Superoxide dismutase polymorphisms in wild populations of herb Paris (*Paris quadrifolia* L., *Trilliaceae*)

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Polymorphism of superoxide dismutase (SOD) was investigated in leaves of herb Paris (*Paris quadrifolia* L., *Trilliaceae*). The plants were collected during the summer and autumn of 2001 from different natural locations in Lithuania and Norway. Crude extracts from leaves were analyzed using electrophoresis in polyacrylamide gel for SOD polymorphism detection. By means of analysis of plants from different locations, some differences in the electrophoretic mobility and the phenotypes of SOD bands were detected. Differences appeared between the Lithuanian and the Norwegian samples and among the Lithuanian samples from different locations as well as inside them. These findings indicate a polymorphism in plants from Lithuania and Norway. Analysis of the results revealed five types of SOD isozyme spectra in both countries. SOD isozyme spectra also differed in leaves, seeds, roots and rootlets.

Key words: enzyme electrophoresis, *Paris quadrifolia*, polymorphism, superoxide dismutase

INTRODUCTION

Herb Paris (*Paris quadrifolia* L., *Trilliaceae*) is widespread in deciduous and mixed forests of Europe and Central Asia (Lietuvos… 1963; Meusel et al., 1965). Its large habitat along the continent of Eurasia with various ecological conditions allows to presume a possibility of polymorphism. Various enzyme systems have been applied for polymorphism detection in different organisms (Asins et al., 1995; Kertadikara et al., 1995). Higher plants possess a number of superoxide dismutase isozymes that have been used as a molecular markers in polymorphism studies (Kertadikara et al., 1995; Pszybylska et al., 1992; Žvingila et al., 1993). Superoxide dismutases (superoxide: superoxide oxidoreductase; SOD; EC 1.15.1.1) are ubiquitous enzymes found in all the aerobes and involved in protection from oxygen toxicity. These metalloproteins catalyze the dismutation of the superoxide radical to molecular oxygen and hydrogen peroxide. The superoxide ($O_2^{\cdot -}$) and hydroxyl (· OH) radicals together with hydrogen peroxide (H_2O_2) are the so-called reactive oxygen species (ROS) that pose a serious threat to all organisms. ROS are also crucial for many physiologic processes and usually exist in the cell in a balance with the antioxidants. However, excess ROS resulting from exposure to environmental oxidants, toxicants, radiation, or numerous biostressors perturbs the cellular redox balance (to a more oxidized state) and disrupts normal biological functions. This condition is referred to as "oxidative stress" and may be detrimental to the organism by contributing to the pathogenesis of disease and aging, and numerous physiologic dysfunctions leading to cell death (Kernodle et al., 2001).

The aim of this work was to investigate whether polymorphism is present within wild populations of herb Paris, using superoxide dismutase as a molecular marker.

MATERIALS AND METHODS

Plant material

Leaves of *Paris quadrifolia* were used in experiments for the detection of SOD polymorphism in different populations. Leaves, rhizome, rootlets and seeds were used for the detection of SOD polymorphism in plant tissues of different organs. The samples were collected in different locations of Lithuania (Fig. 1) and Norway during the summer and autumn of the year 2001. Five different natural locations of herb Paris in Lithuania were chosen: Joniškis district,

Fig. 1. Map showing the populations of herb Paris (*Paris quadrifolia* L.) examined in Lithuania: *1* – Joniškis, *2* – Labanoras wood, *3* – Kairėnai, *4* – Vingis (Vilnius), *5* – Trakai, *6* – Varėna

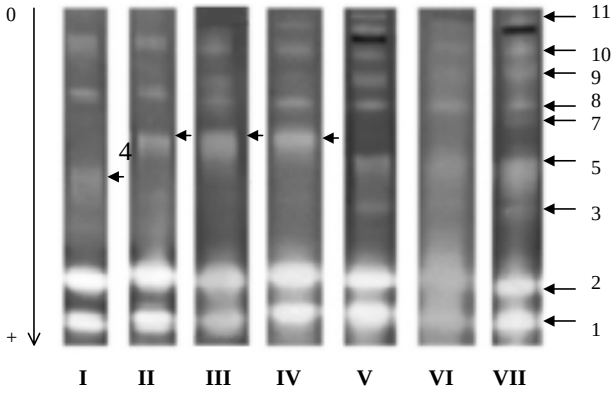


Fig. 2. Electrophoregrams in native – PAGE (9%) of SOD from leaves of Paris herb (*Paris quadrifolia* L.) collected in different locations: I – Joniškis, II – Trakai, III – Varėna, IV – Labanoras, V – Kairėnai, VI – Hørte (Norway), VII – Vingis. Bands are numerated from fastest to slowest

Švenčionys district (Labanoras forest), Trakai district, Varėna district, and Vilnius district (Botanical Garden of Vilnius University in Kairėnai and Vingis). In Norway the samples were collected from 16 different natural locations. Randomly sampled plants were stored at –18 °C until further analysis. From each location at least three plants were taken for the preparation of crude extract and tested using polyacrylamide gel electrophoresis.

Only one population from Norway was used for comparison with the other populations from Lithuania, because the intensity of bands at the catodic zone was very low in all the samples from Norway and it was impossible to make a reasonable comparison among them. The low quality of electrophoregrams from the Norwegian material may be caused by storage conditions as the plants were collected in 2001 and kept at –18 °C for almost a whole year. Since the electrophoregrams from Hørte population were most intensive (Fig. 2), they were used as representative ones for all the Norwegian population in this investigation.

Crude extract

For crude enzyme extract, 1–2 leaves of each plant were grinded in a mortar with sand in the precooled extraction buffer. One millilitre of 0.1 M potassium phosphate extraction buffer (pH 8.2) was taken for 1 g (fresh weight) of leaves (Beauchamp et al., 1971). The homogenate was centrifuged at 13000× g for 10 min. The supernatant was used for further analysis. All procedures were performed at 4 °C.

When homogenizing rhizomes and rootlets, for 1 g (fresh weight) of tissue 3 ml of extraction buffer and for 1 g of seeds 4 ml of the extraction buffer were taken. Subsequent sample preparations for electrophoresis were the same as described above.

Polyacrylamide gel electrophoresis and enzyme detection

SOD was analyzed on 4% concentrating and 9% discontinuous nondenaturing polyacrylamide gels (PAG) by vertical gel electrophoresis (Davis, 1964) (at 200 V, 40 mA) for about 3 h at 4 °C (Beauchamps et al., 1971) using Tris-glycine buffer (pH 8.3). About 30 µl of crude extract from leaves and 20 µl from other organs were loaded in each lane. The zones of SOD activity were detected by staining the gel in a following mixture: 100 ml 0.1 M Tris-HCl buffer (pH 8.5), 15 mg tetranitro blue tetrazolium, 15 mg phenazine methosulphate and 20 mg magnesium chloride, incubating for an hour at 37 °C in the dark.

SOD densitograms were made using a DM-1 densitometer with a white filter No. 5.

RESULTS AND DISCUSSION

Six to nine zones of superoxide dismutase activity were observed in our experiments (Figs. 2, 3). Previous papers of other researches also indicated SOD polymorphisms in other plant species: three zones of SOD activity were identified in pea leaves (Palma et al., 1998), four zones of SOD were detected in sunflower leaves (Palomo et al., 1999). In diploids, an enzyme band is coded by one or two copies of an allele. It is therefore difficult to determine the exact genotype and allele frequency in a polyploid without genetic analysis of crosses (Ny-

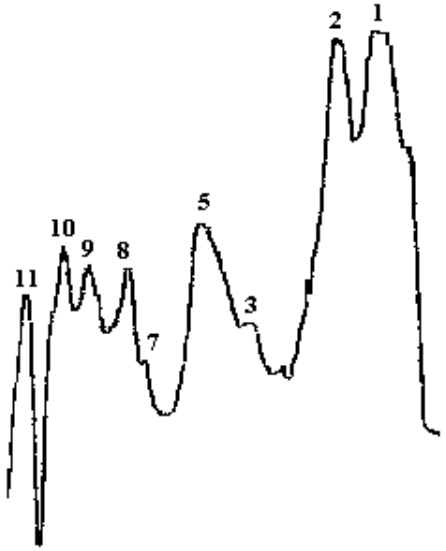


Fig. 3. Densitogram of superoxide dismutase from leaves of herb Paris (*Paris quadrifolia* L.) (Vingis location). Numbers of peaks correspond with numbering of bands in electrophoregram in Fig. 3

berg Berglund et al., 2001). Since it is complicated to distinguish allozymes (genetically determined forms of enzymes) from isozymes (any multiple forms of enzymes) in polyploids without extensive analyses of segregating progeny, we used the term enzyme bands instead of allozymes and isozymes. The zones of SOD activity were distributed to the fastest anodic, the slowest catodic and the medium mobility bands. Only one two-banded phenotype was observed in the anodic zone. Three phenotypes were distinguished in the medium mobility zone. Three phenotypes were observed in the catodic zone of SOD activity as well (Fig. 2).

General analysis of the zymograms revealed five types of SOD isozyme spectra (Fig. 4). Zymograms of *P. quadrifolia* from Joniškis population were attributed to the first SOD isozyme spectrum. Zymograms of herb Paris from Trakai and Varėna populations were attributed to the second type and from Labanoras to the third type of SOD isozyme spectrum. The fourth type of spectrum included zymograms of herb Paris from Kairėnai and Hørte (Norway) populations, while the Vingis population had its own type of SOD isozyme spectrum. The anodic zone showed no polymorphism in all spectra, probably due to a low variation of frequencies at this locus. The medium mobility band of the first spectrum type was faster than in the rest zymograms of the other spectra. A certain difference in rapidity at the medium mobility zone was observed inside the Labanoras population. This could indicate a polymorphism within the population. Additional experiments are needed to prove it. As mentioned above, three phenotypes were found at the catodic zone of SOD activity. The first phenotype was three-banded, the second four-banded, while the third had five bands.

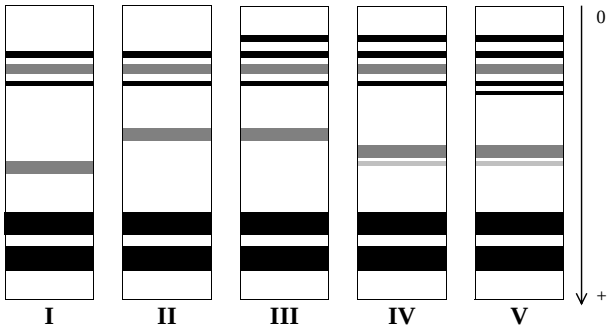
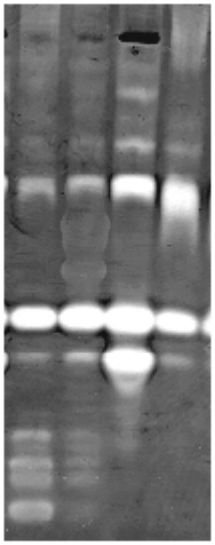


Fig. 4. Types of SOD isozyme spectra in leaves of herb Paris (*Paris quadrifolia* L.):
I – Joniškis, II –Trakai and Varėna, III – Labanoras, IV – Kairėnai and Hørte (Norway), V – Vingis

SOD polymorphism in Lithuanian populations of herb Paris was compared with that in the Norwegian populations. Anodic two-banded zones showed no polymorphism. Activity of catodic bands in Norwegian populations was weaker in comparison with Lithuanian populations. Therefore a precise estimation of SOD activity and mobility was impossible because of a poor quality of electrophoregrams from the Norwegian plants. Thus it was difficult to make a comparison both within the Norwegian and Lithuanian populations.

We have performed 9% PAGE to define the SOD banding pattern in tissues of different organs from herb Paris. The leaves, rhizome, rootlets and seeds of two plants from Vingis were analyzed. The rhizome and rootlets showed the same banding pattern, however, it differed from the banding pattern in leaves and seeds (Fig. 5). The main difference appeared in five additional anodic bands not observed earlier in zymograms from leaves. A variation was also defined at the anodic zone: the faster band was less intensive as in leaves. Moreover, SOD activity was weaker in the medium mobility and catodic zones in underground organs of the plants. This may depend on a variety of reasons such as dosage effect of gene copies, gene silencing or difference in kinetic activity (Nyberg Berglund et al., 2001). Besides, SOD activity at the catodic zone was stronger in plants collected during the summer and autumn of 2001 than in plants collected in April 2002. This difference can be related to the maturity stage of the plants collected at the begining and end of vegetation. SOD electrophoresis in tissues of different organs of herb Paris, as in maize (Baum et al., 1981), indicates a dependency of isozyme activities



I II III IV

Fig. 5. Electrophoregrams in native PAGE (9%) of SOD from tissues of different organs of herb Paris (*Paris quadrifolia* L.):
I – rootlets, II – rhizome, III – leaves, IV – seeds

relative to the plant tissue and development stage. Changes in the pattern of SOD isozymes reveal regulatory mechanisms controlling the synthesis of SOD in response to different oxidative stimuli and providing an adequate protection of plants during plant growth and development Scandalios, 1993.

Thus, our work revealed SOD polymorphism in the wild populations of herb Paris as well as among different organs of this plant.

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SUPEROKSIDDISMUTAZĖS POLIMORFIZMO TYRIMAI NATŪRALIOSE KETURLAPĖS VILKAUOGĖS (*PARIS QUADRIFOLIA* L., *TRILLIACEAE*) POPULIACIJOSE

S a n t r a u k a

Superoksiddismutazės (SOD) polimorfizmas buvo tiriamas keturlapės vilkauogės (*Paris quadrifolia* L., *Trilliaceae*) lapuose. Tyrimams augalai surinkti Lietuvos bei Norvegijos gamtinėse vilkauogės augimvietėse. SOD polimorfizmui nustatyti buvo ruošiami grubūs augalų ekstraktai, kurie ištirti baltymų elektroforezės poliakrilamidiniame gelyje metodu. Buvo nustatyta, kad elektroforezinis izoformų judrumas skiriasi Lietuvos ir Norvegijos vilkauogėse, taip pat augaluose iš skirtingų Lietuvos vilkauogių populiacijų. Palyginus Lietuvos ir Norvegijos augalų lapų elektroforegramas, nustatyti penki SOD izofermentų spektrai. Atlikus iš lapų, sėklų, šaknų bei šakniastiebių išskirtos SOD analizę, nustatyta, kad skirtingose augalo dalyse SOD izofermentų spektrai skiriasi.

Raktažodžiai: fermentų elektroforezė, *Paris quadrifolia*, polimorfizmas, superoksido dismutazė