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Change of peroxidase enzyme activities in annual cuttings during rooting

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ABSTRACT The peroxidase enzyme (POD) activities (U/mg protein) were measured by spectrophotometric method in different organs of some annual ornamental plant cuttings during rooting period. The examined species were *Lantana camara L.* "Schneewittchen", *Lantana camara L.* "Prof. Raoux", *Heliotropium arborescens L.* and *Helichrysum stoechas (L.) Moench*. Rooting of cuttings was more than 90% at all species. The POD enzyme activities increased from the top towards the base of cuttings at all species and in all measuring dates. The highest POD activities were in the base measured one week after cutting, these values decreased in connection with the root formation in all cases. In the case of Helichrysum stoechas the rooting took much longer time while it was shown in the ten times lower POD enzyme activities in all measuring dates.
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KEY WORDS

peroxidase
annual
bedding plant
rooting

In the recent years annual ornamental plants are commonly used as bedding and as balcony plants (Herneth 2000; Sygenta 2002). Some of these plants are propagated vegetatively, and others generatively (Nau 1995). The shoot cutting as propagating method is applied in those cases where the species has good rooting potential and usually it shows seasonality (Guerriero and Loreti 1975; Schmidt and Tóth 1996; Erbil 1997). The seasonal changes might be caused by some root-promoting factors isolated by Fadl and Hartmann (1966, 1967). It is well known that the peroxidase enzyme activity plays an important role in root initiation set up by Hartman et al. (1990). Some authors found increased peroxidase activity by the end of endodormancy (Lasheen and Chaplin 1971; Marquat et al. 1999). In the experiments of Kenis (1976), besides more enzymes observed that the peroxidase and polyphenoloxidase enzyme activity increased between the end of dormancy and bud burst. Guskov et al. (1988) found correlation between the peroxidase activity of the rooting zone and the rooting of hardwood cuttings where the peroxidase activity was higher in difficult-to-root plants than in readily rooting ones. Above mentioned data suggest that peroxidase activity and rooting potential might be associated. In our preliminary experiment our aim was to examine the change of peroxidase enzyme activity during rooting procedure by some annual bedding plants.

Materials and Methods

During our examination the following bedding and balcony plants were tested:

- *Lantana camara L.* 'Schneewittchen'
- *Lantana camara L.* 'Prof. Raoux'
- *Heliotropium arborescens L.*
- *Helichrysum stoechas (L.) Moench*

Cuttings of above-mentioned species were collected from Margaret Island in September, 2001. This is the time for starting the propagation of mother plants. The leaves from the lower nodus were removed and nodus was pushed under the medium. The fresh cuttings (with 4-8 leaves) were placed into cell trays in Stender propagating medium. Rooting hormone was not used. Cell trays were covered with polyethylene folie in greenhouse. Plant protection was not necessary.

First sampling was carried out at the day of cuttings preparation, the second sampling was one week later and the third one 3 weeks after the collection. The enzyme analyses were made of the standard of 5 plant samples.

One g of the part of the plants were homogenized in an ice chilled mortar with pestle, with quartz powder in 1 ml ice cold extraction buffer: 0.1 M Na-acetate buffer pH=5.0, containing 10 mg/ml polyvinylpyrollidone, 200 mg/ml saccharose, 0.35 mg/ml bovine serum albumin, 100 mg/ml Triton X-100. The crude extract was centrifuged with 13000 rpm at 4°C for 15 minutes. Supernatants were analysed.

The total peroxidase activity (U/ml) was measured by a spectrophotometric method at λ=460 nm by a Varian DMS 100 UV-visible spectrophotometer using H_2O_2 as a substrate and ortodiazidine as a chromogen reagent (ε=11.3) (Srivastava et al. 1983). Protein contents were determined by Bradford method (Bradford 1978). Three replications of each experiment were performed.

Results

As we observed, cuttings of all species collected in autumn rooted well (90-95%). There were differences between the rate of root initiation, 95% of the 2 varieties of *Lantana camara L.* and 90% of *Heliotropium arborescens L.* rooted within 4 weeks. Cuttings of *Helichrysum stoechas (L.) Moench* needed 6 weeks for the same result. 3 weeks after propagation callus was formed on the base of cuttings and small roots appeared. Meantime even the top of the shoots

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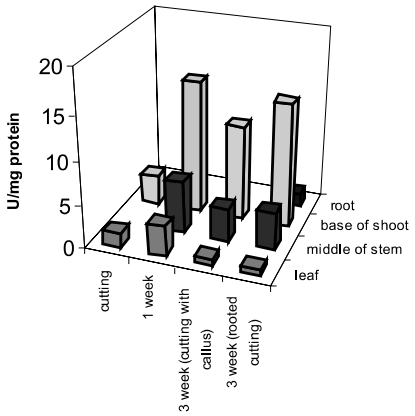

Figure 1. Peroxidase enzyme activities of different organs of *Lantana camara* L. "Schneewittchen" in 3 measuring time.

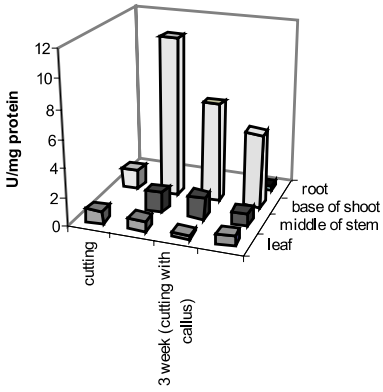

Figure 3. Peroxidase enzyme activities of different organs of *Heliotropium arborescens* L. in 3 measuring times.

began to grow.

Comparing the POD activities of the given species rather great differences could be detected (Figs. 1, 2, 3 and 4). The POD enzyme activities of *Lantana* varieties and *Heliotropium* were ten times higher than it was determined in any organ of *Helichrysum.*

Slow decrease of POD enzyme activity was observed in the leaves of all species in the course of time.

From the shoot tip to the base enzyme activities increased by all species and in all measuring times. In the case of rooted cuttings almost the same POD activities were detected in roots as in leaves by all species.

The most conspicuous change in POD activities was observed 1 week after propagation. In the base of cuttings of *Lantana* varieties and *Helichrysum* 5 times higher and in the case of *Helichrysum* 20 times higher POD activities were measured.

Discussion

After propagation the level of POD enzyme activity increased rapidly in the cutting from the shoot tip in the direction of cutting base. The POD enzyme activity reached the maximum level in all cases during the callus formation in the base of cuttings. With the beginning of root initiation this level decreased and finally reached the original POD activities of the given samples. The lower POD enzyme activities were measured by the slowly rooting *Helichrysum stoechas* (L.) *Moench.*

Based on our results we can conclude that the POD enzyme activities during root initiation are in close connection with the different rooting ability of the different plant species.

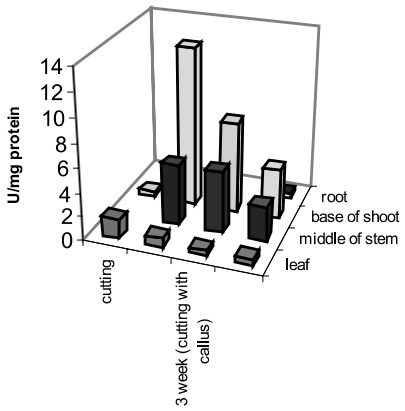

Figure 2. Peroxidase enzyme activities of different organs of *Lantana camara L.* 'Prof. Raoux' in 3 measuring time.

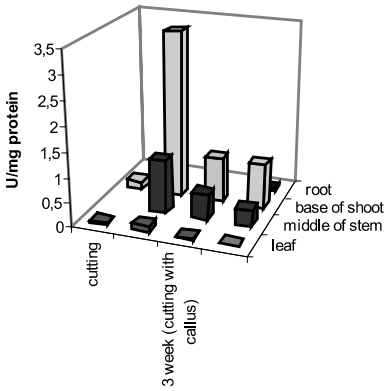

Figure 4. Peroxidase enzyme activities of different organs of *Helichrysum stoechas (L.) Moench* in 3 measuring times.

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