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BRIEF COMMUNICATION

Nitrogen and *Azotobacter chroococcum* enhance oxidative stress tolerance in sugar beet

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Abstract

After treatment with increased quantities of nitrogen and *Azotobacter* strains, activities of antioxidant enzymes superoxide dismutase, peroxidase and catalase, content of chlorophylls and carotenoids, soluble proteins and dry matter in leaves of sugar beet increased.

Additional key words: carotenoids, catalase, chlorophylls, dry matter, peroxidase, proteins, superoxide dismutase.

Oxidative metabolism of normal cells and different stress situations generate highly reactive oxygen species. These active oxygen species include superoxide anion radical, hydrogen peroxide, hydroxyl radical and singlet oxygen (Štajner *et al.* 1995a). Oxidative damage inflicited by these active free radicals is referred to as oxidative stress (Sies 1991). Some major molecular targets of these agents are DNA, proteins, saccharides and lipids (Navari-Izzo 1994). In plants active oxygen and oxygen radicals inhibit chloroplast development (Halliwell 1987), decrease seed viability and root growth, stimulate leaf abscission and desiccation, damage membranes of leaves and roots by their peroxidation (Bowler *et al.* 1992), *etc.*

Several enzyme systems exist within the cell for the neutralization and thus for control of free radical formation. Superoxide dismutase (SOD) catalyzes the transformation of superoxide radicals, though hydrogen and other peroxides are neutralized by the action of catalase (C) and various peroxidases (P). Also non-enzymatic antioxidants such as carotenoids could be involved in scavenging singlet

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Abbreviations: C - catalase, P - peroxidase, SOD - superoxide dismutase.

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oxygen and so diminish oxidative stress (Elstner *et al.* 1994). Increasing nitrogen concentration and inoculation with different fungi and bacteria could produce a benefit to inoculated plants (Caba *et al.* 1994, Michail *et al.* 1994). Therefore the aim of our study was to compare oxidative stress tolerance linked to SOD, C, P and photosynthetic pigments in plants treated with nitrogen and *Azotobacter* with oxidative stress tolerance in untreated sugar beet plants.

Three strains of *Azotobacter chroococcum* (A-1, A-2, and A-3) available from the Department of Microbiology, Institute of Field Crops and Vegetables, University of Novi Sad were used as inoculum. The culture suspension of the bacteria contained 10^{15} -10^{17} living cells per m^3. The sand, experimental pots and plants seeds were sterilized. Each pot containing sugar beet seeds was inoculated with 30 cm^3 of *Azotobacter* culture suspension. Two groups of plants served as control: A-O, plants without inoculation, and A-A, plants inoculated with 30 cm^3 autoclaved *Azotobacter* culture suspension. Six replications were used for each variant. The inoculated plants of sugar beet (*Beta vulgaris* var. *saccharifera* cv. KW Maja) were cultivated in sand culture in a greenhouse at 28 °C under natural irradiance. The plants were supplied with Reid-York nutrient solution containing 0 (N-0), 7.5 (N-1), or 15.0 (N-2) μmol m^{-3} NH_4NO_3 for the first 4 weeks. They were then supplied with distilled water. SOD, C and P activities were determined in 100 mol m^{-3} phosphate buffer (pH 7) extracts from leaves of 30-d-old sugar beat. The SOD activity was determined according to inhibition of transformation of adrenaline to adrenochrome at pH 10.2. The P activity was determined with the guaiacol method at 470 nm. The C activity was determined at 240 nm *via* H_2O_2 content decrease and soluble proteins with Folin-Ciocaltey reagent (Misra and Fridovics 1972, Simon *et al.* 1974, Kock *et al.* 1994).

Contents of chlorophylls (Chl) and carotenoids (Car) were determined in acetone extracts spectrophotometrically using extinction coefficients according to Holm (1954) and Wettstein (1957).

Enzyme activities depended both on nitrogen concentration in nutrient solution and strain of the bacteria (Table 1). Plants supplied with nitrogen showed increased SOD, C and P activities. The increase depended on nitrogen concentrations and at N-2 the highest increase in enzyme activities was observed. *Azotobacter* strains used caused also an increase in enzyme activities. Inoculation with A-3 increased the enzyme activities the most at all N supplies. This observations is in agrement with the findings of Hong *et al.* (1990) that activities of nitrogen assimilation enzymes in inoculated sugar beet depend on the bacteria strain and nitrogen supply. Our previous investigation in the nitrogen fixing plant alfalfa showed a correlation between nitrogen fixing ability and antioxidant enzyme activities (Štajner *et al.* 1992) and hence also sugar beet inoculated with *Azotobacter* might possess some nitrogen fixing ability.

High activities of antioxidant enzymes (especially of SOD) are linked with oxidative stress tolerance (Štajner *et al.* 1995a) and with synergic effect of C and P (Seel *et al.* 1992, Navari-Izzo and Izzo 1994).

Also pigment contents were enhanced by nitrogen and *Azotobacter* supply (Table 1). A similar effect was observed by Perez-Soba *et al.* (1994) in Scots pine.

Table 1. Effect of nitrogen on antioxidant enzyme (SOD, P, C) activities, pigment and soluble protein contents and dry matter in sugar beet leaves inoculated with *Azotobacter chroococcum*.

Nitrogen supply	Inoculation	SOD [nmol(O_2^-) kg^{-1} (f.m.) s^{-1}] × 10^3	P [nmol(H_2O_2) kg^{-1} (f.m.) s^{-1}] × 10^3	C [nmol(H_2O_2) kg^{-1} (f.m.) s^{-1}] × 10^3	Chlorophyll *a* [mg kg^{-1}(d.m.)]	Chlorophyll *b* [mg kg^{-1}(d.m.)]	Carotenoids [mg kg^{-1}(d.m.)]	Soluble proteins [mg kg^{-1}(d.m.)]	Dry matter [g kg^{-1}(d.m.)]
N - 0	A_0	1.73 ± 0.3	0.08 ± 0.01	0.77 ± 0.1	51.1 ± 8.0	33.30 ± 6.0	11.28 ± 4.0	10.6 ± 3.0	14.3 ± 2.0
	A_1	2.35 ± 0.2	0.08 ± 0.01	0.83 ± 0.1	58.4 ± 9.0	38.00 ± 8.0	12.00 ± 3.0	10.5 ± 2.0	18.0 ± 4.0
	A_2	4.32 ± 0.3	0.10 ± 0.05	0.93 ± 0.2	62.3 ± 7.0	38.40 ±12.0	12.20 ± 6.0	12.0 ± 5.0	15.2 ± 6.0
	A_3	4.32 ± 0.3	0.11 ± 0.06	1.15 ± 0.3	68.5 ±10.0	39.00 ±10.0	13.50 ± 6.0	12.0 ± 5.0	19.4 ±10.0
N - 1	A_0	7.82 ± 0.2	0.15 ± 0.08	3.36 ± 1.0	420.0 ± 5.0	150.60 ± 5.0	20.60 ± 5.0	14.7 ±11.0	93.6 ±10.0
	A_1	9.01 ± 0.4	0.21 ± 0.08	3.82 ± 1.0	434.5 ± 8.0	158.70 ± 8.0	25.10 ± 4.0	16.1 ±10.0	117.8 ±11.0
	A_2	8.54 ± 0.5	0.14 ± 0.07	3.89 ± 1.0.	450.2 ±13.0	170.20 ± 7.0	28.20 ± 8.0	15.2 ±11.0	107.0 ±11.0
	A_3	10.17 ± 0.6	0.17 ± 0.01	5.35 ± 2.0	460.3 ±15.0	180.50 ± 8.0	30.00 ± 9.0	16.0 ±12.0	123.3 ±13.0
N - 2	A_0	8.11 ± 0.6	0.14 ± 0.07	4.51 ± 2.0	566.2 ±11.0	210.50 ±10.0	33.20 ±10.0	17.3 ±13.0	109.6 ± 9.0
	A_1	9.08 ± 0.6	0.32 ± 0.09	5.35 ± 3.0	570.0 ± 9.0	280.75 ±12.0	35.10 ±11.0	18.0 ±10.0	121.6 ±12.0
	A_2	8.82 ± 0.8	0.15 ± 0.10	6.29 ± 3.0	610.3 ±12.0	300.00 ±14.0	35.80 ± 8.0	17.0 ±10.0	110.8 ±10.0
	A_3	10.58 ± 0.8	0.18 ± 0.09	7.60 ± 4.0	618.4 ±11.0	310.20 ±16.0	37.10 ± 9.0	18.6 ±12.0	114.8 ±12.0

The N-2 supply increased the pigment contents the most and the A-3 strain was most effective. Increased pigment contents were in agreement with increased dry matter accumulation (Table 1). Among all parameters examined the soluble proteins content was less influenced by nitrogen supply and *Azotobacter* strains.

Thus the increased nitrogen concentrations and inoculation with *Azotobacter* improved oxidative stress defence ability in sugar beet leaves.

References

Bowler, C., Van Montagy, M., Inze, D.: Superoxide dismutase and stress tolerance. - Annu. Rev. Plant Physiol. Plant mol. Biol. **43**: 83-116, 1992.

Caba, J.M., Lluch, C., Ligero, F.: Genotypic variability of nitrogen metabolism enzymes in nodulated root of *Vicia faba*. - Soil Biol. Biochem. **26**: 785-789, 1994.

Elstner, E.E., Schempp, H., Preibisch, G., Hippeli, S., Oswald, W.: Biological sources of free radicals. - In: Free Radicals in the Environment, Medicine and Toxicology. Pp. 13-45. Richelieu Press, London 1994.

Halliwell, B.: Oxidative damage, lipid peroxidation and antioxidant protection in chloroplast. - Chem. Phys. Lipids **44**: 327-340, 1987.

Halliwell, B., Gutteridge, J.M.S.: Free Radicals in Biology and Medicine. - Clarendon Press, Oxford 1989.

Holm, G.: Chlorophyll mutations in barley. - Acta agron. scand. **4**: 457-471, 1954.

Hong, Z., Gašić, O., Popović, M., Lukić, V., Sarić, M., Sarić, Z., Govedarica, M.: Activity of nitrogenase and nitrogen assimilation enzymes in sugar beet (*Beta vulgaris* L.) inoculated with *Azotobacter chroococcum*. - Period. Biol. **92**: 279-284, 1990.

Kock, M., Štajner, D., Gašić, O., Schlee, D.: Alachlor-induced changes of superoxide dismutase activity in seeds of young plants of wheat (*Triticum aestivum*). - Biológia (Bratislava) **49**: 365-370, 1994.

Michail, S.H., Abd El-Rehim, M.A., Kamara, A.M., Saleh, M.M.: Detoxification of alfatoxins produced by *Aspergillus flavus*, isolated from paddy grains by using cetain natural and chemical materials. - Acta phytopathol. entomol. hung. **29**: 7-13, 1994.

Misra, H.P., Fridovich, I.: The role of superoxide anion in the autooxidation of epinephrine and a simple measurement for superoxide dismutase. - J. biol. Chem. 247: 456-459, 1972.

Navari-Izzo, F., Izzo, R.: Induction of enzyme activities and antioxidant production in barley plants as a results of SO_2 fumigation. - Plant Sci. **96**: 21-40, 1994.

Perez-Soba, M. Stulen, I., Van der Eerden, L.J.M.: Effect of atmospheric ammonia on the nitrogen metabolism of Scots pine (*Pinus sylvestris*) needles. - Physiol. Plant. **90**: 629-636, 1994.

Seel, W.E., Hendry, G.A.F., Lee, A.J.: Effects of desiccation on some activated oxygen processing enzymes and antioxidants in mosses. - J. exp. Bot. **43**: 1031-1037, 1992.

Sies, H.: Oxidative Stress: Oxidants and Antioxidants. - Academic Press, London 1991.

Simon, L.M., Fatrai, Z., Jonas, D.E., Matkovics, B.: Study of metabolism enzymes during the development of *Phaseolus vulgaris*. - Biochem. Physiol. Pflanzen **166**: 387-392, 1974.

Štajner, D., Popović, M., Gašić, O., Misković, B.: Comparative study of nitrogen and oxygen metabolism enzymes in Yugoslav cultivars of alfalfa (*Medicago sativa* L.). - Biol. Plant. **34**: 77-83, 1992.

Štajner, D., Gašić, O. Matković, B., Varga, Sz.I.: Metolachlor effect on antioxidants enzyme activities and pigments content in seeds and young leaves of wheat (*Triticum aestivum* L.). - Agr. med. **125**: 267-273, 1995a.

Štajner, D., Mimica-Dukić, N., Gašić, O.: Adaptability to drought in sugar beet cultivars. - Biol. Plant. **37**: 107-112, 1995b.

Wettstein, D. von: Chlorophyll-letale und der submikroskopishe Formwechsel der Plastiden. - Exp. Cell Res. **12**: 427-506, 1957.