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EFFECT OF PHYTOHORMONES ON SHOOT AND ROOT REGENERATION IN ROSE UNDER *IN VITRO* CONDITIONS

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Abstract

In vitro propagation of rose has played a very important role in rapid multiplication of cultivars with desirable traits and production of healthy and disease free plants. Murashige and Skoog medium supplemented with various concentrations and combinations of Indole-3-butyric acid (IBA), Naphthylacetic acid (NAA), Benzylaminopurine (BAP), and Indole-3- acetic acid (IAA) were employed for shoot regeneration in this study, whereas MS½ and full strength medium containing different amounts of IBA (0.5 and 1mg/l) were used for root induction. The highest multiple shoot formation was recorded in MS medium containing IAA (1 mg/l) and BAP (4 mg/l) with 30 g/l sugar. Cytokinin (BAP) in addition to auxin (IBA) caused the maximum bulbus formation which turned into elongated shoots, while the NAA was observed to have antagonistic effect on shoot formation. Highest numbers of roots were produced on MS½ strength + IBA 0.5 mg/l. The aim of this study was to determine growth and development of rose on different growth media, to shorten the multiplication time of virus-free plant material.

INTRODUCTION

The genus *Rosa* includes over 100 species distributed throughout the world (Xing *et al*., 2010). Chromosome numbers in this genus range from 2n=2x=14 to 2n=8x=56 (Udom *et al*., 2009). Roses have been one of the world's most popular ornamental plants for a long time and are being grown globally as cut flowers potted plants and in home gardens. The flowers vary greatly in size, shape and color. Traditionally, roses are multiplied asexually by grafting buds onto root-stocks. This is extremely time consuming and labor intensive, and has a significant impact on the product cost. These bottlenecks make rose a very appropriate candidate for improvement through biotechnological approaches. Various tissue culture techniques, such as micropro-pagation, may decrease propagation time; also it could virtually eliminate the need for grafting onto rootstocks (Wang *et al*., 2002). Micropro-pagation has been shown to be a highly effective method of rapidly propagating disease-free, uniform rose plants (Pati *et al*., 2005; Ozel and Arsalan, 2006). Moreover rapid propagation with micropropagation also minimizes the time requi-red for introduction of new cultivars into the commercial market thus increasing the availa-bility of plants with improved horticultural characteristics (Previati *et al*., 2008).

Rate and mode of shoot multiplication are the two major elements which determine the success of a micropropagation protocol. Other factors which effect the shoot multiplication of rose include the genotype of the plant, growth medium, status of the media, growth regulators, organic and inorganic elements, and physical factors (Pati *et al*. 2005). The first report about multiplication of shoots and roots of the rose was published by Elliot (1970). Rose proliferation is most commonly done employing Murashige and Skogs (MS) medium (Davies 1980). Substantial work has been done to determine the optimum concentrations of components of growth media for *in vitro* rose tissue culture. Rout *et al*. (1999) reported that cytokinins play vital role in propagation of shoot and multiplication of rose. Different concentrations of auxins have been investigated to induce root development (Pati *et al*. 2005). It has been seen that lower concen-trations of auxins (IAA, IBA or NAA) in medium facilitate the root induction from excised micro shoots, whereas higher concentrations can lead to reduction in the root induction (Hassegawa, 1980). Benzylaminopurine (BAP) has also been found to be an essential component of medium for rose culture, as it plays important role in bud proliferation and shoot multiplication (Ibrahim and Debergh 2001). Various reports also mention

BAP to be important for initiation of auxiliary buds formation in roses (Hassegawa, 1980; wulster and Sacalis, 1980; Bressan *et al.* 1982).

In this study, we describe an efficient tissue culture technique to yield large number of shoots from nodal explants of rose. Rapid growth and development of rose was established by using different growth media to shorten the multiplication time of virus-free plant production.

MATERIALS AND METHODS

Nodal explants containing lateral buds of actively field-grown 'Perfume Delight' rose were used for multiplication. Fresh plant material (lateral buds) was collected from the rose plant grown in garden at Nuclear Institute of Agriculture, (NIA) Tando Jam. The collected material was washed several times with tap water, alcohol and sodium hypochloride. The sterilized explants were washed 2-3 times with sterile distilled water to remove disinfecting solution. Then the explants were trimmed down to 1 cm long prior to transfer MS medium (Murashige & Skoog,1962) augmented with different growth regulators (BAP, IBA, IAA and NAA). Seven different media were used in this shoot multiplication study (Table 1). Explants were sub cultured to fresh medium every 4 weeks. After this period excised single shoot from multiple shoots were transferred to different concentrations of MS and IBA for root induction. All these operations were carried out under aseptic conditions and cultures were incubated at 25± 2^{o} C with 16 hours photo period (Thorpe, 1981). Rooted plantlets were acclimatized and transplanted to field for screening (Table-2). The data recorded was statistically analyzed using Duncan's Multiple Range Test (Steel & Torrie, 1980), to check the level of significance between the treatments.

Table -1: Different media used for shoot multiplication

Treatments	Media components
A	MS + 30 g/l sugar
B	MS + 0.5 mg/l BAP + 30g /l sugar
C	MS + 0.1 mg/l IBA + 4 mg BAP + 30 g/l sugar
D	MS + 0.5 mg/l NAA + 0.5 mg/l BAP + 30 g/l sucrose
E	MS + 0.1 mg/l NAA + 2 mg/l BAP + 30 g/l sucrose
F	MS + 1 mg /l IAA + 4 mg /l BAP + 30 g/l sugar
G	MS + 0.5 mg /l IAA + 4 mg /l BAP + 30 g/l sugar

Table- 2: Effect of medium strength and auxin concentration on *in vitro* rooting

Treatments	Media components
A	MS½ + 0.5 mg/l IBA + 30 g /l sugar
B	MS½ +1 mg/l IBA + 30 g/l sugar
C	MS full + 0.5 mg/l IBA + 30 g/l sugar
D	MS full + 1 mg/l IBA + 30 g/l sugar

RESULTS AND DISCUSSIONS

Shoot multiplication: Statistical analysis of the data showed that the significant differences were observed in the growth of shoot and root in response to different growth regulators (Table 3 & 4). Two types of growth responses were observed on the shoot induction medium, the axillary shoots and the bulbus formation (Fig 3a & b). The earliest and vigorous multiple axillary shoots (9.66) proliferated on modified MS medium containing 1mg/l IAA, 4mg/l BAP and 3g/l sugar after 23 days (Fig 1 & 2). The augmentation of MS medium with IAA along with the cytokinin was found to be supportive for the vigorous shootlets formation but it also increase the length of the shootlets that of observed 8.66cm with 1 mg/l of IAA and 8cm with 0.5mg/l IAA. However the addition of IBA with BAP induced the bulbus formation that turned the duration of axillary shoots formation to 26 days. It was observed that the addition of NAA (0.1 and 0.5 mg/l) with BAP slowed down the process of axillary shoot formation up to 45-47 days and the number of shootlets were only 1.33. Cytokinin is the most crucial components for the shoot formation (Vijaya *et al.,* 1991). In present study BAP was found to be the stimulating agent for the shoot formation and IAA supported the BAP for multiple shoot formation. Cytokinin and auxin in appropriate ratio are required for efficient shoot

regeneration as reported by Maurya (2013) and Canli *et al*. (2009). The findings of the present study are in conformity with the findings of Pati (2005); Ozel and Arsalan (2006) and Castilon *et al*. (2006). Similar effect of media combinations have also been reported by Nam *et al.* (2011); Asadi *et al*. (2009) and Taylor *et al.* (2005).

Table -3: Effect of different growth regulators on shoot induction and multiplication

Treatments	Number of days for axillary shoots	Multiplication shoot	Shoot length (cm)
A	37.00 c	3.66 d	4.66 d
B	35.00 c	4.66 c	5.66 c
C	26.66 d	7.66 b	7.00 b
D	45.00 b	1.66 e	2.66 e
E	47.66 a	1.33 e	2.33 e
F	23.00 e	9.66 a	8.66 a
G	27.33 d	7.66 b	8.00 a
SE	0.94	0.17	0.39
LSD (5%)	2.06	0.38	0.85

Table-4: Effect of medium strength and auxin concentration on in vitro rooting

Source	No. of days taken for visible root formation	Number of roots
A	14.33 d	11.00 a
B	19.00 c	9.66 b
C	30.00 b	6.66 c
D	34.66 a	6.00 d
SD	0.45	0.27
LSD (5%)	1.10	0.66

Fig -1: Effect of different growth regulator concentrations on plant regeneration

Fig -2: Regeneration in 1mg /l IAA+ 4mg /l BAP+30g/l sugar

Rooting: To establish complete rose plants, regenerated shoots were excised and transferred to rooting medium on MS½ and MS full strength with IBA 0.5 mg/l and 1 mg/l. The visible roots formation was started after 14 days for the application of MS½ + 0.5 mg/l IBA + 30 g /l sugar (Table 4). The highest roots were observed on MS½ strength medium supplemented with 0.5 mg/l IBA (11.00) followed by rooting on same strength of MS medium containing 1.0 mg/l IBA (9.66) (Fig 3c). In this research, reduced salt concentration increased rooting in MS medium, which is in accordance with (Salekjalali *et al*., 2011) who also reported that the reduced salt concentration generally increases rooting in MS medium. Different concentrations of auxin have been investigated for root induction in many of the previous studies (Udom *et al.,* 2009). Among the MS basal salts concentration used in this study, MS½ strength was found to be more effective for *in vitro* rooting than full strength MS medium on the same concentration7of IBA.

Table-5: Mean square performance for shoot multiplication of rose explants under different growth regulators.

Sources	DF	No. of days for axillary shoots	Multiplication shoot	Shoot length (cm)
Replications	2	5.905	2.0476	2.2857
Treatments	6	267.540**	31.0952**	18.6349
Error	12	1.349	0.0476**	0.2302
CV		3.36	4.20	8.61

Fig -3a, b & c: Bulbuls formation on MS+0.1mg/l IBA +4mg BAP+30g/l sugar c) rooting

Table -6: Effect of medium strength and auxin concentration on *in vitro* rooting.

Sources	No. of days taken for visible root formation	No. of roots
MS½ + 0.5 mg/L^{-1} IBA + 30 g/L^{-1} sugar	14.33d	11.00a
MS½ + 1.0 mg/L^{-1} IBA + 30 g/L^{-1} sugar	19.00c	9.66b
MS full + 0.5 mg/L^{-1} IBA +30 g/L^{-1} sugar	30.00b	6.66c
MS full +1.0 mg/L^{-1} IBA + 30 g/L^{-1} sugar	34.66a	6.00d
SD	0.45	0.27
LSD	1.10	0.66

Table -7: Mean square performance for root induction rose shootlets under different growth regulators.

Sources	DF	No. of days taken for visible root formation	Number of roots
Replications	2	1.750	2.3333
Treatments	3	267.222**	62.5278**
Error	6	0.306	0.1111
CV		2.26	3.48

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