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; Pszybyska 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 (O2−) and hydroxyl (·OH) radicals together with hydrogen peroxide (H2O2) 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

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.

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

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 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 beginning 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.
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.

References