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Original article

Peroxidase and catalase activities are involved in direct adventitious shoot formation induced by thidiazuron in eastern white pine (*Pinus strobus* L.) zygotic embryos

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Abstract

We reported establishment of an efficient plant regeneration procedure through direct adventitious shoot (DAS) formation from cotyledons and hypocotyls of eastern white pine (*Pinus strobus L.)* mature embryos in this investigation. Multiple DASs were initiated from cotyledons of embryos on PS medium containing N_6-benzyladenine (BA), thidiazuron (TDZ), or kinetin (KIN). Among different concentrations of casein enzymatic hydrosylate (CH) and glutamine used in this study, 500 mg l^{-1} CH or 600 mg l^{-1} glutamine induced the highest frequency of DAS formation. Rooting of regenerated shoots was obtained on PS medium supplemented with 0.01–0.1 μM indole-3-acetic acid (IAA) with the highest frequency on medium containing 0.01 μM IAA. No DASs were obtained on medium without TDZ. Measurement of peroxidase (POD) and catalase (CAT) activity during direct shoot induction and differentiation demonstrated that the lowest POD activity appeared in the 5–6th week of culture and lowest CAT activity occurred in the 7–8th week of culture on medium with TDZ. No such a change in POD and CAT activities was observed on medium without TDZ. These results demonstrated that POD and CAT activities were involved in DAS formation induced by TDZ in eastern white pine.

Keywords: Catalase; Direct organogenesis; Peroxidase; Conifer; Plant growth regulators; Seeds

1. Introduction

In vitro plant regeneration has facilitated crop improvement, mass propagation of elite families and genotypes, and germplasm conservation, in particular for in vitro mass propagation of economically important crops [11,16,25,35] and forest tree species [4]. Investigation of in vitro processes of either dedifferentiation or differentiation in a number of plants have been focused on the effect of the donor plant genotype, plant growth regulators, and physiological stages of explant source [1,25]. The lack of efficient regeneration protocols in some important agricultural plants and trees is a major impediment to their improvement via genetic engineering, therefore, it is important to continue research towards understanding factors involved in in vitro plant regeneration [4,11,25].

In recent years, there has been a growing interest in the functional significance of plant growth regulators in growth, development and differentiation of plant cells [38,42]. Several reports have suggested a link between activities of certain enzymes and organogenic callus. Direct shoot organogenesis and root organogenesis is a special way of morphogenesis in plant [21]. The pathway of plant morphogenesis depends on the species and the culture condition. It is well known that differentiation of somatic cells is one of the most useful phenomenons for understanding plant development. A variety of genes expression and protein synthesis are involved in the shoot organogenesis that is a biologically complex developmental and differentiation process. Tian et al. [36] have investigated the release of peroxide radicals and hydrogen peroxide, and the activities of antioxidant enzymes during organogenesis. During the differentiation and development of strawberry callus, superoxide dismutase (SOD)

Abbreviations: BA, N6-benzyladenine; CH, casein enzymatic hydrosylate; IAA, Indole-3-acetic acid; IBA, Indole-3-butyric acid; KIN, Kinetin; NAA, α-Naphthaleneacetic acid; TDZ, Thidiazuron.

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activity increased in the early regeneration culture and decreased thereafter, and catalase (CAT) activity constantly declined while peroxidase (POD) decreased during the 5-days culture and gradually increased thereafter differentiation and development of shoot buds from strawberry callus [36]. Their results suggested that H_2O_2 was correlated with the morphogenetic process in strawberry callus, and may actually serve as a messenger in the process of bud primordium formation [36]. However, the activities of antioxidant enzymes during organogenesis have not been investigated in eastern white pine.

The effects of auxin and cytokinin on shoot induction, differentiation, and development have been investigated in pigeon pea and apples tree [30,41]. In apple, it has been reported that TDZ combined with 2,4-dichlorophenoxyacetic acid (2,4-D) enhanced more cell divisions than TDZ with indole-3-butyric acid (IBA) [41]. One day exposure to either TDZ and IBA or TDZ and 2,4-D, followed by culture on hormone free medium, initiated cell determination towards shoot regeneration [41]. TDZ with IBA or with indole-3-acetic acid (IAA) determined cell fate to shoot development, whereas TDZ and 2,4-D application led to the decline of shoot development [41]. The type of auxin, the timing of its application, and the length of explant exposure to the specific auxin are critical for the activation and progression of the developmental program in explants [30,41]. There is no report about the effect of TDZ on direct shoot organogenesis in eastern white pine.

Eastern white pine (*P. strobus* L.) produces the most valuable softwood lumber in eastern North America and is used extensively for interior trim, window sashes, doorframes, and for intricate carpentry. It is also grown extensively as Christmas trees in the southern foothills of the Appalachians. Micropropagation via organogenesis [10,13,28,40] or somatic embryogenesis [14] have been reported in eastern white pine. However, the frequency of plant regeneration was relatively low. Recently, Somatic embryogenesis in *P. strobus* has been dramatically improved and became an important clonal propagation tool potentially applicable in tree improvement programs [12,17,18].

Our studies have focused on direct adventitious shoot (DAS) induction from cotyledons of mature zygotic embryos, for which the choice of growth regulators, media, and concentrations of casein enzymatic hydrolysate and glutamine has been examined. The aims of the investigation reported here were to establish a reliable micropropagation protocol enabling high frequency induction of multiple shoot structures via direct organogenesis and to elucidate the relationship between POD and CAT activities and DAS formation.

Table 1
The basal media used in tissue culture in eastern white pine

Chemical formula (mg l^{-1})	PS	LP [21]	SH [23]	TE [20]
$Ca(NO_3)_2.4H_2O$	1100	0	0	556
KNO_3	2150	1900	2500	340
$CaCl_2.2H_2O$	350	1760	200	85
NH_4NO_3	250	1200	0	400
$MgSO_4.7H_2O$	200	370	400	720
KCl	100	0	0	1900
KH_2PO_4	170	340	0	170
$NH_4 H_2PO_4$	0	0	300	0
$ZnSO_4.7H_2O$	15.6	0	1.0	25.8
$MnSO_4.H_2O$	22.5	2.23	10.0	25.35
H_3BO_3	6.2	0.63	5.0	6.2
KI	0.83	0.75	1.0	0.83
$Na_2MoO_4.H_2O$	0.25	0.025	0.1	0.25
$CoCl_2.6H2O$	0.025	0.025	0.1	0.025
$CuSO_4.7H_2O$	0.025	0.025	0.2	0.025
$FeSO_4.7H_2O$	27.8	13.9	15.0	27.8
NaEDTA	37.3	0	20.0	37.3
Myo-inositol	1000	1000	1000	1000
Nicotinic acid	0.5	0.5	0.5	0.5
Pyridoxine HCl	0.5	0.5	0.5	0.5
Thiamine HCl	0.1	0.1	0.1	0.1
Glycine	0.1	0.1	0.1	0.1
PH	5.7	5.7	5.7	5.7

2. Results

2.1. Induction of DASs

For inducing DAS formation from mature embryos of eastern white pine, we pretreated embryos on a medium containing 5 μM IAA, 3 μM IBA, and 3 μM BA for 1 week (Tables 1 and 2) followed by culture of embryos on a medium supplemented with 3 μM IAA, 6 μM BA and 6 μM TDZ for 2 weeks

Table 2
Procedure for DAS formation and plantlet regeneration in eastern white pine. The basal media used for differentiation of DASs from cotyledons include LP [22], MS [8], SH [9], TE [33], and PS (see Section 4). All other steps are on PS medium

Plant growth regulators	Procedure of plant regeneration				
	Pretreatment	Induction	Differentiation	Elongation	Rooting
NAA	0	0	0	0	0.01–10 μM
Indole-3-acetic acid (IAA)	5 μM	3 μM	0	2 μM	0.01–10 μM
IBA	3 μM	0	0	0	0.01–10 μM
BA	3 μM	6 μM	1–9 μM	1 μM	0.5 μM
TDZ	0	6 μM	1–9 μM	0	0
KIN	0	0	1–9 μM	0	0
Myo-Inositol	0.5 g/l	0.5 g/l	0.5 g/l	0.2 g/l	0.2 g/l
Sucrose	30 g/l	20 g/l	20 g/l	20 g/l	10 g/l
Culture time	1 weeks	2 weeks	5 weeks	5 weeks	6 weeks