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LIPID COMPOSITION OF PEA (*PISUM SATIVUM* L.) AND MAIZE (*ZEA MAYS* L.) ROOT PLASMA MEMBRANE AND MEMBRANE–BOUND PEROXIDASE AND SUPEROXIDE DISMUTASE

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Abstract — Plasma membrane was isolated from roots of pea and maize plants and used to analyze POD and SOD isoforms, as well as lipid composition. Among lipids, phospholipids were the main lipid class, with phosphatidylcholine being the most abundant individual component in both pea and maize plasma membranes. Significant differences between the two plant species were found in the contents of cerebrosides, free sterols, and steryl glycosides. Most maize POD isoforms were with neutral and anionic pI values, but the opposite was observed in pea. While both anionic and cationic SOD isoforms were isolated from maize, only two anionic SOD isoforms were detected in pea.

Key words: Lipid composition, superoxide dismutase, peroxidase, plasma membrane, pea, maize

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INTRODUCTION

The plasma membrane (PM) of root cells has numerous physiological roles comprising cell wall biosynthesis, hormone action, and signalling processes during disease, plant development, and programmed cell death (Neil et al., 2002; Mika and Lüthje, 2003; Vuletić et al., 2005; Schopfer and Liszkay, 2006). In all of these processes, reactive oxygen species (ROS) such as superoxide anion radicals ($O_2^{\cdot-}$), H_2O_2, and hydroxyl radicals (·OH) play a pivotal role. A number of environmental stresses lead to enhanced production of ROS within PM that may cause oxidative damage. Hydroxyl radicals can initiate lipid peroxidation in a radical chain reaction leading to increased membrane leakage and cell death. Proteins embedded in the membrane may also be damaged by ROS, leading to loss of enzyme activity and transport processes. The role of extracellular antioxidant enzymes in regulation of ROS concentrations in the apoplast is important. Evidence for PM-bound POD and SOD activity in higher plants has been reported (Karpinska et al., 2001; Hadži-Tašković Šukalović et al., 2003; Vuletić et al., 2003; Kukavica et al., 2005). It has been shown that protein–lipid interaction is crucial for localization of membrane proteins and, consequently, their function (Escriba et al., 1997; van Klompenburg et al., 1997; Benfenati et al., 1998; Berglund et al., 2000; van Voorst and Kruijff, 2000). In the present study, we compared the IEF profile of PM-bound POD and SOD isoforms of pea and maize roots with the lipid composition of these two membranes. POD and SOD coexistence in PM of pea and maize roots would implicate their specific role in the antioxidative protection of membrane constituents, as well as in the redox communication between apoplast and symplast, which is part of signalling processes.

MATERIALS AND METHODS

Plant growth

Pea (*Pisum sativum* L.) and maize (*Zea mays* L.) seedlings were grown in hydroponic culture with continuous aeration in a growth chamber with day/night temperatures of 21°C/16°C, a 16-h photoperiod, a photon flux density of 400 μmol m^{-2} s^{-1}, and 70 to 75% relative humidity. Light was provided by fluorescent tubes (Osram L140W/20)

and incandescent lamps (Philips 25-W). Seeds were pre-germinated on moistened paper and then placed in plastic pots filled with a half-strength aerated Hoagland's No. 2 solution that was renewed every 3 days (H o a g l a n d and A r n o n, 1950). At day 14, roots of intact plants were washed with distilled water and collected for PM isolation and biochemical analyses.

Isolation of plasma membrane

PM was isolated using a two-phase partition system. Roots were cut into pieces and immediately ground using a Braun blender in 2 volumes of an extraction medium consisting of 50 mM TRIS-HCl, pH 7.5, 0.25 M sucrose, 3 mM Na_2EDTA, 10 mM ascorbic acid, and 5 mM diethyldithiocarbamic acid. The homogenate was filtered through four layers of a nylon cloth and centrifuged at 10,000g for 10 min. The supernatant was further centrifuged at 65,000g for 30 min to yield a microsomal pellet, which was resuspended in 2 ml of a resuspension buffer (5 mM K-phosphate, pH 7.8, 0.25 M sucrose and 3 mM KCl). The PM was isolated by loading microsomal suspension (1 g) onto an aqueous two phase polymer system to give a final composition of 6.5% (w/w) Dextran T 500, 6.5 % (w/w) polyethylene glycol, 5 mM K-phosphate (pH 7.8), 0.25 M sucrose, and 3 mM KCl. The PM was further purified using a two-step batch procedure. The resulting upper phase was diluted fourfold with 50 mM TRIS- HCl, pH 7.5, containing 0.25 M sucrose, and centrifuged for 30 min at 100,000g. The resultant PM pellet was resuspended in the same buffer containing 30 % ethylene glycol and stored at –80 °C for lipid analyses. All steps of the isolation procedure were carried out at 4 °C.

In order to check the purity of the PM of maize and pea roots, the activity of the vanadate-sensitive ATPase as a marker enzyme was determined (N a v a r i-I z z o et al., 1993). Cytochrome c oxidase, NADH cytochrome c reductase, and NO_3^--sensitive ATPase activities were used as markers of mitochondria, endoplasmic reticulum, and tonoplast, respectively (N a v a r i-I z z o et al., 1993). Tests with the markers showed that, as a mean value of the isolations performed, ATPase specific activity in both maize and pea was 66% higher in the PM than in the microsomal fraction; vanadate inhibited ATPase activity by 88% in the PM fractions and by 35% in the microsomal ones. The addition of KNO_3 negligibly reduced ATPase activity in the PM fractions (6 and 4% inhibition in maize and pea, respectively). The specific activities of marker enzymes such as cytochrome c oxidase and NADH cytochrome c reductase in the upper phase of both PM were 4 and 8%, respectively, of those determined in the lower phase.

Lipid extraction and separation

Lipids were extracted from PM suspension by addition of boiling isopropanol followed by chloroform: methanol (2:1 v/v) containing butylhydroxytoluol (50 μg ml^{-1}) as an antioxidant. The solvent mixture was then washed with 0.88% KCl to separate the chloroform phase. The upper water phase was re-extracted with chloroform and the chloroform phases combined and dried under a stream of N_2. Total lipids were fractionated into neutral lipid, glycolipid, and phospholipid (PL) fractions on Sep-Pack cartridges (Waters, USA) and sequentially eluted with 20 ml of chloroform: acetic acid (100:1 v/v) for neutral lipids, 10 ml acetone and 10 ml of acetone: acetic acid (100:1 v/v) for glycolipids, and 7.5 ml of methanol: chloroform: water (100:50:40 by vol) for PL (Q u a r t a c c i et al., 2001). Chloroform (2.25 ml) and water (3 ml) were added successively to the eluate containing PL to obtain phase separation and facilitate their recovery. Separation of individual lipids was performed by TLC (Silica Gel 60, 0.25 mm thickness; Merck, Germany) with the following solvent mixtures: petrol ether: ethyl ether: acetic acid (80: 35:4 by vol) for neutral lipids (free sterols, FS, and sterol esters), chloroform: methanol: water (65:25:4 by vol) for glycolipids (steryl glycosides and cerebrosides), and chloroform: methanol: acetic acid: water (85:15:10:3.5 by vol) for PL. After development, bands were located with iodine vapors. Individual lipids were identified by chromatography with authentic standards. Quantitative analyses of sterols, cerebrosides, and PL were performed as reported earlier (N a v a r i-I z z o et al., 1993) using cholesterol, glucose, and KH_2PO_4 as standards, respectively. All procedures were performed in the

presence of silica gel from TLC.

Fatty acid analysis

Fatty acid methyl ester derivatives from PL were obtained as previously described (Quartacci et al., 1997) and separated by GLC on a Dani 86.10 HT gas chromatograph equipped with a 60-m x 0.32-mm SP-2340 fused silica capillary column (Supelco Sigma-Aldrich, USA) coupled to a flame ionization detector (column temperature of 175°C). Both the injector and detector were maintained at 250°C. Nitrogen was used as the carrier gas at 0.9 ml min^{-1} with a split injector system (split ratio 1:100). Heptadecanoic acid was used as the internal standard.

Determination of POD and SOD isoforms

For determination of POD and SOD isoforms on native gel, the same amounts of PM proteins were loaded. Native electrophoresis was performed on 5% stacking and 10% running gel with a reservoir buffer consisting of 0.025 M TRIS and 0.192 M Gly (pH 8.3) at 24 mA for 120 min. IEF was carried out in 7.5% polyacrylamide gel with 3% ampholite in a pH gradient from 3 to 9. Markers for isoelectrofocusing with pI range of 3.6-9.3 (Sigma) were used to determine pI values of POD and SOD isoforms.

To assay POD activity, gels were incubated with 10% 4-chloro-α-naphthol and 0.03% H_2O_2 in 100 mM K-phosphate buffer (pH 6.5). Determination of SOD activity on gels was performed according to Beauchamp and Fridovich (1971). After incubation in a reaction mixture (0.1 M EDTA, 0.098 mM nitroblue tetrazolium, 0.030 mM riboflavin, and 2 mM TEMED in K-phosphate buffer, pH 7.8) for 30 min in the dark, gels were washed in distilled water and illuminated with white light. Band density, expressed in relative units, of different POD and SOD isoforms after separation on IEF gel was determined using TotalLab software (Nonlinear Dynamics, UK). Protein content was measured by the method of Bradford (1976), with bovine serum albumin as a standard.

Statistical analysis

Results, unless differently specified, are the

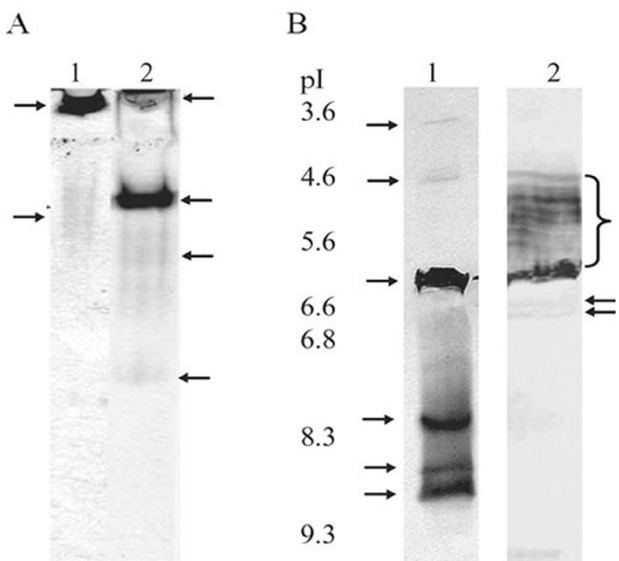

Fig. 1. Native-PAGE stained for POD activity. (A) POD isoforms from pea (lane 1) and maize plasma membrane (lane 2) were separated. (B) Isoelectrofocusing stained for POD activity from pea (lane 1) and maize (lane 2) plasma membrane. Arrows indicate different POD isoforms.

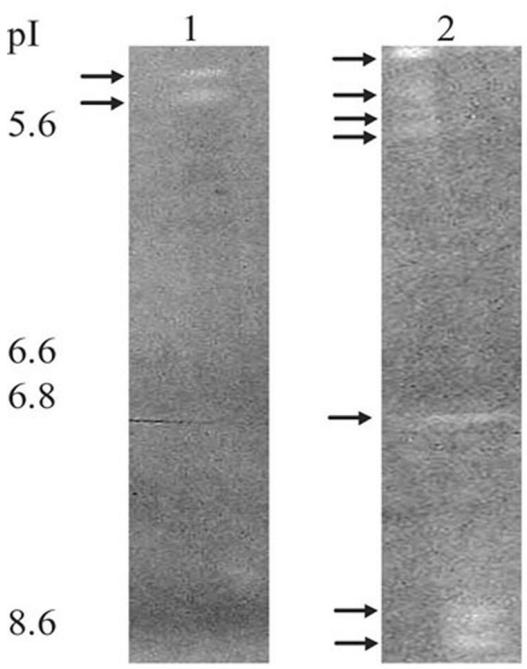

Fig. 2. Isoelectrofocusing of SOD isoforms bound to plasma membrane isolated from pea (lane 1), and maize (lane 2) roots. Arrows indicate different SOD isoforms.

means of three replicates of three independent experiments (n = 3). Values for means followed by different letters are significantly different at $P \leq 0.05$ (Mann-Whitney test).

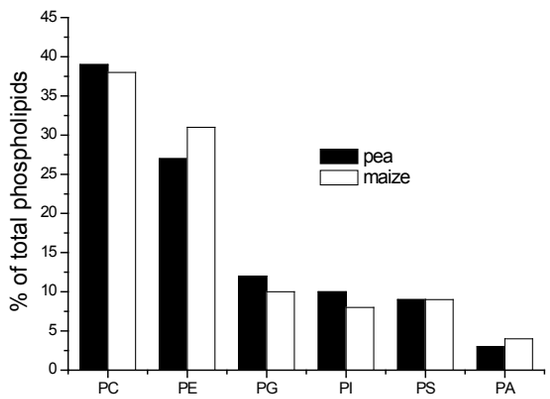

Fig. 3. Phospholipid composition of pea and maize root plasma membrane. PC-phosphatidylcholine, PE-phosphatidylethanolamine, PG-phosphatidylglicerol, PI-phosphatidylinositol, PS-phosphatidylserine, PA-phosphatidic acid.

RESULTS

Native PAGE of PM proteins isolated from 14-day-old pea and maize roots showed one POD isoform with low mobility in pea and two POD isoforms in maize (Fig. 1A). Several weak bands were noticed in both species as well (Fig. 1A). The IEF profile of pea POD showed that the low mobile isoform included three cationic isoforms (pI 8.3, 8.8 and 9.0) and one neutral POD isoform. Two weak anionic (pI 4-5) isoforms were also detected on gels.

Table 1. Lipid content and composition of pea and maize root plasma membrane, and free sterols to phospholipids molar ratio. FS-free sterols, CER-cerebrosides, SG-steryl glycosides, ASG-acylated steryl glycosides, PL-phospholipids. *indicates significant differences between pea and maize by Mann-Whitney U test (** $p<0.005$,* $p<0.05$).

	PM pea		PM maize	
Lipid class	μmol/mg$_{prot}$	mol %	μmol/mg$_{prot}$	mol %
FS	0.057±0.006**	14	0.096±0.002**	18
CER	0.077±0.007**	19	0.109±0.02**	21
SG	0.034±0.004*	9	0.048±0.004*	9
ASG	0.023±0.006	5	0.032±0.01	6
PL	0.218±0.026	52	0.235±0.04	46
total	0.413±0.039		0.512±0.08	
FS/PL	0.27		0.39	

In maize, PM several isoforms with pI from 6.8 to 5.6 and two weak bands with pI 6.8 were detected (Fig. 1B).

Separation of PM-bound SOD isoforms by IEF from pea roots showed the presence of two anionic isoforms (pI of about 5.5) (Fig. 2). Maize PM contained several bands, both anionic and cationic: in addition to one strong anionic band with pI of about 5, three weak bands (pI of about 5.6), one neutral (pI of about 7.2) and two cationic SOD isoforms (with pI of about 8.6) were observed (Fig. 2). Total POD and SOD activities calculated as the sum of individual band densities showed that POD activity was higher in pea (346 relative units) than in maize (277 relative units). On the contrary, total SOD activity in pea PM was three times lower (82 relative units) than in maize (242 relative units).

Total lipid contents of maize and pea PM, calculated as the sum of each lipid class detected and expressed on a protein basis, were not significantly different (Table 1). Phospholipids were the major lipid class in both species and accounted for about half of the total lipids, with no difference in their amounts. In pea and maize, steryl lipids represented 28 and 33%, respectively, of total PM lipids, and among them FS accounted for 49% and 54%, respectively. Plasma membrane isolated from pea roots contained cerebrosides in a quantity of 0.077 μmol/mg of protein, a value 30% lower than the content detected in maize (0.109 μmol/mg of protein). However, their molar percentage was similar in pea and maize (19 and 21% of total lipids, respectively) (Table 1). Among PL, phosphatidylcholine (PC) represented the main compound in both plants, followed by phosphatidylethanolamine (PE) (Fig. 3). As for cerebrosides, their percentage did not differ between the two species. Fatty acid compositions of PM total lipids showed that linoleic (18:2) and palmitic (16:0) acids were the most abundant fatty acids in both pea and maize, the unsaturation degree being similar (Table 2). A significant difference in the amount of 18:3 and 14:0 was found between species.

DISCUSSION

A wide variation in lipid composition of isolated

Table 2. Fatty acid composition of pea and maize plasma membrane total lipids. 14:0-myristic acid, 16:0-palmitic acid, 16:1-palmitoleic acid, 18:0-stearic acid, 18:1-oleic acid, 18:2-linoleic acid, 18:3-linolenic acid, 20:0-behenic acid.

Fatty acid (%)	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	% unsaturation
PM pea	0.64	22	1.0	5.0	5.2	60	4.85	1.24	72
PM maize	1.35	22.5	1.45	6.84	3	63	0.84	1.72	69

PM from different species and from different organs within a species has been reported (Uemura and Steponkus, 1994; Quartacci et al., 2001, 2002; Wu et al., 2005). Our results show that PL were the most abundant lipid class in PM of pea and maize roots, accounting for 49 and 52% of total lipids, respectively (Table 1). The main PL identified in pea and maize was PC, as was found for PM of other plants (Navari-Izzo et al., 1993; Renault et al., 1997). The PC to PE molar ratios of pea (1.4) and maize (1.2) were similar to those reported for root PM of pea (1.5) and maize (1.2) (Hernandez and Cook, 1997; Bhon et al., 2001). Plant PM are known to be rich in steryl lipids, especially FS (Wu et al., 1998; Mansour et al., 2002; Wu et al., 2005). We observed that FS accounted for 14 and 18 mol % of total lipids in pea and maize, respectively, levels which are compatible with the above cited values. The FS to PL molar ratio of maize roots (0.38) was similar to that reported by Granmougin-Ferjani et al. (1997). In PM of pea and maize roots, a relatively high amount of cerebrosides (20%) was determined compared with previous reports for wheat and maize roots (Bohn et al., 2001; Quartacci et al., 2001).

Peroxidase isoforms from pea and maize had different mobility on native PAGE gels and different pI values (Fig. 1). While pea PM contained highly cationic POD isoforms, maize PM contained neutral and slightly anionic isoforms. We detected several SOD isoforms in maize PM and two anionic SOD isoforms in pea (Fig. 2). Two anionic (pI 5.5) and two cationic (pI 8.6) SOD isoenzymes were detected in maize PM by Kukavica et al. (2005). Analysis of the extracellular and cytosolic SOD isoforms from Scotch pine showed that the extracellular isoforms have distinctly higher isoelectric points than those reported for cytosolic SOD (Schinkel et al., 1998). Karpinska et al. (2001) found CuZn-SOD with high isoelectric points (10.2) in PM of the same species. The significant differences found in lipid composition of pea and maize (PC to PE and FS to PL molar ratios, cerebroside and conjugate sterol amounts, Table 1) indicate that lipids may affect binding of differentially charged POD and SOD isoforms to PM. *In vivo* functioning of a particular enzyme is likely to be determined by its location in a particular tissue or compartment (cellular or subcellular), which in turn may depend in part on its ionic nature, as was shown for cell wall (Penel et al., 2000; Carpin et al., 2001).

Lipid-protein interaction has been demonstrated to be specific, positively charged amino acids being bound to negatively charged PL, which might determine orientation of membrane proteins within the membrane (van Klompenburg et al., 1997). Preferential binding of proteins to different PL has been documented by *in vitro* studies with the fluoresceine derivative spectrin, which binds phosphatidylserine (O' Toole et al., 2000) and dynamine, which binds to phosphatidylinositol and phosphatidic acid (Burger et al., 2000). It has also been reported that differences in lipid composition of the PM can affect the activity of membrane proteins such as H^+-ATPase (Cooke et al., 1994; Granmougin-Ferjani et al., 1997).

The plant PM contains high amounts of polyunsaturated fatty acids (Navari-Izzo et al., 1993; Bohn et al., 2001). In both pea and maize PM, we detected a high degree of fatty acid unsaturatin (Table 3). Oxidative stress may cause peroxidation and consequent changes in membrane permeability (Quartacci et al., 2001; Berglund et al., 2002). Membrane repair mechanisms involving POD have been demonstrated for the peroxisomal membranes (Jespersen et al., 1997). POD activity of PM was previously observed in maize roots, and several functions have been postulated for PM-bound POD (Hadži-Tašković Šukalović et al., 2003;

Mika and Lüthje, 2003; Mojović et al., 2004; Vuletić et al., 2005). Although most of these functions were also demonstrated for soluble and cell wall–bound POD (Hiraga et al., 2001; Kawano, 2003), there may be some functions specific to membrane-bound POD, namely the membrane-protective function. For CuZn-SOD with high isoelectric points, it has been proposed that in the phloem it acts as a regulator of H_2O_2 pulses, being involved in transmission of systemic signals in wounding or pathogen responses (Karpinska et al., 2001). Our results showed that POD and SOD activities are intrinsic to pea and maize root PM (Figs. 1 and 2). Total POD activity, calculated as the sum of individual band densities on IEF gel, was higher in pea than in maize PM. On the other hand maize root PM had cationic SOD isoforms and higher total SOD activity than in pea PM.

POD and SOD coexistence in the PM would provide a complementary enzymatic system in addition to NADPH oxidase, their role being to defend against ROS and maintain the $H_2O_2/O_2^{\cdot-}/{\cdot}OH$ balance.

Abbreviations used:

PM, plasma membranes; POD, peroxidase; SOD, superoxide dismutase; PL, phospholipids; FS, free sterols, CER, cerebrosides; SG, steryl glycosides; ASG, acylated steryl glycosides; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; PA, phosphatidic acid; IEF, isoelectro-focusing.

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REFERENCES

Benfenati, A. H., Greengard, P., Brunner, J., and *M. Bahler* (1998). Electrostatic and hydrophobic interaction of synapin I and synapin II fragments with phospholipid bilayers. *J. Cell Biol*. **108**, 1851-1862.

Berglund, A., Norberg, P., Quartacci, M., Nilsson, R., and *C. Liljenberg* (2000). Properties of plant plasma membrane lipid models- bilayer permeability and monolayer behavior of glucosylceramide and phosphatidic acid in phospholipid mixture. *Physiol. Plant*, **109**, 117-122.

Berglund, A. H., Quartacci, M. F., Calucci, L., Navari-Izzo, F., Pinzino, C., and *C. Liljenberg* (2002). Alterations of wheat root plasma membrane lipid composition induced by copper stress result in changed physicochemical properties of plasma membrane lipid vesicles *Biochim. Biophys. Acta - Biomembranes*, **1564**, 466-472.

Bohn, M., Heinz, E., and *S. Luthje* (2001). Lipid composition and fluidity of plasma membranes isolated from corn (*Zea mays* L.) roots. *Arch. Biochem. Biophys*. **387**, 35-40.

Burger, K. N., Demel, R. A., Schmid, S. L., and *B. de Kruijff* (2000). Dynamin is membrane-active: lipid insertion is induced by phosphoinositides and phosphatidic acid. *Biochemistry*, **39**, 12485-12493.

Beauchamp, C., and *I. Fridovich* (1971). Superoxide dismutase: improved assay and assay applicable to acrylamide gels. *Anal. Biochem.* **44**, 276-287.

Bradford, M. M. (1976). A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Anal. Biochem.* **72**, 248-254.

Carpin, S., Crevecoer, M., de Meyer, M., Simon, P., Greppin, H., and *C. Penel* (2001). Identification of a Ca^{2+}-pectate binding site on an apoplastic peroxidase. *Plant Cell* **13**, 511-520.

Cooke, D. T., Burden, R. S., James, C. S., Seco, T., and *B. Sierra* (1994). Influence of sterols on plasma membrane proton-pumping ATPase activity and membrane fluidity in oat shoots. *Plant Physiol. Biochem.* **32**, 769–773.

Escribá, P. V., Ozaita, A., Ribas, C., Miralles, A., Fodor, E., Farkas, T., and *J. A. García-Sevilla* (1997). Role of lipid polymorphism in G protein-membrane interactions: Nonlamellar-prone phospholipids and peripheral protein binding to membranes. *PNAS* **94**, 11375-11380.

Grandmougin-Ferjani, A., Schuler-Muller, I., and *M. A. Hartmann* (1997). Sterol modulation of the plasma membrane H^+ATPase activity from corn roots reconstituted into soybean lipids. *Plant Physiol*. **113**, 163-174.

Hadži-Tašković Šukalović, V., Vuletić, M., and *Ž. Vučinić* (2003). Plasma membrane–bound phenolic peroxidase of maize roots: *in vitro* regulation of activity with NADH and ascorbate. *Plant Sci.* **165**, 1429-1435.

Hernandez, L., and *D. Cook* (1997). Modification of the root plasma membrane lipid composition of cadmium-treated *Pisum sativum*. *J. Exp. Bot.* **48**, 1375-1381.

Hiraga, S., Sasaki, K., Ito, H., Ohashi, Y., and *H. Matsui* (2001). A large family of class III plant peroxidases. *Plant Cell Physiol.* **42**, 462– 468.

Hoagland, D. R. and *D. I. Arnon* (1950). The water-culture meth-

od for growing plants without soil. *Calif. Agr. Exp. Sta. Circ.* **347**, 1-39.

Jespersen, H. M., Kjærsgård, I. V. H., Østergaard, L., and *K. G. Welinder* (1997). From sequence analysis of three novel ascorbate peroxidases from *Arabidopsis thaliana* to structure, function and evolution of seven types of ascorbate peroxidase. *Biochem. J*. **326**, 305–310.

Karpinska, B., Karlsson, M., Schinkel, H., Streller, S., Suss, K.H., Melzer, M., G. and *G Wingsle* (2001). A novel superoxide dismutase with a high isoeletric point in higher plant. Expression, regulation, and protein localization. *Plant Physiol.* **126**, 1668-1677.

Kawano, T. (2003). Roles of the reactive oxygen species-generating peroxidase reactions in plant defense and growth induction. *Plant Cell Rep*. **21**, 829–837.

Kukavica, B., Vučinić, Ž., and *M. Vuletić* (2005). Superoxide dismutase, peroxidase and germin-like protein activity in plasma membranes and apoplast of maize roots. *Protoplasma,* **226**, 191-197.

Mansour, M. M. F., Salama, K. H. A., Al-Mutawa, M. M., and *A. F. Abou Hadid* (2002). Effects of NaCl and polyamines on plasma membrane lipids of wheat roots. *Biol. Plantarum*, **45**, 235-239.

Mika, A., and *S. Lüthje* (2003). Properties of guaiacol peroxidase activities isolated from corn root plasma membranes. *Plant Physiol.* **132**, 1489–1498.

Mojović, M., Bačić, G., Vučinić, Ž., and *M. Vuletić* (2004). Oxygen radicals produced by plant plasma membranes: An EPR spin-trap study. *J. Exp. Bot.* **55**, 2523-2531.

Navari-Izzo, F., Quartacci, M., Melfi, D., and *R. Izzo* (1993). Lipid composition of plasma membranes isolated from sunflower seedlings grown under water-stress. *Physiol. Plantarum* **87**, 508-514.

Neill, S., Desikan, R., and *J. Hancock* (2002). Hydrogen peroxide signaling. *Curr. Opin. Plant Biol.* **5**, 388–395.

O'Toole, P. J., Morrison, I. E. and *R. J. Cherry* (2000). Investigations of spectrin-lipid interactions using fluorescein phosphatidylethanolamine as a membrane probe. *Biochim. Biophys. Acta* **1466**, 39-46.

Penel, C., Carpin, S., Crèvecoeur, M., Simon, P., and *H. Greppin* (2000). Binding of peroxidase to Ca^{2+}-pectate: Possible significance for peroxidase function in cell wall. *Plant Perox. Newslett.* **14**, 33-40.

Quartacci, M. F., Forli, M., Rascio, N., Dalla, M., Vecchia, F., Bochicchio, A., and *F. Navari-Izzo* (1997). Desiccation-tolerant *Sporobolus stapfianus*: lipid composition and cellular ultrastructure during dehydration and rehydration. *J. Exp. Bot.* **48,** 1269–1279.

Quartacci, M., Cosi, E., and *F. Navari-Izzo* (2001). Lipids and NADPH-dependent superoxide production in plasma membrane vesicles from roots of wheat grown under copper deficiency or excess. *J. Exp. Bot.* **52**, 77-84.

Quartacci, M. F., Glisic, O., Stevanovic, B., and *F. Navari-Izzo* (2002). Plasma membrane lipids in the resurrection plant *Ramonda serbica* following dehydration and rehydration. *J. Exp. Bot.* **53**, 1–8.

Renault, S., Shukla, A., Giblin, M. E., MacKenzie, S. L., and *M. D. Devine* (1997). Plasma membrane lipid composition and herbicide effects on lipoxygenase activity do not contribute to differential membrane responses in herbicide-resistant and -susceptible wild oat (*Avena fatua* L.). *J. Agr. Food Chem.* **45**, 3269-3275.

Schopfer, P., and *A. Liszkay* (2006). Plasma membrane-generated reactive oxygen intermediates and their role in cell growth of plants. *Plant and Animal Cell Enlargement* **28**, 73– 81.

Schinkel, H., Streller, S., and *G. Wingsle* (1998). Multiple forms of extracellular superoxide dismutase in needles, stem tissues and seedlings of Scotch pine. *J. Exp. Bot.* **49**, 931-936.

Uemura, M., and *P. Steponkus* (1994). A contrast of the plasma membrane lipid composition of oat and rye leaves in relation to freezing tolerance. *Plant Physiol*. **104**, 479-496.

van Klompenburg, W., Nilsson, I. M., von Heijne, G., and *B. de Kruiff* (1997). Anionic phospholipids are determinants of membrane protein topology. *EMBO J*. **16**, 4261-4266.

Vuletić, M., Hadži-Tašković Šukalović, V., and *Ž. Vučinić* (2003). Superoxide synthase and dismutase activity of plasma membranes from maize roots. *Protoplasma* **221**, 73-77.

Vuletić, M., Hadži-Tašković Šukalović, V., and *Ž. Vučinić* (2005). The Coexistence of the oxidative and reductive systems in roots: The role of plasma membranes. *Ann. N.Y. Acad. Sci.* **1048**, 244-258.

van Voorst, F., and *B. de Kruijf* (2000). Role of lipids in the translocation of proteins across membranes. *Biochem. J.* **347**, 601–612.

Wu, J. D. M., Seliskar, J., and *J. L. Gallagher* (1998). Stress tolerance in the salt marsh plant *Spartina patens*: impact of NaCl on growth and root plasma membrane lipid composition. *Physiol. Plantarum* **102**, 307-317.

Wu, J. D. M, Seliskar, D. M., and *J. L. Gallagher* (2005). The response of plasma membrane lipid composition in callus of the halophyte *Spartina patens* (Poaceae) to salinity stress. *Am. J. Bot.* **92**, 852-858.

ЛИПИДНИ САСТАВ ПЛАЗМА МЕМБРАНА КОРЕНА ГРАШКА (*PISUM SATIVUM*, L) И КУКУРУЗА (*ZEA MAYS*, L) И МЕМБРАНСКИ ВЕЗАНЕ ПЕРОКСИДАЗНЕ И СУПЕРОКСИД ДИСМУТАЗНЕ ИЗОФОРМЕ

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У раду је одређен липидни састав плазма мембрана (ПМ) изолованих из корена грашка и кукуруза и анализиране су плазма мембрански везане пероксидазне (POD) и супероксид дисмутазне (SOD) изоформе. Резултати су показали да су фосфолипиди најзаступљенија класа липида у плазма мембранама обе биљне врсте, док су значајне разлике пронађене у садржају цереброзида, слободних стерола и стерил гликозида. Пероксидазне изоформе везане за ПМ корена грашка су катјонске за разлику од пероксидаза везаних за ПМ корена кукуруза чије су pI вредности неутралне и анјонске. Две анјонске SOD изоформе су детектоване на ПМ грашка, а на ПМ кукуруза и анјонске и катјонске SOD изоформе. Различити изоензимски састав POD и SOD у ПМ корена грашка и кукуруза дискутовани су у односу на разлике у липидном саставу ПМ две биљне врсте.