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Full Length Research Paper

Effect of spermidine on ornamental bromeliad cultured *in vitro*

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Plants of the ornamental bromeliad, *Aechmea blanchetiana* (Baker) L.B. Smith obtained from seeds germinated *in vitro*, were treated with spermidine (Spd) at various concentrations (0, 10, 50 and 250 µM) or in the presence of 1.07 mM α-naphthaleneacetic acid (NAA) + 22.20 mM 6-benzyladenine (BA) or 0 mM NAA + 0 mM 6-BA for shoot induction and 0, 1.07 and 5.37 mM of NAA for root induction. The number of shoots, fresh and dry weight of shoots on shoot induction and fresh and dry weight of shoots and roots on root induction, and activity of peroxidases were evaluated. Polyamine Spd was not effective on shooting induction, rooting, mass accumulation of fresh and dry weight and had no influence on the activity of the enzyme peroxidase, and had a deleterious effect on dry matter accumulation in the treatment for shooting induction. Spd improved the qualitative and quantitative responses of *in vitro* rhizogenesis of *A. blanchetiana* as compared to the free polyamine medium. Peroxidase activity was higher in leaves of plants subjected to shoot induction, with 6-BA+NAA. The exogenous spermidine did not have effect on peroxidase activity.

Key words: Bromeliad, micropropagation, peroxidase activity, polyamine.

INTRODUCTION

Many exogenous and endogenous factors regulate the formation of adventitious roots such as Ca^{2+}, sugars, auxin, polyamines, ethylene, nitric oxide, hydrogen peroxide, carbon monoxide, cyclic guanosine monophosphate (cGMP), mitogen-activated protein kinase (MAPKs), peroxidase, etc. These mediators are thought to function as signals and mediate auxin signal transduction during the formation of adventitious roots (Li et al., 2009).

Polyamines (PAs) are low molecular mass, aliphatic, nitrogenous polycations playing a role in a variety of biological processes. The diamine putrescine (Put), polyamines spermidine (Spd) and spermine (Spm) have been found in all higher plants analysed, as well as in other organisms, indicating their universal distribution (Couée et al., 2004). Kuznetsov et al. (2002) has reported that PAs can be classified into two categories based on their biological effect. The first comprises Put and cadaverine, which stimulate cell elongation and root formation, like auxins and gibberellins. The second includes Spd and Spm, which similar to cytokinins, control cell division, organogenesis and plant senescence. In addition, PAs are also closely related with the growth and differentiation of plant cells. However, in the morphogenesis of different species or different explants, Spd, Spm and Put have been observed to exhibit dissimilar effects, and the mechanism by which the PAs could affect the morphogenesis of cultured plants is still unclear (Zhu and Chen, 2005).

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Abbreviations: BA, 6-Benzyladenine; **NAA,** α-naphthaleneacetic acid; **Spd,** spermidine.

Table 1. Shoot number (SN), fresh (LFM) and dry (LDM) matter of leaves of *A. blanchetiana* cultured *in vitro* in 6-BA + NAA free medium (FM) or in 1.07 mM NAA + 22.20 mM 6 BA shoot induction medium (SIM).

Spd (µM)	SN		LMF		LDM	
	FM	SIM	FM	SIM	FM	SIM
0	1.00Ab*	10.81^{Ba}	1.06^{Aa}	1.27^{Aa}	0.096^{Aa}	0.067^{Ab}
10	1.00Ab	14.06^{ABa}	0.59^{ABb}	1.42^{Aa}	0.069^{ABa}	0.074^{Aa}
50	1.00Ab	15.44^{Aa}	0.62^{ABb}	1.56^{Aa}	0.071^{ABa}	0.089^{Aa}
250	1.00Ab	15.79^{Aa}	0.54^{Bb}	1.54^{Aa}	0.061^{Bb}	0.085^{Aa}

*Means with the same capital letter within the column and smaller letter within the row are not significantly different by Tukey's test ($P \leq 0.05$).

Currently, many studies have sought to correlate possible biochemical differences with the tissue regeneration processes, since visual identification is subjective and applied only after prolonged periods of cultivation. Thus, biochemical markers may help to identify early regenerative processes during differentiation, growth and somatic embryogenesis. The activities of various antioxidant enzymes such as catalase (CAT, EC 1.11.1.6), peroxidase (POX, EC 1.11.1.7) and polyphenoloxidase (PPOX, EC 1.10.3.1), along with biochemical parameters (such as reducing and non reducing sugars, starch, total proteins, phenols and proline contents) were studied in regenerating and non-regenerating calluses (Shriram et al., 2008).

Aechmea blanchetiana (Baker) L. B. Smith is a bromeliad native to northeastern Brazil, also known as Porto Seguro bromeliad. They are herbaceous or epiphytes, rhizomatous, perennials growing 60 to 90 cm height, with attractive leaves and flowering stems. Furthermore, they are widely used as ornamental plant in gardens and parks (Kanashiro et al., 2009). The present work was designed to determine the effect of Spd on *A. blanchetiana* (Bromeliaceae) in the induction of rooting and shooting phases during the *in vitro* culture of *A. blanchetiana* on the basis of morphogenetic observations and peroxidase activity measurements.

MATERIALS AND METHODS

A. blanchetiana plantlets were obtained from seeds germinated *in vitro* and cultured for 160 days on Murashige and Skoog (1962) (MS) modified medium with half concentration of macronutrients and supplemented with 30 g L^{-1} sucrose and 7 g L^{-1} agar.

The treatments consisted of modified MS medium with or without plant growth regulators: 1.07 mM α-naphthaleneacetic acid (NAA) + 22.20 mM 6-benzyladenine (BA) (Galvanese et al., 2007) and Spd at concentrations of 0, 10, 50 and 250 µM for shoot induction and 0, 1.07 and 5.37 mM of NAA and 0, 10, 50 and 250 µM of Spd for root induction. MS medium was modified with half strength macronutrients, liquid (without agar) and supplemented with 30 g L^{-1} sucrose. Spd was sterilized by filtration with a millipore filter system. The cultures were maintained in a room with photoperiod of 12 h (28 lumen m^{-2} s^{-1}), temperature of 25 ± 2°C and culture media were changed every 30 days. The study design was in a factorial scheme (2 x 4 for shoot induction and 3 x 4 for root induction), with each plot consisting of 10 vessels with six plants each. Peroxidase activity was determined according to Lima et al. (1999). Supernatants (extracts) were obtained following homogenization of fresh tissue samples in 0.2 M phosphate buffer, pH 6.7 and centrifugation at 4°C. Activity determination was accomplished with 20 mM hydrogen peroxide, 4 mM aminoantipyrine and 10 mM phenol, and the results were expressed in µmol of H_2O_2 decomposed g^{-1} min^{-1}.

Data were analyzed by analysis of variance (ANOVA) and Tukey test ($p < 0.05$), using Assistat 7.6 (Silva and Azevedo, 2009). The parameters analyzed were the number of shoots, fresh and dry weight of shoots for shoot induction and fresh and dry weight of shoots and roots for root induction, as well as the peroxidase activity.

RESULTS AND DISCUSSION

The polyamine Spd used alone on MS medium did not promote shoot induction. However, when associated with the plant growth regulators, 6-BA and NAA, it stimulated the growth of shoots in *A. blanchetiana* plants cultured *in vitro*, with an increase of approximately 50% in the number of new shoots. Spd showed a deleterious effect on dry and fresh weight of leaves, especially when used at higher concentration of spermidine (Table 1). The use of exogenous polyamines represents a simple way to enhance endogenous polyamine content and, in some cases, to stimulate plant regeneration (Takeda et al., 2002). It was expected that the exogenously application of Spd, would be able to induce shoot formation, since PAs, including Spd, have been suggested to be implicated in morphogenic processes, such as somatic embryogenesis and regeneration, either indirectly through the release of nitric oxide or through inhibition of ethylene biosynthesis (Venkatachalam and Bhagyalakshmi, 2008). According to Wang et al. (2009), the exogenous addition of PAs, mainly Spd or Put increase the endogenous levels of PAs and also promoted the frequency of conversion of protocorm-like body to shoots, as demonstrated in *Dendrobium huoshanense*.

Spd alone or associated with 1.07 mM NAA had no effect on rooting treatment of *A. blanchetiana* (Table 2). Moreover, together with 5.37 mM NAA, Spd exogenous had a significant effect on the induction of roots and in fresh and dry matter of roots and fresh matters of leaves (Table 2). Some reports show that treatment with auxin

Table 2. Fresh and dry matter of roots (FMR and DMR) (g) and leaves (LFM and LDM) of *A. blanchetiana* cultured *in vitro* in medium free and with growth regulators for 120 days.

Spd (µM)	FMR			DMR			LFM			LDM		
	NAA (mM)			NAA (mM)			NAA (mM)			NAA (mM)		
	0	1.07	5.37	0	1.07	5.37	0	1.07	5.37	0	1.07	5.37
0	0.062^{Ab}*	$0.217A^{ab}$	0.420^{Ba}	0.011^{Ab}	0.039^{Aa}	0.058^{Ba}	0.680^{Ab}	1.007^{Ab}	2.365^{Ba}	0.048^{Ab}	0.119^{Aa}	0.144^{Aa}
10	0.042^{Ac}	0.275^{Ab}	0.652^{ABa}	0.007^{Ab}	0.034^{Ab}	0.069^{ABa}	0.425^{Ac}	1.631^{Ab}	2.605^{ABa}	0.034^{Ab}	0.110^{ABa}	0.121^{Aa}
50	0.090^{Ab}	0.202^{Ab}	0.628^{ABa}	0.010^{Ab}	0.025^{Ab}	0.064^{ABa}	0.617^{Ab}	1.415^{Aab}	2.273^{ABa}	0.040^{Ab}	0.097^{ABa}	0.117^{Aa}
250	0.053^{Ab}	0.170^{Ab}	0.807^{Aa}	0.008^{Ab}	0.017^{Ab}	0.090^{Aa}	0.470^{Ab}	1.025^{Ab}	3.085^{Aa}	0.037^{Ab}	0.064^{Bb}	0.145^{Aa}

*Means with the same capital letter within the column and smaller letter within the row are not significantly different by Tukey's test ($P \leq 0.05$).

Table 3. Peroxidase activity (µmol H_2O_2 decomposed g^{-1} min^{-1}) of *A. blanchetiana* plants cultured *in vitro*. Plants were grown in auxin (NAA)-free or in NAA-containing medium for 120 days.

Spd (µM)	NAA			NAA			NAA + 6 BA	
	Peroxidase activity in root			Peroxidase activity in leaves			Peroxidase activity in leaves	
	0	1.07	5.37	0	1.07	5.37	0 mM NAA + 0 mM 6-BA	1.07 mM NAA + 22.20 mM 6-BA
0	0.38^{Aa}*	0.49^{Aa}	0.64^{ABa}	3.02^{bA}	6.20^{aA}	3.57^{abA}	1.00^{bA}	3.27^{aA}
10	0.61^{Aa}	0.58^{Aa}	0.40^{Ba}	5.80^{aA}	2.43^{bB}	4.61^{abA}	1.02^{bA}	3.18^{aA}
50	0.43^{Aa}	0.46^{Aa}	0.45^{Ba}	3.26^{aA}	2.68^{aB}	3.92^{aA}	0.50^{bA}	3.18^{aA}
250	0.64^{Aab}	0.55^{Ab}	0.94^{Aa}	3.84^{aA}	2.43^{aB}	2.91^{aA}	0.64^{bA}	3.69^{aA}

*Means with the same capital letter within the column and smaller letter within the row are not significantly different by Tukey's test ($P \leq 0.05$).

(indolbutiric acid, IBA) and polyamine (putrescine) promoted root number per cutting in *Corylus avelana*, while the use of diamine does not induce the formation of roots (Cristofori et al., 2010).

Peroxidase activity was lower in roots of *A. blanchetiana* when compared with leaves (Table 3), combined or not with NAA. The use of the growth regulators, NAA + 6-BA, increased peroxidase activity. The application of exogenous Spd did not promote changes in peroxidase activity in roots of plants cultivated with NAA or NAA + 6-BA; however, when 1.07 NAA was applied, the peroxidase activity was decreased. In this study, the enzyme did not alter the exogenous Spd, and the results show that the enzyme could act by signaling the morphogenesis and plant growth processes in *A. blanchetiana* cultured *in vitro*.

There was no correlation between the increased activity of the enzyme and the formation of roots. Other studies show a correlation between the increase in peroxide value and formation of roots. Higher concentrations of H_2O_2 were required during the formation and development of adventitious roots in cucumber and mung bean which functioned as a signal molecule, involved in the auxin-induced formation of adventitious roots (Li et al., 2009). Libik et al. (2005) found a higher concentration of H_2O_2 in morphogenic callus and consequently supported the hypothesis that H_2O_2 may be produced in excess due to the disruption of oxidative balance which in turn may promote the expression of genes responsible for the initiation of morphogenesis. In this study, we found no correlation between peroxidase activity and organogenesis, even with the addition of Spd, as recently demonstrated for other species; although polyamine metabolism and regulation are complex processes involving multiple genes,

single-gene genetic transformation could be sufficient to obtain important modifications in polyamine balance (Cristofori et al., 2010).

In the present study, exogenously added polyamine Spd was not enough to stimulate *A. blanchetiana* rooting. However, the incorporation of polyamines to the *in vitro* auxin rooting media improved the qualitative and quantitative responses of *in vitro* rhizogenesis of *A. blanchetiana* as compared to the original protocol. The polyamine Spd was not effective on shoot induction, rooting, fresh and dry mass accumulation and had no influence on the activity of the enzyme peroxidase, and also had a deleterious effect on dry matter accumulation in the treatment for shoot induction.

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