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RESEARCH ARTICLE

EFFECT OF POLYAMINES ON IN VITRO GROWTH, SHOOT MULTIPLICATION AND ROOTING IN WRIGHTIA TOMENTOSA ROEM ET SHULT

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ABSTRACT

Effect of exogenous polyamines (putresine, spermine and spermidine) was examined on *in vitro* shoot bud proliferation, multiplication and rooting in *Wrightia tomentosa*. Addition of polyamines in the concentrations ranging from 0.1 mM to 1.0 mM in MS medium in combination with 5.0 mgl^{-1} BAP stimulated axillary shoot bud proliferation and rate of multiplication. Maximum number of bud proliferation (9.61 shoot buds/explant) was observed on 1.0 mM spd followed by 1.0 mM spm and 0.5 mM spm forming 9.59 and 9.39 shoot buds/explants, respectively as compared to 5.0 mgl^{-1} BAP (3.80 shoot buds/explant) which served as control. The shoot length was also improved significantly on all the concentrations of polyamines as compared to control. Maximum shoot length (2.52 cm) was observed in explants treated with 0.5 mM spm which was significantly higher to control (1.48 cm). Addition of polyamines at various concentrations influenced the rate of multiplication significantly. Highest rate of shoot multiplication was obtained on 0.5 mM spm (3.93-fold) as compared to 2.98-fold on control. There was no significant increase in shoot length when polyamines were added to multiplication medium as compared to control except by spd at 1.0 mM concentration. Addition of polyamines in rooting medium had no significant positive effect in terms of rooting percentage, number and length.

Abbreviations

BAP, Benzyl amino purine

INTRODUCTION

Wrightia tomentosa (Roxb.) Roem et Shult. (family: Apocyanaceae) locally known as "Dudhi" or "Khirna" is an endangered tree species of Aravallis in south-east Rajasthan (Sharma, 1983). It is a small deciduous tree growing up to 15 m height. It is a tree of choice for foresters as it provides good quality wood used by local artisans for turnery, carving and toy making. The dried bark of the tree is used as a substitute of "Kurchi" (*Holarrhena antidysenterica*) bark for the alkaloid conessine, and also for snake bite, scorpion sting and menstrual and renal disorders.

The tree has been over-exploited by local artisans and craftsmen for the valuable wood used for making toys, reducing its population to an extreme low. Micropropagation protocol for *W. tomentosa* has been reported by Purohit *et al*. (1996).

In plants polyamines have been implicated in regulation of a multitude of growth and developmental processes including DNA replication, transcription of genes, cell division, embryogenesis, nodule development, organogenesis, pollen development, floral development, fruit development and ripening, rhizogenesis, senescence and abiotic stress tolerance (Evans and Malmberg, 1989; Bais and Ravishankar, 2002; Kaur-Sawhney *et al*., 2003). Polyamines are known to play a major role in proliferation and growth of plant cells, since application of exogenous polyamines stimulates development in several higher plants suggesting that endogenous concentration of these amines could be growth limiting (Galston and Flores, 1991). Polyamines show stimulatory effects on phytohormonal signaling, endogenous growth and synergism (Jang *et al*., 2002). They act as promoters of growth and survival and are essential factors for cell viability, protein phosphorylation, post transcriptional modification and for the formation of secondary metabolites (Kusano *et al*., 2008).

Polyamines have been demonstrated to have a promoting effect on a variety of morphogenic processes such as somatic embryogenesis in *Citrus sinensis* (Wu *et al*., 2009), *Mamordica charantia* (Paul *et al*., 2009), shoot regeneration from cotyledonary explants of *Cucumis sativus* (Zhu and Chen, 2005), *Lagenaria ciceraria* (Shyamali and Hattori, 2007), from protocorm-like bodies in *Dendrobium 'Sonia'* (Saiprasad *et al*., 2004) and *D. officinale* (Wei *et al*., 2010) from nodal and shoot tip explants in *Corylus avellana* (Nas, 2004), cotton (Ganesan and Jayabalan, 2006), *Bixa orellana* (Parimalan *et al*., 2011), *Stevia rebaudiana* (Guruchandran and Chinnagounder, 2013), and from callus in Pine (Tang *et al*., 2004) and petiole derived callus in *Mamordica charantia* (Thiruvengadam *et al*., 2012).

Promotive effect of polyamines on shoot multiplication was observed in *Achras sapota* (Purohit *et al*., 2007), cucumber (Vasudevan *et al*., 2008), sugarcane (Umashankar *et al*., 2011) and *Dendrobium* cultivars (Kumari and George, 2011).

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Rooting differentiation and growth are often critical and difficult to perform during *in vitro* propagation of woody species (De Clerk, 1996; Kevers et al.,1997). Among rooting differentiation and growing factors, polyamines have recently acquired relevance in relation to rhizogenesis (Couee et al.,2004). Polyamines can stimulate or inhibit microshoot rooting depending on the type and concentration of polyamine and rooting phase, while polyamine inhibitors can improve rooting in some cases (Arena *et al.*, 2005).

Polyamines have been found to play critical role on rhizogenesis of *Pinus virginiana* (Tang and Newton, 2005), apple rootstock MM 106 (Naija *et al.,* 2009) and *Citrus sinensis* (Mendes et al.,2011).

The present study was aimed to investigate the effects of polyamines in improving the efficiency of shoot proliferation, their multiplication and *in vitro* rooting of micropropagated *W. tomentosa*. Effect of L-arginine serving as precursor of polyamines and D-arginine, an inhibitor of arginine decarboxylase was also analysed on the process of shoot multiplication in *W. tomentosa*.

MATERIALS AND METHODS

Seed germination and selection of explants

Mature follicles of *W. tomentosa* were collected from various trees growing at Kevda-Ki-Nal and Loira in the months of Feb-March. Pods were kept in shade and seeds were separated from floss in dehiscing pods. Seeds from the same tree were collected, cleaned, weighed and stored after drying. The seedlings were raised fresh when required for experimentation. Seeds were first rinsed in 90% alcohol for 30 seconds then with 0.1 % mercuric chloride for 10 minutes, followed by thorough washing with sterile distilled water. Surface sterilized seeds were allowed to remain in sterile water upto an hour for soaking. Finally seeds were transferred to water agar (0.8%). Radical started emerging within 48 h of incubation when kept in dark and warmer area. Vessels containing germinated seeds were transferred to light conditions.

For axillary bud proliferation, the cotyledonary nodes from seedlings were kept vertically on MS medium fortified with 5mgl^{-1} BAP and different concentrations (0.1, 0.5 and 1.0 mM) of put, spd and spm. Observations were recorded after 21 days. For multiplication proliferating groups of shoots were cultured on MS medium containing 2 mgl^{-1} BAP and various concentrations (0.1, 0.5 and 1.0 mM) of put, spd and spm and L- arg and 0.1µm and 1.0µm D-arg. These were subcultured upto three passages onto the same medium without separation of proliferating shoots. Results were recorded after three passages in terms of shoot elongation and multiplication rate (calculated by dividing the number of shoots obtained after one subculture by number of shoots inoculated initially). The experiments with polyamines and inhibitors were repeated thrice.

Elongated shoots measuring more than 1.5 cm obtained from multiplying cultures were used for root induction. Excised shoots with IBA treatment were implanted on rooting medium. Shoot bases were dipped in 200mgl^{-1} of pre-autoclaved aqueous IBA solution for 10 minutes. Rooting medium containing 1/4 strength MS salts with sucrose (1%) and agar (0.6%) as recommended by Tak (1993) was considered as standared rooting medium (SR Medium). Polyamines, put, spd and spm were added in rooting medium in the concentrations of 0.1, 0.5 and 1.0 mM to observe their effect on root induction. All the data were subjected to statistical analysis using standard procedures.

RESULTS AND DISCUSSION

A large corpus of literature gives ample evidence that polyamines play a major role in morphogenic processes in plant tissue culture (Umashankar *et al*., 2011; Thiruvengadam *et al*., 2012). In the present study effect of exogenous polyamine on the three different stages of micropropagation, shoot bud proliferation, multiplication and rooting was investigaed.

During shoot bud proliferation, all the three polyamines *viz.*, put, spd and spm when added in different concentrations along with optimum BAP produced significantly higher number of shoots as compared to BAP (5.0 mg l^{-1}) alone taken as control. As the concentrations of polyamines were increased from 0.0 to 1.0 mM, production of mean number of shoot buds per node increased gradually. Among all the concentration of three polyamines, 1.0 mM spd was found to be the best for shoot induction and at this concentration maximum shoot bud proliferation (9.61 shoots per node) was achieved. This was followed closely by 1.0 mM spm (9.59 shoots per node) and 0.5 mM spm (9.39 shoots per node) (Table 1). Significant improvement in the shoot length of the proliferated shoots was also observed on all the concentrations of polyamines as compared to control. Maximum shoot length of initiated shoots (2.52 cm) was observed in cotyledonary explants treated with 0.5 mM spm, which was significantly superior to control. Prolonged maintenance of cultures on media containing polyamines increased number of shoots, without any deterioration. Similar type of promoting effect of polyamines on shoot shoot proliferation and elongation have been reported in several dicot plants, such as *Lagenaria ciceraria* (Shyamali and Hattori 2007), *Cucumis sativus* (Zhu and Chen, 2005), *Corylus avellana* (Nas, 2004) cotton (Ganeshan and Jayabalan,2006) and *Bixa orellana* (Parimalan et al.,2011). Same kind of observations were also found by many other workers (Wei *et al*., 2010; Thiruvengadam *et al*.,2012; Guruchandran and Chinnagounder, 2013).

Table 1 Effect of polyamines on shoot bud proliferation from cotyledonary node of *W. tomentosa*

MS+BAP(mg/l) + Polyamine(mM)	Mean number of shoots per node @	Mean Shoot length (cm)	Callus intensity
Control	3.80(1.95)d	1.48c	+++
Putrescine 0.1	7.12(2.67)c	2.28ab	++
Putrescine 0.5	8.12(2.85)abc	2.33ab	+++
Putrescine 1.0	8.44(2.91)abc	2.0b	+++
Spermidine 0.1	7.61(2.76)bc	2.15a	++
Spermidine 0.5	9.0(3.00)ab	2.03b	+++
Spermidine 1.0	9.61(3.10)a	2.15ab	+++
Spermine 0.1	8.63(2.94)abc	1.88b	++
Spermine 0.5	9.39(3.06)a	2.52a	+++
Spermine 1.0	9.59(3.10)a	2.03b	+++
SEm±	0.11	0.16	
$CD_{.05}$	0.30	0.46	
$CD_{.01}$	0.40	0.61	

@ Figures in parentheses are $\sqrt{\times}$ transformed values.

Means followed by different letters in the same column differ significantly.

Cultures, established from cotyledonary explants were used to study the effect of polyamine on multiplication where 2.0 mgl^{-1} BAP served as control. Addition of polyamines influenced the rate of multiplication significantly (Table 2). The best response (3.93-fold) of shoot multiplication was achieved when BAP at 2mg l^{-1} concentration was combined with 0.5 mM spm as compared to control (2.98-fold). This was followed by 1.0 and 0.1 mM spm, 1 mM put and 0.1, 1.0, 0.5 mM spd. There was no significant increase in shoot length when polyamines were added to multiplication medium as compared to control (2.51 cm) except by spd at 1.0 mM concentration (2.98 cm). Similarly, enhancenment of shoot multiplication was observed in polyamines supplemented regeneration medium in *Achras sapota* (Purohit *et al.*, 2007), cucumber (Vasudevan *et al.*, 2008), sugarcane (Umashankar *et al.*, 2011) and *Dendrobium* cultivars (Kumari and George, 2011). Kumari and George (2011) observed that polyamines, spm and spd hastened shoot multiplication at 0.25, 0.5 and 1 mM concentration in '*Dendrobium* Rungnappa Red'and 'Miss Snow White' cultivar.

Enhanced effect of polyamines on multiple shoot bud induction per explant and multiplication rate could be ascribed to their stimulatory effect on cell division (Bais and Ravishankar, 2002) and/or the inhibitory effect on ethylene production (Bais et al., 2000).

L-arg which serves as precursor of polyamine put was found to enhance the frequency of plantlet regeneration in taro (*Colocasia esculenta*) (Sabapathy and Nair, 1992) but in the present study, addition of L- arg to standard medium did not have any significant effect on shoot multiplication. D-arg, an inhibitor of put biosynthesis, led to the decrease in multiplication rate as compared to control,which was similar to the results observed by Tian *et al.* (1994) and Zhu and Chen (2005) during adventitious shoot formation from cotyledons of *Cucumis melo* and *C. sativus*, respectively. The regenerated microshoots on polyamine supplemented medium were healthy and greenish.

Table 2 Effect of polyamines on shoot multiplication and shoot elongation in cotyledonary node derived cultures of *W.tomentosa*

SM medium + polyamines(mM)	Multiplication Rate (in folds)	Shoot elongation (in cm)
Control	2.9833±0.0764	2.5167±0.1155
Putrescine 0.1	2.2000±0.1732	2.1333±0.4726
Putrescine 0.5	2.6000±0.3041	2.0167±0.4311
Putrescine 1.0	3.3833±0.5346	2.0167±0.3055
Spermidine 0.1	3.1667±0.0289	2.7500±0.1323
Spermidine 0.5	3.1000±0.0500	2.6333±0.1756
Spermidine 1.0	3.1333±0.1041	2.9833±0.1528
Spermine 0.1	3.3833±0.2517	1.9333±0.4752
Spermine 0.5	3.9333±0.6212	2.4167±0.5346
Spermine 1.0	3.4667±0.4537	2.0833±0.1528
L-Arginine 0.1	2.8333±0.2021	1.9500±0.4272
L-Arginine 0.5	2.9500±0.2000	2.1167±0.6048
L-Arginine 1.0	2.8667±0.1443	2.6000±0.0866
D-Arginine0.1µM	2.1967±0.0451	2.5833±0.1528
D-Arginine1.0µM	2.2000±0.0500	2.2833±0.2754
GM	2.9598±0.5440	2.3344±0.4294
Se	0.1626	0.1987
CD5%	0.4696	0.5740
CD1%	0.6327	0.7734
CV	9.51	14.75

rooted on polyamines containing medium were more healthy, with negligible callus. Several studies have reported the enhancing effect of polyamines on rooting (Arena et al., 2005; Mendes et al., 2011; Thiruvengadam et al., 2012) but in the present study it was not the same case. Exogenous polyamine was found not to increase either root number or root length. On the other hand, polyamines- put (0.5 mM) and spm (0.5-1.0 mM) (Table 3), expressed mild inhibitory effect on rooting in *W. tomentosa*. Only put at 0.5 mM produced number of roots equal to control. Significantly taller shoots were obtained on medium containing polyamines and the best concentration was found to be spm at 0.5 mM concentration. Shoots

Table 3 Effect of polyamines on rooting in IBA pulse treated shoots of *W.tomentosa* from CN cultures

SR + IBA + Polyamines (mM)	Percent rooting response	Mean number of roots @	Mean root length	Mean shoot length(cm)
Control	90	3.97(1.99)	1.48a	3.82c
Putrescine 0.1	10	3.58(1.89)	1.22b	3.98abc
Putrescine 0.5	58	3.97(1.99)	1.22b	4.28a
Putrescine 1.0	90	3.18(1.79)	1.18c	3.96abc
Spermidine 0.1	92	3.38(1.84)	1.16c	3.84bc
Spermidine 0.5	83	3.15(1.78)	1.38ab	4.18ab
Spermidine 1.0	100	2.75(1.66)	1.16c	3.84bc
Spermine 0.1	83	3.76(1.94)	1.36ab	3.66cd
Spermine 0.5	33	3.94(1.99)	1.44a	4.20b
Spermine 1.0	58	3.38(1.84)	1.32bc	3.42d
SEm±		0.09	0.06	0.13
$CD_{.05}$		NS	0.17	0.37
$CD_{.01}$		NS	0.22	0.56

@ Value in parentheses are $\sqrt{\times}$ transformed values.
Mean followed by different letters in the same column differ significantly.

The present study highlights the promotive effect of polyamines on shoot proliferation and multiplication during *in vitro* propagation of *W. tomentosa*. The results in this study clearly demonstrate that high frequency regeneration from cotyledonary node explants and multiplication in *W. tomentosa* could be achieved by applying exogenous polyamines.

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