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Research Article

Peroxidase and IAA-Oxidase Activities During Rooting in Cuttings of Three Poplar Species

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Abstract: Peroxidase (PO: E. C 1.11.1.7) and IAA. Oxidase (IAA-O) enzyme activities were investigated during rooting in cuttings of *Populus nigra* L., *Populus alba* L. and *Populus tremula* L. (*Salicaceae*), and the relations between enzyme activities and rooting ability were examined comparatively. PO activity started to increase the early stages of primordium formation and reached the highest level before root emergence. However, no apparent correlation was found between the activity of PO and the ability of the cuttings to form roots. IAA-O activity was observed only in root cuttings of *P. alba* and *P. nigra.* Activity increased after day 6 and reached the highest level at the stages of root emergence. A positive correlation between rooting and IAA-O activity was observed.

Key Words: Peroxidase, IAA. Oxidase, Rooting, Poplar

Üç Kavak Türünün Çeliklerinde Köklenme Sırasındaki Peroksidaz ve IAA-Oksidaz Aktiviteleri

Özet: *Populus nigra* L., *Populus alba* L. ve *Populus tremula* L. (*Salicaceae*) çeliklerinde köklenme sırasındaki Peroksidaz (PO: E. C 1.11.1.7) ve IAA-Oksidaz (IAA-O) enzim aktiviteleri saptanmış ve köklenme yeteneği ile enzim aktiviteleri arasındaki ilişkiler karşılaştırmalı olarak incelenmiştir. PO aktiviteleri Primordium oluşumunun erken evrelerinde artmaya başlamış ve kök çıkışından önce maksimum düzeye ulaşmıştır. Fakat, çeliklerin kök oluşturma yeteneği ile enzim aktiviteleri arasında bir ilişki bulunamamıştır. IAA-O aktiviteleri sadece *P. alba* ve *P. nigra*'nın köklenen çeliklerinde saptanmış ve aktivite 6. günden sonra artarak kök çıkışının erken evrelerinde maksimum düzeye ulaşmıştır. IAA-O aktivitesi ile köklenme arasında pozitif bir korelasyon olduğu görülmüştür.

Anahtar Sözcükler: Peroksidaz, IAA-Oksidaz, Köklenme, Kavak

Introduction

Rooting is a crucial step in the propagation of woody species and there is great variation in the rooting ability of different species. During rooting and primordium formation many biochemical and physiological changes and an increase in the activities of several enzymes. Peroxidases (donor: H2O2 oxidoreductase: E.C 1.11.1.7) are involved in many developmental processes in plants and can act as an IAA-oxidase (1). In this capacity they may function in growth regulation and other physiological processes (2, 3, 4). Peroxidases are known to be good physiological markers of rooting in many species and there is substantial evidence that peroxidase activities in plant cuttings are related in some way to rooting (5,6,7). Peroxidase isoenzyme can catalyse the oxidation of indolyacetic acid (IAA) and such activity is termed auxin-oxidase (IAA-O). Some authors have suggested that IAA-O activity influences rooting by IAA catabolism (9, 10).

Earlier studies with cuttings of *Populus nigra* L. (10,11) and *Populus tremula* L. (12) have shown that some peroxidases increase during the rooting of cuttings. In this study, peroxidase (PO) and IAA-O enzymes activities were studied during rooting in cuttings from three different poplar species [Populus alba L., Populus nigra L., Populus tremula L. (Salicaceae)] and the relationship between the enzyme activities and rooting ability was investigated. The present study was undertaken to obtain further information on the activities of enzymes in poplar cuttings.

Materials and Methods

Three rooting experiments were carried out on different dates during the winter of 1993-1994. Sixty cuttings from all three poplar species were prepared for each experiment. Cuttings of the whole of the young shoot (15-20 cm in length, with 6-7 nodules) were taken

from *P. alba*, *P. nigra* and *P. tremula* (from C.U. campus, Zara and Hafik/SİVAS) and were placed in distilled water in a beaker. The rooting and growth of the cuttings took place in darkness at 25°C. The percentage of cuttings showing root and primordium formation were recorded every other day and sections from the basal end of the cuttings were taken in order to investigate enzyme activities.

Enzyme extraction and purification: The PO extraction procedures were adapted from Dalet and Cornu (2). The enzyme was extracted from 500 mg of samples by homogenising with a Fisher model-300 homogeniser for 3 minutes in 10 ml of cold 0.05 M Sodium-phosphate (PH: 5.5) buffer containing 15 mg of $MgCl_2$. The homogenate was filtered through two layers of cheesecloth and the residue was washed twice with 0.05 M sodium phosphate (PH: 5.5). The combined filtrates were centrifuged at 20.000xg for 20 min. The crude extracts were dialysed overnight at 40°C with 0.05M sodium phosphate (PH: 5.5).

The dialysed extracts were placed in a chromatography column (1.5x18 cm) containing Sephadex G_{50} prewashed with the same buffer (flow rate 1ml/min.). The purified extracts were assayed for PO and IAA-O activities according to the method of Srivastava and Huystee (18). The reaction mixture for the PO assay (0.5 ml enzyme sample, 1ml 40 mM guaiacol, 0.5ml 10 mM H202 and 1ml phosphate buffer) was incubated at 25 OC for 10 minutes and the change in absorbance at 460 nm was measured using a Hitachi model-220 spectrophotometer.

In order to determine IAA-O activity, the reaction mixture (0.1ml 0.32 nm IAA, U.16 mm 2.4 Dichlorophenol, 0.16 mM Mn Cl_2, 0.01 M H_2O_2 ,0.1 ml phosphate buffer and 0.5 ml enzyme sample) was incubated at 30°C for 15 min and absorbance was measured at 530 nm. The IAA oxidase activity was expressed as micrograms IAA destroyed per millilitre of solution (23). The quantity of IAA was estimated by reference to a calibration curve.

A protein analysis was carried out according to the method of Lowry et al.(21).

Results

Rooting was observed only on cuttings of *P. nigra* and *P. alba* although primordium development was determined in all three species. The root formation and the number of roots in the cuttings varied according to time and species, and rooting ability differed between the species (Table 1). In the cuttings of *P. nigra,* primordium formation appeared by the 4th day and roots emerged from the basal parts on the 8th day. The rooting rate and the number of roots were higher than in *P. alba* and *P. tremula,* although the number of primordia was smaller. Rooting was complete before the buds broke. After the 16th day, there was no change in the rooting rate or root number (Table 1). In the *P. alba* cuttings, primordium formation appeared by the 10th day and primordia were numerous (av. 29) and clear. The emergence of the first root was observed on the 16th day and the root number increased in the following days (Table 1). In the *P. tremula* cuttings, primordia developed on the 10th day and were numerous (av.31) and clear. Rooting did not take place and, after the 18th day, the cuttings started to decay from the basal parts..

Table 1. Primordium and root formation in the cuttings of Populus alba, Populus tremula and Populus nigra, (±: Standard error)

Culture days	Populus alba			Populus tremula			Populus nigra		
	Average of primordium number	Rooting%	Average of root number	Average of primordium number	Rooting%	Average of root number	Average of primordium number	Rooting%	Average of root number
0	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	11.70±0.10	-	-
8	-	-	-	-	-	-	13.20±0.30	33.3±1.30	0.80±0.34
10	21.00±0.20	-	-	25.75±0.25	-	-	9.25±0.45	78.0±0.16	5.55±0.05
12	21.05±0.05	-	-	28.50±0.30	-	-	8.05±0.05	89.0±0.80	7.37±0.37
14	21.85±0.65	-	-	28.80±0.80	-	-	6.01±0.38	90.0±0.60	7.90±0.30
16	21.30±0.30	40.0±1.80	0.40±0.25	29.45±1.05	-	-	5.96±0.95	91.0±0.10	7.96±0.46
18	21.01±0.45	46.6±0.80	1.64±0.95	29.45±0.65	-	-	5.96±0.45	91.0±0.25	7.96±0.72
20	18.60±0.30	66.1±1.15	2.03±1.15	29.25±0.95	-	-	5.96±0.30	91.0±0.10	7.98±0.25

PO activity

P. nigra: PO enzyme activity showed a large increase after the 4th day and the increase continued through to the 6th day during primordium formation. It reached the highest level during the rooting period (10th). After the completion of rooting, enzyme activity slightly declined, and then remained at almost the same level (Table 2, Fig. 1).

Table 2. Peroxidase and IAA-O activities in cuttings of *P. alba, P. nigra* and *P. tremula* (± standard error). In each column values with the same letter are not significantly different from each other at a probability level of 0.05

Culture days	Populus alba			Populus tremula			Populus nigra		
	Protein (mg/ml)	Peroxidase (U/mg.prot.)	IAA-Oxidase (µg/mg.prot)	Protein (mg/ml)	Peroxidase (U/mg.prot.)	IAA-Oxidase (µg/mg.prot)	Protein (mg/ml)	Peroxidase (U/mg.prot)	IAA-Oxidase (µg/mg.prot)
0	0.400±0.15a	131±0.10a	0.000	0.333±0.01a	113±0.25a	0.000	0.403±0.01a	122±0.00a	0.000
2	0.400±0.10a	152±0.25a	0.000	0.333±0.01a	131±0.10a	0.000	0.404±0.14a	159±0.00a	0.000
4	0.412±0.18a	193±0.30a	0.000	0.343±0.05a	171±0.10a	0.000	0.412±0.46a	214±0.30a	0.000
6	0.443±0.30b	309±0.05a1	0.045±0.03a	0.350±0.00a	249±0.95a	0.001±0.05a	0.440±0.00b	481±0.13a	0.000
8	0.465±0.25b	1095±0.78b	0.133±0.09b	0.385±0.15a	561±0.10b	0.004±0.10a	0.467±0.30b	686±0.20b	0.137±0.02a
10	0.483±0.41b	1445±1.60b	0.196±0.07b	0.430±0.05b	921±0.20b	0.010±0.90a	0.465±0.25b	791±0.57b	0.324±0.05b
12	0.489±0.10b	1708±3.50b	0.280±0.01b	0.439±0.00b	973±0.13b	0.010±0.20a	0.454±0.15b	724±0.30b	0.432±0.07b
14	0.472±0.27b	1663±2.20b	0.305±0.14b	0.445±0.01b	1122±0.30bc	0.000	0.440±0.14b	673±0.10b	0.436±0.08b
16	0.469±0.15b	1631±4.15b	0.443±0.03c	0.425±0.02b	926±1.16b	0.000	0.478±0.25b	784±1.00b	0.433±0.01b
18	0.463±0.30b	1414±0.20b	0.445±0.08c	0.420±0.0b	835±2.20b	0.000	0.476±0.30b	744±0.21b	0.420±0.02b

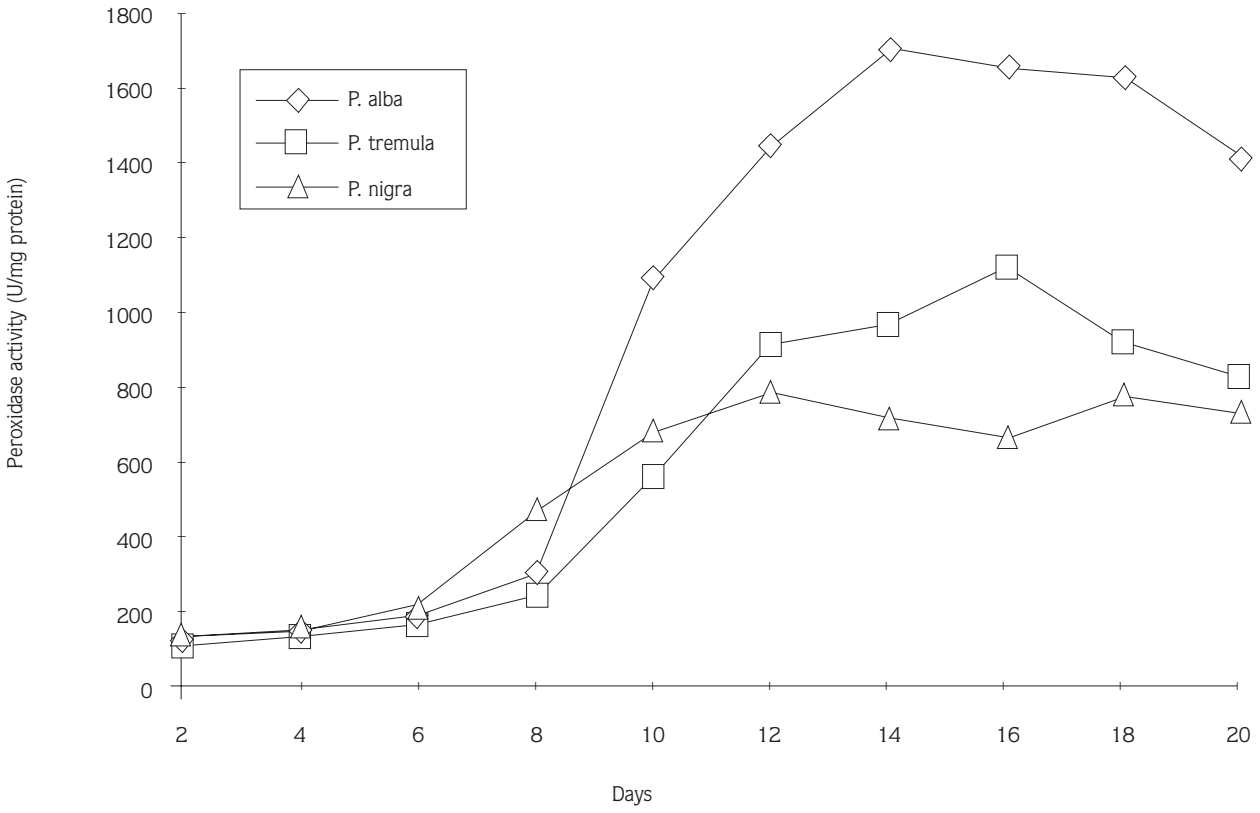

Figure 1. Peroxidase activity in cuttings of P. alba, P. nigra and P. tremula

P. alba: PO enzyme activity was very low at the beginning, and started to increase after the 4th day. The increase continued through to the 10th day and reached the highest level during rooting on the 12th day. The enzyme activity decreased gradually after the 16th day (Table 2, Fig. 1).

P. tremula: There was little activity until the 6th day. Activity started to increase when the buds broke, and continued throught to the 14th day. It reached the highest level on the 14th day and then decreased (Table 2, Fig. 1).

IAA-O activity

P. nigra: There was no IAA-O activity until primordium formation. Enzyme activity showed a large increase after the 6th day and reached the highest level on 14th day, and then remained at almost the same level (Table 2, Fig. 2).

P. alba: At first, no IAA-O activity was determined. After the 4th day, a little activity became apparend. Enzyme activity started to increase from Day 6, reached its highest level on the 14th day, and then remained at almost the same level during the rooting period (Fig. 2)

P. tremula: There was no significant activity in the cuttings of this species. A little activity was determined during primordium formation on the 12th day.

Discussion

Peroxidase has a number of very important roles in plants. It plays a role in the defence response of a plant to pathogen attack (22), in the biosynthesis of lignin, suberin and some phenolics, and in cell division and differentiation (21). Previous studies have shown that PO activity increases during cell division and primordium formation (2, 3, 5). Haissing (6) suggested that PO plays a role in the formation of cofactors which are necessary for root initiation. In our study, PO activity increased during rooting or primordium formation periods, in all cuttings, although the rooting capacities were very different. However, no apparent correlation was found between PO activity and rooting ability. Our results are in agreement with the earlier findings (5, 23).

IAA-O activity is related to IAA catabolism in cuttings. Dalet and Cornu (2) concluded that IAA-O regulates the endogenous IAA concentration and promotes rooting. In

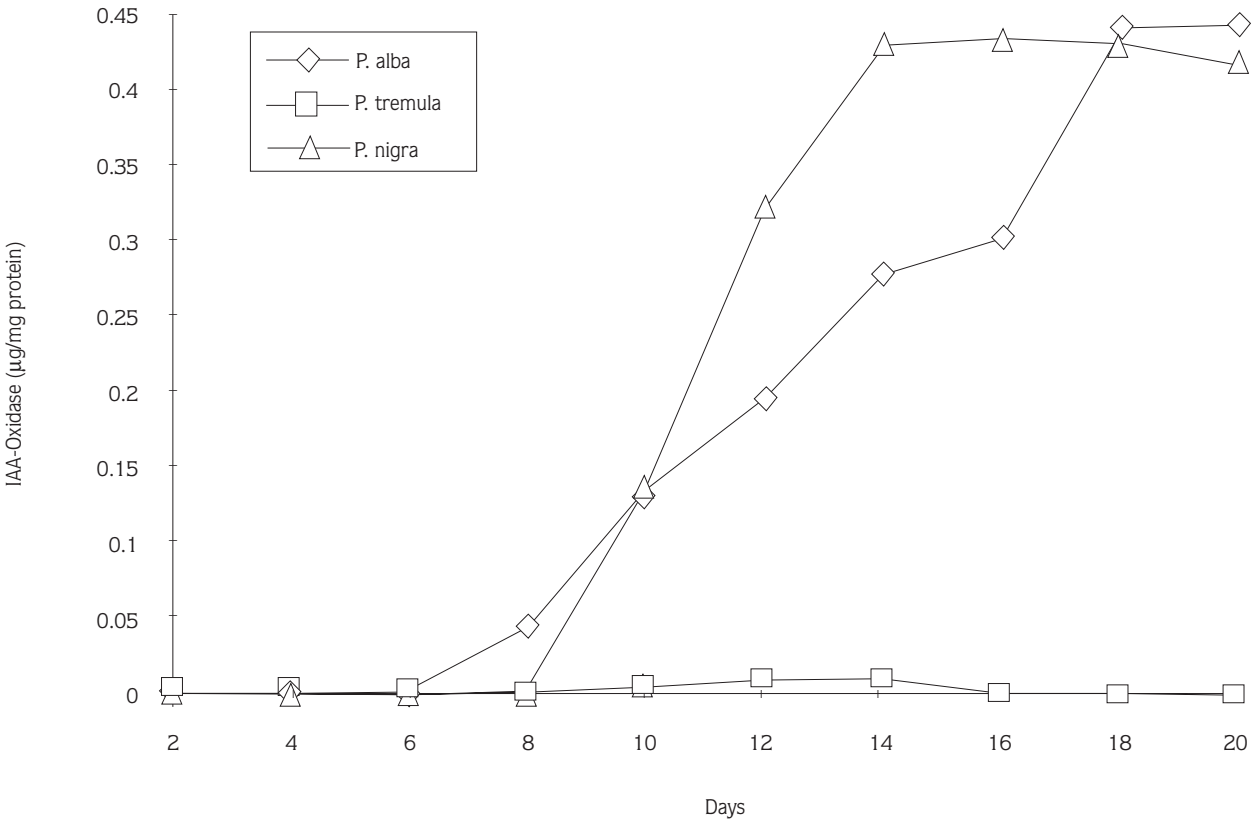

Figure 2. IAA-Oxidase activity in cuttings of P. alba, P. nigra and P. tremula

addition, previous studies have shown that IAA-O is localised in the tissue, where root initials are first formed, and promotes rooting (11, 12). Auxin treatment induces an increase in IAA-O and peroxidase activities in roots (13, 14). In our experiments, IAA-O activity was observed during rooting and primordium formation but there was no IAA-O activity in the unrooted *P. tremula* cuttings, in which bud burst occurred before primordium formation. These findings are in agreement with earlier findings. It can be speculated that the increased IAA-O activity was responsible for rooting or at least for the emergence of roots. We suggest that foliar originated IAA is localised in the basal parts of the cuttings and inhibits rooting. IAA-O enzyme changes influence rooting by IAA catabolism.

In conclusion, the results suggest that PO activity doesn't affect rooting ability or rooting percentage, but can play a role in cell division. IAA-O is related to rooting and probably affects root formation by IAA catabolism. In the initial stage, oxidation products of IAA may promote root formation, especially when linked with the presence of phenolic substances.

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