Adaptability to drought in sugar beet cultivars

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Abstract

The effects of NaCl and polyethylene glycol (PEG) on superoxide dismutase (SOD) and peroxidase (P) activities, lipid peroxidation (LP) and proline content in seeds and leaves of drought tolerant (FC-506 and MS-100) and drought sensitive (MS-612 and MS-13) sugar beet cultivars were examined. After PEG and NaCl treatment in tolerant cultivars both in seeds and leaves SOD activity mainly increased, though P activity increased only in leaves of tolerant cultivars. In drought sensitive cultivars the decrease of SOD and P activity was mostly observed. LP increased in seeds and leaves of all examined cultivars. The proline content increased in the leaves of examined cultivars and was significantly higher in drought tolerant plants. On the other hand, in the seeds only slight increase in proline content was found. The results obtained indicated that drought tolerance could be correlated with high proline content and enzymatic defense against lipid peroxidation.

Key words: lipid peroxidation, NaCl, peroxidase, polyethylene glycol, proline, superoxide dismutase

Introduction

Water stress alters several metabolic pathways in higher plants and other organisms. One of them is oxidative injury (Hallivell 1984), which could brings lipid peroxidation (Monk *et al.* 1989, Štajner *et al.* 1993b) and damage all constituents of plants cells (Hailstones and Smith 1988). By the action of antioxidant enzymes, such as superoxide dismutase (SOD; EC 1.15.1.1.), and peroxidase (P; EC 1.11.1.8.) as well, oxidative injury could be diminished (Monk *et al.* 1989, Larson *et al.* 1988).

The accumulation of free proline is one of the most notable response to the water stress in nitrogen metabolism disturbance (Aspinal and Paleg 1981). Several authors provided evidences that the ability of high proline accumulation is positively correlated to drought resistance (Singh *et al.* 1973, Pinter *et al.* 1979, Gašić and Mimica-Dukić 1986) though the exact role of proline is still matter of dispute.

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Abbreviations: LP - lipid peroxidation; MDA - malonyldialdehyde; P - peroxidase; PEG - polyethylene glycol; SOD - superoxide dismutase; TBA - thiobarbituric acid.

The aim of this study was to determine relationship between SOD and P activities, LP and content of proline in drought tolerant and drought sensitive sugar beet cultivars exposed to water stress induced by NaCl and PEG. Obtained results could be important for understanding biochemical events providing protection against oxidative damage essential for the sugar beet production.

Materials and methods

Seeds and young plants of four Yugoslav sugar beet cultivars drought tolerant (FC-506, MS-100) and drought sensitive (MS-13, MS-612) were exposed to NaCl and PEG solutions. 10 g of dry seeds were placed in distilled water (control), 2.6 M NaCl and 0.05 M PEG-6000 solutions for 48 h. Young plants were obtained after 21 d of growth in Hoagland nutritious solution and subsequently transferred in distilled water (control), 2.6 M NaCl and 0.05 M PEG for 48 h. After this period all determination were performed. For the experiment second leaf was used. Results represent the average of two experiments with samples taken in triplicate form.

For the determination of SOD activity, the enzyme was extracted by crushing plant tissue (seeds and leaves) in 0.1 M K_2HPO_4, medium ratio (1:5), and subsequently centrifuged at 15 000 *g* for 10 min. All steps in the preparation of extract were carried out at 0 - 4 °C. In supernatant a mixture of chloroform:ethanol (3:5) was added before measurement of the methods based on the inhibition of the transformation of adrenalin to adrenochrome at pH 10.2 (Misra and Fridovics 1972, Matkovics *et al.* 1977).

For the P measurement the grinding medium was 0.1 M phosphate buffer (pH 7), medium ratio (1:5), and after centrifugation the supernatant aliquots were used to measure enzyme activity according to Simon *et al.* (1974).

Lipid peroxidation was measured at 37 °C as MDA production at 535 nm with TBA as described by Placer *et al.* (1966) and Gidrol *et al.* (1987).

Free proline was extracted with 0.12 M sulphosalicylic acid end estimated by the acid-nynhidrin method of Bates *et al.* (1973).

Values are given as means ± SEM of 4 measurements.

Results and discussion

After NaCl treatment of sugar beet seeds SOD activity declined in most of examined cultivars (Table 1), though SOD activity in genotype MS-100 was higher for about 99 % comparing to the control. In leaves of examined genotypes NaCl caused the increase of SOD activities in cultivars FC-506, MS-612 and MS-100 but in cultivar MS-13 it was lower for about 8 % then in control. Similarly, after PEG treatment of seeds SOD activities increased only in cultivars FC-506 and MS-100. Similar effect was also observed in drought tolerant and sensitive wheat cultivars exposed to water stress (Štrbac *et al.* 1993). Our results confirmed the statment about SOD as essential component in organism stress defence mechanism (Bowler *et al.* 1992). It is also obvious that young plants possessed higher stress tolerance (higher SOD) than seeds because the content of carotenoids which also participate in stress defence (Krinsky 1989) is also much higher in young plants.

Table 1. Effect of NaCl and PEG on superoxide-dismutase (SOD) activity in sugar beet seeds leaves. Mean of 4 experiments ± S.E.

Cultivar	SOD [%] Seeds Control	NaCl	PEG	Leaves Control	NaCl	PEG
FC-506	100	92.88 ± 1	44.25 ± 2	100	234.48 ± 16	216.41 ± 7
MS-612	100	80.57 ± 4	77.58 ± 9	100	107.40 ± 12	74.53 ± 6
MS-13	100	89.02 ± 4	81.51 ± 5	100	81.95 ± 6	64.01 ± 6
MS-100	100	199.15 ± 1	156.32 ± 6	100	189.93 ± 4	188.00 ± 7

It is obvious that LP increased after NaCl and especially PEG treatment in all seeds and leaves. The smallest increase in LP after PEG treatment both in seeds and leaves, was observed in cultivars FC-506 and MS-100 (Table 2).

Table 2. Effect of NaCl and PEG on lipid peroxidation (LP) in sugar beet seeds and leaves.

Cultivar	LP [%] Seeds Control	NaCl	PEG	Leaves Control	NaCl	PEG
FC-506	100	101.91 ± 1	133.32 ± 8	100	101.63 ± 6	145.11 ± 1
MS-612	100	102.19 ± 2	220.19 ± 7	100	101.16 ± 4	243.40 ± 5
MS-13	100	136.17 ± 5	243.75 ± 10	100	110.77 ± 4	281.76 ± 5
MS-100	100	116.17 ± 4	174.42 ± 12	100	107.02 ± 5	176.11 ± 8

Drought tolerant cultivars have smaller LP than drought sensitive, what means that drought tolerant cultivars of sugar beet had high SOD activity and low LP. Such relationship between SOD activity and LP was first observed in drought tolerant mosses by Dhindsa and Matove (1991) and later confirmed by other autors (Smirnoff and Colombe 1988, Štajner *et al.* 1993a,b).

In seeds treated with NaCl the decrease of P activities was observed in all examined cultivars. On the other hand, in leaves of MS-612 and MS-13 P activities were on the control level while in the leaves of MS-100 and FC-506 it was about 50 % higher than control. PEG treatment similarly affected P activities as NaCl treatment. In leaves of cultivars MS-100 and FC-506 it increased for 48 % and 24 % respectively (Table 3).

Presented results indicate that peroxidase also participate in drought defence mechanisms.

In the seeds exposed to NaCl, a slight decrease in proline content was recorded. On the other hand, after PEG treatment an increase in proline level was found. The highest increase showed cultivar MS-100 which is know as drought tolerant one (Table 4).

Table 3. Effect of NaCl and PEG on peroxidase activity (P) in sugar beet seeds and leaves.

Cultivar	P [%] Seeds Control	NaCl	PEG	Leaves Control	NaCl	PEG
FC-506	100	54.28 ± 2	28.57 ± 5	100	150.00 ± 16	124.00 ± 8
MS-612	100	66.67 ± 3	33.33 ± 9	100	100.00 ± 12	74.00 ± 7
MS-13	100	93.75 ± 5	81.25 ± 6	100	100.00 ± 10	66.67 ± 5
MS100	100	75.00 ± 2	90.00 ± 8	100	152.00 ± 1	148.00 ± 9

In the leaves of examined cultivars a strong increase in proline accumulation was found, both after NaCl and PEG treatments. Extremely enhanced proline content was found in drought tolerant cultivars FC-506 and MS-100, exposed to PEG.

Table 4. Effect of NaCl and PEG on proline accumulation in sugar beet seeds and leaves.

Cultivar	Proline content [%] Seeds Control	NaCl	PEG	Leaves Control	NaCl	PEG
FC-506	100	76.83 ± 1	108.14 ± 10	100	750.92 ± 9	6 277.10 ± 10
MS-612	100	93.78 ± 6	109.62 ± 12	100	320.41 ± 12	5 285.04 ± 8
MS-13	100	80.13 ± 4	118.29 ± 8	100	176.02 ± 7	2 900.13 ± 7
MS-100	100	70.15 ± 2	125.24 ± 7	100	512.80 ± 5	9 492.40 ± 12

The obtained results are in agreement with the presumptions that the ability of proline accumulation is positivelly correlated with drought tolerance (Singh *et al.* 1973, Karamanos *et al.* 1983, Goyai *et al.* 1985, Mali and Mehta 1977) and in contrast to those that consider proline accumulation as a symptom of stress with no adaptive significance (Hanson 1980, Aloni and Rosenshtein 1984). In addition, from the presented results is obvious that proline accumulation is more rapid and intensive in the leaves, which have also higher SOD activity and lower LP, than in the seeds in plants exposed to water stress both by NaCl and PEG. The results which demonstrated that different plant organs exhibit tolerance diversity against lower water supply, have been already reported (Singh and Paleg 1972, Rogozinska and Flasinke 1987).

The results presented, indicate that sugar beet cultivars FC-506 and MS-100 could be used in breeding water stress resistant hybrids.

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