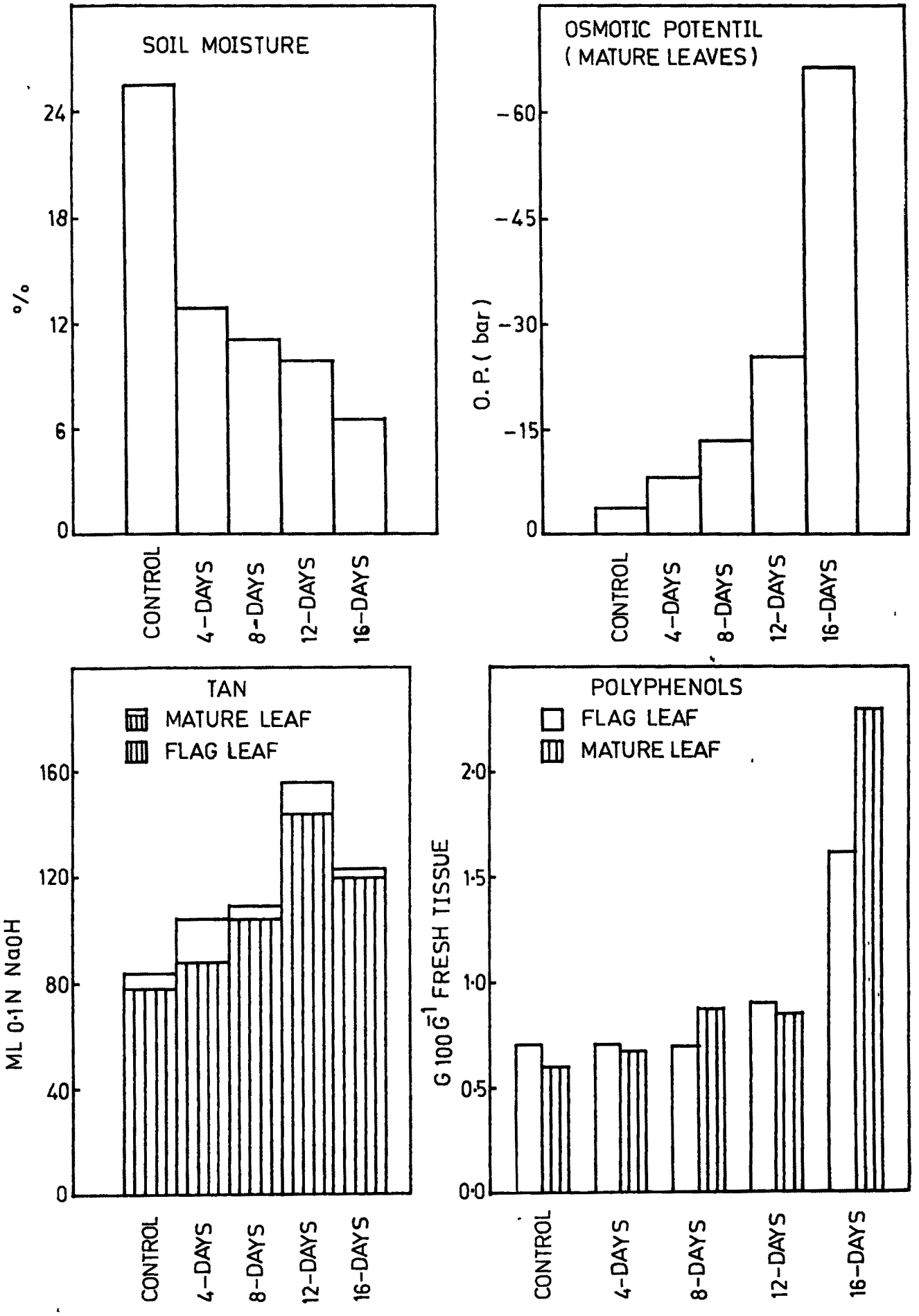


FIG.4·2 EFFECT OF WATER STRESS ON OSMOTIC POTENTIL, TAN AND POLYPHENOLS IN THE LEAVES OF P. miliaceum.

retention capacity at all conditions of water stress. While the plant can retain about 40% water in the leaves at 12 days stress. This indicates that prosomillet possesses a good drought tolerance potential.

iii) TAN (Titratable Acid Number) :

It is evident from Table 4.1 and Fig. 4.2 that Titratable leaf juice acidity in proso millet leaves is significantly increased when exposed to 4, 8 and 12 days of water stress which clearly indicates the stimulation of organic acid synthesis due to water stress. Even the leaf juice acidity of 16 days water stressed plants is higher than that of well irrigated plants. Our findings are in agreement with those of Ramati et al., (1979) and Ford & Wilson et al., (1981) who reported accumulation of organic acids in Panicum ripens and P.maximum respectively. Further they have also reported that organic acids like malate and succinate accumulate in stressed leaves but there is decrease in the level of aconitate. No change in oxalate content was observed due to increase in water stress. In P.miliaceum, there is slight decrease in organic acid content when exposed to 16 days water stress, but it is still higher than that in control. This may be due to reduced rate of respiration in those plants..

Organic acids have been shown to play a prominent role in osmotic adjustment (Osmond, 1963). But according to Ford & Wilson (1981) et al., role of organic acids in osmotic adjustment is relatively

small than solute accumulation. Lawlor and Fock, (1977) observed an increase in labelling in succinate, fumarate and aconitate in water stressed sunflower leaves. Thus an increase in organic acid content in water stressed leaves appears to be an adaptive feature.

iv) Total Polyphenols :

It is clear from Table 4.1 and Fig.4.2 that even the total Polyphenols in the flag and mature leaves of proso millet increase to a considerable extent when exposed to water deficit. These findings are different from those of Tsai and Todd (1972) who observed about 25% decline in the phenolic contents in both resistant and susceptible varieties of wheat due to water stress. According to them if phenolic compounds are involved in cellular injury following drought stress it might be due to release of these substances in to cytoplasm rather than to increased synthesis. Todd (1972) further claims that such a release might be affecting enzymatic activities. However, our findings are in agreement with those of Brachet and Bichaut (1972) who recorded that simultaneous action of atmospheric and edaphic moisture deprivation produces an increase in the synthetic rate of phenols. Talha et al., (1975) also reported that there was considerable increase in the alkaloid content of Cartharanthus roseus due to moisture deficit. The anatomical structure of leaves of Impatiens balsamica suffering from water stress has a greater number of

tannins and raphide sacs (Todd et al., 1974). However, the exact role of polyphenols during the water stress is not completely understood. The increase in total polyphenol content may be possibly due to induction of secondary metabolism under stress conditions.

v) Chlorophylls :

The changes in chlorophyll content fresh weight basis in proso millet leaves during water stress are recorded in Table 4.1 and Fig.4.3 . The values of chlorophylls depicted in the Table 4.2 are expressed on dry weight basis according to suggestions of Sestak et al., (1971) for water stress studies. It can be seen that in proso millet the total chlorophyll content is reduced due to water stress. The values of chlorophyll on fresh weight basis recorded in Table 4.1 indicate that total chlorophyll content goes on increasing as plants are exposed to 4, 8, 12 and 16 days water stress. This is possibly due to loss of moisture from leaves and increasing dry weight of it with increasing period of water stress. This is more clear when we took the values of chlorophylls when expressed on dry weight basis. It is evident that the trend shown by chlorophylls when expressed on dry weight basis is exactly oppsite to that when expressed on fresh weight basis.

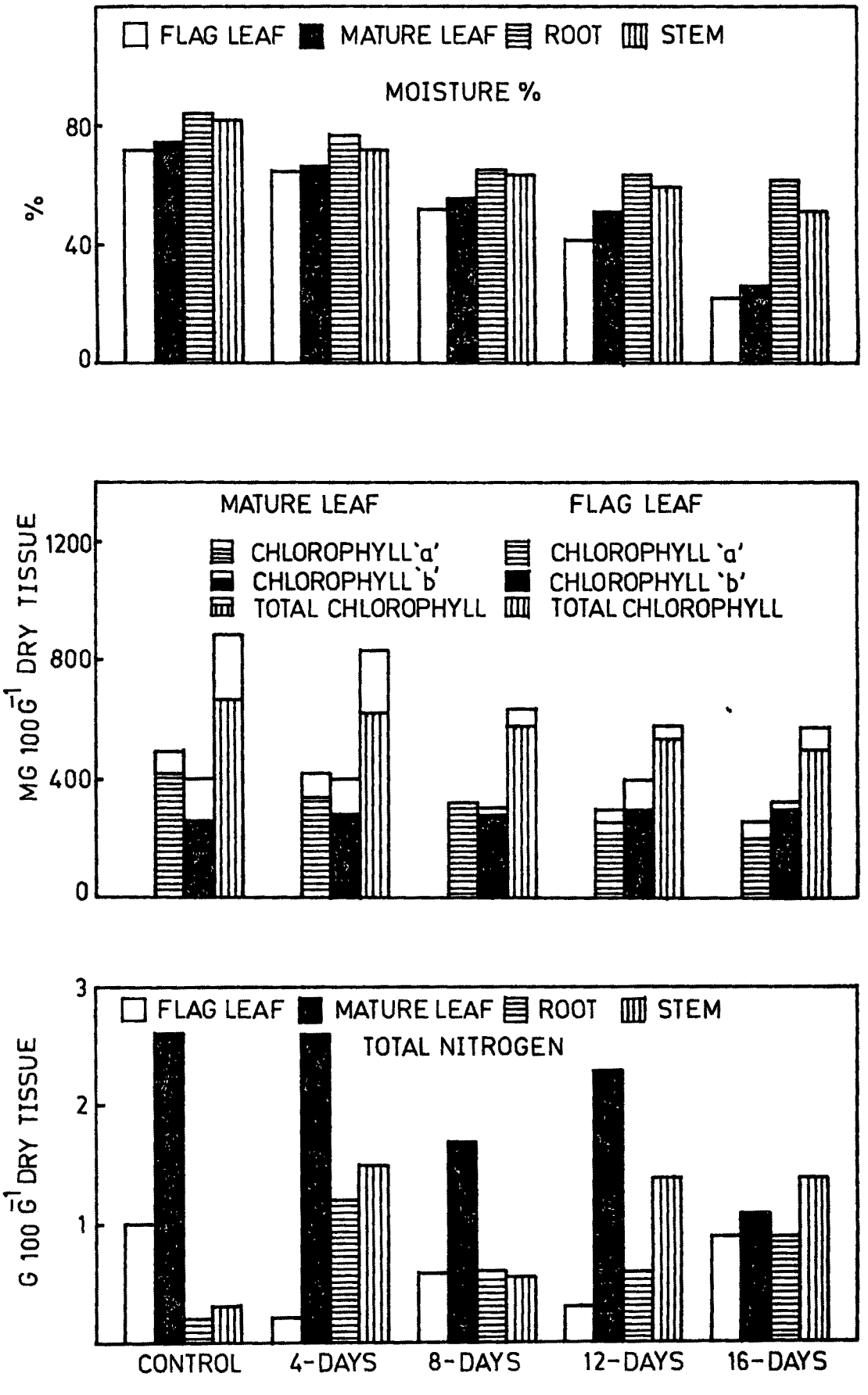


FIG43 EFFECT OF WATER STRESS ON MOISTURE CONTENT AND ORGANIC CONSTITUENTS IN DIFFERENT PARTS OF P. miliaceum .

The stability of chlorophyll molecules has long been considered as an essential parameter of drought resistance. There are various methods to determine chlorophyll stability index (Mathew and Ramadasan, 1973). The validity of such methods has been doubted by Levitt, (1972). One thing is certain that loss of chlorophylls during drought is harmful to the plant. Wilson et al (1968) noticed that occurence of single drought during grain filling stage of maize hastened leaf senescence. In the experiment of Asana and Basu (1963), the yellowing of the ears due to water stress was also evident.

According to Virgin (1963), even rather small water deficit caused strong inhibition of chlorophyll a formation. He further stated that this effect was due to decreased rate of formation of protochlorophyll a. Further it was found that such inhibition was reversible. Maranville and Paulsen (1970) also found that drought reduced chlorophyll content as well as light absorption. Bourgue (1971) studied the effects of small water deficit on chlorophyll accumulation in elongated leaves of Canavalia enciformis L. He observed that at the low relative humidity (25%) very slow chlorophyll accumulation occured. Singh et al., (1973) and Dnysen and Freeman (1974) also noticed an impaired synthesis of chlorophylls due to water stress in barley and wheat respectively. Tkachur and Aerov (1974) investigated effect of drought on optical and radiant energy absorption of winter wheat leaves. It was

observed that due to sudden drought a 12% decrease in radiẽnt energy absorption took place. These workers considered these changes to be related to change in chlorophyll content of the leaves.

The state of Plant plastid apparatus under the condition of water stress was investigated by Kushnirenko et al., (1971). They found that drought conditions affected to lesser degree of quantity of segments strongly bound with the lipoprotein complex perticularly chlorophyll b. According to them the drought resistant plant are characterised by least changes in the pigment content especially of the strongly bound chlorophyll form. Sanchez, et al (1983) reported that water stress in maize leaves reduces chlorophyll content, stomatal conductance and photosynthesis but the nitrogen content of the leaves is not affected. Evidently, the stress induced loss of chlorophyll is not mediated by a lack of nitrogen. Losses of upto 40% of leaf chlorophyll content were insufficient to affect the rate of photosynthesis. In proso millet also we can see that the plants exposed to water stress (16 days) the flag leaves and mature leaves still retain about 76 and 65% of chlorophylls respectively. Thus such retaintion of chlorophylls even after stress can be considered as a drought resistant feature of the plant and insufficient to affect the rate of photosynthesis. It may be useful in the process of drought recovery when water becomes available.

Table 4.1 : Organic constituents of root, stem and leaves of P.miliaceum during water stress (Fresh weight basis).

Organic constituents:	Organ	Water stress				
		Control	4 days	8 days	12 days	16 days
Soil moisture %	-	26.00	13.00	11.00	10.00	6.50
Moisture %	Root	84.52	76.80	66.50	64.80	62.80
	Stem	83.12	72.50	64.50	59.50	53.30
	Flag leaf	71.50	64.50	52.50	41.50	23.00
	Mature leaf	74.50	66.00	56.00	51.75	25.50
Osmotic Potential (bar)	Mature leaf	- 3.92	- 8.45	-13.19	-25.43	-66.38
Chlorophyll a*	Flag leaf	118.40	121.80	141.90	149.40	159.06
	Mature leaf	124.60	141.60	142.10	141.50	201.88
Chlorophyll b*	Flag leaf	73.50	97.60	137.20	174.10	235.84
	Mature leaf	103.00	140.60	136.00	140.60	232.40
Total chlorophylls*	Flag leaf	191.90	219.40	279.10	323.50	394.90
	Mature leaf	227.60	282.10	278.00	282.10	434.20
Chlorophyll a:b	Flag leaf	1.61	1.25	1.03	0.86	0.67
	Mature leaf	1.21	1.01	1.04	1.00	0.86
TAN**	Flag leaf	78.31	87.35	105.42	144.58	123.50
	Mature leaf	84.34	105.42	108.43	156.63	120.48

Table 4.1 : (Contd...)

Organic constituents	Organ	Water stress				
		Control	4 days	8 days	12 days	16 days
Polyphenols***	Flag leaf	0.71	0.69	0.69	0.90	1.62
	Mature leaf	0.60	0.67	0.88	0.86	2.30
Reducing Sugars***	Root	0.065	0.19	0.72	0.66	0.76
	Stem	0.22	1.014	0.96	0.87	0.30
	Flag leaf	0.99	2.27	1.53	1.84	1.84
	Mature leaf	0.53	1.79	1.96	2.05	2.44
Starch***	Root	6.04	11.20	11.16	9.54	7.8
	Stem	8.27	15.39	9.36	6.12	6.04
	Flag leaf	14.58	10.42	10.62	12.07	13.99
	Mature leaf	9.36	10.30	11.86	11.86	18.02

* Values in mg 100^{-1} g fresh tissue.

** Values in ml of 0.1 N NaOH required to neutralize the acids in 100 g of fresh tissue.

*** Values in g 100^{-1} g fresh tissue.

vi) Carbohydrates :

The effect of water stress on reducing sugars and starch contents on fresh weight basis and dry weight basis is recorded in Table 4.1/4.2 and Fig. 4.4 respectively. It appears that both reducing sugars as well as starch content increase under conditions of water stress. It appears from the Table 4.2 (on dry weight basis) both reducing sugars as well as starch contents slightly increase when plants exposed to water stress (4 and 8 days) therefore, it is decreased when exposed to 16 days water stress. This might represent a gradual fall in the overall metabolic order of the plant. When expressed on dry weight basis however, stem and to some extent leaves show deviation in the effect of water stress. It can be seen that with increasing the intensity of water stress there is continuous fall in the level of reducing sugars in the stem which can be observed it in the leaves also but only under severe drought conditions.

According to Slatyer (1969) carbohydrate metabolism is affected by drought through direct and indirect effect on photosynthesis and through several intermediate components and processes. Attempts have been made to explain the role of sugars in drought tolerance. Maximov, (1929) suggested two possibilities (1) the accumulation of sugars might protect the protoplasm from coagulation and dessication and (2) the high concentration might prevent visible wilting for a long time

inspite of an increasing water deficit. Iljin (1929) suggested that plants grouped ecologically, their sugar content increases with the dryness of the habitat. Maranville and Paulsen (1970) studied alternation of carbohydrate composition of corn seedlings during moisture stress. They found that water stress decreased starch concentration markedly. They further observed that this was due to acceleration of starch hydrolysis and not due to impairment of starch synthesis. It is evident from the results of proso millet that the starch content of the root is slightly increased and that up to the 8 to 12 days of water stress (fresh and dry weight basis). However, when expressed on dry weight basis the starch content of all parts continuously decreases the lowest value being in the plants of 16 days stress.

Lee et al., (1974) reported that drought stress decreases reducing sugars, sucrose and starch concentration in both drought tolerant and susceptible varieties of pea. Barlow et al., (1976) found that in corn seeding suffering from induced water stress the increase in soluble carbohydrate concentration was inversely related to both rate of leaf elongation and total dry matter accumulation. A decrease in starch content due to water stress was evident in the experiments of Parker (1970) and Stewart (1971). Murty and Srinivasudu, (1968) observed that the drought resistant rice variety had higher sugar concentration than susceptible variety

even under conditions of drought. Thakur et al., (1980) studied the water stress effect of carbohydrate metabolism of resistant and susceptible cultivars of zea mays. They observed that at 1st leaf stage, starch content of the resistant cultivar was lower than that of susceptible cultivar but reducing sugar content was much higher. At the 3rd leaf stage starch contents were similar in two cultivars but sugar contents were higher in the resistant. Differential changes in root and shoot carbohydrate as affected by increasing levels of stress were also observed. According to Vora et al., (1974) accumulation of sugars under water stress indicates a protective role of sugars. Ackerson (1981) found that increase in leaf carbohydrates helps in osmotic adjustment during water stress in cotton. Considering the above views it appears that proso millet also possess a moderate drought resistance potential as the sugar content registered increase when plants are subjected to water stress. Ford et al., (1981) observed the accumulation of reducing sugars and total sugars in stressed leaves of Panicum maximum and considered that contribution of carbohydrate to the osmotic adjustment was relatively small than accumulation of solutes.

vii) Total nitrogen :

It is clear from Table 4.2 and Fig.4.3 that total nitrogen content is increased in proso millet stem and roots, but there is a slight decrease in it in flag and mature leaves.

There is extensive literature regarding the effect of water stress on nitrogen metabolism. Wadleigh and Richards (1951) have reviewed the literature on this point for several vegetable crops and concluded that most experimental evidence shows that for a given level of fertility, decreasing moisture apply is associated with a definite increase in total nitrogen content. Several workers (Barnett and Naylor, 1966; Rahman et al., 1971) have shown such increase in total nitrogen content.

At the same time there are few reports where decrease in total nitrogen content due to water stress has been recorded. Povov (1969) stated that drought has a deleterious effect on the biological removal of soil nutrients by the plants especially of nitrogen. Pande and Singh (1969) observed that limited moisture supply reduces nitrogen content in rice. Samuels (1972) observed a varietal difference regarding the nitrogen content on exposure to drought. Thus tobacco, Tomato, and corn exhibited increase in nitrogen due to water stress. On the other hand in sugarcane, the nitrogen level was considerably lower due to water deficit. Affinity of key enzyme in nitrogen metabolism nitrate reductase is also considerably affected by water stress, (Slukhaing et al., 1973 and Plant, 1974). In proso millet leaves perhaps such inhibition of nitrogen metabolizing enzymes may occur. Which may be resulting in decrease in nitrogen content. The form in which nitrogen accumulate in stem and roots of proso millet plants under water stress is not investigated. However, high nitrogen content can contribute to synthesis of aminoacid like proline.

Alternatively it may be true that nitrogen accumulation in stem and root systems and the corresponding deficiency of this macronutrient in the leaves indicates that probably the transport of nitrogen metabolites from the root to stem and finally to the leaves might have affected by the water stress conditions. Thus it is the translocation process which is affected by water stress.

viii) Proline :

It can be seen from Table 4.2 and Fig.4.4 that free proline is accumulated continuously during the water stress period in all three parts of plants. Water stress not only influences the nitrogen uptake but also affects the protein metabolism. It was observed by Stutte and Todd (1969) that water deficit causes qualitative changes in proteins. According to Gates (1964) protein synthesis may be interrupted in stressed plants. Feeding with ^{14}C labelled serine. Morchiladze (1969) observed that lack of water hindered the aminoacid incorporation into the protein of grape leaves but increase its transformation in to the other free aminoacids.

Proteolysis is a common feature in cut plants that are allowed to wilt (Dove, 1968). Thus the content of free aminoacids is increased considerably due to water stress (Barlow et al., 1976). According to Naylor (1972) there is

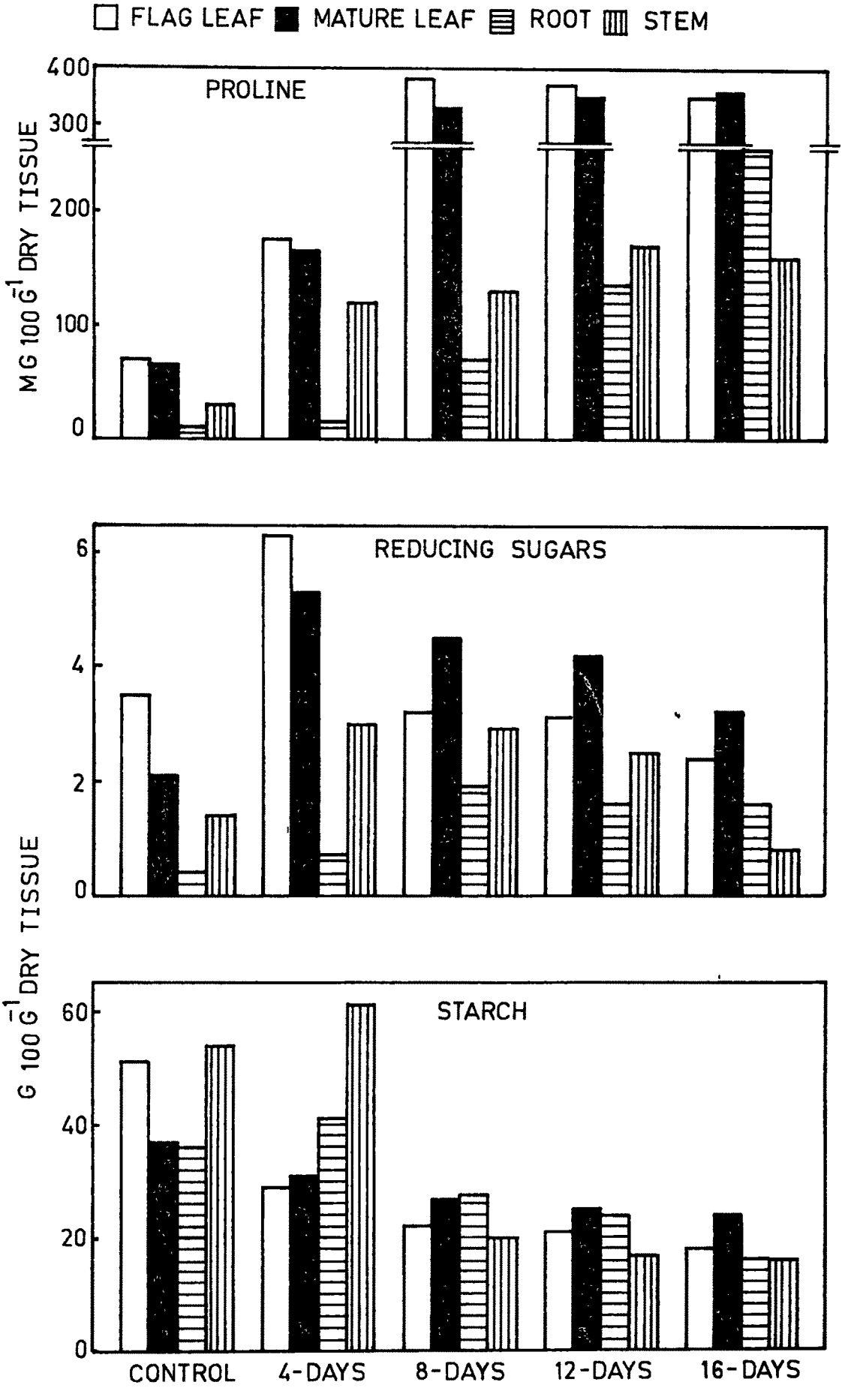


FIG.4·4 EFFECT OF WATER STRESS ON PROLINE, REDUCING SUGARS AND STARCH CONTENT IN DIFFERENT PARTS OF *P. miliaceum*.

no uniform hydrolysis of proteins due to water stress because the number and amounts of the aminoacids do not reflect the hydrolysis of the "average" protein of the cell. There is an especially marked accumulation of free proline, (Barnett and Naylor, 1966; Singh et al., 1973; Rajgopal et al., 1977; Iwai et al., 1979; Richard and Thurling, 1979; Palfi and Pinter, 1980; Thakur and Rai, 1982; Ilahi and Doerffling et al., 1982) when plants are exposed to water stress.

From Table 4.2 it is clear that the proline increases to a significant extent in all three parts of proso millet plants following exposure to water stress. The effect of water stress on proline content in proso millet plant is so significant that the proline content has increased 50 to 100 fold over that of control in the plants exposed to 16 days watersstress. This is not surprising because Barnett and Naylor, (1966) noticed a 10 to 100 fold increase in proline content due to water stress in Cyndon dactylon. Baskin and Baskin (1974) reported a 115% increase in the total amountof amino acids due to water stress in Astragales tennesseensis of which proline accounted for about 30% increase. Palfi et al., (1974) carried out extensive investigation regarding the effect of dehydration on proline accumulation. They observed that not all species accumulate proline under water deficit. These are : Beta vulgaris, Spinacia oleracea; Chenopodium album, Rumex scutatus, Cucurbita pepo, Cucumis

sativus, Zea mays, Phaseolus vulgaris, Allium sativum, A.cepa and Lactuca sativa. Among the 60 plants studied majority of herbaceous mesophytic, cultivated plants belonging to families Solanaceae, Luguminosae, Cruciferae, Umbelliferae, Compositae and Graminae accumulate proline under water deficit. Depending on these observations Palfi et al., (1974) classified the species as "proline accumulating or proline" type if the stage of microsporogenesis with accumulation the amount of free proline in the leaves at the time of a strong water deficit attains at least 1% of the dry matter. From present observations in proso millet it can be concluded that proso millet belongs to proline accumulating type.

The proline accumulation is not merely a product of proteolysis but it may be arising from synthesis and interconvertion of other amino acids. In stressed green leaves glutamate is generally considered as a major carbon donor to proline synthesis (Barnett and Naylor, 1966; Morris et al., 1969; Larher et al., 1970; Singh et al., 1973). However, the experiments of Stewart and Boggess (1977) indicate that both glutamate and arginine appear to contribute carbon to proline. Wrench et al., 1977 observed that arginine is quantitatively the more important precursor. Recently Stewart et al., (1977) suggested that proline accumulation results from inactivation by water stress of normal mechanism by inhibiting proline oxidation.

Though a great deal of work has been done regarding the accumulation of proline during water stress the exact role of proline in drought resistance is not well understood. According to Levitt (1972) water deficit in plant results in accumulation of protein hydrolysis products to a sufficient degree to be toxic. Tolerance of protein loss could be induced by accumulation of protein loss. According to Palfi et al (1974) proline increases considerably the amount of strongly bound water in the leaves. In addition it is highly water soluble compared with other protein forming amino acids, it is the most stable amino acid as regards resisting 'oxidative' acid hydrolysis and it stores up reducing energy its formation.

Proline may be single source and precursor of hydroxy proline in the structural protein of cell walls, participating in the cell extension process and may serve as energy material for respiration. It also stimulates absorption of oxygen by plant tissues (Savitskaya, 1976). According to Bluum and Ebercon (1976) free proline accumulation during water stress is correlated signfficantly with post stress recovery rating, free amino concentration and dark respiration rate. They further claim that accumulation of free proline in water stressed Sorghum leaves is related to the ability of cultivar to recover upon the relief of stress possibility by way of prolines role as a source of a respiratory energy in recovering plant. Tyankova (1967) observed that application of proline helped wheat plants to recover from drought.

Table 4.2 : Changes in organic constituents of root, stem and leaves of P.miliaceum during water stress (dry weight basis).

Organic constituent	Organ	Water stress				
		Control	4 days	8 days	12 days	16 days
Moisture %	Root	545.9	310.0	198.5	184.1	168.8
	Stem	492.4	263.6	181.7	146.9	114.1
	Flag leaf	250.9	181.7	110.5	70.9	29.9
	Mature leaf	292.2	194.1	127.3	107.3	34.2
Chlorophyll 'a'*	Flag leaf	415.58	343.48	398.70	255.47	206.79
	Mature leaf	488.43	416.01	322.57	292.91	268.50
Chlorophyll 'b'	Flag leaf	257.99	275.23	288.80	297.71	306.59
	Mature leaf	403.76	413.36	308.72	391.04	309.09
Total Chlorophylls*	Flag leaf	673.57	618.42	587.30	553.01	513.37
'a:b'	Mature leaf	892.19	829.37	631.04	583.95	577.20
Chlorophyll	Flag leaf	1.61	1.25	1.03	0.86	0.67
	Mature leaf	1.21	1.01	1.04	1.00	0.86
Total Nitrogen***	Root	0.22	1.19	0.62	0.61	0.86
	Stem	0.26	1.45	0.57	1.40	1.40
	Flag leaf	0.97	0.19	0.63	0.31	0.88
	Mature leaf	2.64	2.60	1.70	2.29	1.10

Table 4.2 : (Contd...)

Organic constituent	Organ	Water stress				
		Control	4 days	8 days	12 days	16 days
Protein***	Root	1.25	6.78	3.53	3.51	27.70
	Stem	1.51	8.28	3.26	7.98	7.98
	Flag leaf	5.52	1.10	3.61	1.76	5.02
	Mature leaf	15.05	15.00	4.01	13.05	6.27
Reducing Sugars***	Root	0.39	0.70	1.87	1.63	1.59
	Stem	1.43	3.01	2.86	2.47	0.80
	Flag leaf	3.47	6.40	3.22	3.15	2.39
	Mature leaf	2.08	5.25	4.45	4.24	3.25
Starch***	Root	35.76	40.76	29.02	23.56	16.38
	Stem	53.83	61.09	20.40	17.38	16.07
	Flag leaf	51.17	29.38	22.35	20.65	18.17
	Mature leaf	36.69	30.28	26.92	24.55	23.96
Proline ***	Root	2.5	12.00	70.00	134.00	257.00
	Stem	30.00	118.00	130.00	169.00	162.00
	Flag leaf	70.00	175.00	381.00	371.00	353.00
	Mature leaf	66.00	165.00	330.00	353.00	362.00

* Values in mg 100^{-1} g dry tissue.
** Values in mg 100^{-1} g dry tissue.
*** Values in g 100^{-1} g dry tissue.

Obraztosova and Nikiforva (1967) found that drought resistant trees were distinguished by a higher content of free amino acids than the nonresistant ones. On the contrary to above observations, Waldren and Teare (1974) stated that proline is not the sensitive indicator of drought stress. However, most of the investigators believe that proline has an important role in the drought resistance mechanism. Thus Baskin and Baskin (1974) suggested that the ability to accumulate proline may be of adaptive value to Astragalus tennesseensis during short periods of drought. Singh et al., (1973) observed that varieties of barley which accumulated larger amount of free proline tended to have leaves which were most drought resistant.

Ilahi Doerffling et al., (1982) observed the accumulation of proline and abscisic acid in 4 days drought resisting cultivar of maize and considered that proline and ABA are possibly biochemical indicators of resistance against drought. In proso millet we can see that the plant has ability to accumulate proline after exposure to water stress (in all treatments) can be considered as a drought resistant feature of plant. This accumulated proline can play a key role during stress recovery.

B. EFFECT OF WATER STRESS ON INORGANIC CONSTITUENTS :

The contribution of minerals to the increase in total plant dry matter is much less than that of photosynthesis,

ranging among different plant species from 2-20% of the total dry weight (Evans, 1972; Milthorpe and Moorby, 1974). The movement of water in the rhizo-sphere facilates the supply of nutrients to the roots. When the movement of soil water ceases because of soil dryness nutrients uptake occures only by diffusion close to the root. This sort of supply becomes limiting in a very short time since the nutrients reserves are quickly deplected (Crafts, 1968). Consequently, increasing water shortage should be associated with a decreasing rate of mineral absorption.

Barber et al., (1963) suggested that mass flow could account for most of the transport of Ca^{2+}, Mg^{2+} and N while P^{+5} and K^{+} are moved mainly by diffusion which is a very sensitive process to soil water content. It is not therefore, surprising that plants subjected to water stress had a lower content in P^{+5} and K^{+} than the control (Marais and Wiersma, 1975) Regarding the nutrients moving mainly by mass flow, the accumulation of Mg^{2+} was reduced by soil water stress (Mederski and Wilson, 1960). Finally the evidence for N is rather contradictory. The increase in the nitrogen content of the tissues with increasing water stress is likely to be associated with effect on nitrate reductase enzyme system (Viets, 1972). In any case the decrease in the microbiological activity of the soil with increasing dryness (Kramer, 1969) cause a in the rate of the decomposition of organic matter. This results in

a decrease in the rate of both ammonification and nitrification of organic matter; and hence in a decrease in the nitrogen availability of soil.

According to Pavlov, (1982) the water deficit in wheat and barley, the uptake of phosphorus and other minerals that move through the soil by means of diffusion is decreased to a degree greater than the uptake of NO_3^- that moves by means of mass flow. This movement depends upon transpiration stream. The N/P ratio increases under a water deficit.

Water deficit in three species of genus Panicum (P. turgidum, P.antidotale, P.coloratum), Panicum ripens and Panicum maximum accumulate inorganic ions such as Na, K, Cl observed by Rahman et al., (1971), Ramati et al., (1971) and Ford (1981) respectively. They considered that accumulation of inorganic ions accounted for osmotic adjustment. According to Pande et Singh, (1981) water stress in Panicum coloratum decreases growth and biomass. However, the work of above workers indicate that drought adversaly affects the nutrient uptake and growth of plant. Effect of water stress on major inorganic constituents of proso millet leaves are recorded in Table 4.3 and Fig. 4.5, 4.6.

i) Phosphorus : Changes in phosphorus contents during water stress in different parts of proso millet plant are recorded in Table 4.3 and Fig. 4.5 . Except few observations

(Takeshi, 1966 and Samuels, 1972) most of the workers have noticed that water stress greatly hampers phosphorus uptake process. Wilson et al., (1968) observed that reduction of phosphorus content due to drought was a common feature in many annual forage legumes and the reduction was of the order of 4 to 68%. The work of Sakanoue and Iguchi (1968) indicated that among phosphorus, Silicon, Potassium, Calcium, magnesium, nitrogen and manganese, the uptake of Phosphorus was most sensitive to drought.

Many workers observed a decrease in phosphorus uptake due to drought in Rice (Pande and Singh, 1969), Apple (Kongstrud, 1969), Sorghum (Eck et al., 1979), Grasses and Legumes (Rahman et al., 1971), Oil palm (Forde, 1972), Onion (Dunham and Nye, 1976). Greenway et al., (1969) reported that the potential above -10.4 atm. in tomato plants affects the uptake and distribution of 32 P in both root and shoot.

The reduction in Phosphorus uptake due to drought leads to further metabolic disturbances as Phosphorus is essential for many processes. Gorden and Bichurina, (1970) reported that phosphorus shortage resulted due to water stress becomes the factor limiting in glycolysis. Thus glycolysis is dropped due to water stress while pentose phosphate pathway predominates. Samuilov and Lebedeva (1973) observed a sharp reduction of nucleotide phosphates and phoric esters of

sugars due to severe drought. According to them such a reduction might be affecting the enzymatic metabolism of the plant.

In proso millet root, stem, flag and nature leaves show increase in phosphorus content due to water stress. These results are different from those observed in Rice, Apple, Sorghum, tomato by different workers. Increasing phosphorus content in proso millet indicate less disturbances in metabolic activities of this plant, during water stress. This might be adaptive feature to overcome drought.

ii) Potassium :

Potassium content in all organs of proso millet considerably increases due to severe moisture stress (Table 4.3 and Fig.4.5) and among these plant parts the accumulation of potassium is quite significant in roots and to some extent in flag leaf.

Present findings are contradictory to those of Richards and Wadleigh (1952) who summarized the existing data on nutrient availability in relation to soil moisture availability and concluded that water stress causes a definite decrease in potassium concentration in the plant parts. Similar observations were made latter by Sakanoue and Iguchi (1968), Gilmore (1971), Mengel and Braunshweing (1972), Varma et al., (1976),

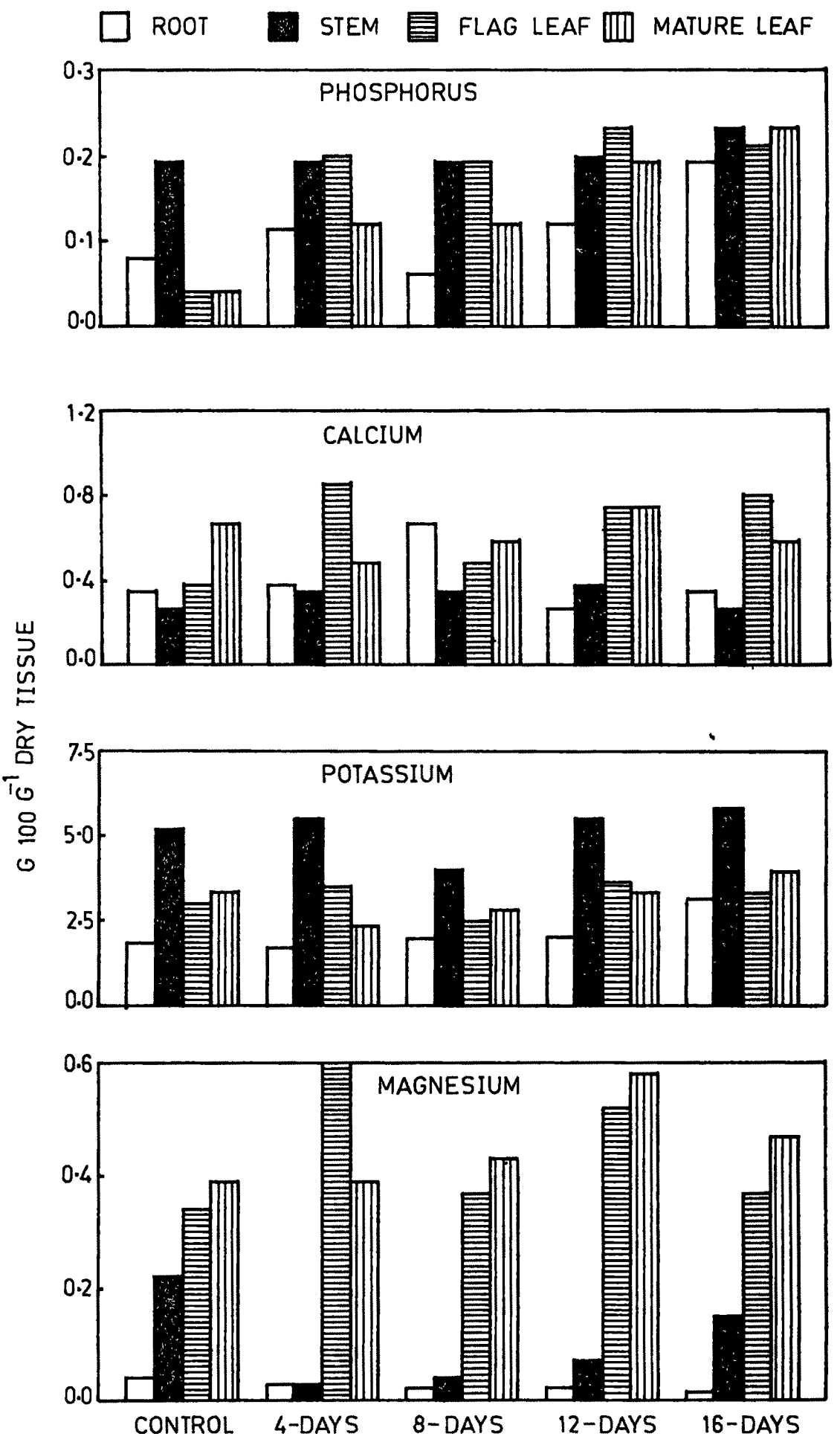


FIG.4·5 EFFECT OF WATER STRESS ON PHOSPHORUS, CALCIUM, POTASSIUM AND MAGNESIUM CONTENTS OF DIFFERENT PARTS OF P- miliaceum .

Martinz-Carrasco & Ramke et al., (1979) and Singh et al., (1979). The experiment of Stewart and Hungata (1966) and Shimomura (1967) indicated that Potassium uptake is only slightly reduced by water stress. Dunham and Nye, (1976) estimated the root absorbing power and suggested that mechanism of Phosphorus uptake was much more sensitive to decreasing water potential than potassium. Kongstrud (1969) observed a varietal difference regarding Potassium uptake during water deficiency. He observed that moisture stress increased potassium in black currants but not in apples. There are also few reports where increase in potassium uptake due to moisture stress affect the nitrogen and phosphorus more than potassium, calcium and magnesium in Sorghum bicolor. It was evident from Takeshi's experiments (1966) that water stressed leaves of Brassica rapa and Vigna sinensis contained more potassium. Singh and Singh (1970) found increase in potassium in the first period of growth of rice due to depleted soil water but later the trend was reversed. In 8 plant species namely Chloris gayana, Panicum turgidum, P.antidotale, P.maximum, P.coloratum, Cryzopsis miliacea, Medicago sativa and Crotolaria aegyptiaca studied by Rahman et al., (1971) it was noticed that the deficiency in moisture content is associated with increase in potassium content during all stages of their growth.

A high rate of potassium uptake by root cells depresses the osmotic potential in the cells and this induces water

uptake. According to Mengel and Kirkby (1980) uptake of water by roots and the ability of the plant to exploit soil water, therefore, depends on the potassium nutritional status of the plant. Shchykina (1965) emphasized that potassium as a fertilizer and also presowing hardening treatment increased drought resistance in maize.

According to Brag (1972) the Potassium content in plant might be helpful in controlling transpiration which should be checked in arid environments. The effect of potassium in regulating transpiration was ascribed to changes in Stomatal aperture. Ford et al., (1981) observed accumulation of potassium, sodium and chloride in Panicum maximum and this accumulation of inorganic ions was accounted for osmotic adjustments. The accumulation of potassium in water stressed proso millet, may be considered as adaptive in the light of the above findings.

iii) Calcium :

It is clear from Table 4.3 and Fig.4.5 that calcium content is increased by water stressed prosomillet Root, Stem and leaves. There is higher accumulation of calcium both in flag and mature leaves than root and stem. Calcium plays an important role in number of metabolic processes. Besides this calcium is found to be involved in mechanism of heat resistance in thermophilic bacteria.

(Ljunger, 1973). In arid lands, dryness and high temperatures are generally associated with each other. However, the exact role of calcium in heat tolerance of higher plants has not been worked out.

Richards and Wadleigh (1952) summarised the existing data on nutrient availability and concluded that water stress caused variable effects on calcium concentration in plants. In Brassica rapa and Vigna sinensis water stress was found to increase calcium content in the leaves (Takeshi, 1966). Kongsturd, (1969) observed that moisture stress increased calcium in black currants but not in apples. Rahman et al., (1971) observed that the calcium content rises with deficiency in soil moisture in different stages of development of some Panicum species. Similar observations were made in case of loblolly pine by Gilmore (1971). In sugarcane calcium content registered increases due to water stress (Samuels, 1972).

In contrast to above observations there are few reports where adverse effects of water stress on calcium uptake are noticed. According to Kunno et al., (1964), the inhibition of calcium and magnesium absorption may be one of the reasons for increased flower and pod shedding caused by water deficit in soybean. Giller (1969) observed symptoms of calcium deficiency in groundnut crop subjected to drought. The work of Singh and Singh (1970) with rice indicated that calcium was greatly reduced in wilting leaves. Vander Boon (1973)

noticed that drought lowered calcium content and raised K/Ca ratio in tomato fruits. In contrast to these observations, employing ^{45}Ca Stewart and Hungate (1966) found no effect of soil moisture on calcium uptake in Phaseolus vulgaris. Similarly Eck et al., (1979) observed that water stress did not affect calcium concentration in leaves of Sorghum bicolor.

In prosomillet calcium uptake is increased by water stress. Like potassium, calcium may be involved in drought resistance mechanism in this plant.

iv) Magnesium :

It is clear from Table 4.3 and Fig.4.5 that magnesium content is decreased in proso millet root and stems while in flag and mature leaf it is increased due to water stress. Sakanaue and Iguchi (1968, a, b, c) found that magnesium uptake is greatly reduced in rice plant under water stress. On the other hand Kongstrud (1969) observed an increase in magnesium content in black currant but not in apple. Gilmore (1971) found decreased amount of this nutrient in the leaves of loblolly pine due to water stress.

Magnesium is an important cofactor in metabolism and is essential for chlorophyll synthesis. Georgieva, et al., (1982) have reported that magnesium deficiency in chloroplast lipids of maize leaves reduce the phospholipids, phosphatidyl-

glycerol and galactosyldiglycerides. The decrease in magnesium content in root and stem and its accumulation in leaves indicates possibly it is translocated towards the leaf to reduce losses in lipids of chloroplast during water stress. This phenomenon of accumulation of magnesium in the leaves, the most active part of the plant may add to the retention of chlorophylls in the leaves even under severe drought conditions. We have already seen that the chlorophyll content of the leaves of proso millet is unaffected due to water stress.

v) Iron :

Water stress increases iron content in root and stem of proso millet. The iron content in mature leaves and flag leaves is increased after four days water stress and there after there is a slight decrease in it when the plants are stressed more. Basiouny and Biggs (1971) and Rahman et al., (1971) observed that iron uptake is hindered due to water stress. Ivanov and Karakash (1965) reported that soil moisture stress increased the content of soluble iron in roots.

Our findings with Proso millet clearly suggest that Iron accumulates in Roots due to stress while there is slight reduction in iron content of both flag as well as mature leaves. This may be due to negative influence of water stress on iron translocation. As iron is an essential element decrease in

Iron content in water stressed leaves can lead to metabolic disorders. However, from the present studies it appears that the level of iron in the leaves of stressed plants may not be producing any deficiency symptoms.

vi) Manganese :

It is clear from Table 4.3 and Fig.4.6 that water stress has caused slight decrease in manganese content of proso millet flag and mature leaves but there is increase in the root and stem. According to Viets, (1972) it is equally important to study the effect of water stress on the major elements (nitrogen, phosphorus, potassium) secondary elements (calcium, magnesium, silicon) as well as minor elements like manganese, iron and boron. Sakanoue and Iguchi (1968 ~~a, b, c~~) carried out extensive investigations to study the effect of low soil moisture on growth and nutrient absorption of rice at various stages of rice growth. They reported that drought increased the absorption of manganese in rice. Working with the same crop Pande and Singh (1969), however, found reduction in manganese content due to moisture stress. Slight decrease in manganese content in water stressed proso millet leaves may not cause any metabolic disorder.

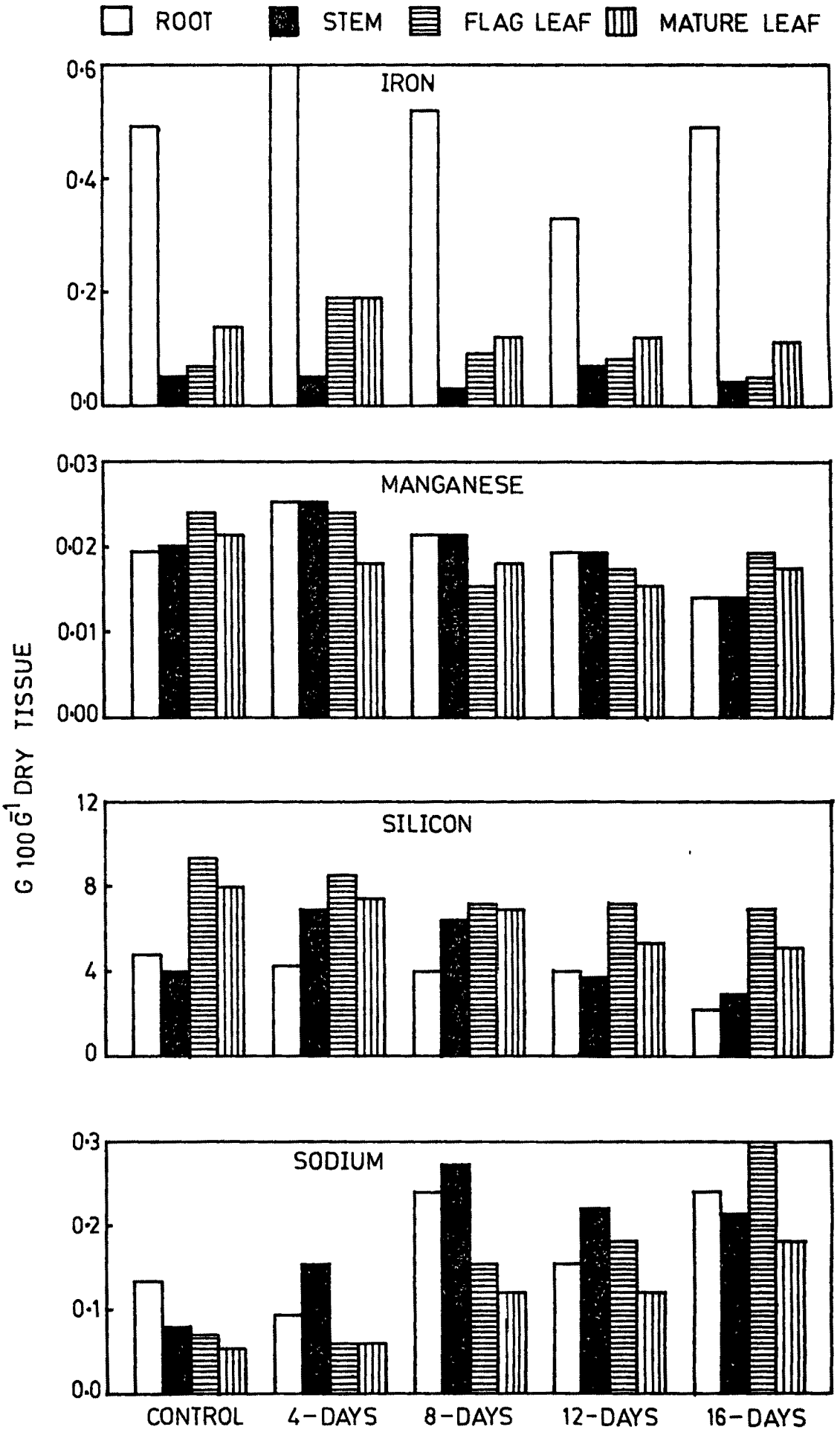


FIG.4·6 EFFECT OF WATER STRESS ON IRON, MANGANESE, SILICON AND SODIUM IN DIFFERENT PARTS OF P. miliaceum.

vii) Silicon :

The silicon content in the water stressed proso millet leaves and roots is considerably decreased but in case of stem it increases when exposed to eight days water stress; there after it decreases significantly in the plants stressed for 12 and 16 days. Jones and Handreck (1965) suggested that the uptake of silicon is a passive non-selective process and the quantities of silica which are absorbed and translocated to the tops can be accounted for in terms of level of silica in the external soil solution and the amount of water transpired. Lanning et al., (1958) have generalized that the more water is absorbed by the plants, the more silica is deposited. Yoshida et al., (1962) believe that silicon distribution within rice plants is related to the transpiration stream which is constantly flowing upward and outward so that silicon is concentrated and precipitated in the leaves as a result of transpiration and the participation of enzymes in silicic acid insolubilization is unlikely. According to Tanaka and Park (1966) along with transpiration stream, metabolic activities of the organ also control the uptake of silicon.

Sakanocie and Iguchi (1968) observed that soil moisture stress considerably decreased silicate concentration in rice. Singh and Singh (1970) on the contrary reported that low soil moisture caused accumulation of silicon in rice. Fox et al.,

(1969) studied the fate of soluble and total silicon in sugarcane. These workers found that young drought stricken sugarcane was very low in total silicon. Thus it is apparent that the uptake of silicon mainly depends upon the supply of water. As during water stress both availability of water and metabolic activities in the leaves are low, the silicon uptake is severely affected. Though the exact role of silicon in the plant metabolism is not clear, the structural role served by silica in rice is very well understood. The silica uptake is hampered by water stress in proso millet and this in turn may cause structural anamolies like leaf rolling in the plants stressed for more than 12 days.

ix) Sodium :

The proso millet plant exposed to water stress shows increased sodium content in all the three parts of plant, (Table 4.3 and Fig.4.6). There is little work on the effect of drought on sodium uptake. Takeshi (1966) found very little difference in sodium contents in the leaves of Brassica rapa and Vigna sinensis due to water stress. Rahman et al., (1971) observed that moisture stress caused rise in plant sodium content at each stage of development in different grass species. According to these workers such increase may be attributed to the great reduction in dry matter at low moisture content. Lawlor and Milford (1973) reported that sodium increases

drought resistance of water stressed sugar beet by altering leaf water balance.

Ramati et al., (1979) and Ford & Wilson (1981) observed the accumulation of sodium, potassium and chloride during water stress in Panicum ripens and Panicum maxicum respectively. They considered that the panicum grass leaves adjusted osmotically to water stress apparently through accumulation of solutes so that there was a decrease in osmotic potential at full turgor. The accumulation of sodium in all three parts of proso millet plant during water stress, may be considered as an adaptive feature in the light of the above findings with other panicum species.

It is evident from the foregoing discussion that water stress brings about several metabolic changes in proso millet plant which are clearly reflected in a changed chemical composition of the plant. Some of these changes indicate stimulation of drought sensitive processes which can adversely affect further growth and development. However, at the same time we can notice that Proso millet plant does posses certain adaptive features. These include changes in the level of proline, organic acids, sugar, under stress condition and this can definitely contribute to osmotic balance as well as post stress recovery processes. Similarly an efficient potassium and sodium uptake mechanism can improve water balance of this plant

Table 4.3 : Changes in inorganic constituents* of P.miliaceum during water stress.

Inorganic constituent	Organ	Water stress				
		Control	4 days	8 days	12 days	16 days
Phosphorus	Root	0.08	0.11	0.06	0.12	0.19
	Stem	0.19	0.19	0.19	0.20	0.23
	Flag leaf	0.04	0.20	0.19	0.23	0.21
	Mature leaf	0.04	0.12	0.12	0.19	0.23
Calcium	Root	0.34	0.37	0.67	0.27	0.34
	Stem	0.27	0.34	0.34	0.36	0.27
	Flag leaf	0.36	0.84	0.47	0.74	00.80
	Mature leaf	0.67	0.47	0.60	0.74	0.60
Potassium	Root	1.84	1.68	2.00	2.00	3.20
	Stem	5.20	5.52	4.00	5.46	5.76
	Flag leaf	2.94	3.50	2.56	3.68	3.36
	Mature leaf	3.28	2.40	2.80	3.36	4.00

...

Table 4.3 : (Contd...)

Inorganic constituent	Organ	Water stress				
		Control	4 days	8 days	12 days	16 days
Magnesium	Root	0.04	0.03	0.02	0.02	0.01
	Stem	0.22	0.03	0.04	0.07	0.15
	Flag leaf	0.34	0.60	0.37	0.52	0.37
	Mature leaf	0.39	0.39	0.43	0.58	0.47
Iron	Root	0.49	0.61	0.52	0.33	0.49
	Stem	0.05	0.05	0.03	0.07	0.04
	Flag leaf	0.07	0.19	0.09	0.08	0.05
	Mature leaf	0.14	0.19	0.12	0.12	0.11
Manganese	Root	0.019	0.025	0.021	0.019	0.014
	Stem	0.020	0.025	0.021	0.019	0.014
	Flag leaf	0.024	0.024	0.015	0.017	0.019
	Mature leaf	0.021	0.018	0.018	0.015	0.017

....

Table 4.3 : (Contd...)

Inorganic constituent	Organ	Water stress				
		Control	4 days	8 days	12 days	16 days
Silicon	Root	4.80	4.20	4.00	4.00	2.00
	Stem	4.00	6.80	6.50	3.60	2.80
	Flag leaf	9.20	8.50	7.20	7.20	7.00
	Mature leaf	8.00	7.50	7.00	5.20	5.00
Sodium	Root	0.13	0.09	0.24	0.15	0.24
	Stem	0.08	0.15	0.27	0.22	0.21
	Flag leaf	0.07	0.06	0.15	0.18	0.30
	Mature leaf	0.05	0.06	0.12	0.12	0.18
Na/K	Root	0.07	0.05	0.12	0.08	0.08
	Stem	0.02	0.03	0.07	0.04	0.04
	Flag leaf	0.02	0.02	0.06	0.05	0.09
	Mature leaf	0.02	0.03	0.04	0.02	0.02

* Values in g 100^{-1} g dry tissue.

under stress conditions. It is essential that such studies should be performed with several improved varieties of proso millet so that it can be seen whether such adaptive features can be exploited for breeding programme to synthesize a drought resistant variety.

C. Stomatal behaviour during water stress :

Stomatal resistance is the major physiological control for reducing water loss and preventing the development deleterious water deficits. Further more, it excerts a predominant influence over net photosynthesis of grasses during water stress (Doley and Trivett, 1974; Ludlow and Ng, 1976). Therefore it is of interest to study the response of stomata of plant to water stress. The effect of water stress on stomatal behaviour in P.miliaceum has been depicted in Table 4.4 and Fig. 4.7

It is evident that the size of the stomatal apparatus in lower epidermis is larger than in the upper one. This is quite significant when the length of stomatal apparatus is considered. However, the breadth of stomatal apparatus (including guard cells) is almost similar on both the surfaces of leaf. It is also clear that with increase in the water deficit level the stomatal apparatus becomes narrower. The measurement of the width of stomatal aperture at 11.0 A.M. indicates that increasing water stress in the proso millet

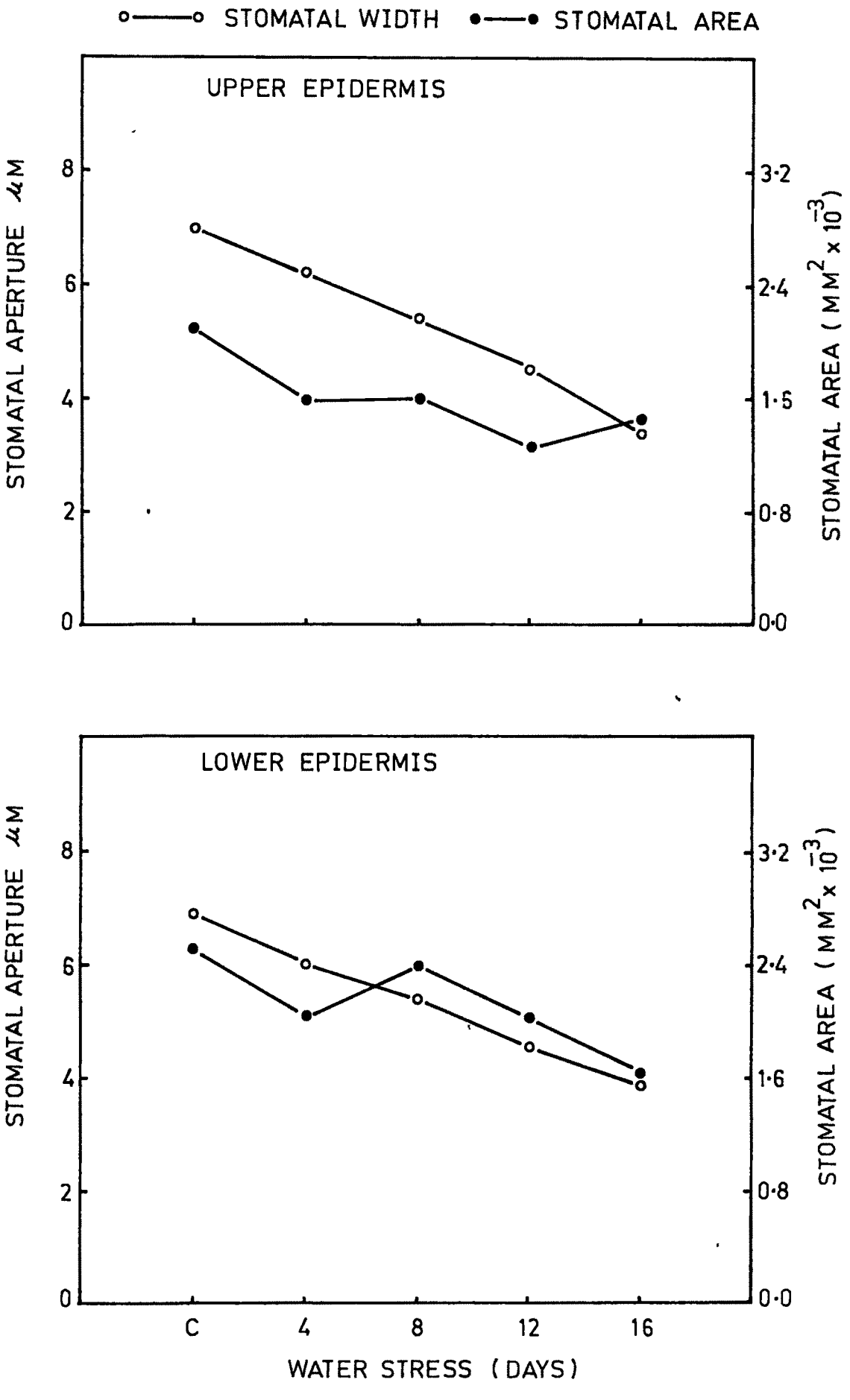


FIG.4·7 EFFECT OF WATER STRESS ON STOMATAL APPARATUS IN P miliaceum .

growth medium influences the opening of stomata very strongly during severe water deficit conditions. It is also evident that water stress for more than 8 days affects the opening of stomatae on the lower epidermis more than that on upper epidermis.

Partial or total stomatal closure caused by water stress may increase epidermal resistance of the leaf to the inward passage of CO_2 affecting the rate of photosynthesis (Hsiao et al., 1976).

Table 4.4 : Effect of water stress on Stomatal apparatus in P.miliaceum.

Sr. No.		WATER STRESS									
		Control		4 days		8 days		12 days		16 days	
		Upper Epidermis	Lower Epidermis	Upper Epidermis	Lower Epidermis	Upper Epidermis	Lower Epidermis	Upper Epidermis	Lower Epidermis	Upper Epidermis	Lower Epidermis
1.	Maximum length (μm)	29.70 ± 2.82	33.48 ± 2.16	26.82 ± 1.50	36.36 ± 1.76	24.58 ± 3.22	40.14 ± 2.79	22.68 ± 1.65	36.9 ± 2.01	27.36 ± 2.25	28.12 ± 2.20
2.	Maximum breadth (μm)	22.50 ± 1.45	22.14 ± 2.91	18.54 ± 1.52	19.08 ± 1.44	20.78 ± 1.50	18.54 ± 1.80	17.64 ± 0.72	17.46 ± 1.41	16.56 ± 1.76	17.21 ± 1.42
3.	Total stomatal area (mm^2) (4 π ab)*	2.1×10^{-3}	2.46×10^{-3}	1.56×10^{-3}	2.18×10^{-3}	1.6×10^{-3}	2.41×10^{-3}	1.26×10^{-3}	2.03×10^{-3}	1.42×10^{-3}	1.55×10^{-3}
4.	Width of stomatal aperture at 11.0 a.m. (μm)	7.60 ± 0.43	6.88 ± 0.65	6.23 ± 0.40	6.01 ± 0.58	5.36 ± 0.11	5.47 ± 0.27	4.46 ± 1.40	4.61 ± 0.48	3.42 ± 0.37	3.96 ± 0.32
5.	Stomatal aperture area (mm^2) (4 π ab)*	0.71×10^{-3}	0.72×10^{-3}	0.53×10^{-3}	0.69×10^{-3}	0.41×10^{-3}	0.69×10^{-3}	0.32×10^{-3}	0.53×10^{-3}	0.29×10^{-3}	0.35×10^{-3}

* Perimeter = 4 π ab. a = 1/2 Stomatal length
b = ,, ,, breadth /i width of stomatal aperture.

Table 4.4 : Effect of water stress on Stomatal apparatus in P.miliaceum.

Sr. No.		WATER STRESS									
		Control		4 days		8 days		12 days		16 days	
		Upper Epidermis	Lower Epidermis	Upper Epidermis	Lower Epidermis	Upper Epidermis	Lower Epidermis	Upper Epidermis	Lower Epidermis	Upper Epidermis	Lower Epidermis
1.	Maximum length (μm)	29.70 ± 2.82	33.48 ± 2.16	26.82 ± 1.50	36.36 ± 1.76	24.58 ± 3.22	40.14 ± 2.79	22.68 ± 1.65	36.9 ± 2.01	27.36 ± 2.25	28.12 ± 2.20
2.	Maximum breadth (μm)	22.50 ± 1.45	22.14 ± 2.91	18.54 ± 1.52	19.08 ± 1.44	20.78 ± 1.50	18.54 ± 1.80	17.64 ± 0.72	17.46 ± 1.41	16.56 ± 1.76	17.21 ± 1.42
3.	Total stomatal area (mm^2) (4 π ab)*	2.1×10^{-3}	2.46×10^{-3}	1.56×10^{-3}	2.18×10^{-3}	1.6×10^{-3}	2.41×10^{-3}	1.26×10^{-3}	2.03×10^{-3}	1.42×10^{-3}	1.55×10^{-3}
4.	Width of stomatal aperture at 11.0 a.m. (μm)	7.60 ± 0.43	6.88 ± 0.65	6.23 ± 0.40	6.01 ± 0.58	5.36 ± 0.11	5.47 ± 0.27	4.46 ± 1.40	4.61 ± 0.48	3.42 ± 0.37	3.96 ± 0.32
5.	Stomatal aperture area (mm^2) (4 π ab)*	0.71×10^{-3}	0.72×10^{-3}	0.53×10^{-3}	0.69×10^{-3}	0.41×10^{-3}	0.69×10^{-3}	0.32×10^{-3}	0.53×10^{-3}	0.29×10^{-3}	0.35×10^{-3}

* Perimeter = 4 π ab. a = 1/2 Stomatal length

b = ,, ,, breadth / width of stomatal aperture.