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PROLINE ACCUMULATION IN PLANTS OF DIFFERENT ECOLOGICAL GROUPS AS A RESPONSE TO WATER DEFICIT

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ABSTRACT

The diurnal changes in proline content were investigated in different plants including a desert shrub *(Retama raetam),* a halophytic grass *(Sporobolus virginicus)* and a cultivated crop *(Zea mays).* Though these species have different values of accumulated proline, the diurnal march in all species exhibited the same trend. Low proline contents were recorded before sunrise then increase considerably by the progress of the day and decrease to low levels after sunset. The curves showing the diurnal march of proline content are parallel to those of the water saturation deficit. Both curves are mirror images of those showing the water content.

The irrigation regime has a prominent effect on the proline content of *Zea* plants. Proline accumulation in water-stressed plants is reversed when the stress situation is eliminated by re-watering the plants. Continuous water supply to *Retama* cut branches results in a notable decrease of profile as compared to natural water-stressed plants. Stress due to salinity causes proline accumulation in *Zea* plants grown in saline soil. The proline content in *Sporobolus* exhibits seasonal variations comparable to those of the evaporative power of the atmosphere.

The conditions of proline accumulation, which is a biochemical response of plants to environmental stresses, and the role of this accumulation are discussed.

INTRODUCTION

Accumulation of proline in wilted plant tissues in amounts greater than could be accounted by proteolysis was first noted by Kemble and MacPherson (1954). Though some amino acids accumulate to an extent (Thompson *et al.* 1966), proline is rather unique among the amino acids. This is due to the fact that proline accumulates consistently in numerous plant species and under a range of environmental conditions. The metabolic effects of wilting which lead to proline accumulation have not been observed with any other amino acid (Stewart, 1981).

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Proline Accumulation

Proline accumulation has been reported in numerous plants during drought stress so that it is considered a general phenomenon in plants (Barnett and Naylor, 1966; Thompson *et al.* 1966 Stewart, 1972 and 1981: Batanouny *et al.* 1981). It seems that proline accumulation is a general response to stress since it also accumulates under salinity and temperature stress (Stewart, 1981). That proline plays some role under saline conditions has been revealed by Stewart and Lee (1974).

Despite the presence of numerous publications dealing with proline accumulation in different plant species, yet studies on desert and halophytic plants natural conditions are fragmentary (*cf.* Batanouny *et al.* 1981). In the present investigation, the phenomenon or proline accumulation has been studied in different species with different ecological amplitudes.

MATERIALS AND METHODS

Three different plant species were used in the present investigation, representing three main ecological groups. These include: a desert shrub *(Retama raetam* (Forssk.) Heywood), a halophytic grass *(Sporobolus virginicus* (L.) Kunth) and a summer field crop (*Zea mays* L.).

The investigated *Retama* shrubs grow under natural conditions in the desert along Cairo - Suez road, Egypt. The diurnal march of proline content was investigated and the water content of the twigs was determined at regular intervals from 6 a.m. to 8 p.m. At the same time, the vapour pressure deficit of the atmosphere was calculated. Comparison of the diurnal march of proline and water content of *Retama* plants and branches with cut ends dipped in water was undertaken. Also, the march of proline and water content in cut starved branches of *Retama* were investigated.

The halophytic grass, *Sporobolus virginus* grows in the salines of the Mediterranean littoral coast to the west of Alexandria. The diurnal march of proline and water content was investigated. The seasonal changes of the proline content in this plant was studied by measuring the proline content in different seasons.

The mesophytic summer field crop, *Zea mays,* was cultivated in the Nile delta and irrigated with the Nile water. The diurnal march of proline and water contents in samples of the fourth leaf was investigated in plants irrigated regularly every two weeks, according to the practice adopted by the natives. The effect of extending the period between the successive irrigations on proline content, water saturation deficit and water content of *Zea* plants was investigated in plants irrigated every week, every two weeks and every three weeks. Under the latter treatment, the diurnal march of the above-mentioned criteria was studied before and after irrigation.

The method used in the determination of free proline content is that adopted by Bates (1973). The water saturation deficit in the *Zea* leaves was determined according the method given by Stoker (1929).

RESULTS

I Diurnal Changes of Proline in Different Plant Species.

The diurnal march of proline and water contents in different plant species is presented in Fig. 1. These plants are : a xerophyte (*Retama raetam*), a mesophyte (*Zea mays*) and a halophyte (*Sporobolus virginicus*). The figure shows also the diurnal march of the vapour pressure deficit (V.P.D.) values during the experimental periods. A glance to the figure shows that the water content values in all the studied species decrease by the progress of the day, reaching minimal values at noon in *Retama* and in the after-noon hours in the other species. The march of the water content is almost related to that of the atmospheric V.P.D. in the case of the mesophyte only. There is a lack of parallelism between the V.P.D. and the water content values in the other plants.

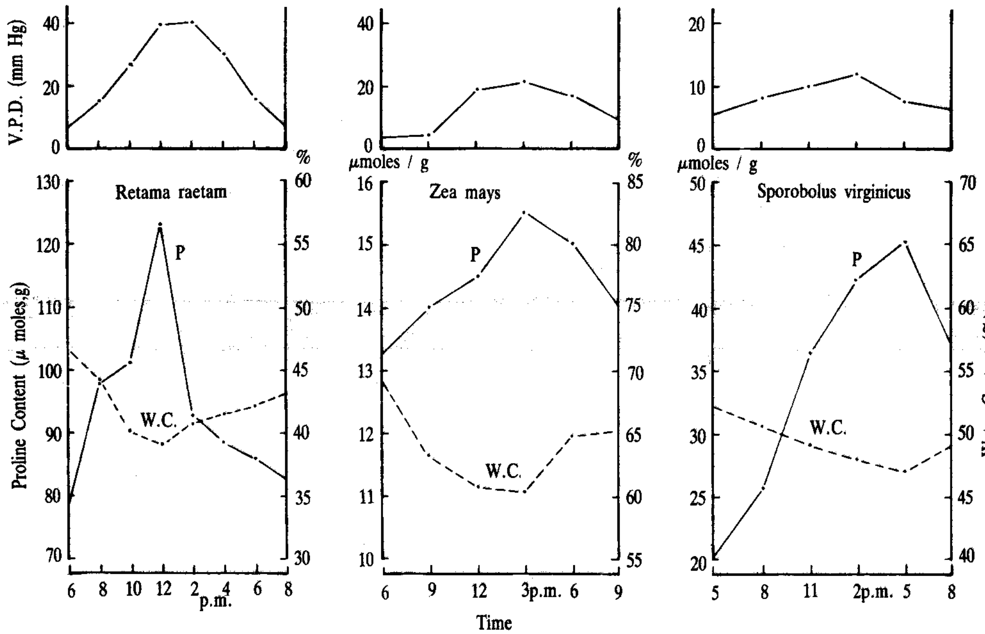

Fig.1 - Diurnal march of proline (P) and water contents (W.C.) of *Retama raetam*. *Zea mays* and *Sporobolus virginicus*. The march of vapour pressure deficit during each experiment is given.

It is clear that the curves showing the diurnal march of proline content are mirror images of those showing the water content. This indicates a close relation between the water status in the plant body and the amount of the accumulated proline.

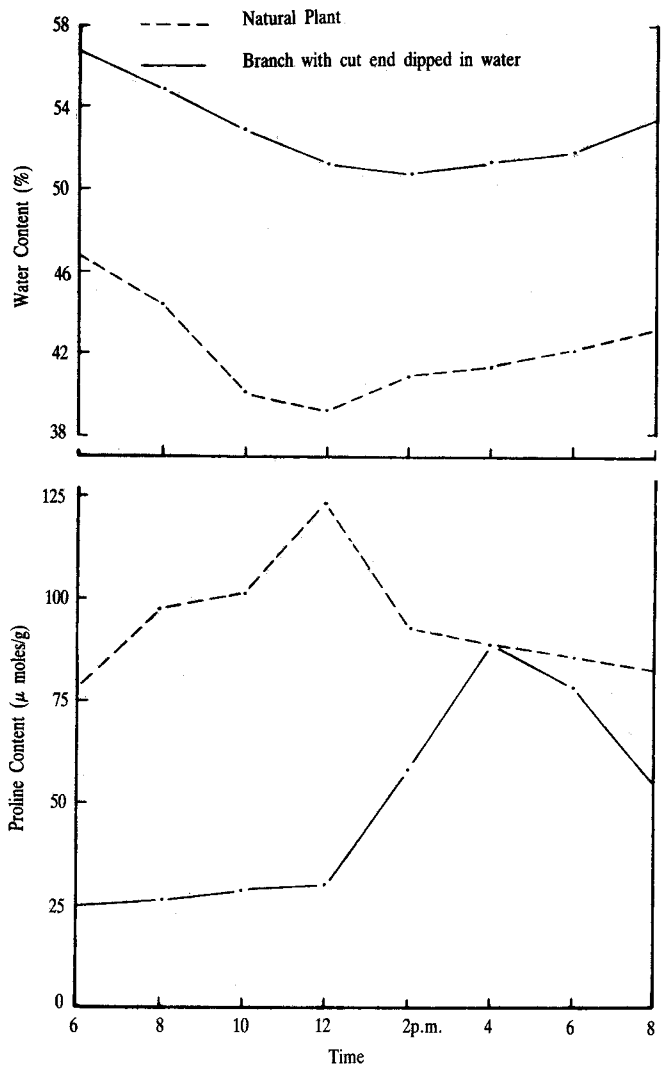

Fig.4 - Comparison of the diurnal march of proline and water contents of *Retama raetam* plants and *R. raetam* branches with cut ends dipped in water.

grows. The data obtained are illustarted in Fig. 4. The cut branch, being dipped in water 12 hrs before the beginning of proline and water contents determination, has higher water content values than the intact branches. In the early morning at 6 a.m., the water content of the cut branches with ends dipped in water was 56.7%, while that of the intact branches was 46.6%. By the progress of the day, the water content values in both cases decreased. However, the minimal values of 50.8% in cut branches was reached at 2 p.m., while it reached 39.2% in intact branches at noon time.

The proline content of the branches supplied with water was very low as compared to that of the intact of branches, being 24.74 μ moles/g in the former and 78.71 μ moles/g in the latter at 6 a.m. The proline content of the intact branches increased progressively reaching a maximum value of 123.13 μ moles/g at noon when the water content attained its lowest value. On the other hand, the cut branches supplied with water have proline content values kept at a low level till noon, not exceeding 29.87 μ moles/g. However, the severity of the atmospheric conditions resulted in a slight decrease of the water content and a considerable increase of proline content to a maximum of 88.95 μ moles/g at 4 p.m. This value is equal to that of intact branches at the same time, despite the wide difference in the water content values.

It is to be noted that the V.P.D. value at 6 a.m. was 6.96 mm Hg and increased by the progress of the day reaching 39.27 and 39.72 mm Hg at noon and 2 p.m., respectively. The V.P.D. values decreased progressively to low values reaching 7.83 mm Hg at 8 p.m. after sunset. Despite the low V.P.D. values after sunset, the water content values in both cases were low and the proline contents were high. This shows that there was some sort of water stress after due to the high water expenditure during the daytime.

V Effect of Starvation of Water on Proline Content.

Five branches of *Retama* were cut and left under natural desert conditions. The proline and water contents were determined at intervals over a period of 11 hours starting from 9 a.m. to 8 p.m. The data obtained are illustrated in Fig. 5. A glance to this figure reveals that the proline content increases and the water content decreases as a result of starvation of water. The curve showing the march of proline content is a mirror image of that of the water content.

Two minutes after cutting the branches, the mean proline content was 63.41 μ moles/g and the water content was 46.9%. Twenty minutes later, the proline content increased to 68.28 μ moles/g and the water content decreased to 41.9%. Decrease of the water content and increase of the proline content continued at a low rate reaching 31.7% and 88.42 μ moles/g at 2 p.m. Later at 4 p.m. a sharp rise of proline content could be observed reaching 145.5 μ moles/g. This occurred despite the slight decrease of the water content to 29.4%. In the period from 4 p.m. to 8. p.m., the proline content exhibited a progressive increase reaching 163.4 μ moles/g, while the water content decreased slightly to 28.6%.

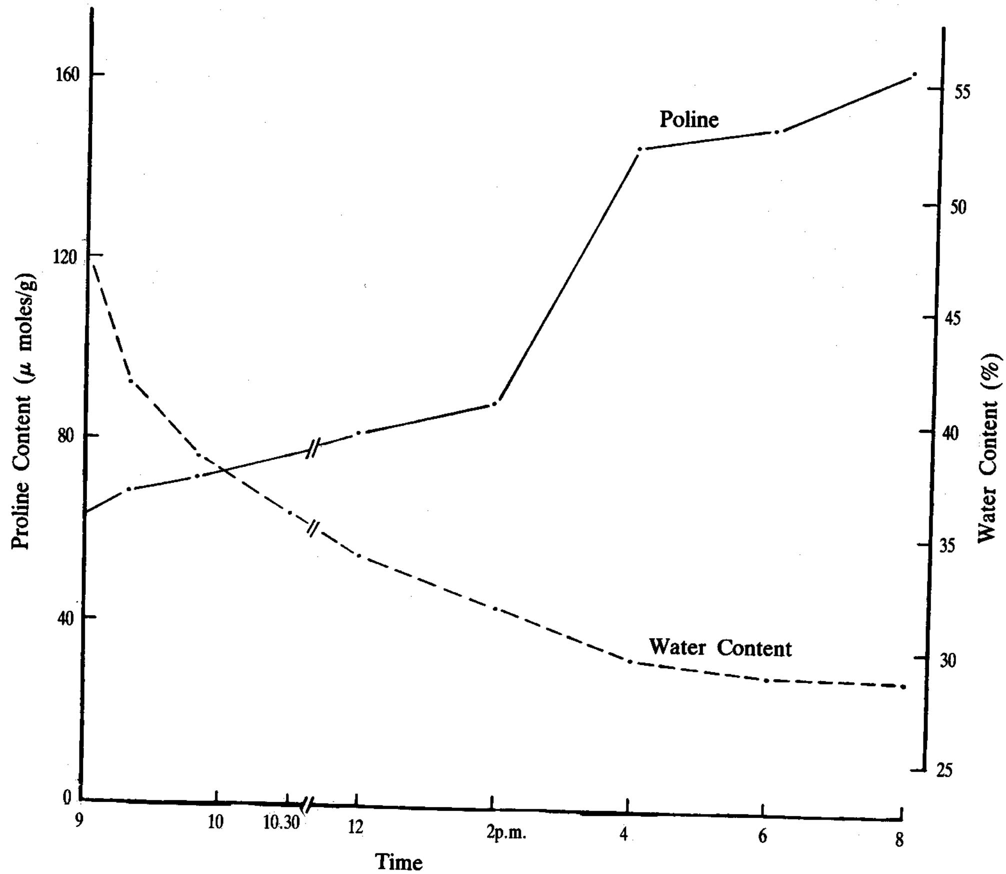

Fig.5- March of proline and water content in *Retama raetam* cut branches starved of water.

DISCUSSION

The present investigation deals with plant species belonging to different ecological groups. These species are: (a) *Retama raetam,* a xerophytic leafless shrub, (b) *Sporobolus virginicus,* a halophytic grass and (c) *Zea mays,* a cultivated summer field crop. Investigation of proline content, water content and water saturation deficit values was undertaken using samples collected from naturally growing desert and halophytic plants and from corn fields. The prevailing climalic aridity, represented by vapour pressure deficit values, was determined simultaneously with sampling of plant material. The results obtained in the present study reveal that different species have different values of accumulated proline. This has nothing to do with the ecological group to which each species is belonging. In some desert plants maximum proline values of 11.16 μ moles/g and 23.3 μ moles/g were obtained from *Francoeria crispa* and *Zygophyllum qatarense,* respectively (Batanouny *et al.* 1981). However, in *Retama,* the proline content ranges from 78.71 to 123.13 μ moles/g, while it ranges from 9.74 to 20.35 μ moles/g in *Zea mays.* This shows that plants could not be classified into groups with respect to their proline content or the amount of accumulated proline content due to stress. However, the different investigated species exhibit the same trend of the diurnal march of the proline content. The coincidence of the proline increase with the water content decrease indicates a relationship. In all the studied species, the curve showing the diurnal march of proline content is a mirror image of that showing the march of the water content. It is noteworthy that the maximum amount of accumulated proline in the different plants was attained at different times of the day. In the desert plant, the maximum was reached at noon and decreased rapidly by the progress of the day despite continuous rise of the evaporative power of the atmosphere represented by high V.P.D. values. This may be attributed to the regulating mechanism of transpiration rate in this plant. It is a well-known fact that desert plants are able to reduce their water expenditure, perhaps through the closure of their stomata, during the daytime despite the intensification of the evaporative power of the atmosphere (*cf.* Batanouny, 1980). Diminution of water loss may decrease the water stress and hence the accumulated proline decreases.

In the mesophytic plant, *Zea mays,* the maximum proline content was attained at the same time of maximum V.P.D. and minimum water content value. But in the case of the halophytic grass, the maximum proline content was reached at 5 p.m., three hours later after the maximum V.P.D. was reached. In such a case, it would be noted that the V.P.D. values are not as high as in the desert; the plant grows in the Mediterranean costal zone. The plant may transpire till the afternoon hours, resulting in water stress which attains its high effect at 5 p.m. Generally, the above-mentioned results evince that accumulation of proline is related to the water status in the plant body.

The results embodied in this study show that the water conditions in the photosynthesizing (transpiring) organs are reflected on the proline content and its accumulation. It has been found that expansion of the interval between two successive irrigations has a prominent effect on the accumulation of proline. The frequency of irrigation affects the water saturation deficit values and the water content of the transpiring organs. Parallelism occurs between the curves showing the diurnal march of the water saturation deficit values and those of the proline. Maximum values of both parameters were obtained at the same time (3 p.m.) under different irrigation treatments.

The results show, also, that irrigation, through its effect in eliminating water stress, resulted in reducing the accumulation of proline. The amount of proline in *Zea* plants before irrigation (irrigation every three weeks) reached values ranging from 15.44 to 20.35 μ moles/g. After irrigation, the proline content values were reduced to levels ranging from 3.88 to 9.16 μ moles/g . It is to be noted that the water saturation deficit decreased considerably after irrigation. Singh *et al.* (1973a and b) report that proline accumulation in water stressed plants is readily reversed if the stress situation is eliminated by rewatering the plants. Also, Stewart (1972) states that the accumulation of non-protein proline caused by wilting is stopped when leaves are rehydrated. Watering desert plants under natural conditions caused a considerable reduction of their proline content (Batanouny *et al.* 1981).

Desert plants should absorb water in amounts comparable to those lost through transpiration, otherwise the water balance becomes negative. This does not occur either all the year around or the whole day due to the deficiency of available water and the high water expenditure. These plants are subject to water deficit in their transpiring organs and they can tolerate such a deficit for relatively longer periods than mesophytes. The degree of water deficit varies in the same plant, being with daily and seasonal variations. Jarvis (1963), Kozlowski (1964) and Slatyer (1967) emphasized that increased total moisture stress was associated with increased leaf water deficits, decreased transpiration and decreased growth. Variations of the water deficit are reflected on the amount of proline content and the degree of its accumulation. In *Retama* plants, the proline content was 78.71 μ moles/g. at 6 a.m. before sunrise. On the other hand in a branch *Retama* with cut end dipped in water overnight under natural atmospheric conditions, the proline content at 6 a.m. was 24.74 μ moles/g, i.e. less than one third of that of the intact branches. The evaporating power of the atmosphere increased by the progress of the day and the V.P.D. values rose from 6.96 mm Hg at 6 a.m. to 39.27 mm Hg at noon. The water content values in intact and cut branches in water decreased. However, the lowest value of the water content of branches with cut ends dipped in water is still higher then the highest water content value of intact branches. This is reflected on the proline content values, being higher in natural plants than in branches supplied with water from their cut ends. By the progress of the day, the proline contents were equal in both cases, then it decreased considerably in the branches supplied with water.

The low proline values in the studied species observed before sunrise may be attributable to different factors. During the night the transpiration is low. Hence the water stress is reduced and the proline accumulation ceases. Moreover, the already accumulated proline would be metabolised by oxidation in the case of carbohydrate depletion in the transpiring organs or incorporated into portein as long as carbohydrate is present (*cf.* Stewart, 1972), *Retama* cut branches starved of water and exposed to natural desert conditions showed accumulation of proline in their tissues. Two minutes after cutting, the proline content was 63.41 μ moles/g and increased to 88.42 μ moles/g after 5 hrs. This rise could be interpreted by the increase of water stress resulting from the decrease of the water content from 46.9 to 31.7%. There is an amazing rise of proline content two hours later reaching 145.5 μ moles/g. This occurred despite the slight decrease of the water control to a level of 29.4%. Such a sharp rise of proline content, 7 hrs after cutting the branch, may be due to many reasons, including: stimulated synthesis of proline, inhibition of oxidation of proline, impaired proline synthesis. The increase of proline formation can be due either increased proteolysis or increased synthesis from precursors or both (Stewart, 1972). Severe water stress in the cut branches after 7 hours can be drastically dehydrating the tissues. This may be a cause of proteolysis which contributes to the proline content. At the same time, proline oxidation is inhibited by water stress (Stewart, 1981).

Though proline accumulates in the tissues of different plants with various ecological amplitudes as a response to water stress, yet the amount of proline could not be taken as a diagnostic feature for plants of different ecological groups. Thereafter, proline-accumulating potential should not be taken as a positive index of drought resistance. Even in cultivars of the same species, Hanson *et al.* (1977) obtained data on two contrasting barley cultivars which demonstrate that reports of a simple positive correlation between proline accumulating potential and drought resistance in barley may be in error.

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تجمع البرولين في نباتات من مجموعات بيئية مختلفة استجابة لنقص المـــاء

كمال الدين حسن البتانوني

عبد الرحمن حسـين حسن و كمال محمد زايـد

تمت دراسة المسار اليومي لمحتوى البرولين في نباتات مختلفة هي : الرتم (شجيرة صحراوية) ، سبوروبولس (نبات نجيلي ملحي) والاذرة (من نباتات المحاصيل) ورغم ان كميات البرولين المتجمعة تختلف باختلاف الانواع إلا ان المسار اليومي لمحتوي البرولين متشابه في هذه الأنواع . ففي الصباح الباكر قبل الشروق تكون كمية البرولين منخفضة ثم ترتفع إلى حد أقصى عند الظهيرة أو بعدها ، بعده يعود إلى النقصان إلى مستوى منخفض بعد الغروب ، والخطوط البيانية التي توضح المسار اليومي لمحتوي البرولين متوازية في مسارها مع تلك التي توضح مسار النقص في التشبع في الماء ، وكلاهما يمثل صورة في المرآة للخطوط البيانية للمحتوي المائي .

ويؤثر معدل الري على كمية البرولين في نبات الاذرة ، كما ان البرولين المتجمع في النباتات الواقعة تحت تأثير نقص الماء يتناقص بعد الري ، وكذلك الامداد المستمر بالماء لفروع الرتم المقطوعة يؤدي إلى نقص كمية البرولين بالمقارنة بكميته في النباتات الطبيعية المتعرضة لنقص الماء .

والأثر الناجم عن الملوحة مدعاة لزيادة تجمع البرولين ، فالاذرة المنزرعة في أرض ملحية تحتوي على كمية من البرولين أعلى من تلك الموجودة في النباتات المنزرعة في أرض غير ملحية .

والمسار الموسمي للبرولين في نباتات سبوروبولس مماثل لمسار قوة التبخير الجوية . وناقش البحث الظروف التي يتجمع تحتها البرولين الذي يعتبر استجابة بيوكميائية للضغوط البيئية ، كما نوقش دور تجمع البرولين .