Trakia Journal of Sciences, No 1, pp 46-51, 2020

Available online at:
http://www.uni-sz.bg

ISSN 1313-7050 (print)
ISSN 1313-3551 (online) **doi:10.15547/tjs.2020.01.009**

Original Contribution

EFFECT OF PHYTOHORMONES AND THEIR DIVERSE CONCENTRATIONS ON REGENERATION OF ROSE (*ROSA HYBRIDA* L.)

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ABSTRACT

Roses most important regularly used for ornamental, medicinal and aromatic rationale in the world. The relevance of plant tissue culture technology to produce planting material of rose in masses depends on the availability of an effective regeneration protocol. The present experiment was done to scrutinize for appropriate basal medium of Murashige and Skoog (1962), phytohormones with their diverse concentrations influence for establish *In vitro* shoot and root induction of rose (*Rosa hybrida* L.). The statically analysis of variation explain that least days to initiation, number of shoots, length of shoot cm, number of leaves, days taken in root initiation and number of roots were significant @ 5% possibility. Increase evidence viewing that experimental conclusion exhibit that minimum days to initiation, utmost number of shoots bottle^{-1}, shoot length bottle^{-1} and number of leaves bottle^{-1} be record within the concentration of MS + NAA 0.5 mgL^{-1} + BAP 2 mgL^{-1}. Hence forward minimum days taken in root initiation, highest roots number recorded at $^{1/2}$MS + NAA 1.0mg/l + IBA 1.0 mg/l respectively. *In vitro* healthy and complete plantlets successfully were shifted in to different pot mixtures, supreme survival % recorded at Soil+sand+FYM (1:1:1).

Key words: Regeneration, (*Rosa hybrida* L.), phytohormones, concentrations, acclimatization

ABBREVIATIONS

MS- Murashige and Skoog, BAP- 6-Benzylaminopurine, NAA- Naphthaleneacetic acid, IBA- indole-3-butyric acid, FYM- Farm Yard Manure.

INTRODUCTION

Rose is a representation of elegance, fondness, sensuality, stimulation, holiness and resource of aesthetic delight for human beings. Belong to the family Rosaceae also genus Rosa, more than 18,000 cultivars, 200 species (1). Even so, Rose is an attractive flower of enormous horticultural importance (2). Diminutiveroses (*Rosa hybrida*L.) are progressively more well-liked flowering pot plants. Tissue culture is widely used reproduction technique in modern agriculture that allows a single cell to grow in to an intact plant (3). Also tissue culture is considered an asexual breeding technique because it involves the cells of solitary parent's plant. Asexual reproduction produces plants that are genetically identical to their parents and to one another (4). All dissimilar cells in a plant necessity develop and works in a coordinated conduct in classify to carry out the different processes needed for plant survival. In normal development, the specialized cells of plants are produced at appropriate times to respond to chemicals that stimulate and regulate growth called hormones. Through tissue culture hormones must be artificially supplied to plants at the appropriate time. The two essential hormones used in tissue culture are auxin and cytokinin. These hormones promote cell division and afterward development of stem, leaves and roots. Since hormones to facilitate and promote stem and leaf development may reduce root growth. Testing developing plants with dissimilar hormones at dissimilar times is often necessary (5). Deliberate that plant micro propagation method be included in which

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plant cells, tissues, organs isolate, surfaces sterilizes and for breed many clonal plantlets incubate during a growth-promoting aseptic environment (5). Genotypes greatly influence the growth and morphogenesis of tissue culture *in vitro* (7). Micro propagation has been revealed to be extremely effectual techniques of fast propagate disease-free, identical plants of roses (8, 9). What's more rapid breeding with micro propagation also minimizes the time required for introduce of newest cultivars into the commercial market, hence growing convenience plants with enhanced horticulture uniqueness (10). Declared that for propagation of rose shoot and multiplication cytokinins play as basic role (11). Scrutinize by (8) for the root development diverse concentrations of auxins be encourage. (3) Deliberatefor the, specialized in plant regeneration such as growth hormone, cytokinin and auxin are commonly used. Explants of Rosa species were commonly cultured on a full- or half-strength Murashige& Skoog.

There are a few studies on the effect of phytohormones and cultural conditions on plant regeneration of commercial rose cultivars. The goal of this present study was to begin a competent protocol on micro propagation by manipulates phytohornmones and diverse concentration for plant regeneration of rose (*Rosa hybrida*L.).

MATERIALS AND METHODS

Experiment conducted at Plant tissue culture laboratory, Plant Pathology Section, Sindh Agriculture Research Institute, Tandojam, Pakistan during 2019. In this experiment nodal explants containing lateral buds were used. Collected Lateral buds of fresh plant materials, the excised and young mature shoot tips washed in running water for ten minutes. Then were cut length 3-4 cm segment and used exterior disinfested 70% ethanol for 30 seconds. Furthered, absorbed in 10 % sodium hypochlorite of laundry commercial bleach solution (5.25% NaOCl) contain 2 drops of Tween-20 emulsifier to assist wet for 20 minutes.

Culture medium and conditions

For the shoot and root aseptic plant material were cultured in bottles containing sterilized MS media Murashige Skoog's 1962 (12) supplement with different Plant growth regulators and their concentrations. Beneath aseptic condition in laminar air flow the sterilized explants were inoculated on sterilized media. All media were supplemented 30gm sugar and activated charcoal (0.5 mg L^{-1}) was added meant for browning control. Before autoclaving medium was attuned at 5.7-5.8, then media was autoclaved at 121 °C plus (15-20 psi) in favor of 20 minutes. Also be maintained uniform culture conditions like 16-hour photoperiod at 25±2 ^{o}C for increase temperature.

Shoot proliferation, Rooting, Acclimatization

For shoot proliferation Lateral bud of nodal explants was transferred in MS medium contained, with different concentrations of phytohormones. While for root induction micro shoots about 2-3 cm long were excised form mother explants were transfer to vigor MS medium with different hormones and their concentrations. Regenerated rooted plants were carefully taken out from culture medium and dipped in systemic fungicide (bavistin, 1.0%, w/v; 2 min), then washed with running tap water. Intend for acclimatation used different soil mixtures. Subsequently plantlets were transferred in green house for further growth.

Treatments feature: The experimentation was lay out with diverse growth hormones, concentrations. The detail concerning treatments is seeing that follow **Table 1**.

Statistical analysis: experiment was performing via Complete Randomized Design (CRD) with three replications and consequences were analysis scholar Edition of Statistix (SWX), Version 8.1 (Copyright 2005, Analytical Software-USA).

RESULTS AND DISCUSSION

Experiment was performed on find out the effect of different phytohormones and diverse concentrations for shoot and root regeneration of (*Rosa hybrid* L.). The statically analysis of assorted result presented significant @5%.

Table 1. *Treatments Detail*

SL. No.	Media/Hormones concentrations for Shoot proliferation	Symbol
1	MS control free hormones	T_1
2	MS+ BAP 0.5 mgL^{-1}	T_2
3	MS+NAA 0.5 mgL^{-1}	T_3
4	MS+BAP 2mgL^{-1+} NAA 0.5mgL^{-1}	T_4
5	MS+IBA 0.1 mgL^{-1}+ BAP 5mgL^{-1}	T_5
Media/Hormones concentrations for Rooting		
1	MS^{-1} control free hormones	T_1
2	½ MS+NAA 1mg/l	T_2
3	½ MS+NAA 2mg/l	T_3
4	½ MS+NAA 1mg/l+IBA 0.5mg/l	T_4
5	½ MS+NAA 1mg/l+IBA 1mg/l	T_5
Potting mixtures/Ratio		
1	Soil alone (Control)	T_1
2	Soil+sand+FYM (1:1:1)	T_2
3	Soil+sand+FYM (2:1:1)	T_3
4	Sand+soil (1:1)	T_4
5	Vermiculate+soil (1:1)	T_5

Table 2. *Effect of different phytohormones and various concentrations on Days to initiation, number of shoots bottle^{-1}, shoot length (cm) bottle^{-1}, and number of leaves bottle^{-1}in rose (Rosa hybridaL.)*
Mean with same column, performance significantly different

Treatments	Phytohormones + Concentrations	Days taken initiation	Numbers of shoots $bottle^{-1}$	Shoot length (cm) $bottle^{-1}$	Number of leaves $bottle^{-1}$
T1	MS control free hormones	19.00a	1.70e	3.10e	2.50e
T2	MS+ BAP 0.5 mgL^{-1}	16.00ab	2.75d	3.95d	4.90d
T3	MS+NAA 0.5 mgL^{-1}	12.00c	5.10b	5.85b	9.90b
T4	MS+BAP 2mgL^{-1+} NAA 0.5mgL^{-1}	7.00d	7.50a	7.20a	11.30a
T5	MS+IBA 0.1 mgL^{-1}+ BAP 5mgL^{-1}	13.50bc	4.40c	4.75c	5.80c
LSD (5%)		1.7176	0.0940	0.0316	0.0477
CV		3.9607	0.2167	0.0729	0.1101

Shoot regeneration

In the shoot experiment, to achieve this ambition diverse concentrations and combination of MS, 6-Benzylaminopurine (BAP), Naphthaleneacetic acid (NAA) and Indole-3-butyric acid (IBA) were hold, result offered in **Table 2.** The progressive results judge that T4 MS+ BAP 2mgl^{-1}+ NAA 0.5mgl^{-1} early days recorded for shoot initiation (7.00). Even as a good number of shoots $bottle^{-1}$ (7.50), maximum length of shoot (cm) $bottle^{-1}$ (7.20), and maximum number of leaves recorded (11.30), flowed by T3 MS+ NAA 0.5mgl^{-1} minimum day for shoot initiation record (12.00), shoot $bottle^{-1}$ (5.10), maximum shoot length (cm) (5.85), uppermost number of leaves $bottle^{-1}$ (9.90) conversely poorer results recorded at MS alone as control, minimum days taken for shoot initiation (19.00), number

of shoot bottle^{-1} (1.70), maximum shoot length (3.10), and maximum leave bottle^{-1} (2.50) respectably. Our results are comparing with many researchers also they reported these positive effects of phytohormones on shoot regeneration. The entirely support results by (13) explained that for shoot regeneration, the initial segment BAP is generated in the most advanced processing. However, combination BAP with NAA is an appropriate process for leaf explants micro propagation and production. (14, 15) reported that micro propagation is the most imperative technique. The nodal segment with multiple axillary buds or meristem proliferation, in which terminal bud are cultured to numerous shoots with no one callus regeneration have improved the malignancy of several rose varieties. In addition, PGR manipulation, interaction of (BA, NAA) and carbonate (sucrose, glucose) have been observed in the reproduction of rose varieties. The proliferation with NAA and BAP significantly amplified number of innovative green leaves and axillary shoots also leaves. Also, outcome supported by (16-19).

Root formation

During the rooting research half vigour MS medium supplement with dissimilar concentrations of IBA and NAA was attempted, results obtainable in **Table 3**. The superlative results for rooting of micro shoots were obtained, minimum days for root initiation (9.00), maximum number of roots (6.70) on T5 ½MS+ NAA 1mgl+ IBA 1mg/l. flowed by T4 ½MS+ NAA 1mg/l+ IBA 0.5mg/l, data recorded least days for root initiation (13.00), and maximum number of roots (4.9). Sincerely again control MS alone recorded lower results, minimum days were recorded for root initiation (20.00) and maximum number of roots (2.70). In the experiment our finding T5 ½MS+ NAA 1mgl+ IBA 1mg/l promote and accelerate root formation of rose (*Rosa hybrida* L.). In fact, high and very low principles were also inhibitory for the roots. Our outcomes are comparing with other researchers; the results settled with (13) ½MS medium contains 1.0 mg L^{-1} IBA proved the optimum root germination of rose. The half strength medium was higher as compared to full strength control medium for rooting. For superior rooting of micro shoots half vigor MS media was precious (20-22). (9-23), IBA is a plant auxin PGR that promotes and accelerate root formation in plants. In accumulation, IBA can be worn on ornamental turf to promote flowers progress and fruit to enhance crop yield. Its efficiency the highest, broadly used in the root seedling. Moreover, referred to helpful result of NAA lying on length amplifies and number of roots (24). Also (25) studied that the half meditation of MS medium vitamins, macro and micro salts contains 0.5 mgL^{-1} IBA was mainly appropriate used for in vitro rooting. (26, 27) the effects of NAA and IBA promoters on rooting *in vitro*were rooted. While (28) was observed that rooting media did not have identical effect of all tested genotypes of rose.

Table 3.*Effect of different phytohormones and various concentrations on Days taken to root initiation and number of roots in rose (Rosa hybridaL.)*
Mean with same column, performance significantly different

Treatments	Phytohormones + Concentrations	Days taken in root initiation	Number of Roots
T1	MS^{-1} control free hormones	20.00a	2.70e
T2	½ MS+NAA 1mg/l	13.00bc	4.50c
T3	½ MS+NAA 2mg/l	16.50ab	3.70d
T4	½ MS+NAA 1mg/l+IBA 0.5mg/l	13.00bc	4.9b
T5	½ MS+NAA 1mg/l+IBA 1mg/l	9.00c	6.70a
LSD(5%)		2.7111	0.0592
CV		6.2518	0.1364

Survival

The regenerated plantlets were transferred in different potting mixtures, mixed in equal proportion results are presented in **Figure 1.** Maximum survival (89%) recorded at T2 soil+sand+FYM (1:1:1) was conclude the

greatest potting mixture. Followed by T5 soil+sand+FYM (2:1:1) survival (75%), soil unaided as control recorded lower survival (40%). Our results similarly finding with other researchers, report by height (84%) plantlets survival percentage were recorded in sand+soil+FYM (1:1:1) (29, 30, 31, 25). As well reported 86.24% survival in the tissue cultured plants of rose (20). Although (78.5%) survival of tissue cultured plants were observed in sand+soil+FYM reported by (19).

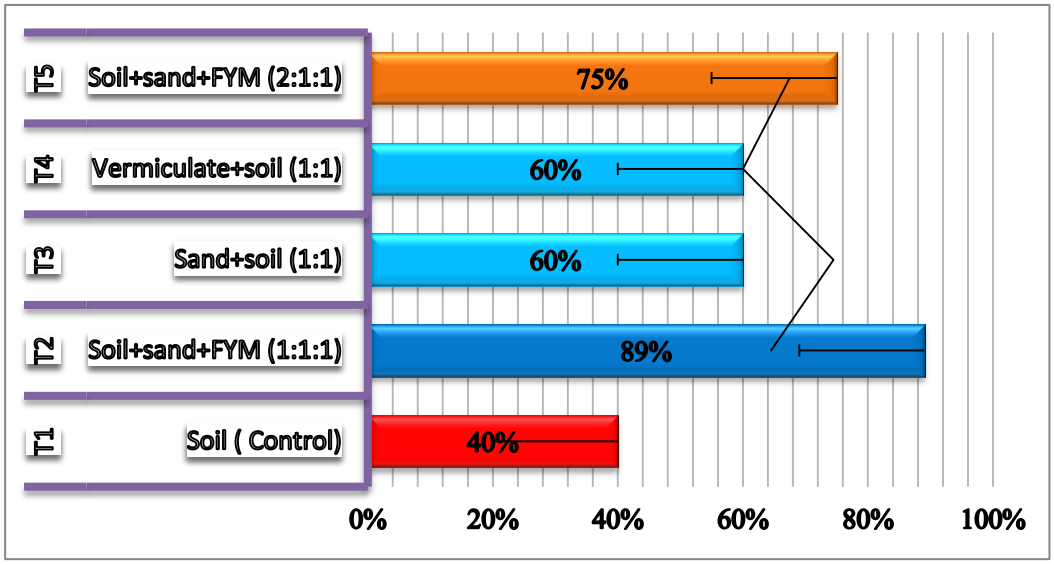

Figure 1. Survival % recorded under dissimilar potting mixtures

CONCLUSION

Present study, graceful and competent method of *In vitro* regeneration for rose has been developed. The disease-free rose plant producing through the help of this protocol. That maybe easily adopted for other species of rose. Indeed, significant result noticed by different phytohormones and their concentrations in rose (*Rosa hybrida* L.). Thus, this experiment clearly viewing that T4 MS+BAP 2mgl^{-1}+ NAA0.5 mgl^{-1} lively perform for shoot regeneration, T5 ^{½}MS+NAA 1mgl+ IBA 1mg/l for root formation and T2 soil+sand+FYM (1:1:1) for survival.

ACKNOWLEDGEMENT

Author thanks to Mr Shahnawaz Palijo and Mr Khalid Hussain Rajput, Incharge of Plant tissue culture laboratory, Sindh Agricutlure Research Insitute, Tandojam, Pakistan, for their cooperation.

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