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BRIEF COMMUNICATION

Peroxidase activity in leaves of *Syngonium podophyllum* following transition from *in vitro* to *ex vitro* conditions

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Abstract

A low activity of peroxidase (POD, EC 1.11.1.7) was found in crude extracts from *in vitro* plants of *Syngonium podophyllum* cv. Butterfly sealed in the multiplication vessels. Removal of the lids from the vessels increased the POD activity. A greater increase in the POD activity was measured when *in vitro* plants were transplanted into a weaning growth medium. The POD activity was further promoted by removal of the phenolic substances from the extracts by polyvinyl-polypyrrolidone (PVPP).

Vulnerability of *in vitro* plants following transfer to *ex vitro* conditions is a well known phenomenon (*e.g.* Preece and Sutter 1991). Hardening or weaning period, therefore, is an important part of acclimatization of the *in vitro* plants prior to further use.

One of the factors which may affect acclimatization of *in vitro* plants to *ex vitro* conditions is reduced lignification of the plant cell wall (Kevers *et al.* 1987, Letouze and Daquin 1987). The low lignification of *in vitro* plants may be induced by ambient climatic conditions, the composition of environmental atmosphere and/or the nutrients in the growth medium (Ziv 1991).

Peroxidases (POD) are considered as a major group of enzymes involved in the final stages of lignin formation (Gaspar *et al.* 1982). It has been shown (Zieslin and Ben-Zaken 1991) that the content of lignin in peduncles of rose flowers correlated with the activity of soluble peroxidases in crude extracts from the peduncles. Results of an additional study (Chabbert *et al.* 1993) showed that the POD activity corresponded also with the content of lignin in the cell walls of those peduncles.

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Abbreviations: f.m. - fresh mass; POD - peroxidase; PVPP - polyvinyl-polypyrrolidone.

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It has been also demonstrated (Zieslin and Ben-Zaken 1993) that the POD activity in crude extracts from peduncles of rose flowers correlated negatively with the content of endogenous phenolic compounds in various parts of the peduncles and was promoted by removal of the phenolic substances form the extracts. Thus, it is possible that hypolignification of *in vitro* plants may stem from low activity of peroxidases involved in the lignification process. This assumption was examined in the present study by measurements of POD activity in extracts from *Syngonium* plants during the first stages of the weaning period.

Plants of *Syngonium podophyllum* cv. Butterfly were grown in vessels on multiplication medium (Miller and Murashige 1976) at temperature 22 °C, irradiance 25 μmol m^{-2} s^{-1}, 16 h photoperiod in tissue culture laboratory. The plants were separated into three groups. After removal of the vessel sealings one groups of plants was transplanted into trays with growth medium (containing 80 % peat moss and volcanic scoria) used for hardening of *in vitro* plants. The second group of plants was kept on multiplication medium in unsealed vessels while the third group of plants was left in the sealed vessels. All three groups of plants were transferred into a greenhouse (Ben-Zur Nurseries, Tirat Yehuda, Israel, day/night temperature 26/18 °C, air humidity 85 % and nutural irradiance) *i.e.* conditions appropriate for hardening of *in vitro* plants. Uniform leaves (blade and petiole) from the three plant groups were used for determination of POD activity.

The activity of soluble peroxidases was measured in crude or polyvinyl-polypyrrolidone (PVPP) purified extracts according to the procedure described previously (Zieslin and Ben-Zaken 1991) with slight modifications. The tissue (200 - 500 mg) was ground in liquid nitrogen, homogenized in sodium phosphate buffer, pH 6.5, and was centrifuged for 20 min at 10 500 *g*. 1.5 cm^3 of the supernatant was combined with 0.05 cm^3 of 1 mM guaiacol solution in ethanol. 0.05 cm^3 of 1 mM H_2O_2 in re-distilled water was added before the spectrophotometric measurements. The absorption was measured at $\lambda = 470$ nm and the POD activity was expressed as a change in absorbance, ΔA min^{-1} g^{-1}(f.m.), at the linear part of the absorption curve. For removal of phenolic substances 50 mg mg^{-1}(buffer) of PVPP was added to the plant tissue before homogenization. The POD activity in the leaves was measured four times: immediately after removal of the sealings from the vessels (day 0) and one, two and three weeks thereafter.

A very low activity of POD was found in crude extracts from leaves of *S. podophyllum* in the sealed vessels prior the transfer into the greenhouse (Fig. 1). A significant increase in POD activity measured in the sealed vessels after one week in the greenhouse was followed by a decrease in the activity to the initial level.

Removal of lids from the vessels resulted in three-fold increase in POD activity after one week in the greenhouse as compared to the activity in plants that remained in the sealed vessels. The increased level of POD activity remained almost constant during additional two weeks in the grenhouse. This three-fold increase in POD activity following exposure of the *in vitro* plants to the ambient atmosphere, emphasizes the inhibitory effect of the *in vitro* atmosphere on the activity of POD and probably also of other enzymes involved in lignification of plant tissues (Haddon and Northcote 1976, Gaspar *et al.* 1982). Thus, it may be one of the factors affecting

the vulnerability of *in vitro* plants during transfer to *ex vitro* conditions (Preece and Sutter 1991, Ziv 1991).

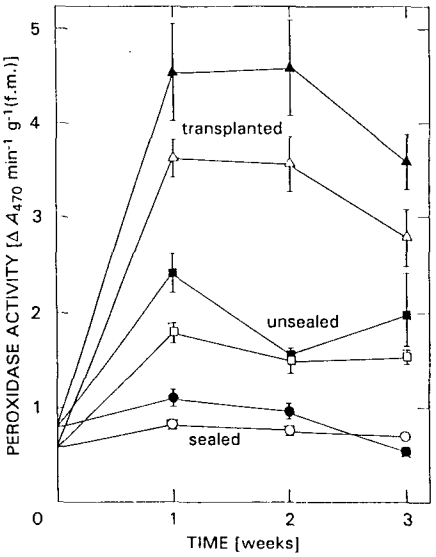

Fig. 1. Peroxidase activity in extracts from leaves of *in vitro* plants of *Syngonium podophyllum* following transfer to weaning conditions (*open symbols* - crude extracts, *closed symbols* - PVPP purified extracts).

The most pronounced six-fold increase in POD activity was measured when the plants were transplanted into the weaning medium. The high POD activity remained during the further week, but it decreased after 3 weeks. This increase in POD activity following plant transfer into the weaning medium may stem from establishment of normal function of the root system, even though the possibility that stimulation of POD activity is due to thigmomorphogenetic effects of hand manipulations during transplanting, similarly to the effect reported previously in *Bryonia dioica* (De Jaegher *et al.* 1985) cannot be excluded.

Removal of phenolic compounds from the crude extracts by PVPP resulted in an elevated activity of POD in the purified extracts. The increase in activity was similar in all three plant groups examined in the present study (Fig. 1). Increased POD activity in the PVPP-purified extracts indicates the presence of inhibitors of phenolic nature similarly to the phenolic inhibitors found in *Pelargonium* (Castillo *et al.* 1991) and *Rosa* plants (Zieslin and Ben-Zaken 1993). However, the increase in POD activity due to removal of phenolic substances by PVPP from the extracts of *in vitro* plants of *Syngonium* was lower than that observed in the rose plants (Zieslin and Ben-Zaken 1993). This difference may indicate a low level of synthesis of phenolic substances in these *in vitro* plants (Hegedus and Phan 1987, Phan 1992).

It offers a possibility that supplementation of various phenolic compounds into *in vitro* culture growth media of various plants may influence lignification of plants *in vitro*. The examination of the content and composition of the various phenolic components in the *in vitro* plants are recently under investigation.

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