Results in the determination of some *Hosta varieties* by the method of isoelectric focusing

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Summary: Mass propagation of 5 newly introduced *Hosta* varieties was carried out by the method of micropropagation. Our aim was to determine exact variety specificity after the micropropagation period in the pattern of peroxidase isoenzymes by isoelectric focusing in pH 3–9 range and to determine that phenological phase of mother plant in which the isoenzyme pattern of mother plant can safely be comparable to the isoenzyme pattern of micropropagated descendants. The isoenzyme patterns of descendants were similar to the mother plants of the same hybrid lines. The older leaves seemed to be not so suitable for examination than newly developed ones despite of the higher activity of peroxidase enzymes. There were big differences in isoenzyme patterns of leaves in different phenological phases. With this quick and easy method *Hosta* varieties could be selected already in the very early stage of micropropagation.

Introduction

Mass propagation of 5 newly introduced *Hosta* varieties was carried out by the method of micropropagation. A major problem of this method is that during micropropagation some variegated varieties segregate to different colourleaf plants in the test tubes or do not develop their specific leaf pattern until the third year of growth in the nursery. Until now the major way of identification was mainly morphological, that can now be extended with a biochemical method *(McMillin,* 1983). *Chung* et al. (1991) reported the examination of isoenzyme diversity in different *Hosta* species to study the relationships between the species. Our preliminary aim was to determine exact variety-specificity after the micropropagation period in the pattern of peroxidase isozymes by isoelectric focusing in the pH 3–9 range. A further aim of this study was to determine at which phenological phase of the mother plant the isozyme pattern of mother plant could safely be compared to the isozyme pattern of micropropagated descendants.

Material and methods

Plant material

The *Hosta* varieties originated from a collection from the Netherland. Mother plants were planted out-door in Hévíz, West-Hungary. Leaves of the above described varieties were collected in April (newly developed) and in August (fully developed). Leaves were put in –23 °C freezer within one hour after collection and were kept there till examination.

Micropropagation technique and acclimatization

Varieties of *Hosta* species: *H. tardiana* 'Devon Green', *H. tokudama* 'Blue Cadet', 'Drummer Boy', 'Samurai' and *H. fortunei* 'Diamond Tiara' were micropropagated according to the method described by *Szafián* et al. (1995). The solidified culture medium contained MS *(Murashige & Skoog,* 1962) macro- and microelements in half strength, MS vitamins and 3 mg/l kinetin and 0.1 mg/l indole-butyric-acid

as growth regulators. Culture conditions were: 21 °C, 16/8-hour light 5000-lux light intensity. Leaves of the varieties were cut directly from test tubes and kept in freezer till examination.

Shoots (2–4 cm long) were taken out of test tubes and planted in small pots in turf and were acclimatized under controlled conditions: 21 °C, 100% vapour and double cover of white plastic in the first week, 21 °C, 70% vapour and simple plastic cover from the second week till rooting. After 6 weeks rooted plants were potted to 9-cm pots and put out-door. Leaves of one-year-old micropropagated plants were cut in August, and kept in freezer till examination.

Sample preparation for isoelectric focusing

100 mg of leaves were homogenised in an ice chilled mortar with pestle, by adding quartz powder in 500 µl ice cold 20 mM Tris-HCl buffer pH 7.8, containing 100 mg/ml Triton X-100, 200 mg/ml sacharose, 2 mM albumin bovine serum. The homogenate was centrifuged with 15000 g at 4 °C for 15 minutes. Supernatants were analysed.

Isoelectric focusing was carried out on a PhastSystem (LKB-Pharmacia, Sweden). In order to develop the pH gradient (pH 3–9) ready-made gels were prerun by 2.5 mA at 10 °C, for 75 Vh, and crude extracts of leaf samples were applied onto the acidic (pH 4.5) end of the gel and run at 2.5 mA at 10 °C for 700 Vh. Gels were stained for peroxidase activity with H_2O_2 as substrate and ortodianisidin as chromogen reagent *(Vallejos,* 1983). Dried gels were scanned by Image Master Laser Densitometer (LKB, Pharmacia, Sweden) and the relative positions of bands were determined.

Results and discussion

The results of isoelectric focusing can be seen on *Figure 1 and 2.*

These preliminary studies showed considerable differences in the isozyme patterns between the descendants of one mother plant which could reflect the possible different leaf patterns. We could find similar patterns of descendants of mother plants of unclear origin. The isozyme patterns of leaves, which kept their characteristic colour, differed from those, which lost this special colour. There were also some differences between the isozyme patterns of colour mother plant and micropropagated descendants of the same colour. There were marked differences in the isosyme patterns between the different phenological phases of leaves. The older leaves didn't seem to be so suitable for examination despite the higher activity of peroxidase enzymes. The reason of this fact may occur because the new leaves developed out-door are in a juvenile stage and therefore the enzyme-activity is more similar to the micropropagated plants in test tubes. The location of the peroxidase bands showed more similarity between the young, developing leaves and the leaves of plants from test

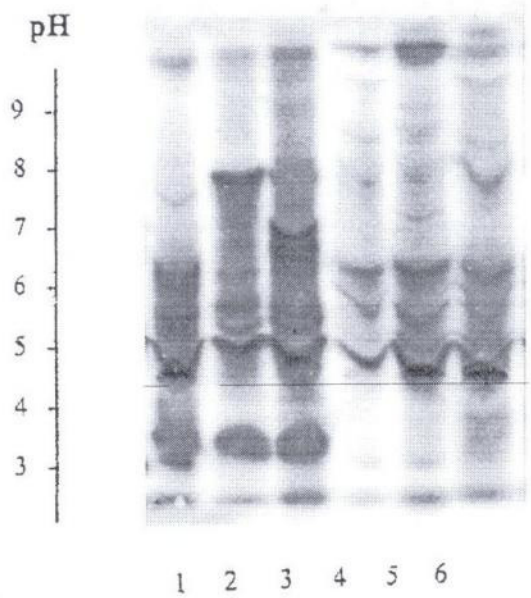

Figure 1 Locations of peroxidase isosyme bands from leaves of mother plant and micropropagated plants from the following *Hosta* varieties:
1.: Motherplant of H. fortunei 'Diamond Tiara'
2.: *H. fortunei* 'Diamond Tiara' micropropagated plant
3.: Albino micropropagated *H. fortunei* 'Diamond Tiara'
4.: Normal colour leaf pattern micropropagated *H. tokudama* 'Samurai'
5.: Green leaved (without yellow edge) micropropagated *H. tokudama* 'Samurai'
6.: Mother plant of *H. tokudama* 'Samurai'

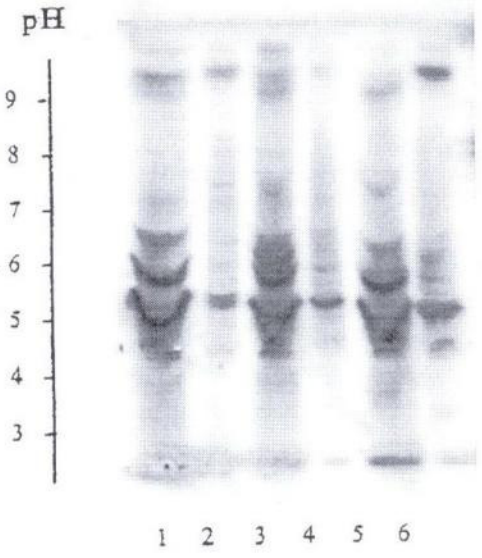

Figure 2 Locations of peroxidase bands from leaves of the mother plant and micropropagated plants from the following *Hosta* varieties:
1.: *H. tardiana* 'Devon Green' motherplant
2.: Micropropagated *H. tardiana* 'Devon Green'
3.: *H. tardiana* 'Blue Cadet' motherplant
4.: Micropropagated *H. tardiana* 'Blue Cadet'
5.: *H. tokudama* 'Drummer Boy' motherplant
6.: Micropropagated *H. tokudama* 'Drummer Boy'

tubes than between the test tube plants and the mature leaves close to senescence in autumn. This may be accounted by the fact that in test tubes plants are restored to the juvenile phase. After studying the isozyme patterns of different *Hosta* varieties this method could become a suitable way of confirming the identity of young micropropagated plants. These preliminary results indicate that with this quick and easy method *Hosta* varieties could be identified in a very early stage of micropropagation.

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