Zavod za zaštitu prirode Crne Gore

Ministarstvo održivog razvoja i turizma

NAUČNI SKUP SA MEĐUNARODNIM UČEŠĆEM ▌INTERNATIONAL CONFERENCE

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20 – 23 Septembar 2011.
Žabljak, Crna Gora / Montenegro

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ANTIOXIDATIVE ACTIVITY OF CELL WALL ISOLATED FROM *PICEA OMORIKA* NEEDELS SHOWS SEASONAL CHANGES

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Abstract

*Picea omorika (*Pančić*)* Purkyně is a Balkan endemic coniferous species and a Tertiary relict of the European flora. After the Ice Age the habitat of this species is a rather narrow one (approx. 10000 km2), occupying exclusively the middle and upper courses around the river Drina. This coniferous species is exposed to subfreezing temperatures that range from -10 to -30°C during the autumn/winter and high temperatures exceeding 30°C during the summer. In the autumn, as temperatures began to decrease, photosynthetic potential decreased as spruce entered dormancy. Cold stress is associated with the accumulation of reactive oxygen species (ROS). Among them, the hydroxyl radical is a very reactive oxygen species with a short half-life, and is considered to be responsible for much of the biological damage during cold stress. This study investigates the ability of cell walls isolated from *P. omorika* needles, during four seasons, to scavenge •OH radical using the Fenton reaction as an "•OH producing" system. Electron paramagnetic resonance spectroscopy using spin-trap DEPMPO was applied to detect hydroxyl radical. The capacity of the cell wall of Serbian spruce needles to scavenge hydroxyl radical is the largest in the autumn and winter. Changes in the molecular size and quantities of cell wall constituents have been considered to be involved in the response to various environmental stresses. The protective roles of cell walls in *P. omorika* needles against cold stress might be additionally attributed to their free radical scavenging capabilities.
Key words: cell wall, *Picea omorika* (Pančić) Purkyně, antioxidative activity.

Introduction

Picea omorika (Pančić) Purkyně is a Balkan endemic coniferous species and a Tertiary relict of the European flora. Before the last glaciation period omorika occupied a large area in Europe. After the Ice Age the habitat of this species is a rather narrow one (approx. 10000 km2), occupying exclusively the middle and upper courses around the river Drina - the present territory of Serbia and Bosnia (JOVANOVIĆ, 1970). The trees of this species grow in a large edaphic and altitudinal range (300-1700 m) and occur exclusively within disturbed and relatively open habitats such as cliffs, forest clearings and vegetation gaps (ČOLIĆ, 1957, ČOLIĆ 1966). Extremely variable light and temperature conditions both in space and in time are characteristic for such type of habitat (BAZZAZ, 1996).

Climate on the mountain Tara is mostly a typical temperate mountain climate, with cold winters and moderately warm summers. *P. omorika* in its natural habitat is exposed to subfreezing temperatures that range from -10 to -30°C during the autumn/winter and high temperatures exceeding 30°C during the summer. Exposure to low temperatures is one of the most important plant abiotic stress factors. Chilling and/or freezing temperatures can cause different damages to plant cells; these include damage to membranes, protein denaturation and accumulation of toxic products (BOWERS, 1994). Lower temperatures also induce accumulation of reactive oxygen species (ROS). Confronted to these damages, some plants are able to adapt through mechanisms based on protein synthesis, membrane composition changes, and activation of active oxygen scavenging systems.

Plant cell walls are complex and dynamic structures composed of many different polysaccharides, including cellulose, hemicelluloses and pectin, proteins and polyphenolics (CARPITA and

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MCCANN, 2000). This complex structure not only is responsible for the structural integrity of the cell, but it also represents the plant's outer barrier against the environment, thus protecting the plant against biotic and abiotic stresses. In the present paper, we have examined the hydroxyl radical scavenging capacities of cell wall isolated from *P. omorika* needles.
Our aim was to evaluate the antioxidant and free radical scavenging activities of isolated cell walls in order to show their possible contribution to the antioxidant capacity of apoplast and cell. To determine antioxidative activity, we employed Electron Paramagnetic Resonance (EPR) spin-trapping spectroscopy.

Material and methods

Needles were collected from, about 80 to 100-year-old, healthy omorika trees, in its natural habitat in the mountain region of Tara (43° 52' N, 19° 26' E, 1100 m), Serbia, in 2003/2004. The needles were collected at approximately the same height of the trees. All needle samples were taken from the trees between 9 and 11 AM, and immediately stored in the liquid nitrogen.
Dry needles (72 h at 80º) were ground into a fine powder. To obtain cell walls, 400 mg of needle powder was homogenized for 5-10 min in 10 ml 80% methanol. The homogenate was stirred for 1 h at room temperature and centrifuged for 5 min at 1500 g. The pellet was reextracted twice with 10 ml 80% methanol. The pellet was subjected to the following washing steps (STRACK et al, 1988, CHEN et al, 2000): 1x (1M NaCL, 0.5% Triton X-100), 2x distilled water, 2x 100% methanol, 2x 100% acetone (each step in 20 ml, 30 min). Finally, isolated cell walls were dried in vacuum.
The ability of cell wall to scavenge •OH radical was tested using the Fenton reaction as an "•OH producing" system. Fenton reaction was performed by combining 0.5 mM H_2O_2 (Renal, Budapest, Hungary) and 0.075 mM $FeSO_4$ (Merck, Darmstadt, Germany). Spintrap DEPMPO was purchased from Alexis Biochemical (Lausen, Switzerland), purified, and tested for hydroxylamine impurities. DEPMPO was added at final concentration of 28 mM. DEPMPO reacts with $•O_2$ - and •OH to form DEPMPO/OOH and DEPMPO/OH adducts, respectively. For all experiments, bidistilled deionized ultrapure (18 MΩ) water was used. Final concentration of cell wall (previously dissolved in water) was 10 mg/mL. Sample with no extract served as a control.
EPR spectra were recorded using a Varian E104-A EPR spectrometer operating at X-band (9.572 GHz) with the following settings: modulation amplitude, 2 G; modulation frequency, 100 kHz; microwave power, 10 mW; time constant, 32 ms; field center, 3,410 G; scan range, 200 G; scanning time, 4 min. Temperature in cavity was 291 K. Recordings were performed using EW software (Scientific Software, Bloomington, IL, US). Samples were drawn into 10 cm long gas-permeable Teflon tubes in order to maintain constant O2 level in the sample (wall thickness 0.025 mm and internal diameter 0.6 mm; Zeus industries, Raritan, NJ, USA). Measurements were performed using quartz capillaries in which Teflon tubes were placed. Recordings were conducted 2 min after the reaction had started. Spectral simulation of each spectrum was performed using WINEPR SimFonia computer program (Bruker Analytische Messtechnik GmbH, Darmstadt, Germany) in order to determine the signal intensity. Antioxidative activity (AA) was calculated as: AA= (Io − Ix)/Io, where Io and Ix are the intensities of EPR spectra obtained in control and samples with cell walls, respectively. Maximal value of AA is 1.

Results and discussion

Much of the oxidative damage to biomolecules can be induced by hydroxyl radical, the most reactive one among ROS species. Hydroxyl radicals generated in Fe^{2+}/H_2O_2 system is trapped by 5-diethoxyphosphoryl-5-methyl-1-pyrroline-N-oxide (DEPMPO) spin-trap forming spin adduct which could be detected by (EPR) spectrometer. The EPR spectrum is inhibited by the presence of hydroxyl radical scavengers, which compete with DEPMPO for hydroxyl radicals.
Seasonal variations in antioxidative capacity of *P. omorika* cell wall are shown in Figure 1. Seasons had a significant effect on the hydroxyl radical scavenging activities of *P. omorika* cell walls. The capacity of the cell wall of Serbian spruce needles to scavenge hydroxyl radical is the largest in the autumn and winter.

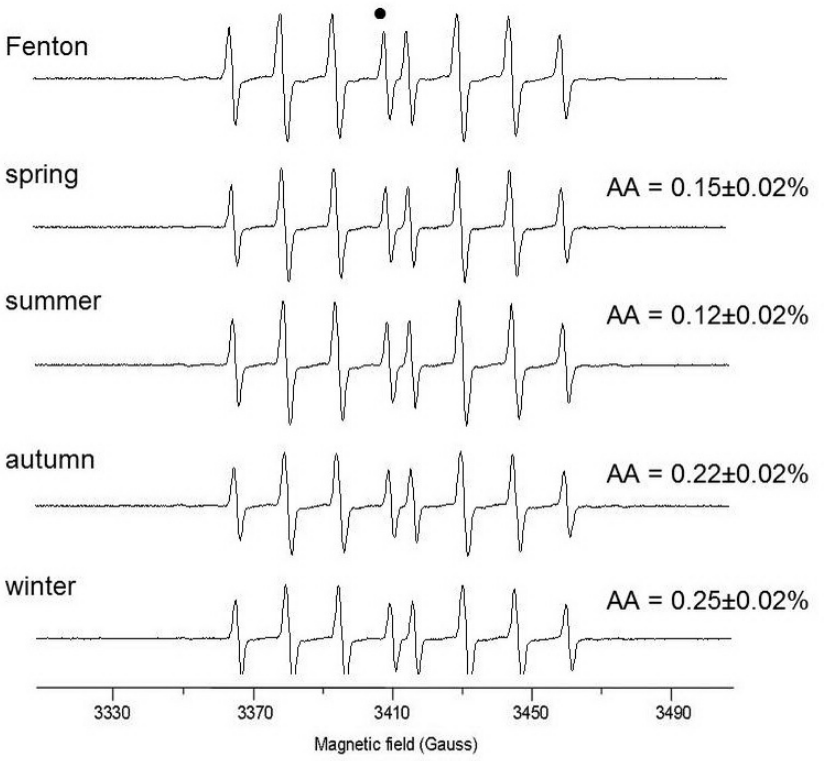

Figure 1. The radical scavenging effects of cell wall isolated from *P.omorika* needles (15 mg/mL) in the Fenton system ($Fe^{2+} + H_2O_2 \rightarrow \bullet OH + OH^- + Fe^{3+}$) during four season. EPR spectra represent the signals of the DEPMPO adduct of •OH generated in the Fenton reaction (Fe^{2+} (0.1 mM) + H_2O_2 (2 mM)). Antioxidative activity (AA) was calculated as: AA= (Io − Ix)/Io, where Io and Ix are the intensities of EPR spectra obtained in control and samples with cell walls, respectively. Maximal value of AA is 1.

Slika 1. Antioksidativna aktivnost ćelijskog zida izolovanog iz četina *P. omorike* (15 mg/mL) u Fenton sistemu ($Fe^{2+} + H_2O_2 \rightarrow \bullet OH + OH^- + Fe^{3+}$) tokom četiri godišnja doba. EPR spektri predstavljaju DEPMPO adukt hidroksil radikala nastalog u Fentonovoj reakciji (Fe^{2+} (0.1 mM) + H_2O_2 (2 mM)). Antioksidativna aktivnost (AA) je izračunata po jednačini AA= (Io − Ix)/Io, gde je Io intenzitet EPR spektra dobijenog u kontrolnij reakciji a Ix je intenzitet spektra dobijenog u prisustvu izolata ćelijskog zida. Maksimalna vrednost za AA je 1.

The differing functions of the cell wall are reflected in the large variation and reorganization in cell wall composition between different cell types and during cell differentiation (COSGROVE, 2005). The ratio and exact composition of the various wall polymers differs phylogenetically between plant taxa (POPPER and FRY, 2003), spatially between the tissues of a plant (LYNCH and STAEHELIN, 1995), and temporally during the development of a plant cell (BRETT and WALDRON, 1996).

Cold acclimation is accompanied by changes in the cell wall. In winter oil-seed rape acclimation at 2°C was associated with increases in leaf tensile stiffness, cell wall and pectin contents, pectin ethylesterase activity, and in low-methylated pectin content (SOLECKA et al., 2008). A wide range of polysaccharides, naturally occurring and isolated from plant sources, have been identified as a free radical or ROS scavengers (ZHU et al., 2009).

Presented results show that cell wall could play a significant role as one of non-enzymatic defense mechanisms in stress induced by chilling and/or freezing temperatures.

Conclusion

Changes in the molecular size and quantities of cell wall constituents have been considered to be involved in the response to various environmental stresses. The protective roles of cell walls in *P. omorika* needles against cold stress might be additionally attributed to their free radical scavenging capabilities.

Acknowledgment: This work was supported by a grant (No. 173017) from the Ministry of Education and Science of the Republic of Serbia.

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