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Micropropagation of Garden Rose (*Rosa Indica L.)* through Different Size of Node Explants

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Abstract

The present investigations were carried out to standardize surface sterilization of explants, suitable explants type for culture establishment, growth regulators for shoot multiplication and rooting and to evaluate suitable hardening media. Of the various concentrations of $HgCl_2$ and NaOCl tried for surface disinfection. The explants *viz.* node were tried. It was observed that complete shoot gave the best results and emerged as suitable explants for Rose culture establishment. Among different concentration of MS medium, ½ MS medium gave early and maximum shoot germination. In all the combination of plant growth regulators, the highest number of shoots regeneration was observed in growth regulator 1.5 mg/l BAP and 1.5mg/l IAA containing medium. The highest shoot initiation was observed in combination MS + 1.5mg/l BAP.

Keywords: Rosa, BAP, IAA, MS media, Node, Micropropagation.

Contents

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1. Introduction

A rose is a perennial plant of the genus *Rosa*, within the family Rosaceae. There are over 100 species [1]**.** Rose plants range in size from compact, miniature roses, to climbers that can reach 7 meters in height. Species from different parts of the world easily hybridize, which has given rise to the many types of garden roses [2]. Roses have been one of the world's most popular ornamental plants for a long time. The flowers vary greatly in size, shape and colour. Tissue culture system in roses has been established [3-8]**.** Recently, *in vitro* flower induction in roses was demonstrated [9]. The majority of ornamental roses are hybrids that were bred for their flowers.

2. Materials and Methods

Shoots of Rose were thoroughly washed with tap water containing Tween- 20 for 10-15 min and Bavistin (1gm/L), followed by running tap water treatment for 30 minute. Shoots were peeled-off and transfer to laminar air flow. The nodes were surface sterilized with the treatment of 0.1 % $HgCl_2$ for 3 minute followed by four washes with sterile water. Surface sterilized node region were inoculated culture vessels containing Murashige and Skoog [10] medium with various concentrations and combinations of BAP and IAA; then incubated in growth room for shoot initiation at 26 ± 2 °C temperature, 50-60 % relative humidity and l6/8 hours (dark/light) photoperiod of 3000 lux fluorescent cool light were used for different experiments under study.

3. Results and Discussion

In the present investigation, attempts were made to study the effect of phytohormones on various aspects of shoot growth. The development of efficient and reproducible regeneration protocol from tissues is prerequisite for the successful application of micropropagation technology for the mass multiplication of plant. Various concentrations and combinations of BAP and IAA were used in MS (1962) medium and observed for the morphogenic responses of explants of pointed gourd. The results of each of these aspects are presented and discussed Herewith considering almost weekly observations.The data on percentage of shoot initiation collected that the highest of shoot formation were observed in node size in 1.0cm (Tab-1), 0.8cm (Tab-2) and 0.5cm (Tab-3) in the Treatment of S3 (90%) fig-1. The lowest percentage of shoot formation was observed in different size 1.0 S2 (60%), 0.8 S2 (50%). In other treatment, (S4 and S2) average percentage of shoot formation was observed 40% to 60%.

In this study, cytokine alone induced as reported by Telgen, et al. [11]. cytokine give the best highest number of shoot formation. The shoots inducted from the multiplication culture were further sub-culture for shoot multiplication. In the present study after 14 days observation of multiple shooting in Size 1.0cm (Tab-4), 0.8cm (Tab-5), 0.5cm (Tab-6) length in treatment M3 is the highest multiple shooting (fig-2). Auxin and cytokine combination of the highest number of multiple shooting was reported Singh and Syamal [12]. After the observation of 14 days the highest roots regenerates from shoot of Explant size 1.0cm in 1.5mg/l the highest regenerates 90%., Auxin has positive effective on root growth.

Figure-1. Shoot Formation on MS medium with 1.5 mg/l BAP

Table-1. Effect of BAP on shooting in different explants size.

Explants size	Treatment	BAP conc. (mg/l)	No. of Explants Inoculated	No. of Explants sprouted	%	Shoot length (cm)	No.of leaves
1.0cm	S1	0.0	10	5	50	0.26± 0.11	3± 1.58
	S2	1.0	10	6	60	0.31± 0.147	2.5± 1.04
	S3	1.5	10	9	90	0.33± 0.325	3.6± 3.96
	S4	2.0	10	4	40	0.4± 0.425	2.5± 2.37

Table-2. Effect of BAP on shooting in different explants size.

Explants size	Treatment	BAP conc. (mg/l)	No. of Explants Inoculated	No. of Explants sprouted	%	Shoot length(cm)	No.of leaves
0.8 cm	S1	0.0	10	5	50	0.24± 0.208	2.6± 2.72
	S2	1.0	10	6	60	0.21± 0.18	2.5± 2.61
	S3	1.5	10	9	90	0.28± 0.126	2.2± 2.40
	S4	2.0	10	7	70	0.27± 0.281	1.8± 1.83

Table-3. Effect of BAP on shooting in different explants size.

Explants size	Treatment	BAP conc. (mg/l)	No. of Explants Inoculated	No. of Explants sprouted	Percentage%	Shoot length(cm)	No.of leaves
0.5 cm	S1	0.0	10	4	40	0.17± 0.095	0.4± 0.40
	S2	1.0	10	5	50	0.24± 0.114	1.6± 1.58
	S3	1.5	10	9	90	0.46± 0.591	2.5± 1.33
	S4	2.0	10	4	40	0.17± 0.095	1.7± 1.68

Figure-2. Multiple Shooting on MS medium with 1.5 mg/l BAP and 1.5 mg/l IAA

Table-4. Effect of shoot multiplication with growth hormone BAP+IAA.

Explant size	Treatment	BAPconc (mg/l)	IAA conc (mg/l)	No.of Explants inoculated	No.of Explants sprouted	Percentage %	Shootin length(cm)	No.of shoots
1.0cm	M1	0.0	0.0	10	5	50	0.62± 0.344	2± 0.70
	M2	1.0	1.0	10	4	40	0.7± 0.375	2± 0.81
	M3	1.5	1.5	10	9	90	0.22± 0.224	2.7± 1.64
	M4	2.0	2.0	10	5	50	0.17± 0.18	2± 0.70

Table-5. Effect of shoot multiplication with growth hormone BAP+IAA.

Explant size	Treatment	BAPconc (mg/l)	IAA conc (mg/l)	No.of Explants inoculated	No.of Explants sprouted	Percentage %	Shootin length (cm)	No.of shoots
0.8cm	M1	0.0	0.0	10	5	50	0.18± 0.083	2.4± 1.14
	M2	1.0	1.0	10	6	60	0.21± 0.116	1.8± 0.752
	M3	1.5	1.5	10	10	100	0.2± 0.105	2.1± 1.197
	M4	2.0	2.0	10	4	40	0.25± 0.129	2± 0.816

Table-6. Effect of shoot multiplication with growth hormone BAP+IAA.

Explant size	Treatment	BAPconc (mg/l)	IAA conc (mg/l)	No.of Explants inoculated	No.of Explants sprouted	Percentage %	Shootin length (cm)	No.of shoots
0.5cm	M1	0.0	0.0	10	4	40	0.17± 0.095	2± 1.411
	M2	1.0	1.0	10	5	50	0.24± 0.114	2.2± 0.836
	M3	1.5	1.5	10	9	90	0.23± 0.12	2.4± 0.881
	M4	2.0	2.0	10	5	50	0.2± 0.070	2.2± 0.836

In this study, rooted plantlet with regenerates in the ratio of 2:1 ; vermiculture and soil. 80% of plant growing in laboratory scale and 91% plant successfully grown in green house fig-3 & fig-4; Tab-7 & 8; Kumar, et al. [13]; Singh and Syamal [12].

Figure-3. Rooting in half strength of MS+1.0mg/l IBA.

Table-7. Effect of IBA hormone on rooting.

Treatment	Explant size	IBA (mg/l)	Total no.of shoots inoculated	No.of Explant sprouted	Percentage of root Induction inlab
R1	1.0cm	1.5	10	9	90%

Figure-4. Acclimatized rose plantlet in soil.

Table-8. Effect of soil +Vermiculture on green house.

Treatment	Ration	Potted plant	No.of Explant sprouted	Percentage of plant	In Green House
A1	2:1	10	9	80%	91%

The present exploration entitled **"*Micro* Propogation Of Rose (*Rosa Indica L.*)"** was carried out at Tissue CultureLaboratory-2, P.S Science and H.D Patel Arts College, Kadi. DuringJanuary - April 2014. The experiment was conducted with a view tostudy micropropogation of rose with responses of phytohormones and explants. The characters studied were Percentage shoot Initiations, Percentage of shoot formation, Average number of leaves, Percentage of average no. of shoots length, average no. of roots, Accamatization. In present experiment node was used as an explant for different experiments. Based on the purpose of study, Murashige and Skoog [10] medium were used as basal media for different studies. Different phytohormone *i.e.* BAP, IAA and IBA were added in basal media.

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