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THE EFFECT OF PROLINE AND INDOLE ACETIC ACID ON TOTAL AMINO ACID CONTENT OF ALFALFA SHOOTS IN THE CASE OF WATER-DEFICIENCY

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Abstract

Basal parts of isolated alfalfa shoots were placed in distilled water, and in aqueous solutions of proline, IAA, and ABA; they were standing there in light during 24 hours. Water uptake and transpiration of the shoots standing in ABA and proline solution were lower and quantity of bound water higher than those of the shoots standing in pure water. After the 24 hours of absorption the shoots were laid out for two days (also in light) subjecting them to a sublethal water-deficiency. This stress caused in the shoots standing previously in distilled water a 410% increase of the total amino acid content as compared to the shoots dried immediately after cutting off. An additional 56% increase of the total amino acid content was observed in the shoots which absorbed previously proline and a 15% increase in the shoots which absorbed previously IAA. According to these result proline may have an important role in the watereconomy of the shoots — as IAA and ABA do.

Key-words: ABA, IAA, *Medicago sativa,* Proline waterdeficiency stress.

Introduction

It was already established that when shoots of herbaceous plants are cut off and laid out in light after loosing the greater part of their water content the total free amino acid concentration considerably increases (LEWITT, 1980; PALEG and ASPINALL, 1981). Under the influence of the stress of water loss the proline is the amino acid the concentration of which increases in the highest degree (BATES et al., 1973; TYMMS and GAFF, 1979). DASHEK and ERICKSON (1981) and FELLENBERG (1981) observed that during the first 6—8 hours of the development of waterdeficiency from the active components of the shoots the concentration of IAA-oxydase and of ABA abruptly increases, and at the same time the quantity of IAA considerably decreases. Besides the high ABA concentration considerably increases the synthesis of free proline in the leaves (PALEG and ASPINALL, 1981; QUARRIE and HENSON, 1982; RAJAGOPAL, 1981; TJANKOVA et al., 1982) established that exogenous proline increases the stomaresistency of leaves. Authors investigated how exogenous ABA, IAA, and proline changes water-economy of isolated alfalfa shoots in the case of abuindant water supply. They also investigated the effect of these substances on the free total amino acid content of the shoots during the development of a sublethal deficiency of water in two days.

Materials and methods

Four weeks old shoots of Medicago sativa var. Europe growth under optimal conditions were cut off and weighed in five groups each containing 50 pieces (fresh weight) .The shoots of one group were immediately dried (dry weight, without any manipulation). The shoots of other four groups were put (basal parts below) in aqueous solutions of ABA (4×10^{-5} M), IAA (10^{-4} M), proline (4.3×10^{-3} M) and in pure distilled water. Absorption was performed 24 hours in constant illumination (22 W m^{-2}), temperature was 26 °C and relative humidity 60 per cent. After 24 hours it was determined by weighing how much water was absorbed. Thereafter the shoots were laid out and constantly illuminated for two days causing sublethal water deficiency. Subsequently the shoots were weighed, then split up and dried at 70 °C. Amino acids were determined from the air dry matter with the aid of a "BIOCAL BC 200" automatic analyser. Soluble total protein was extracted from the live-wilted shoots with tris-buffer (pH 7.5) and determined according to Lowry et al. (1951).

Results and discussion

The IAA solution induces epinastic flexoions of alfalfa shoots proving the entry and efficiency. Closing of the stomata was observed in every two hours by microscope. In 6 hours ABA caused a total closing, while proline caused about a 50% decrease of the stomatal opening. Accordingly the agents found entrance into the shoots. Quantity of the absorbed water also shows considerable vitality of the isolated shoots during the 24 hours incubation (Table 1.).

Table 1. Effect of biologically active substances and the proline on water economy and on soluble total protein content of isolated alfalfa shoots in the case of considerable water deficiency. Control: The shoots were dried immediately after cutting off.

Treatment of isolated alfalfa shoots	Data relate to 100 g fresh weight of alfalfa shoots					Total protein as related to the weight after water deficiency mg/g
	water uptake	bound water	evaporated water	after two days of water deficiency		
	in one day			wilting weight	dry weight	
Control	—	—	—	—	19.3	20.4
Water absorption live-wilted	117.2	12.8	104.4	46.5	19.3	19.6
ABA absorption and live-wilted	80.1	15.4	64.7	53.6	19.3	19.5
IAA absorption and and live-wilted	121.6	13.1	108.5	46.7	19.0	20.4
Proline absorption and live-wilted	89.2	17.9	71.3	52.4	19.5	20.7

(Average deviation being below ±5 per cent; n=4).

According to the data in Table 1. the shoots in ABA solution absorbed 31.6% less water than the controls standing in pure water. It is surprising, however, that also absorption of proline caused a considerable (24%) decrease of water absorption;

this was already mentioned by FELLENBERG (1981) and RAJAGOPAL (1981). Order of quantity of the water evaporated by the shoots corresponds to the quantity absorbed. Quantity of bound water is the highest in the presence of proline which shows that proline is effective not only trough the control of the stomatal openings but also through other physiological factors (PALEG and ASPINALL, 1981; TYANKOVA, 1982). According to VAN DE DIJK (1981) in connection with drought-resistance it should be considered that an identical level of „outer water deficiency" produces a lower grade of "inner water deficiency" in varieties better adapted to drought tolerance. Table 2. shows changes in the free amino acid content.

Table 2. Changes in free amino acid content in isolated alfalfa shoots as effected by water, ABA, IAA and proline solutions in the case of considerable water deficiency. Control was dried immediately after cutting off.

Amino acids	Control	Substances absorbed for 24 hours			
		Water	ABA	IAA	Proline
		mg/g dry matter			
Asp	1.50	4.51	4.57	4.52	5.76
Thr	0.59	1.76	1.21	1.34	1.67
Ser	0.95	3.31	3.68	3.57	3.65
Asn	1.66	4.24	3.75	4.28	5.17
Gln	1.30	2.55	2.45	3.54	8.40
Pro	0.35	12.03	17.16	13.29	26.64
Glu	2.08	6.47	6.54	6.41	8.37
Gly	0.58	3.46	3.45	3.83	3.58
Ala	1.13	3.18	3.63	4.15	4.32
Val	0.51	1.79	2.05	3.40	3.16
Cys	0.36	1.17	1.28	1.27	1.55
Met	0.34	1.09	1.15	1.60	1.63
Ile	0.42	2.26	2.28	2.75	2.77
Leu	0.66	2.85	3.14	3.66	3.84
Tyr	0.49	2.15	2.06	2.02	2.73
Phe	0.47	2.03	2.15	2.56	3.18
Trp	0.20	0.92	0.89	1.15	1.16
Lys	0.51	1.52	1.62	1.87	1.93
His	0.53	1.88	1.76	2.33	2.41
Arg	0.40	2.47	2.54	3.31	4.34
Total amino acids	15.03	61.64	67.36	70.85	96.26

(Average deviation being below ± 5 per cent; $n=4$).

According to the data of Table 2. during the development of lethal water deficiency the total amino acid content of the shoots absorbing previously pure water increased 410% as compared to that of the shoots fixed immediately after cutting off. The concentration of proline became thirtyfourfold. The highest differences were produced by the water deficiency. Exogenous proline caused an additional 56% increase of the total amino acids. At the same time IAA caused a 15% while ABA

a 9% additional increase of the total amino acids as compared to the shoots standing in pure water. In the case of ABA increase of the total amino acids is nearly totally attributed to the increase of proline.

Authors conclude that in alfalfa shoots proline induces in the water economy and thereafter during the development of the water deficiency in the total amino acid content similar changes as do IAA and ABA. Moreover, opposite tendencies of the effects of IAA and ABA seem to becombined in the effect of proline. Authors' data prove that proline considerably facilitates tolerance of water deficiency. At the proline-type plants (PÁLFI et al., 1974) we have established that a high proline concentration is advantageous and we are supporting that as follows:

1. The hygroscopic nature of proline and its water-fixing capacity is the highest among all the protein-forming amino acids. Its water-solutibility is standing in the first place, too: at syntheses and transaminations, the most frequently involved glutamic acid is soluble 192-times, and aspartic acid 300-times more poorly in water than proline does. It may therefore be in the tissues of plants in a dissolved, active state, even in the more and more decreasing water.

2. During hydrolysis the free amino acids with 6n HCl, for 24 hours, at 110 °C, under pressure — in the presence of KNO_3 as oxidizer — every protein-forming amino acid was decomposed, except for proline. The proline-stability is extremely high.

3. Proline, during its being formed from glutamic acid, is storing reducing energy coming from photosynthesis and that gets released after the water deficiency being ceased and proline reverting to glutamic acid again. Owing to this redox property, proline has a respiration-influencing role, as well (LEWITT, 1980; PALEG and ASPINALL, 1981).

4. The high concentration of free proline in the tissue and pollen — as compared with the other amino acids — is favourable to growth because it is less toxic, as we have proved that with germinating experiments carried out under sterile conditions and with an oat coleoptil test (PÁLFI et al., 1974, 1981; PINTÉR et al. 1979).

5. In addition, proline is an important component of the proteins of the cell wall during growth and division of the cells. But following its incorporation it is converted to hydroxyproline (DASHEK and ERICKSON, 1981; FELLENBERG, 1981; LEWITT, 1980; PALEG and ASPINALL, 1981; PÁLFI et al., 1974; TYANKOVA et al., 1982).

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