Qatar Univ. Sci. Bull. (1984) 4: 57-66

WATER CONDITIONS AND PROLINE CONTENT IN SHADE AND SUN PLANTS

By

K. H. BATANOUNY*, A. H. HASSAN
Faculty of Science, Cairo University, Egypt

Y. M. ABU SITTA
Faculty of Science, Girls Branch. Al-Azhar Univ.

Key words: Proline content, Shade and Sun plants, Water Conditions.

ABSTRACT

Different parameters of the water conditions (Water content, water saturation deficit, osmotic pressure of the cell sap and partial osmotic pressure due to sugars), chlorophyll and proline contents were investigated in sun and shade plants of *Sesbania sesban, Ocimum basilicum* and *Chenopodium murale*. The total chlorophyll content per unit fr. wt. is higher in shade than in sun plants. In shade, a considerable decrease of chlorophyll a/b ratio is observed in *Sesbania* and *Chenopodium;* but this ratio increases slightly in *Ocimum*. The different parameters measured exhibit a wide difference in sun and shade plants. The diurnal march of the proline content, water saturation deficit and the osmotic pressure show almost the same trend; being low before sunrise and increasing by the progress of the day. Their march is a mirror image of that of the water content of the photosynthesizing organs. The partial osmotic pressure due to sugars contributes more to the total osmotic pressure of the cell sap than electrolytes in sun and shade. The rise of the osmotic pressure at midday and afternoon hours is mainly due to the rise of the partial osmotic pressue of the sugars, particularly in sun plants.

INTRODUCTION

It is a well-known fact that the consequences of shading are the net influence of a set of concomitant variables operating on a series of independent plant functions. Many shade factors other than light are implicated in affecting the water status in the plant body, especially the photosynthesizing organs. Plants that normally grow in full sunlight, usually known as heliophytes, may grow fairly well under shade. However, their water conditions exhibit different pictures in sun and shade plants.

In the present investigation, three mesophytes, namely *Sesbania sesban* (L.) Merril, *Ocimum basilicum* L. and *Chenopodium murale* L., are used to study the effect of shading on the various water conditions. Diurnal changes of proline content were investigated in sun and shade plants to detect the water status in these plants. Proline accumulation has been found to be induced by water stress in plants (*cf.* Barnett & Naylor. 1966; Thompson *et al.* 1966, 1977; Singh *et al.* 1973 a and b; Huang & Cavalier 1979; Batanouny & Ebeid 1981).

The present paper reports the effect of shading on various criteria of the water conditions, chlorophyll and proline contents in the above mentioned plants. The diurnal changes of these components have been followed at intervals during the daytime.

* **Present address:** University of Qatar, P.O.Box 2713 Doha, Qatar.

MATERIALS AND METHODS

The studied plants are growing in the botanical garden of the Botany Department in the Cairo University Compass, Giza. These species are: (1) *Sesbania sesban* (L) Merril which is grown as a hedge, (2) *Ocimum basilicum* L. which is cultivated as an ornamental plant and (3) *Chenopodium murale* L. which grows as a weed in the garden.

It happened that a part of the garden is completely protected from direct sunrays by the neighbouring building. The three studied species grow in the shaded area as well as in other parts of the garden fully exposed to direct sunrays. All the investigated plants are full grown and those growing in shade have not been exposed to direct sunrays all over their lives. The soil moisture in the shaded and sunny areas is kept almost at equal levels and the plants were irrigated in both areas to eliminate water stress resulting from soil drought.

The investigated criteria include: leaf area, chlorophyll content, osmotic pressure values of the cell sap, the partial osmotic pressure of the sugars in the cell sap, water content of the transpiring organs, water saturation deficit and proline content. With the exception of the leaf area and the chlorophyll content, all the criteria were investigated at 3-hr intervals over the period from 6 a.m. before sunrise to 6 p.m. after sunset. The air temperature was meassured at 2-hr intervals in sun and shade during the experimental period. The water saturation deficit was estimated according to the method adopted by Stocker (1928), while the proline content was estimated according to the method given by Bates (1973).

RESULTS

Air Temperature

During the experimental period in December, 1982, the air temperature showed no difference in both sites before sunrise and after sunset. However, the values of air temperature varied widely during the daytime between sun and shade (Table 1). The maximum difference was observed at noon reaching 5^0C. It is to be noted that air temperature in the sunny habitat was measured while the bulb of the thermometer was shaded. This means that the measured values represent the air temperature in both sites eliminating the influence of direct sunrays.

Soil Moisture Content

The shaded and sun plants are a few metres apart and the soils supporting them have almost the same physical and chemical properties. The soil moisture content at the root zone of the sudied species in both sites did not differ widely. It ranged from 26.1 to 27.2% and from 25.8 to 27.2% in shade and sun, respectively.

Leaf Area

Fig. 1 shows representative shade and sun leaves (the third and fourth leaves) of the studied species. It is evident that the leaf area in sun plants is much smaller than that of shade plants. Taking the fourth leaf as an example, one finds that in *Sesbania,* the average leaf area is 16.2 cm^2 in the sun and 38.8 cm^2 in shade. In *Ocimum,* the leaf area is 10 cm^2 in the sun and 22.2 cm^2 in shade, while it is 12.9 cm^2 in the sun and 28.5 cm^2 in shade in *Chenopodium.* It is clear also (Fig. 1) that the outline of shade *Ocimum* and *Chenopodium* leaves is more nearly entire than that of sun leaves.

The fresh and dry weights as well as the total area of twigs including both leaf surfaces are given in Table 2. The ratios of area: fresh weight are determined.

The area per unit weight in sun plants is obviously lower than that in shade ones. In *Sesbania,* this value is 55.9 in the sun and 64.4 in shade, in *Ocimum* it reaches 30.7 in the sun and 43.5 in shade, while in *Chenopodium* it is 57.4 in the sun and 64.6 in shade. The same trend could be observed if the values are calculated on the bases of area per unit oven dry weight.

Table 1-Air temperature (^0C) in sunny and shaded habitats during the experimental period in December, 1982

Time	6 a.m.	9	12	3 p.m.	6
Sunny site *	11	18	25	21	18
Shaded site	11	14	20	19	18

* the bulb of the thermometre was shaded and not exposed to direct sunrays

Table 2-Weight and area parameters in twigs (down to the fourth node) of sun and shade plants

Species	Condition	Fr.wt. (g)	Dry wt. (g)	Total surface area * $(cm)^2$	Area/Fr.wt. (cm^2/g)
Sesbania sesban	Shade	5.80	1.03	373.2	64.4
	Sun	2.37	0.47	132.4	55.9
Ocimum basilicum	Shade	8.40	0.83	365.4	43.5
	Sun	5.42	0.45	166.5	30.7
Chenopodium murale	Shade	3.82	0.44	247.1	64.6
	Sun	2.72	0.35	156.2	57.4

* including the stem and both surfaces of the leaves

Table 3-Chlorophyll and carotenoids content of sun and shade leaves.

Species	Condition	Chlorophyll			Chlorophyll ratio	Carotenoids (g/g fr. wt.)
		a	b	Total		
Sesbania sesban	Shade	1.36	0.49	1.85	2.78	0.37
	Sun	0.98	0.21	1.19	4.67	0.33
Ocimum basilicum	Shade	0.60	0.18	0.78	3.33	0.23
	Sun	0.55	0.17	0.72	3.24	0.33
Chenopodium murale	Shade	0.95	0.32	1.72	2.47	0.31
	Sun	0.84	0.25	1.09	3.36	0.27

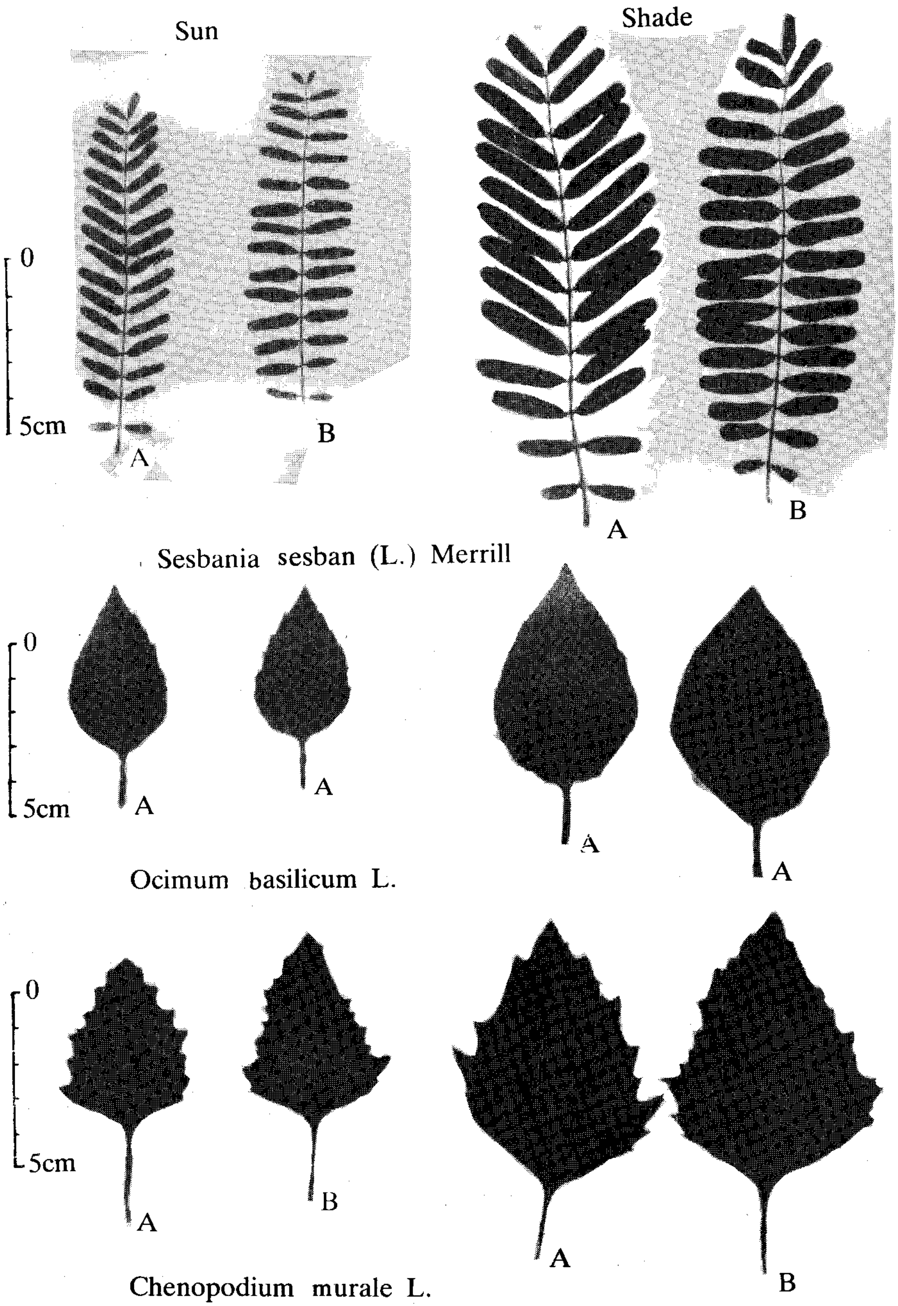

Fig. 1. Sun and shade leaves of the studied species (A: the third leaf and B: the fourth leaf)

Chlorophyll Content

The data given in Table 3 show that the total fresh weight related chlorophyll content of leaves is higher in shade than in sun leaves. However, the difference is not remarkable in the case of *Ocimum*. The increase of the total chlorophyll content in shade leaves compared with that in sun leaves is contributed by the different components of chlorophyll (a or b) in the studied plants.

It is interesting to note that the chlorophyll a : b ratio decreases by shading in *Sesbania* and *Chenopodium;* but it shows a slight increase in *Ocimum*. The carotenoids exhibit different trends in the studied species. Shade plants of *Sesbania* and *Chenopodium* have higher carotenoids content than sun plants. The situation is reversed in *Ocimum;* being 0.23 mg/g in shade and 0.33 mg/g in sun.

Water Content

The water content of the transpiring organs in sun and shade show different values all over the experimental period. Values are higher and their diurnal variations are wider in sun than in shade plants. (Fig. 2). The difference between water content values in sun and shade leaves increases with the progress of the day. However, the difference does not increase considerably in *Ocimum.*

The mean water content values range from 81.9% in shade leaves to 76.1% in sun leaves in *Sesbania,* from 90.6% in shade to 88.7% in the sun in *Ocimum* and from 88.6% in shade to 85.2% in the sun in *Chenopodium.*

Water Saturation Deficit

The water saturation deficit is more pronounced in sun than in shade plants. The average values range from 18.1 to 21.8% in *Sesbania,* from 15 to 17.6% in *Ocimum* and from 19.7 to 26.1% in *Chenopodium,* in shade and sun, respectively. The differences between the water saturation deficit values in shade and sun plants increase with the progress of the day (Fig. 2). The maximum value of water saturation deficit reached 33.1% in *Chenopodium* and 27.5% in *Sesbania* at 3 p.m. and 20.3% in *Ocimum* at noon.

Osmotic Pressure

Examination of Fig. 2 reveals the consistent difference between the osmotic pressure values in shade and sun plants; values being higher in sun thanin shade plants. The average values range from 8.54 atm in shade to 11.25 atm in the sun in *Sesbania,* and from 5.5 atm in shade to 7.14 atm in the sun in *Ocimum*. in *Chenopodium,* the range is wider; the average ranges from 11.17 atm in shade to 15.27 atm in the sun.

The maximum osmotic pressure was attained in sun plants three hours earlier than in shade ones, except in *Ocimum* where it was reached at the same time in shade and sun. The osmotic pressure due to sugars represents more than 50% of the total osmotic pressure of the cell sap in shade and sun plants in all the studied species. The highest contribution is observed in *Chenopodium* reaching 73%.The rise of the total osmotic pressure at midday and afternoon hours is mainly due to the rise of the partial osmotic pressue of sugars. An observation of significance is the amount of contribution of electrolytes to the osmotic pressure of the cell sap. In shade, this amount decreases in midday and afternoon and then increases after sunset. The reverse is observed in sun plants.

Proline Content

The average proline contents in shade leaves are lower than those in sun leaves in all the studied species (Fig. 2). The average values range from 12 to 15.2 µ mole/g in *Sesbania,* from 3.3 to 4.1 µ mole-g in *Ocimum* and from 4.45 to 11.3 µ mole/g in *Chenopodium,* in shade and sun plants, respectively. Fig. 2 shows that diurnal variations of the proline content are wider in magnitude in sun than in shade plants. The difference between the proline contents of sun and shade plants increases with the progress of the day. The maximum proline content was attained at noon in all the plants except in *Sesbania* growing in the sun where the maximum was reached at 3 p.m. amounting to 18.3 µ mole/g.

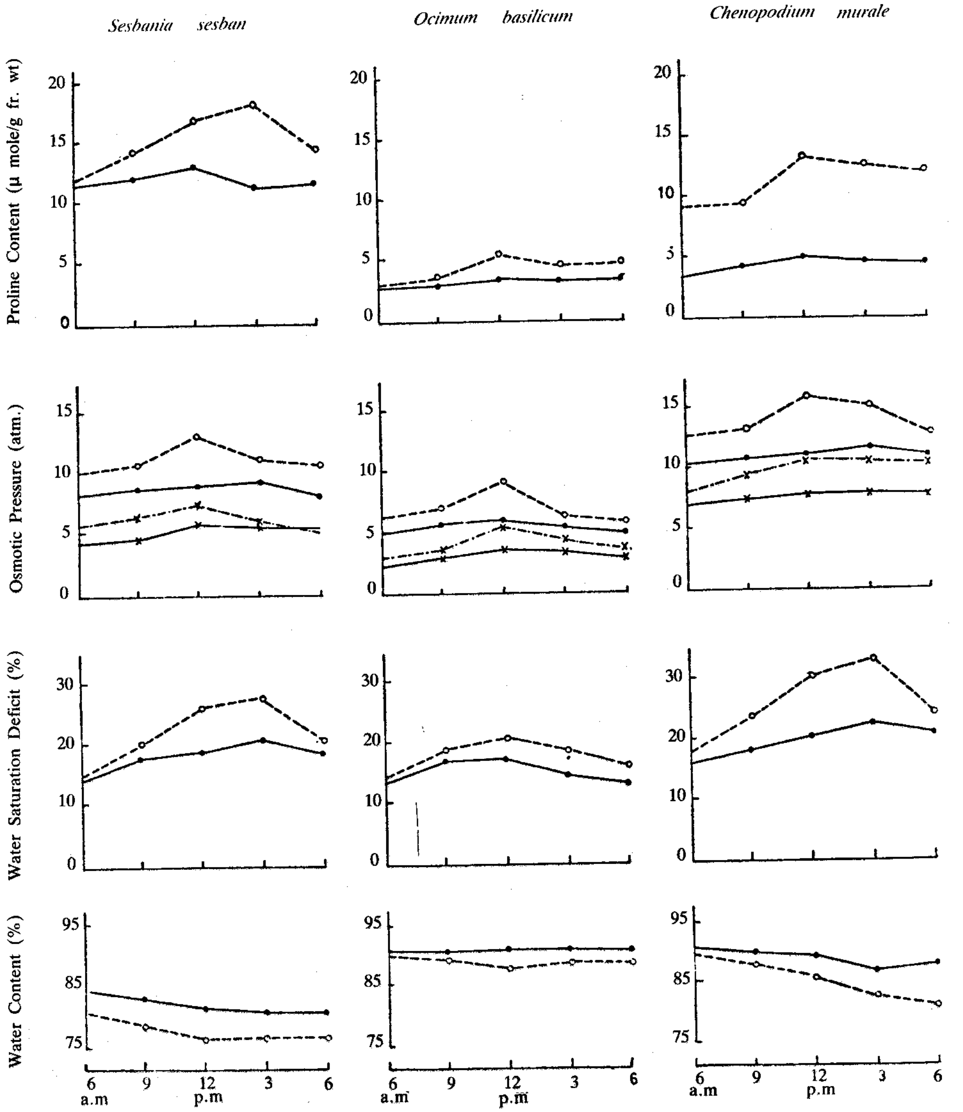

Fig. 2. **Diurnal changes in proline content, osmotic pressure of the cell sap, water saturation deficit and water content of the leaves of different plants in sun (o – o) and shade (•–•). - -x partial osmotic pressure of sugars in sun, x — x partial osmotic pressure of sugars in shade).**

DISCUSSION

The studied plants, in sun and shade, are growing in the same soil type and are irrigated at reasonably narrow intervals to assure adequate soil moisture supply to the plants in both sun and shade plots. It would appear in the case of the plants sampled that radiation was the principal limiting factor. It is noteworthy that the present investigation was undertaken on a sunny winter day in December with a maximum difference of air temperature between sun and shade of 5^0C at noon. Wider differences would be expected on hot summer days. However, the differences in the measured parameters in sun and shade are considerable in the present experiment.

Shade leaves in all the studied plants have larger areas and higher chlorophyll contents than the sun leaves. The ratio area: fresh weight in shade is higher than in the sun leaves down to the fourth node have more total area per unit weight in shade plants than in sun plants. This would show that sun leaves are thicker and the internodes are shorter and with thicker diameters than the shade organs. McClendon and McMillen (1982) found that morphogenetic control of leaves tends to increase the leaf area in shade in order to intercept more light, but there is a genetic or physiological limitation to the total leaf volume (fresh weight) as well as a resource limitation. They state that the leaf thickness is the primary respondent to illuminance, with leaf area and total leaf weight varying according to resources.

The decrease in chlorophyll content that accompanies bright light conditions has its beneficial aspects, for it results in less light being absorbed and more transmitted. The excess absorbed light would be converted into heat which affects the internal water balance and the photosynthesis-respiration balance.

The results show a considerable decrease of the value of chlorophyll a-b ratios in shade leaves in *Sesbania* and *Chenopodium*. However, the ratio increased in the shade in *Ocimum*. It would appear that in shaded *Sesbania* and *Chenopodium,* the increase in chlorophyll a as a result of reduced light intensity is more than the increase in chlorophyll due to reduced photoxidation in lower light conditions. In case of *Ocimum,* where the ratio a-b increased in the shade, it seems to be due to less synthesis of chlorophyll a than to the reduction of photoxidation of chlorophyll b in the shade. It is notable that the concentrations of both chlorophyll a and b of all the studied plants were observed to increase under low light conditions. However, Floyd & Noble (1980) state that the increased synthesis of chlorophyll a plays a greater role in the value of the a-b ratio than the increase of chlorophyll b under low light conditions or increased grana thylakold formation in some species though not in others. They suggest a relation in some chlorophyll concentration and the light environment in a deciduous forest.

The results obtained in the present investigation reveal clearly that light and its concomitant variables affect the water statues in the plant body. This is evident from the data of the water content and the water saturation deficit of the photosynthesizing organs. The water contents of comparable leaves in shade and sun plants are different, particularly at noon and the afternoon hours. This is reflected in the values of the water saturation deficit being higher in sun than in shade conditions.

In addition to low water content and higher water saturation deficits, the sun plants have higher osmotic pressure values than the shade ones. There is a consistent difference between the values of osmotic pressure in sun and shade. It is to be noted that sugars contribute the major part of the total osmotic value. They are more effective in raising the amount of the osmotic pressure than electrolytes. Moreover, the contribution of electrolytes decreases at midday and in the afternoon hours in the shade and increase after sunset. The contrary is observed in sun plants. The increase in the total osmotic values in sun plants may be ascribed to different factors including the rise in sugar content due to photosynthesis and the rise in electrolytes due to absorption resulting from the transpiration stream and the decrease in the water content of the tissues. The slight rise of the osmotic value of the cell sap in shade plants during the daytime is attributed to sugars, but not to the same extent as in sun plants.

It is clear from the collected data that light radiation and its concomitant variables affect the water status in the plant body. Consequently, there is some sort of stress in sun plants, which is not manifested in shade plants. This can be evinced from the diurnal march of the proline content in sun and shade plants.

Results obtained in this study indicate that different species growing under the same environmental conditions have different values of accumulated proline. However, they showed the same trend of the dirunal march of proline content. The coincidence of the proline increase with the decrease in water content and the increase in water saturation deficit and the osmotic pressure of the cell sap indicate the relationship.

Shading through its effect on the water status of the plants resulted in reducing the accumulation of proline in the photosynthetic organs. The low proline content in shade plants may be attributable to different factors including a low transpiration, hence the water stress is reduced and the proline content remains low.

The lowest values of proline were observed before sunrise. This may be ascribed to reduced water stress during the night and to the metabolic oxidation of the proline already accumulated in the case of carbohydrate depletion in the transpiring organs or incorporation thereof into protein as long as carbohydrate is present (*cf.* Stewart 1972). Schobert (1980) found that proline synthesis in the diatom *Phaeodactylum tricornutum* under stress is greatly reduced in the dark, indicating that the reducing power produced during photosynthesis might be involved in the biosynthetic pathway.

There is no wide difference in the proline contents of shade and sun plants before sunrise in the case of *Sesbania* and *Ocimum.* However, the difference at that time is considerable in *Chenopodium.* In all the studied plants, the difference between proline contents in shade and sun plants increases with the progress of the day and starts decreasing in the late afternoon hours. Proline accumulation is a measure of stress response. Differences in proline contents in sun and shade are comparable to those in water saturation deficit. Proline has been shown to reflect closely the water status of the material being studied (Waldern & Teare 1974, Hanson & Nelsen 1978 and Quarrie 1980). Quarrie (1980) found that there were significant negative correlations between proline concentration and shoot water content (as a percentage of shoot fr. wt.) for seven genotypes of spring wheat. However, the data presented by Hanson *et al.* (1977) indicate that proline accumulation in barley may be a measure of drought responses only and not of drought resistance.

ACKNOWLEDGEMENTS

The spectrophotometer used in the determination of proline is a donation from the Alexander von Humboldt Foundation. The authors wish to thank the Foundation for the generous donation.

REFERENCES

Barnett, N.M. and **A.W. Naylor** 1966. Amino acid and proline metabolism in Bermuda grass during water stress. Plant Physiol. 41: 1222-1230.

Batanouny, K.H. and **M.M. Ebeid** 1981. Diurnal changes in proline content of desert plants. Oecologia (Berl.) 51:250-252.

Bates, L.S. 1973. Rapid determination of proline for water stress studies. Plant and Soil 39:205-207.

Floyd, B.W. and **R.D. Noble** 1980. Intraseasonal variation in chlorophyll content and chloroplast ultrastructure of selected plant species in a deciduous forest. Can. J. Bot. 58:1504-1519.

Hanson, A.D. and **C.E. Nelsen** 1978. Betaine accumulation and (^{14}C) formate metabolisn in water stressed barley leaves. Plant Physiol. 62:305-312.

Hanson, A.D., C.E. Nelsen and **E.H. Everson** 1977. Evaluation of free proline accumulation as an index of drought resistance using two contrasting barley cultivars. Crop Sci. 17: 720-726.

Huang, A.C. and **A.J. Cavalieri** 1979. Proline oxidase and water stress-incuced proline accumulation in spinach leaves. Plant Physiol. 63:531-535.

Mc Clendon, J.H. and **G.G. Mc Millen** 1982. The control of leaf morpholigy and the tolerance of shade by woody plants. Bot. Gaz. 143:79-83.

Quarrie, S.A. 1980. Genotypic differences in leaf water potential, Abscisic acid and proline concentrations in Spring Wheat during drought stress. Ann. Bot. 46:383-394

Schobert, B. 1980. The function of proline accumulation in the diatom *Phaeodactylum tricornutum* during water stress. In: Spanswick RM, Lucas WJ, Dainty J. (eds) Plant Membrane Transport, pp 487-488. Elsevir/North-Holland Biomedical Press. Amsterdam.

Singh, T.N., D. Aspinall, L.G. Paleg and **S.F. Boggess** 1973 a. Stress metabolism. II. Changes in proline concentration in excised plant tissue. Australian J. Biol. Sci. 26:57-63.

Singh, T.N., L.G. Paleg and **D. Aspinall** 1977 b. Stress metabolism. I. Nitrogen metabolism and growth in the barley plant during water stress. Australian J. Biol. Sci. 26:45-56.

Stewart, C.R. 1972. Proline content and metabolism during rehydration of wilted excised leaves in the dark. Plant Physiol. 50:679-681.

Stewart, C.R. and **J.A. Lee** 1974. The role of proline accumulation in halophytes. Planta 120:279-289.

Stewart, C.R., C.J. Morris and **J.F. Thompson** 1966. Changes in amino acid content of excised leaves during incubation. II. Role of sugar in the accumulation of proline in wilted leaves. Plant Physiol, 41:1585-1590.

Stocker, O. 1928. Das Wasserhaushalt aegyptischer Wuesten-und Salzpflanzen. Bot. Abhandlungen (Jena) Hft 13, 200

Thompson, J.F., C.R. Stewart and **C.J. Morris** 1966. Changes in amino acid content of excised leaves during incubation. 1. The effect of water content of leaves and atmospheric oxygen level. Plant Physiol. 41:1578-1584.

Waldern, R.P. and **L.K. Teare** 1974. Free proline accumulation in drought-stressed plants under laboratory conditions. Palnt and Soil 40:689-692.

حالة الماء وتجمع البرولين في نباتات الظل والشمس

كمال الدين حسن البتانونى ـ عبد الرحمن حسين حسن
ويسرية محمد ابو ستة

قدرت المعايير المختلفة التى توضح حالة المـاء في جسم النبات وهى المحتـوى المـائى ، النقص في التشبـع ، الضغط الأسمـوزي للعصـير الخلوي ، الضغط الأسموزي الجزئى الناجم عن السكريات الذائبة ، محتوى الكلوروفيل والبرولين . وأجريت هذه الدراسة على ثلاثة أنواع نباتية هى السسبان ، الريحان والزربيح . وقد تم تقدير هذه المعايير في النباتات التي تعيش طوال حياتها في الظل وكذلك التى تتعرض للشمس بصورة طبيعية خلال اليوم طوال حياتها ، وأوضحت الدراسة أن المحتوى الكلي للكلوروفيل في الظل أعلى منه في النباتات المعرضة للشمس . وتبين أن نسبة كلوروفيل أ إلى كلوروفيل ب تنقص في الظل في نباتى السسبان والزربيح بينما ترتفع قليلا في نبات الريحان ، واتضح ان المعايير المقاسة تباينت قيمتها في نبات الظل والشمس .

وأظهرت دراسة المسار اليومي لمحتـوى البرولـين والنقص في التشبع والضغط الأسموزى أنها تتخذ مسـارات متماثلة ، فتكـون منخفضة قبـل شروق الشمس وتزداد قيمتها عند الظهيرة . واتخذ مسار هذه المعايير صورة مقلوبة للمحتوى المائى للأوراق والفروع الخضراء .

وتبين ان الضغط الأسموزي الجزئى الناتج عن السكريات الذائبـة يساهم في الضغط الأسموزي الكلى بقـدر اكبر من الضغط الأسمـوزى الجزئي النـاتج عن الألكتروليتات وذلك في حالتى نباتات الظل ونباتـات الشمس . ولذا فـإن ازدياد الضغط الأسموزي الكلي في منتصف النهار وبعد الظهيرة ناتج أساسا عن ازدياد الضغط الأسموزى الجزئي للسكريات وخاصة في نباتات الشمس .