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RESEARCH ARTICLE

IN VITRO MULTIPLICATION OF ROSE (*Rosa bourboniana*)

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ABSTRACT

A protocol for *in vitro* multiplication of red rose (*Rosa bourboniania*) was developed. Multiple shoots were induced from nodal segments and shoot tips. MS (Murashige and Skoog) medium supplemented with various combinations of BAP (6-Benzyl Amino Purine), NAA (1-Naphthylene Acetic Acid), IAA (Indole Acetic Acid) were used to study the *in vitro* growth response. Culture initiation media were used for further multiplication. MS medium incorporated with 2.0 mgl^{-1} BAP, 30 mgl^{-1}, $Ad.SO_4$ (Adenine sulphate) and 3% sucrose was found to be the most suitable medium for culture initiation and multiplication. Growth regulator combinations did not seem to influence multiple shooting and further growth in this species.

INTRODUCTION

Roses are part of human culture and acquired the position as "Queen" of flowers. In addition to their aesthetic beauty, the economical and medicinal values of roses emphasize the need for their improvement (Mahmood, 1996). Red rose (*Rosa bourboniania*) is cultivated for flowers and products such as rose attar and rose essence that is used as ingredient of perfumes, scents, shampoo, face cream, lotion and bath soaps. This industry has great growth potential if high yielding and disease resistant plants are used for cultivation. Micropropgataion is one of the most important methods of tissue culture that offers vast economic significance in commercial floriculture (Soomro *et al*., 2003; Pati *et al*., 2006). In the present study, high yielding as well as aromatic and attractive flower bearing, naturally occurring variant plants of red rose were selected as mother plants and a highly reproducible protocol for *in vitro* multiplication was developed.

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MATERIALS AND METHODS

Shoot bits of about 15 cm were collected from elite plants, removed the leaves and washed in soap solution and then in running tap water for 10 minutes. Surface sterilization and the rest of the culturing procedure were carried out inside the laminar air flow chamber. The shoot bits were briefly rinsed with 70% ethanol followed by 0.1% mercuric chloride for 5 minutes. After washing thrice in sterilized distilled water, single noded explants were inoculated as one/test tube

containing medium. Three replicates of 20 numbers each were setup for the experiment. Three initiation media were prepared based on MS (Murashige and Skoog, 1962) medium containing 30 gl^{-1} and 8 gl^{-1} agar. The medium was variously supplemented with BAP, NAA and IAA.

Medium 1 was prepared according the formulation of Arnold *et al*. (1995). Medium contained ¾ strength of MS macronutrients with 1856 mgl^{-1} ammonium nitrate, full strength micronutrients with zinc sulphate heptahydrate and manganese sulphate altered to 21.2 and 33.8 mgl^{-1} respectively, 3% sucrose, 0.6% agar, 1.0 mgl^{-1} BAP and 0.005 mgl^{-1}NAA. Medium 2 was prepared by adding 2.0 mgl^{-1}BAP and 30.0 mgl^{-1} $Ad.SO_4$. A combination of BAP (3.0 mgl^{-1}) and IAA (0.3 mgl^{-1}) was incorporated in medium 3. The concentration and combinations of growth regulators are shown in Table 1. pH of the medium was adjusted to 5.8 before autoclaving. The cultures were maintained at 26 ± 2^0C under 16 h light/ 8 h dark and at a light intensity of 1500 lux.

Weekly observations were made for growth response. Parameters assessed were bud initiation, bud elongation, number of buds/explant, number of leaves/shoot and shoot elongation. After 4 weeks, the newly developed shoots were excised and sub-cultured on to fresh initiation medium and also on to the other two initiation media. Cultures were incubated as for culture initiation, and multiplication rates were observed.

RESULTS

The explants inoculated on the three different shoot media (Table 1) had an initial response of bud bursting. Bud elongation, leaf development, leaf expansion and shoot elongation were the subsequent growth responses of the cultures. Medium 2 that contained 2.0 mgl^{-1} of BAP supported a fast response and comparatively uniform growth of the cultures. Cultures on medium 1 (BAP+NAA) and medium 2 (BAP+IAA) showed similar response only during the bud bursting phase (80% and 96% respectively). Further development of medium 1 group was slow and similar to cultures of medium 3 (Table 2, Fig. 1 & Fig. 3). Poor response was obtained from the third group (medium 3) for all the growth phases. Excepting very few cultures (8%), there was no remarkable effect on shoot growth in this group. Number of buds/explant (2 to 3), number of leaves/shoot (5), shoot length

Table 1. Composition of various shoot multiplication media used for the in vitro shoot development of *Rosa bourboniana*

MS Basal medium	Plant growth regulators (mgl^{-1})			Sucrose (gl^{-1})	Agar (gl^{-1})
	BAP	NAA	IAA		
1*	1	0.005	-	30	6
2	2	-	-	30	8
3	3	-	0.3	30	8

Table 2. Effect of different concentration and combinations of plant growth regulators on in vitro multiplication of *Rosa bourboniana*

Medium	1	2	3
No. of cultures initiated	50	50	50
Percentage of cultures with bud development	80	96	46
Percentage of cultures with leaf development	54	96	40
Percentage of cultures with shoots >0.5 cm & shoot length (Mean ± SEM)	32 0.531 ± 0.12	90 0.682 ± 0.005	16 0.525 ± 0.16
Percentage of cultures with shoots >1.0 cm & shoot length (Mean ± SEM)	20 1.08 ± 0.032	94 1.757 ± 0.026	8 1.225 ± 0.025
No. of leaves/shoot (Mean ± SEM)	3 ± 0.258	5.02 ± 0.098	3 ± 0.408

Fig. 1. Growth of cultures grown on medium 1 after 4 weeks of incubation

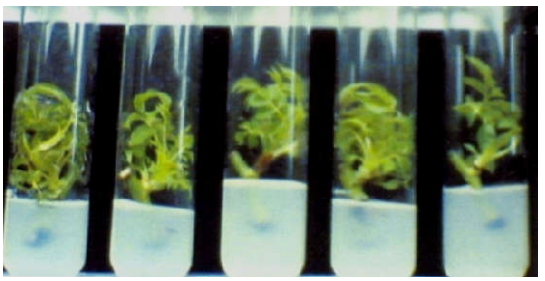

Fig.2. Growth of cultures grown on Medium 2 after 4 weeks of incubation

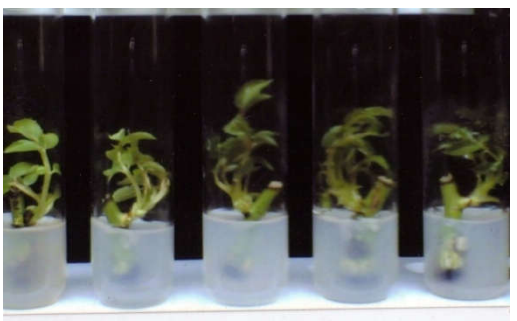

Fig.3. Growth of cultures grown on medium 3 after 4 weeks of incubation

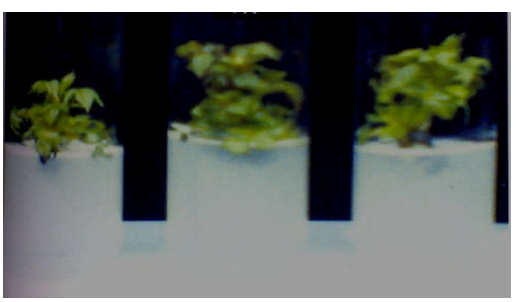

Fig. 4. Multiple shoot development of cultures after 4 weeks of sub-culturing that is initiated and sub-cultured on medium 2.

(1.76 ± 0.026 cm) and percentage of cultures that developed long shoots (94) were high with comparatively uniform growth for cultures grown on medium 2 (end of 4th week), Table 2, Fig. 2. Sub-culturing of the newly developed shoots from this group for further multiplication on the same medium induced multiple shoots at a rate of 7-8/culture (Fig. 4). Further multiplication rate was also poor for the cultures initiated on medium 1&3 (2 fold). However, these cultures sub-cultured on to Medium 2 showed a slight increase (3-fold) in growth.

DISCUSSION

Culture initiation and multiplication of Rosa bourboniana does not seem to be influenced by the presence of both auxin and cytokinin. There are reports explaining the influence of type and combination of growth regulators on multiplication and rooting of rose (Kumar et al., 2001, Pati et al., 2001a, b). In those studies, thidiazurone exposed shoots had a higher multiplication rate. In the present study, the cytokinin BAP combined with an auxin did not seem to have any profound effect on multiplication or growth. The medium containing BAP alone was highly responsive compared to the medium containing combinations with NAA or IAA. Number of leaves/culture was more for those grown on medium containing cytokinin alone (medium 2) than the auxin-cytokinin exposed ones (media 1&3). Regardless of the media, cultures with higher number of leaves had greater shoot length than the rest of the shoots, indicating a direct relationship between number of leaves and length of the shoots. The highest number of long shoots with well expanded leaves was obtained from cultures of medium 2 and the response was consistent during further multiplication phases also. This shows that presence of auxin did not have any effect on shoot multiplication or shoot elongation in red rose. The results explain the effectiveness of endogenous auxin levels for multiplication and growth and the irrelevance of exogenous auxin supplement. The present study was successful in standardizing a simple and efficient protocol for *in vitro* multiplication of red rose (*Rosa bourboniana*) using either shoot tips or nodal segments.MS medium containing BAP is

highly effective for both initiation and further multiplication.

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REFERENCES

Arnold, N.P., Binns, M., Cloutier, D.C., Barthakur, N. N. and Pellerin, R. 1995. Auxins, salt concentrations and their interactions during in vitro rooting of winter-hardy and hybrid tea roses.. *Hort. Sci.,* 30 (7): 1436–1440.

Kumar A., Sood A., Palni UT., Gupta A. K., Palni, M. S. 2001. Micropropagation of Rosa damascena Mill.from mature bushes using thidiazurone. *J. Horticult. Sci. Biotechnol.,* 76 (1): 30-34.

Mahmood, N. 1996. The anti-HIV activity and mechanisms of action of pure compounds isolated from Rosa damascene. *Biochem Biophys. Res. Comm.,* 229: 73-79.

Murashige, T and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant.,* 15: 473-497.

Pati, P.K., Sharma, M., Sood, A. and Ahuja, P. S. 2001a. Direct shoot regeneration from leaf explants of *Rosa damascena* Mill. In Vitro Cell. *Dev. Biol. Plant.,* 40 (2): 192-195.

Pati, P. K., Sharma, M. and Ahuja, P. S. 2001b. Micropropagation, protoplast culture and its implications in the improvement of scented rose. *Acta Hortic.,* 547: 147-148.

Pati, P.K., Rath, S.P., Sharma M., Sood, A. and Ahuja, P.S. 2006. In vitro propagation of rose: a review. *Biotechnol. Adv.,* 24: 94–114.

Soomro, R., Yasmin, S. and Aleem, R. 2003. In vitro propagation of *Rosa indica*. *Pak. J. Biol. Sci.,* 6 (9): 826-830.

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