BIOLOGIA PLANTARUM 46 (1): 141-143, 2003

BRIEF COMMUNICATION

Stress tolerance parameters in different genotypes of soybean

Dj. MALENČIĆ*, M. POPOVIĆ* and J. MILADINOVIĆ**

*Faculty of Agriculture, University of Novi Sad, Trg D. Obradovića 8, YU-21000 Novi Sad, Yugoslavia**
*Research Institute of Field and Vegetable Crops, M. Gorkog 30, YU-21000 Novi Sad, Yugoslavia***

Abstract

Free proline content, superoxide-dismutase activity, and lipid peroxidation were measured in sixteen Yugoslav and introduced genotypes of soybean. More tolerant genotypes with higher free proline content and high superoxide-dismutase activity, and low lipid peroxidation were chosen. The selected genotypes could be used in field production, as well as in breeding.

Additional key words: lipid peroxidation, proline, superoxide dismutase.

Water deficit stress is the primary factor limiting yield in soybean. Drought decreases leaf gas exchange, N_2 fixation and accumulation, seed number and size, and the length of the seed fill period (Purcell and King 1996). In addition, leaves close stomata under water stress and the imbalance between photosynthetic electron transport and CO_2 fixation rates may result in the over-reduction of the electron transport chain components and facilitate the transfer of electrons to O_2 and generation of highly reactive oxygen species (ROS). The ROS include superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$), and singlet oxygen ($^1O_2$) (Scandalios 1993). ROS can react with unsaturated fatty acids to cause peroxidation of essential membrane lipids in the plasmalemma or membrane of intracellular organelles. Antioxidant defence systems have co-evolved with aerobic metabolism to counteract oxidative damage from ROS. This includes antioxidant enzymes such as superoxide-dismutase (SOD), catalase (CAT), and peroxidase (PRX), as well as α-tocopherol, β-carotene, ascorbic acid, reduced glutathione (GSH), *etc.* (Nizamuddin 1987).

One of the common responses to water deficit is the production and/or accumulation of proline (Pro), in the free form. This amino acid is perhaps the most widely distributed compatible osmolyte (Taylor 1996). However, the precise role of Pro accumulation is still a matter of debate especially in higher plants. According to some authors (Larher *et al.* 1993, Rentsch *et al.* 1996, Taylor 1996) accumulated Pro acts as a cytosolute, an osmoprotectant and a protective agent for cytosolic enzymes and various cellular structures. In addition to this, Pro synthesized during water deficit may serve as an organic nitrogen reserve that can be utilized during recovery. There is also evidence that the degradation of Pro in mitochondria is directly coupled to the respiratory electron transport system and ATP production (Taylor 1996). Under some circumstances, and in some plants, Pro can be accumulated until it constitutes more than 10 % of the dry mass of the stressed tissue (Paleg *et al.* 1981).

In order to achieve an improvement of plant adaptations to different types of stresses, the aim of our study was to select genotypes of soybean which are able to accumulate a larger quantities of free Pro, and at the same time have a good resistance to toxic action of oxygen radicals.

Sixteen genotypes of soybean [*Glycine max* (L.) Merr.] were grown on experimental fields at the Institute of Field and Vegetable Crops at the location of Rimski Šančevi, near Novi Sad, in 2000. The trial was set in a complete randomized block design in four replications. Leaves for the biochemical assays were collected in the stage of full blossoming.

Received 9 November 2001, *accepted* 7 January 2002.

Abbreviations: CAT - catalase; GSH - reduced glutathione; MDA - malonyldialdehyde; Pro - proline; PRX - peroxidase; ROS - reactive oxygen species; SOD - superoxide dismutase.

Fax: (+381) 21 450 857, e-mail: malencic@polj.ns.ac.yu

Free Pro was extracted from 0.5 g of fresh leaf tissue into 10 cm^3 of 3 % sulfosalicylic acid. Pro content was determined spectrophotometrically (UV/visible spectrophotometer model *6105*, *Jenway*, Dunmon, UK) at 520 nm following the ninhydrin method described by Bates *et al.* (1973), using pure proline (*Merck*, Darmstadt, Germany) as a standard.

For the determination od SOD activity, one g of plant material was homogenized with 5 cm^3 0.1 M K_2HPO_4 at pH 7.0. After centrifugation at 15 000 *g* for 10 min at 4 °C, aliquots of the supernatant were used for enzyme activity measurements. SOD activity was determined by the method of Misra and Fridovich (1972), based on the autocatalytic transformation of epinephrine-adrenochrome at pH 10.2.

Lipid peroxidation was measured as malonyldialdehyde (MDA) production, spectrophotometrically at 532 nm with thiobarbituric acid (TBA). The total amount of TBA-positive substance is given as nmol (MDA) g^{-1}(f.m.), as described by Placer *et al.* (1966).

The obtained results on the free Pro content, SOD activity and LP were analysed using analysis of variance. The least significant difference (LSD) values at $P = 0.05$ and $P = 0.01$ were calculated.

The results obtained have shown significant differences among examined genotypes. The free Pro content varied from 0.76 to 1.38 µmol(proline) g^{-1}(f.m.). The highest content of free Pro was recorded in French genotype Labrador, while the least was in Chinese genotype JJ 9309-10-2. In average, Chinese soybean genotypes possessed the lowest capability for Pro accumulation. Besides Labrador, other French genotype Essor, as well as US Holfax and Yugoslav genotype Balkan showed high free Pro content. In many plants, Pro has been shown to accumulate under abiotic stresses and genes encoding proteins involved in Pro biosynthesis are upregulated under these conditions (Rentsch *et al.* 1996). It seems that under semi-arid conditions, genotypes with higher Pro content such as Labrador, Essor, Holfax and Balkan, posses better predisposition to water stress and a better capability to osmoregulation, enzyme stability and accumulation of nitrogen.

The leaf SOD activity ranged from 223.85 U g^{-1}(f.m.) (KJ 96010) to 683.33 U g^{-1}(f.m.) (Erie). Lipid peroxidation occurred in all investigated genotypes, ranging from 256.63 nmol(MDA) g^{-1}(f.m.) (Flint), up to 352.08 nmol(MDA) g^{-1}(f.m.) (Essor) (Table 1).

Results obtained for SOD activity are in agreement with the results of other authors (Bao *et al.* 1989) who reported SOD activity in different Chinese soybean genotypes, ranging from 397.00 to 696.00 U g^{-1}(f.m.). As the formation of ROS is favoured under water stress conditions, one can expect some adaptive changes in the active oxygen scavenging enzymes to mitigate the situation (Baisak *et al.* 1994). Among the enzymes responsible for this, SOD is of the greatest importance. This is the enzyme that catalyzes the dismutation of the superoxide radical ($O_2^{\cdot -}$) to molecular oxygen and water (Giannopolitis and Ries 1977), thus preventing the formation of other, more toxic oxygen species such as $OH^{\cdot}$ radicals. According to Baisak *et al.* (1994), SOD activity is clearly increased depending upon the degree of water stress indicating that the leaf has the enhanced capacity to scavenge $O_2^{\cdot -}$ during water stress. In our study, Erie, Flint, Labrador and Ravnica genotypes featured significantly higher SOD activities than the other genotypes.

Table 1. Free proline (Pro) content [µmol g^{-1}(f. m.)], superoxide dismutase (SOD) activity [U g^{-1}(f.m.)], and lipid peroxidation (LP) [nmol(MDA) g^{-1}(f.m.)] in soybean genotypes.

Genotypes	Origin	Pro	SOD	LP
Vojvodjanka	YU	0.83	350.0	332.99
Ravnica	YU	1.04	396.77	344.92
Afrodita	YU	0.81	313.19	288.52
Balkan	YU	1.21	339.0	332.99
Erie	US	0.98	683.33	313.68
Holt	US	0.90	279.54	259.23
Holfax	US	1.25	351.86	265.96
Flint	US	0.79	512.50	256.63
Armor	FR	0.89	366.36	345.35
Essor	FR	1.12	339.0	352.08
Junior	FR	0.85	340.50	311.40
Labrador	FR	1.38	431.70	289.60
KJ 96010	CH	0.77	223.85	279.62
JJ 9330	CH	0.93	338.87	271.16
96009	CH	0.77	352.15	273.98
JJ 9309-10-2	CH	0.76	290.48	303.92
$LSD_{0.05}$		0.286	38.51	33.36
$LSD_{0.01}$		0.395	53.24	46.12

The lipid peroxidation was lower in most of US and Chinese genotypes. Yugoslav genotype Afrodita and French Labrador also showed low lipid peroxidation of 288.52 and 289.60 nmol(MDA) g^{-1}(f.m.), respectively. Previous experiments (Hailstones and Smith 1988) provided evidence of an association between lipid peroxidation and declining vigour in seeds of soybean and cabbage. This means that ROS may react with unsaturated fatty acids of cell membranes both in seeds and leaves, which results in different degree of peroxidation. MDA, being main end-product of lipid peroxidation, can cause the cross-linking and polymerisation of membrane components. Owing to its diffusibility it can also react with free amino groups in protein and DNA bases. All these effects may explain the mutagenic and genotoxic power of MDA (Štajner *et al.* 1993a,b). It seems from our results that the genotypes which have the lowest lipid peroxidation (Holt, Holfax and Flint) are more resistant to the harmful effect of toxic oxygen species. In spite of the enhanced activities of SOD in some genotypes (Ravnica, Armor), the rate of lipid peroxidation increased. This means that scavenging system against ROS is less effective in some genotypes, and the activity of SOD is not enough to prevent oxidative damage, which is in agreement with findings of some other authors (Baisak *et al.* 1994).

The main objective of our study was to select soybean genotypes for field production and potential breeding and selection, using biochemical parameters of importance to water and oxidative stress tolerance. On the basis of our results it can be concluded that genotypes such as Labrador, Essor, Holfax and Balkan accumulate higher content of free Pro, expressing increased water stress tolerance. In the same time, genotypes such as Erie, Holfax, Flint and Labrador have the best antioxidant ability (high SOD activity and low rate of lipid peroxidation). These genotypes could be used in field production, as well as for breeding.

References

Baisak, R., Rana, D., Acharya, P.B.B., Kar, M.: Alterations in the activities of active oxygen scavenging enzymes of wheat leaves subjected to water stress. - Plant Cell Physiol. **35**: 489-495, 1994.

Bao, X., Bingchang, Z., Qinhua, L., Jingliang, L.: Study on the superoxide dismutase of soybean seeds from different species in subgenus soya. - In: Pascal, A.J. (ed.): Proceedings of World Soybean Research Conference IV. Pp. 434-437. Orientacion Grafica Editora S.R.L., Buenos Aires 1989.

Bates, L.S., Waldren, L.P., Teare, J.D.: Rapid determination of free proline for water stress studies. - Plant Soil **39**: 205-207, 1973.

Giannopolitis, C.N., Ries, S.K.: Superoxide dismutases 1. Occurrence in higher plants. - Plant Physiol. **59**: 309-314, 1977.

Hailstones, M.D., Smith, M.T.: Lipid peroxidation in relation to declining vigour in seeds of soya (*Glycine max* L.) and cabbage (*Brassica oleracea* L.). - J. Plant Physiol. **133**: 452-456, 1988.

Lahrer, F., Leport, L., Petrivalsky, M., Chappart, M.: Effectors for the osmoinduced proline response in higher plants. - Plant Physiol. Biochem. **31**: 911-922, 1993.

Misra, H.D., Fridovich, I.: The role of superoxide anion in the autooxidation of epinephrine and a simple measurement for superoxide dismutase. - J. biol. Chem. **247**: 3170-3175, 1972.

Nizzamuddin, A.: NADPH-dependent and $O_2^{\cdot -}$-dependent lipid peroxidation. - Biochem. Educ. **15**: 58-62, 1987.

Paleg, L.G., Douglas, T.J., van Daal, A., Keech, D.B.: Proline, betaine and other organic solutes protect enzymes against heat inactivation. - Aust. J. Plant Physiol. **8**: 107-114, 1981.

Placer, Z.A., Cushman, L.L., Johnson, B.C.: Estimation of product of lipid peroxidation malonyldialdehyde in biochemical systems. - Anal. Biochem. **16**: 359-364, 1966.

Purcell, L.C., King, C.A.: Drought and nitrogen source effects on nitrogen nutrition, seed growth, and yield in soybean. - J. Plant Nutr. **19**: 969-993, 1996.

Rentsch, D., Hirner, B., Schmelzer, E., Frommer, W.B.: Salt stress-induced proline transporters and salt stress-repressed broad specificity amino acid permeases identified by suppression of a yeast amino acid permease-targeting mutant. - Plant Cell **8**: 1437-1446, 1996.

Scandalios, J.G.: Oxygen stress and superoxide dismutases. - Plant Physiol. **101**: 7-12, 1993.

Štajner, D., Gašić, O., Matkovics, B., Kraljević-Balalić, M., Varga, Sz.I.: Lipid peroxidation and reduced glutathione in wheat seeds and their F1 hybrids. - Cereal Res. Commun. **21**: 181-185, 1993a.

Štajner, D., Varga, I., Štrbac, D., Matkovics, B., Gašić, O., Kastori, R.: Change in malondialdehyde, hydroxyl radical, reduced glutathione and protein content in wheat seeds germinated in polyethylene glycol-6000 solutions. - In: Fehér, J., Blázovics, A., Matkovics, B., Mézes, M. (ed.): Role of Free Radicals in Biological Systems. Pp. 3-8. Akadémiai Kiadó, Budapest 1993b.

Taylor, C.B.: Proline and water deficit: ups, downs, ins, and outs. - Plant Cell **8**: 1221-1224, 1996.