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Proline Metabolism During Water Stress in Sweet Pepper (*Capsicum annuum* L.) Plant

By

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With 2 Figures

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Summary

El Sayed H. 1992. Proline metabolism during water stress in sweet pepper (*Capsicum annuum* L.) plant. – Phyton (Horn, Austria) 32 (2): 255–261, 2 figures. – English with German Summary.

The influence of water stress on proline metabolism was studied in 3-month-old sweet pepper plants at four levels of water stress. Leaf water potential was drastically decreased in all treatments. Though leaf area and relative water content were decreased, this was marked only in very severe stress conditions. In stress treatments proline accumulation was observed both in leaves and roots, particularly the latter. The enzymes, proline dehydrogenase and proline oxidase, were inhibited under stress conditions; proline oxidase was more inhibited in roots than in leaves. The significance of the relative activities of these two enzymes is discussed.

Zusammenfassung

El Sayed H. 1992. Prolinstoffwechsel bei Paprika (*Capsicum annuum.* L.) unter Wasserstreß – Phyton (Horn, Austria) 32 (2): 255–261, 2 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Der Einfluß von Wasserstreß auf den Prolinstoffwechsel wurde an 3 Monate alten Paprikapflanzen untersucht, wobei 4 Grade von Wasserstreß angewandt wurden. Das Wasserpotential der Blätter war bei allen Versuchsanordnungen sehr stark reduziert. Obwohl die Blattfläche und der relative Wassergehalt abnahmen, war dies nur unter sehr starken Streßbedingungen ausgeprägt. Unter Streßbedingungen wurde eine

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Prolinanreicherung in den Blättern, besonders aber in der Wurzel beobachtet. Die Enzyme *Prolin-Dehydrogenase und Prolin-Oxidase waren unter* Streßbedingungen inhibiert, wobei dies bei Prolin-Oxidase mehr für die Wurzel als für die Blätter zutraf. Die Signifikanz der relativen Aktivitäten dieser zwei Enzyme wird diskutiert.

Introduction

A wide range of mechanisms for maintaining turgor by osmotic adjustment occurs in plants under water stress. One of these may be the accumulation of amino acids (JONES & al. 1980, MUNNS & WEIR 1981, TURNER & STEWART 1986), including higher concentrations of proline (BOGGESS & STEWART 1980, STEWART & VOETBERG 1985, HANDA & al. 1986). A higher level of proline during water stress, due to enhanced synthesis, is well established (BARNETT & NAYLOR 1966). There are also reports indicating the inhibition of proline oxidation in stressed plants (STEWART & al. 1977). Both proline oxidase and proline dehydrogenase are reported to catalyse proline oxidation and proline oxidase inhibition in water stressed spinach plants (HUANG & CAVALIERI 1979). Little information is available however, on the levels of DNA proline dehydrogenase in water stressed plants, although its occurence in leaves of higher plants is well established (MAZELIS & FOWDEN 1971, MAZELIS & CREVELING 1974, MCNAMER & STEWART 1974, STEWART & LAI 1974).

In the present study, the occurence and relative activities of both proline oxidase and proline dehydrogenase in sweet pepper (*Capsicum annuum* L.) was investigated under different regimes of water stress.

Materials and Methods

Cuttings of sweet pepper (*Capsicum annuum* L.) lenght 12–15 cm; diameter 0.8–1.2 cm, with 3 to 4 active buds were maintained in earthen pots (12″ × 15″) containing 4.0 kg of clay soil and farmyard manure (32:21). Three month old plants were subjected to water stress by daily adding the required volume of water in the morning to give 75%, 50%, 25%, 12.5% of field capacity or by withholding water and stress treatments were denoted as mild, moderate, severe and very severe respectively. Soil moisture content was determined by taking soil samples between 11 h and 12 h, and drying them in an oven at 120° C. Values are expressed as percentages on a wet weight basis. Leaf area and physiological variables were studied in the third, fourth, and fifth leaves from the apex and are given as mean values. The leaf area was measured by plotting the margins of the leaf on graph paper. The leaf relative water content was determined by the method of TURNER (1981). The leaf water potentials were measured by a dye method (KNIPLING 1967).

Free proline from roots and leaves was extracted in aqueous sulphosalicylic acid and estimated using ninhydrin according to the method of BATES & al. (1973). Proline dehydrogenase was extracted in 100 mol m^{-3} phosphate buffer (pH 8.0) containing 1.0 mol m^{-3} cysteine and 0.1 mol m^{-3} EDTA and assayed by following NAD reduction in 3.0 cm^3 of reaction medium containing 100 mol m^{-3} $Na_2CO_3 - NaHCO_3$ buffer (pH 10.3), 20 mol m^{-3} L-proline and 10 mol m^{-3} NAD^+, according to the method of RENA &

SPLITTSTOESSER (1975). Proline oxidase was assayed by following DCPIP reduction in mitochondrial preparations in 3.0 cm^3 reaction medium containing 50 mol m^{-3} Tris – HCl (pH 8.5), %5.0 mol m^{-3} $MgCl_2$, 0.5 mol m^{-3} FAD, 1.0 mol m^{-3} KCN, 1.0 mol m^{-3} phenazine methosulphate, 0.06 mol m^{-3} DCPIP, and 100 mol m^{-3} proline according to the method of HUANG & CAVALIERI (1979).

Results and Discussion

The shoots of 3 month old plants possessed 3 to 4 branches with 10 fully expanded leaves. The root system was about 23 cm long with 8 branch roots. The plants began to wilt on the 3rd and 5th day under very severe stress conditions, respectively. Hence, in the present study, observations were made on plants after 5 d of stress.

The soil moisture content ranged from 12.9% to 2.5% in very severe stress treatments. Leaf area and relative water contents decreased in all stress treatments, but a drastic reduction was observed only with the very severe stress treatment. Leaf water potential was drastically decreased in all stress treatments (from –19.9 bars under mild stress to –27.7 bars under very severe stress treatments) (Fig. 1).

The levels of free proline, proline oxidase, and proline dehydrogenase are presented in Fig. 2. The proline content increased in roots and leaves with increase in intensity of the water stress. It increased steadily from mild do very severe stress in both roots and leaves. The proline accumulation in response to stress was greater in roots than in leaves.

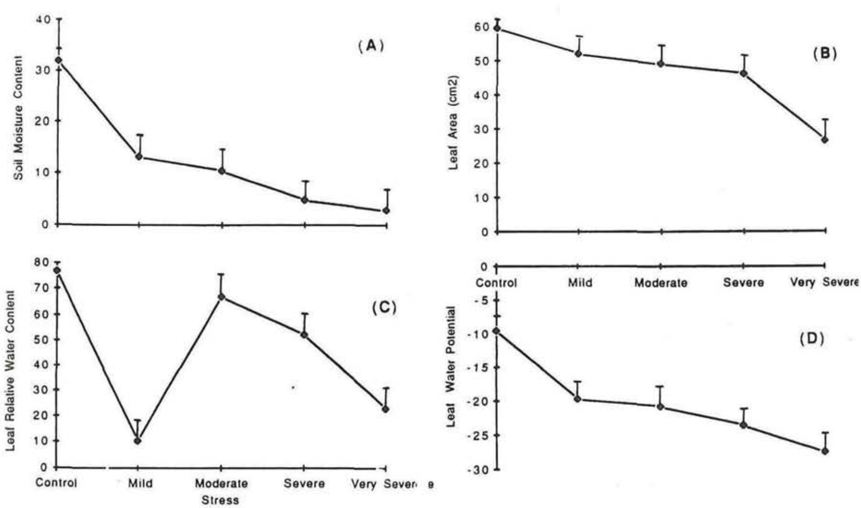

Fig 1. Percentage soil moisture content (A), lear area (cm^2) (B) relative water content (C), and water potential (bars) (D) in control and 5-days stress plants of *Capsicum annuum* L. Values are means of five replicates ± S.E.

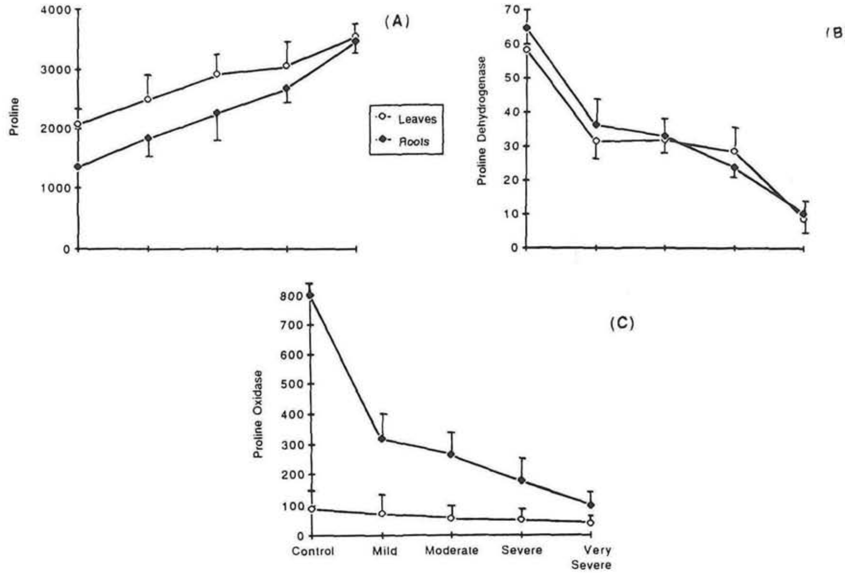

Fig 2. The levels of proline (mol Kg^{-1} dry weight) (A) and activity of enzymes proline dehydrogenase (B) and oxidase (C) (nano katals g^{-1} dry weight) in control and 5-d stressed leaves and roots of *Capsicum annuum* L. plants. Values are means of three replicates ± S.E.

The activities of proline dehydrogenase and proline oxidase decreased as the intensity of stress increased. There was an almost 85% inhibition of proline dehydrogenase activity in very serverely stressed roots and leaves. In control plants proline oxidase activity was higher in roots than in leaves, and under stress conditions the inhibition was greater in roots than in leaves. In stressed leaves, proline dehydrogenase was inhibited to a greater extent than proline oxidase.

The moderate decrease in leaf area and relative water content under mild to severe stress treatments in contrast to the dramatic decrease under very severe stress treatments indicates partial turgor maintenance in these leaves, since leaf expansion and area depend on the turgor of the cells. Furthermore, despite a drastic reduction in leaf water potential, the turgor maintenance, may be due to osmotic adjustment as a result of accumulation of solutes, including higher levels of proline under stress conditions (JONES 1978, JONES & TURNER 1980, TURNER & STEWART 1986).

Proline accumulation in water stressed plants (BANSAL & NAGARAJAN 1986, NEWTON & al. 1986, BINZEL & al. 1987, REDDY 1987) is due to enhanced synthesis (HANDA & al. 1986), and decreased oxidation (STEWART & al. 1977). Furthermore, this proline accumulation was found to be independent of the levels of ABA, which accumulates more rapidly in stressed leaves (STEWART & VOETBERG 1987). The oxidation of proline was catalysed by two enzymes, proline oxidase (HUANG & CAVALIERI 1979) and proline dehydrogenase (RENA & SPLITTSTOESSER 1975). The former is localized in the inner mitochondrial membrane and requires oxygen as the electron acceptor, while the latter is localized in the cytosol and occurs as an active site on a protein which also functions as pyrroline 5 carboxylate (P5C) reductase. P5C reductase and proline dehydrogenase catalyse reactions with the same reactions and co enzymes, but working in opposite directions (RENA & SPLITTSTOESSER 1975). In the present study, inhibition of both proline oxidizing enzymes was observed in leaves and roots of water stressed plants. Proline dehydrogenase activity was inhibited to a greater extent than proline oxidase. More proline accumulation in response to stress was observed in roots than in leaves, in contrast to earlier observations (BARNETT & NAYLOR 1966, SINGH & PALEG 1972). ROGOZINSKA & FLASINSKE (1987) have also observed higher proline levels in leaves than in roots of oil seed rape plants. The higher proline accumulation in roots may be explained by the higher inhibitory rates of both proline dehydrogenase and proline oxidase.

Several investigators have proposed the beneficial effects of proline accumulation as a compatable osmotic solute (HANDA & al. 1986), as a protein stabilizing or solubilizing factor under limiting cell water conditions (BOGGESS & STEWART 1980), and as a source of reduced nitrogen and carbon (TAYLOR & al. 1982). The precise physiological significance of proline in stressed plants however, has yet to be fully elucidated.

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Recensio

LOVETT DOUST Jon & LOVETT DOUST Lesley (Eds.) 1990. Plant Reproductive Ecology. Patterns and Strategies. – Gr. 8°, XIII + 344 Seiten, 25 Abb.; brosch. – Oxford University Press New York, London. – £ 19,50. – ISBN 0-19-506394-5.

Der vorliegende Band ist die Paperback-Ausgabe eines 1988 erschienenen Buches. Die 15 von Spezialisten im jeweiligen Fach geschriebenen Kapitel sind zu drei Abschnitten gruppiert. Da es nicht möglich ist, hier alle Beiträge zu besprechen, seien als Hinweise auf den Inhalt die Titel angeführt.

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