ACTA AGROBOTANICA
vol. 36, z. 1-2 – 1983
59-65

Influence of water stress and abscisic acid on free proline accumulation in barley leaves

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(Received October 26, 1981)

Abstract

Rapid accumulation of free proline was found in barley leaves under conditions of osmotic stress at the level of -8.3 bars, but not before 9 days of its duration. The threshold value of osmotic stress for the start of a rapid accumulation of proline was about -6 bars. The excised leaves of barley subjected to water stress by wilting accumulated free proline more rapidly. In the course of 24 hours free proline content increased about 30-fold, while the water content in the leaves decreased 3-fold only. We found also that excised barley leaves with full turgor treated with abscisic acid (10^{-4}M) accumulated free proline as early as after 3 hours and subsequently after 24 hours free proline content increased 10-fold.

INTRODUCTION

Water stress produces in plants a number of metabolic changes which adapt the organism for further functioning under such unfavourable conditions. One of the metabolites arising as the result of these changes is the amino acid proline. Many investigators noted a rapid and considerable accumulation of proline in leaves of plants subjected to water stress (Barnett and Naylor, 1966; Waldren and Teare, 1974) as well as in osmotic stress caused by salinity of the medium (Göring and Thien, 1978; Covalieri and Huang, 1979; Tal et al., 1979). It is supposed that accumulation of this amino acid is genetically controlled and may be a factor adapting the plant to water stress conditions (Blum and Ebercon, 1976). Singh et al. (1972) found a simple relation between proline accumulation and the yield stability index in various barley varieties under water stress conditions. These authors suggest that accumulation of this amino acid may serve as a useful test for selection purposes in cereal breeding.

Another compound which under water stress conditions accumulates rapidly in leaves is abscisic acid (Hsiao, 1973). This hormone controls among other things the transpiration process by way of inducing the closure of stomates

(Mittelheuser and Stevenick, 1969; Jones and Mansfield, 1970). It was found that barley leaves exposed to the action of exogenous abscisic acid accumulate large amounts of proline independently of the effect of water stress (Rajagopal and Anderson, 1978). The function of abscisic acid in the process of proline formation was established by Stewart (1980) who demonstrated that abscisic acid stimulates the reaction of free proline release from glutamic acid.

The present paper is the first step in more extensive research aimed at establishing the relation between free proline accumulation in leaves and resistance of plants to water stress. The present study was undertaken to determine the free proline content in barley leaves subjected to osmotic stress owing to the saltiness of the medium; to water stress due to gradual dehydration as well as in leaves subjected to the action of abscisic acid.

MATERIAL AND METHODS

Barley plants (*Hordeum vulgare* L.) var. 'Lubuski' were cultivated in glasshouse conditions on a modified Knop medium (Krzywański et al., 1976). Seedlings 21-days old were used for the three different experiments.

Experiment I. Barley seedlings in hydroponic culture were subjected to osmotic stress by introducing into the medium an osmotically active substance such as a mixture of $NaCl + CaCl_2$ in a volume ratio 1 : 1. Four levels of osmotic stress were distinguished: -2.7, -4.5, -6.3, -8.3 bars according to the earlier adopted method (Zielińska and Borys, 1979). The free proline content was determined in leaves of plants after a definite duration of osmotic stress (3, 6, 9, and 12 days) and in leaves of plants not exposed to stress (control -0.7 bars).

Experiment II. Barley seedlings grown on sand supplied the experimental material. The excised leaves were subjected to water stress by gradual dehydration by way of transpiration at 21°C. Free proline content and the percentual water content in the leaves were determined after 6, 12 and 24 h of water stress as well as in leaves not exposed to stress (control).

Experiment III. Barley seedlings grown on sand served as experimental material. The detached leaves were immersed in a 10^{-4}M abscisic acid solution and in distilled water (control). They were then exposed to light of 450 - 500 lux intensity (mercury vapour tubes). The leaves took up abscisic acid with the transpiration current. After 3, 6 and 24 h of transpiration the amount of free proline and the percentual water content in the leaves were determined.

Free proline content was determined by the method developed by Bates et al., (1973). It consists in colorimetric determination of the amount of the coloured reaction product of proline with ninhydric acid. The amount of proline was calculated from the earlier plotted standard curve and expressed in mg/g of fresh leaf weight.

RESULTS

Osmotic stress evoked by the salinity of the medium caused free proline accumulation in barley leaves (Fig. 1). Accumulation of this amino acid depended on the stress level and its duration. The most pronounced reaction was not observed until nine days after exposure to the strongest stress (-8.3 bars). The content of free proline increased drastically as indicated by its sevenfold increase as compared with the control. Further action of osmotic stress did not produce any more changes in free proline content which proved the same after 12 days. These results allow to establish the threshold osmotic stress value for rapid accumulation of free proline in barley leaves, which amounts to -6 bars (Fig. 1).

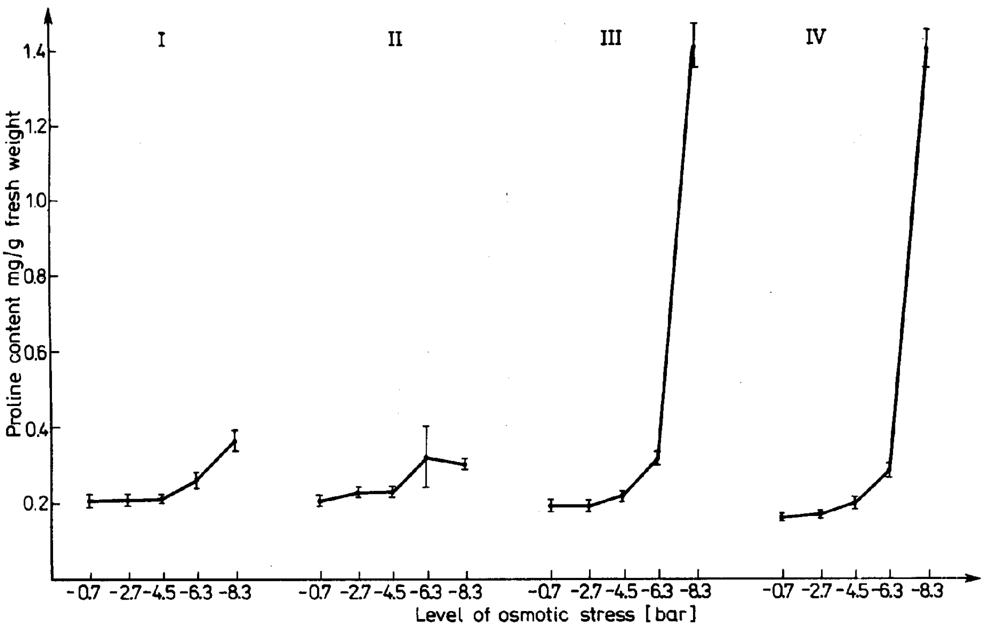
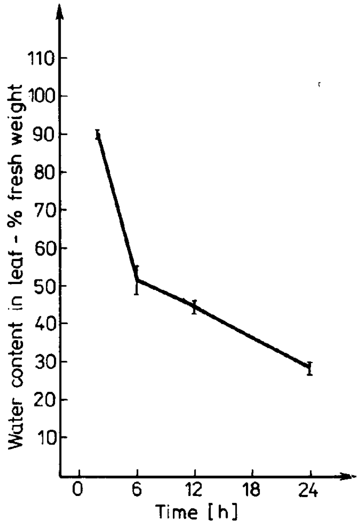


Fig. 1. Effect of increasing osmotic stress and its duration on free proline accumulation in barley leaves. I, II, III, IV – duration time of stress 3, 6, 9 and 12 days, respectively

Water stress arising as the result of gradual loss of water in the excised barley leaves, like osmotic stress, also caused a high accumulation of free proline. Within 24 h the percentual water content in the leaves decreased more than threefold (Fig. 2) and the free proline content increased about thirty times (Fig. 3), whereas in fully hydrated leaves (control) the proline level rose only slightly (Fig. 3). This is evidence that the metabolic changes in the detached leaves with full turgor influenced but little the increase of the free proline content.

The main cause of the high free proline accumulation was the water stress in the leaf, and actually the metabolic changes due to this stress.



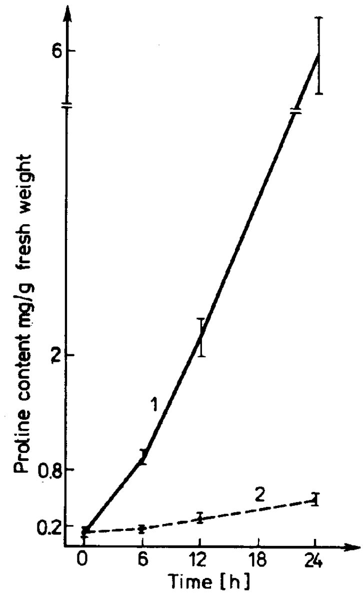


Fig. 2. Water content in excised barley leaves during dehydration

Fig. 3. Effect of gradual decrease of water content in barley leaves on proline accumulation: 1 — free proline content in excised leaves subjected to gradual dehydration, 2 — free proline content in excised leaves with full turgor

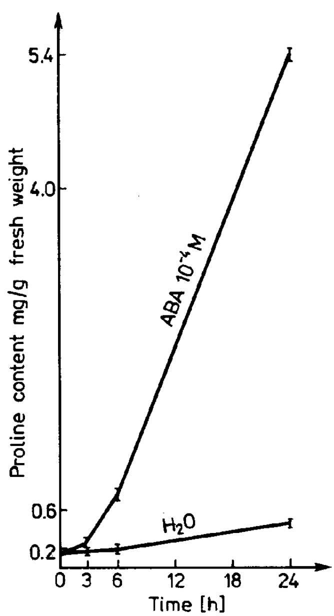


Fig. 4. Effect of abscisic acid on free proline accumulation in excised barley leaves

The excised barley leaves treated with abscisic acid also accumulated considerable amounts of free proline. The action of abscisic acid was noticeable as early as 3 h after placing the leaves in the solution. The free proline content increased proportionally to the time of exposure to this hormone (Fig. 4). After 24 h the amount of proline increased tenfold as compared with the control, whereas the water content remained at a constant level of about 90 per cent of fresh weight of the leaves.

DISCUSSION

The here presented results confirm in general earlier ones in experiments with maize (G ö r i n g and T h i e n, 1978), marsh halophytes (C o v a l i e r i and H u a n g, 1979) and the evergreen desert shrub *Simmondsia chinensis* (T a l et al., 1979). In all these plants an increase of salinity of the medium exceeding the threshold value caused free proline accumulation. The threshold value was, however, different for various plant species, and the amount of accumulated proline was proportional to the degree of salinity. Some authors (C o v a l i e r i and H u a n g, -1979, T a l et al., 1979) call attention to the dynamic character of free proline accumulation.The latter starts a certain time (lag period) after exposure of the plant to stress. When the stress factor is removed the free proline level remains for some time unchanged and then rapidly falls. It was, namely, noted that water stress stimulates free proline formation from glutamic acid with simultaneous inhibition of its oxidation (S t e w a r t, 1977; 1978). Inhibition of protein biosynthesis under conditions of water stress might also lead to an increased accumulation of free proline (S t e w a r t, 1978). Many investigators believe that free proline is a factor in the adaptation of the plant to stress conditions. It is supposed that it is a storage form of nitrogen, a source of energy and reduction force because it undergoes rapid change to glutamic acid (S t e w a r t et al., 1966; S t e w a r t, 1972). Free proline accumulates in the cytoplasm (S t e w a r t and L e e, 1974; G ö r i n g and T h i e n, 1978). It is believed that, as neutral metabolite (compatible organic solutes) it may ensure osmotic equilibrium between the cytoplasm and the cell vacuole, making possible undisturbed metabolism under conditions of water stress.

The results obtained agreeing with the reports of R a j a g o p a l and A n d e r s o n (1978) confirm the role of abscisic acid in free proline accumulation. Bearing in mind the fact that abscisic acid accumulates very quickly in leaves subjected to water stress (H s i a o, 1973), R a j a g o p a l and A n d e r s o n suggest that this substance is the direct factor evoking free proline accumulation. The results of investigations on the mechanism of action of abscisic acid on free proline accumulation (S t e w a r t, 1980) demonstrated that abscisic acid stimulates free proline formation from glutamic acid. Under normal conditions this synthesis is controlled by a negative feedback consisting in that the accumulated proline inhibits its own further synthesis (Boggess et al., 1976). According to the hypothesis of Stewart (1980), abscisic acid blocks this control mechanism, this leading to a considerable uncontrolled accumulation of free proline. The studies of Stewart (1980) did not confirm, however, the convincing assumption that abscisic acid may also contribute to an enhanced free proline accumulation by way of inhibition of protein biosynthesis.

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Wpływ stresu wodnego oraz kwasu abscysynowego na akumulację wolnej proliny w liściach jęczmienia

S t r e s z c z e n i e

Stwierdzono szybką akumulację wolnej proliny na poziomie stresu osmotycznego wynoszącego −8,3 bara, dopiero po 9 dniach jego trwania. Wartość progowa stresu osmotycznego dła zapoczątkowania akumulacji wolnej proliny wynosiła około −6 barów. Znacznie wcześniej wystąpiła akumulacja wolnej proliny w odciętych liściach jęczmienia na skutek stresu wodnego (odwodnienie liści). W czasie 24 godzin zawartość wolnej proliny zwiększyła się około 30 krotnie, podczas gdy zawartość wody w liściu zmniejszyła się tylko 3-krotnie. Nie stwierdzono akumulacji wolnej proliny w odciętych liściach w pełni uwodnionych. Natomiast w liściach odciętych w pełni uwodnionych, ale poddanych działaniu kwasu abscysynowego (stężenie − 10^{-4}M) już po 3 godzinach stwierdzono dość wysoką akumulację wolnej proliny. Ilość wolnej proliny wzrastała proporcjonalnie do czasu działania kwasu abscysynowego i w ciągu 24 godzin osiągnęła wartość 10-krotnie wyższą od kontroli. W świetle przeprowadzonej dyskusji należy sądzić, że kwas abscysynowy jest przyczyną sprawczą w mechanizmie prowadzącym do akumulacji wolnej proliny.