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Effect of Plant Growth Hormones on Shoot and Root Regeneration in Rose under *In Vitro* Conditions

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Abstract

Background: Rose is a commercially important shrub. This research aimed to observe the influence of different plant growth hormones on development of shoots and roots of *Rosa Indica* L. in tissue culture.

Methods: Various concentrations of N6-Benzylaminopurine (BAP), 1-Naphthaleneacetic acid (NAA), Indole-3-acetic acid (IAA), and Indole-3-butyric acid (IBA) were used in the study. The different concentrations of BAP (2.00, 3.00, and 4.00 mg l^{-1}) and IAA (2.00 and 3.00 mg l^{-1}) were tested for shoot induction. While varying concentrations of IAA and IBA were analyzed for root proliferation.

Results: The results of the study indicated that the fastest shoot initiation (17.77 days), the highest number of shoots $bottle^{-1}$ (3.55), the maximum shoot length (4.72 cm), and the utmost number of leaves $bottle^{-1}$ (53.67) were observed on MS media containing 3.00 mg l^{-1} BAP, 3.00 mg l^{-1} IAA, and 30 g l^{-1} sugar, while the highest number of shoots $bottle^{-1}$ were produced under MS + 2.00 mg l^{-1} BAP + 3.00 mg l^{-1} IAA + 30 g l^{-1} sugar. Regarding root induction, the maximum number of roots (4.67) and root length (2.60 cm) were observed under half strength MS media supplemented with 30 g l^{-1} sugar.

Conclusion: The study suggested that MS media containing 3.00 mg l^{-1} BAP and IAA could be used for tissue culturing rose plants. For root induction, half-strength MS media, along with sugar, could be used. The study gives an insight into potential media compositions for the propagation of rose. The suggested media can have promising uses in commercial multiplication of this important plant.

Introduction

Rose is a commercially important shrub, belonging to genus Rosa of *Rosaceous* family. It has a significant role in horticulture trade in Pakistan and contributes up to 50% towards the same. However, floriculture techniques are in initial stages in the country, and therefore, the country's share in international floriculture market remains only 3%. Rose is very popular among flowers because of its fragrance and beauty [1]. The genus *Rosa*, constituting more than 150 species and 1400 cultivars, are grown all over the world for rootstocks and floral displays [2]. Hence, rose can be listed among the top demanded cut flowers globally and has a great economic value. The major rose producing nations include Colombia, Kenya, Netherlands, Italy, Israel, and the United States [3].

Tissue culture techniques, such as micropropagation, can be employed for rapidly multiplying the plant material. This approach may also abolish the requirement for grafting onto rootstocks [4]. Therefore, micropropagation has been widely recognized as a tool for rapidly multiplying disease-free and uniform rose plants [5, 6]. Further, micropropagation can also be used in rose breeding for introducing new cultivars having ameliorated horticultural characteristics [7].

The success of micropropagation protocols hinges on the rate and mode of shoot multiplication. The parameters like species, inorganic salts and organic components, genotype under consideration, and media composition impact the shoot multiplication. Further, the role of physical factors such as temperature and light is also important [5]. Propagation of plant material by tissue culture has traditionally been deemed as an essential strategy to proliferate plants which are otherwise difficult to multiply through seed and other natural methods [8].

Currently, propagation of roses is typically done through pruning on commercial level; however, other methods such as sprouting and grafting can also be used. Nevertheless, all these approaches are laborious and time consuming. The slow rates of multiplication as well as influence of climatic factors are significant limitations to the conventional expansion [9]. Moreover, some pink species are difficult to distinguish for use in such practices. On the other hand, tissue culture has become a popular alternative for rose vegetation [10]. Micropropagated plants have several other advantages too. These plants are better suited for the production of cut flowers as they are more compact. Moreover, they branch relatively better and form abundant flowers [9]. Important attributes of *in vitro* expansion also include its excellent qualitative potential and the possibility of production of healthy and disease-free plants all the year-round [11].

The growth and development of plant tissues is a tightly regulated phenomenon. Specialized cells are formed at the right time during the development. The plant development is regulated through hormones and therefore, such hormones must be artificially supplied at a certain appropriate time in tissue culture. The two most important categories of hormones used in tissue culture are cytokinin and auxin [12]. These hormones promote cell division and specialization, and the growth of stems, leaves, and roots. It is essential to introduce different hormones in the tissue culture at a particular time as they have different roles and the hormone enhancing stem and leaf growth and development can, on the other hand, impede root formation. Tissue culture practices should reduce the time required to bring new cultivars into the market and thereby increase the availability of plants having better agronomic characteristics [7].

For *in vitro* rose tissue culture for commercial purposes or for research, a responsive and fast shoot organogenesis protocol is needed [2]. Therefore, the current study was planned to develop an effective tissue culture method for rose. Different hormone concentrations were used in the media and an effect on the *in vitro* proliferation of rose was recorded. The main objective of the study was to establish a protocol for the production of a large number of shoots from the nodal explants of rose under controlled conditions. This research is of significant importance for the commercial multiplication of roses.

Methods

The research was conducted at Tissue Culture Research Laboratory, Plant Breeding & Genetics Division, Nuclear Institute of Agriculture Tando Jam. The experiment was performed using a complete randomized design with three replications. The rose explants were picked from the agricultural farm of the institute. Various concentrations of plant growth hormones, i.e., N6-Benzylaminopurine (BAP), 1-Naphthaleneacetic acid (NAA), indole-3-acetic acid (IAA), and indole-3-butyric acid (IBA) were tested.

Five different concentrations, as given below, were employed for shoot induction.

1. MS + 2.00 mg l^{-1} IBA + 2.00 mg l^{-1} IAA + 30 g per liter sugar
2. MS + 2.00 mg l^{-1} BAP + 3.00 mg l^{-1} NAA + 30 g per liter sugar
3. MS + 2.00 mg l^{-1} BAP + 3.00 mg l^{-1} IAA + 30 g per liter sugar
4. MS + 4.00 mg l^{-1} BAP + 2.00 mg l^{-1} IAA + 30 g per liter sugar
5. MS + 3.00 mg l^{-1} BAP + 3.00 mg l^{-1} IAA + 30 g per liter sugar

Moreover, five different compositions were used for root induction. The media compositions were as following

1. ½MS + 30 g per liter sugar
2. MS + 0.50 mg l^{-1} IBA + 30 g per liter sugar
3. MS + 1.50 mg l^{-1} IBA + 30 g per liter sugar
4. MS + 3.00 mg l^{-1} IAA + 2.00 mg l^{-1} IBA + 30 g per liter sugar
5. ½MS + 3.00 mg l^{-1} IAA + 3.00 mg l^{-1} IBA + 30 g per liter sugar

The pH of the media was set to 5.8 before autoclaving (121^{o}C and 15 lbs for 2 hours) [13]. Nodal explants containing lateral buds of rose were utilized for multiplication. The explants were cut in 3-4 cm long pieces and then disinfected using 70 % ethanol for 30 seconds. Subsequently, the explants were inundated in 10% sodium hypochlorite solution of commercial laundry

bleach (5.25% NaOCl) containing Tween-20 [2]. The growth media was solidified using gel rite (5 g l^{-1}) as a solidifying agent. Cultures were placed in a growth chamber with an air temperature of 25°C and 16 hours per day photoperiod provided by cool white fluorescent lamps [14].

The study involved the following steps. The plants were selected for micropropagation. From these plants, nodal sections were prepared and transferred to the appropriate culture medium. Then, multiple shoots were induced (usually 5-6 shoots each in 4-5 weeks). Lastly, the shoots were stimulated for rooting.

Statistical analysis

The experimental data were analyzed through factorial design of analysis of variance (ANOVA) under the linear model to determine the statistical differences among different concentrations of plant growth hormones employing Student Edition of Statistics [15]. The least significant difference (LSD) test was applied among different combinations [16].

Results

Days taken to shoot initiation

The different compositions of media yielded significant differences for days taken to shoot initiation. The development of shoots was seen in the shortest period of 17 days under the media composition of MS + 3.00 mg l^{-1} BAP + 3.00 mg l^{-1} IAA + 30 g per liter sugar. Moreover, MS media supplied with 2.00 mg l^{-1} BAP + 3.00 mg l^{-1} IAA + 30 g per liter sugar produced shoots in 22.0 days. Contrarily, the slowest shoot initiation was observed under the media composition of MS + 2.00 mg l^{-1} IBA + 3.00 mg l^{-1} IAA + 30 g per liter sugar (31.11 days).

Number of shoots bottle^{-1}

The maximum number of shoots bottle^{-1} (3.55) were observed under MS media having 2.00 mg l^{-1} BAP + 3.00 mg l^{-1} IAA and 30 g per liter sugar, followed by media containing MS + 3.00 mg l^{-1} BAP + 3.00 mg l^{-1} IAA + 30 g per liter sugar (3.43). However, the minimum number of shoots bottle^{-1} (1.22) were produced under the media composition of MS + 2.00 mg l^{-1} IBA + 2.00 mg l^{-1} IAA + 30 g per liter sugar.

Concentrations	Days to shoot initiation	Number of shoots bottle^{-1}	Shoot length (cm)	Number of leaves bottle^{-1}
MS + 2.00 mg l^{-1} IBA + 2.00 mg l^{-1} IAA + 30 g l^{-1} sugar	27.00 b	1.67 c	2.91 d	32.00 c
MS + 2.00 mg l^{-1} BAP + 3.00 mg l^{-1} NAA + 30 g l^{-1} sugar	31.11 a	1.22 c	2.43 e	21.55 d
MS + 2.00 mg l^{-1} BAP + 3.00 mg l^{-1} IAA + 30 g l^{-1} sugar	22.00 c	3.55 a	3.75 c	33.00 c
MS + 4.00 mg l^{-1} BAP + 2.00 mg l^{-1} IAA + 30 g l^{-1} sugar	24.10 c	2.56 b	4.41 b	45.22 b
MS + 3.00 mg l^{-1} BAP + 3.00 mg l^{-1} IAA + 30 g l^{-1} sugar	17.77 d	3.43 a	4.72 a	53.67 a
SE	0.9361	0.2056	0.0374	0.9481
LSD (5%)	2.1586	0.4742	0.0864	2.1863

Table 1: Effect of plant growth hormones on days to shoot initiation, shoot length, number of shoots, and number of leaves for rose micropropagation under different concentrations of BAP, IAA, and NAA (Means followed by different letters in the same column are significantly different).

Shoot length

The shoot length of the plantlets was also seen to vary among different compositions of media. The longest shoot length of 4.72 cm was achieved under the full-strength MS media supplied with 3.00 mg l^{-1} BAP + 3.00 mg l^{-1} IAA and 30 g per liter sugar. MS media containing 4.00 mg l^{-1} BAP + 2.00 mg l^{-1} IAA and 30 g per liter sugar also produced good shoot length of 4.41 cm. The shortest shoots were observed in MS media having 2.00 mg l^{-1} BAP, 3.00 mg l^{-1} NAA, and 30 g per liter sugar (2.43 cm).

Number of leaves bottle^{-1}

The highest number of leaves bottle^{-1} (53.67) were recorded under the MS media supplemented with 3.00 mg l^{-1} BAP + 3.00 mg l^{-1} IAA + 30 g per liter sugar. Moreover, the compositions MS + 4.00 mg l^{-1} BAP + 2.00 mg l^{-1} IAA + 30 g per liter sugar and MS + 2.00 mg l^{-1} BAP + 3.00 mg l^{-1} IAA + 30 g per liter sugar produced 45.22 and 33.0 leaves bottle^{-1}, respectively. The minimum numbers of leaves were produced under MS + 2.00 mg l^{-1} BAP + 3.00 mg l^{-1} NAA + 30 g per liter sugar (21.55 leaves).

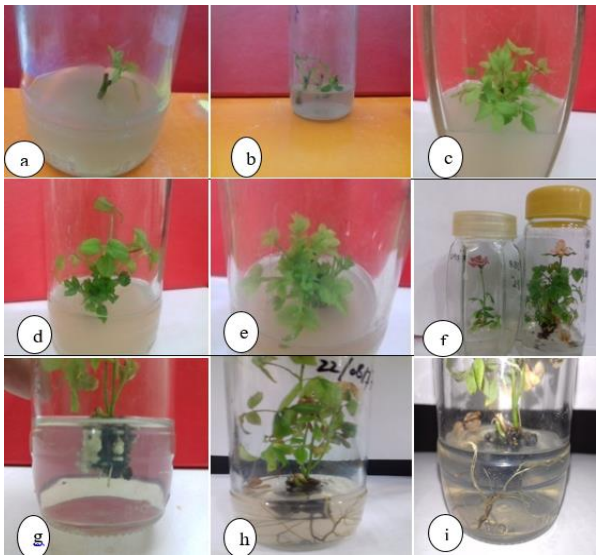

Figure 1: (a) and (b) show shoot initiation in plantlets; (c), (d), and (e) demonstrate the development of leaves and shoots; (f), (g), (h), and (i) indicate root induction and development.

Number of roots bottle^{-1}

Root parameters were also investigated using various concentrations of IBA and IAA in MS media. The maximum number of roots bottle^{-1} were formed in half-strength MS media supplemented with 30 g per liter sugar followed by full-strength MS media having 0.50 mg l^{-1} IBA + 30 g per liter sugar. These concentrations of media produced 4.67 and 4.43 roots bottle^{-1}, respectively. The least number of roots bottle^{-1} (10) were observed on half-strength MS media containing 3.00 mg l^{-1} IAA + 3.00 mg l^{-1} IBA + 30 g per liter sugar.

Root length

The longest roots of 2.60 cm were observed on ½MS media containing 30 g per liter sugar followed by 2.19 cm

long roots in ½MS media supplied with 30 mg l^{-1} IAA, 3.00 mg l^{-1} IBA, and 30 g per liter sugar. Contrarily, the full-strength MS media containing 1.50 mg l^{-1} IBA + 30 g per liter sugar yielded shortest roots (1.01 cm).

Concentrations	Number of roots $bottle^{-1}$	Root length (cm)
½MS + 30 g l^{-1} sugar	4.67 a	2.60 a
MS + 0.50 mg l^{-1} IBA + 30 g l^{-1} sugar	4.43 a	1.72 d
MS + 1.50 mg l^{-1} IBA + 30 g l^{-1} sugar	3.55 b	1.01 e
MS + 3.00 mg l^{-1} IAA + 2.00 mg l^{-1} IBA + 30 g l^{-1} sugar	2.77 c	2.01 c
½MS + 3.00 mg l^{-1} IAA + 3.00 mg l^{-1} IBA + 30 g l^{-1} sugar	1.00 d	2.19 b
SE	0.1317	0.0220
LSD (5%)	0.3038	0.0506

Table 2. Effect of plant growth hormones on the number of roots and root length for rose micropropagation under different concentrations of IBA and IAA (Means followed by different letters in the same column are significantly different).

Discussion

This research aimed to observe the influence of different plant growth hormones on shoot and root development in rose. Commercial propagation of rose through tissue culture carries significant importance as conventional approaches of propagation are extremely slow and tedious. Contrarily, tissue culture can facilitate the rapid propagation of this commercially important plant. In this study, optimal shoot development, the maximum shoot length, and the highest number of leaves $bottle^{-1}$ were shown by plantlets grown under media supplied with 3.00 mg l^{-1} BAP. While the maximum number of shoots $bottle^{-1}$ were produced by seedlings grown in 2.00 mg l^{-1} BAP. These observations indicated an essential role of BAP in shoots and leaves formation. Earlier, BAP has been used for shooting from nodal explants in MS medium for *in vitro* development of various plants [47].

Soomro *et al*. [17] noticed that BAP, IAA, and sugar are important components for shoot proliferation and multiple axillary shoots formation. They recorded profuse shoot development in media having 1.00 mg l^{-1} IAA, 4.00 mg l^{-1} BAP, and 30 g per liter sugar. IAA was also proposed to aid copious shootlets formation as it enhanced the length of shootlets in the presence of cytokinin. While, BAP and IBA, in combination, led to bulbous formation. The presence of NAA (0.1 and 0.5 mg l^{-1}) along with BAP was also seen to decelerate the axillary shoot development significantly. In our study, BAP showed a stimulating response to shoot formation while IAA assisted the role of BAP for multiple shoots development. Parallel to the proposed role of BAP in shoot development, Pittet and Moncousin [18] also used MS media supplied with BAP and IBA for shoot initiation [19]. Similarly, Norton and Boe [20] employed MS media and attained good proliferation under 0.1-2.5 mg l^{-1} concentration of BAP.

Nizamani *et al*. [2] reported the maximum number of roots and root length under half-strength MS media containing 30 g per liter sugar and 2.00 mg l^{-1} IBA. IBA is an auxin plant growth regulator involved in stimulating and accelerating the root formation. Therefore, it is widely used for improving the rooting of tissue cultured plantlets. Our results were in agreement with the reports of Ozel and Arsalan [6] and Chakrabarty *et al*. [21]. Moreover, Sultana *et al*. [22] also proposed that medium containing ½MS supplied with 10 mg l^{-1} IBA provided the best induction in rose. However, in our study, plants cultured in half-strength MS media supplied only with sugar also production excellent rooting.

Auxins have been reported to be significant role-players in root formation. Low concentrations of these growth regulators produce better rooting. Many studies have proposed IAA as well as the combinations of IAA and IBA in lower concentrations for inducing root formation [23, 24]. In some studies, combinations of two auxins have been found more effective in root formation rather than either of the auxins alone [25]. Kosh-Khui and Sink [26] mentioned that optimum root formation was achieved under 0.1 mg l^{-1} NAA and 0.05 mg l^{-1} of either IBA or IAA. However, parallel to our results, Ibrahim and Debergh [27] achieved root formation in the absence of auxin. In another study, Kanakis and Demetriou [28] reported optimal root formation in plants grown in media containing NAA (2.0 mg l^{-1}).

In agreement with our observation of highest root formation in the absence of any growth hormones, Davies [29] attained 100 % rooting in many genotypes of the rose by employing MS media devoid of growth regulators when supplied with 40 g/l of sucrose. In our experiment, quick root formation was observed under half-strength MS medium provided with 30 g per liter of sugar. Media supplemented with a low concentration of IBA also showed similar results; however, root length was seen to decrease significantly in this treatment. Nevertheless, Soomro *et al*. [17] reported maximum rooting under ½MS + 0.5 mg l^{-1} IBA + 30 g per liter sugar. Importantly, a composition having no supplementation of IBA was not used in their study, which was employed in ours. We observed that the absence of IBA or IAA in the media stimulated higher root formation. This observation was also parallel to the report of Salekjalali *et al*. [30]. Moreover, it was seen in this study that the half-strength MS media favored higher root development against full-strength media in agreement with the previous research of Nak-Udom *et al*. [31].

Disparate reports have been published over the years regarding the impact of full and half strength media in the initiation of shoots and roots. Kim *et al*. [24] documented excellent shoot proliferation in full-strength MS media, whereas, better rooting was observed under ¼ strength [32]. On the other hand, Iapichino [33] and Ancora [34] employed ½ strength MS media for optimal rooting. Our results supported the use of full-strength MS media for shoot formation while ½ strength MS media was found excellent for rooting.

This study explored various media compositions for tissue culture of rose. Rose is a commercially important plant and optimized strategies for its rapid multiplication can have economic outcomes. Therefore, the concentrations suggested in this study can be employed for excellent shooting and rooting to propagate rose.

It was concluded that micropropagation of *Rosa indica* can be accomplished from stem source as explants under aseptic conditions. The concentration of MS + 3.00 mg l^{-1} BAP + 3.00 mg l^{-1} IAA + 30 g per liter sugar proved better for shoot induction, while the composition of MS +

2.00 mg l^{-1} BAP + 3.00 mg l^{-1} IAA + 30 g l^{-1} sugar also produced good shooting. For root induction, the media containing ½MS + 30 g per liter sugar and MS + 0.5 mg l^{-1} IBA + 30 g per liter sugar yielded excellent results. The proposed media concentrations can be used on large scale for commercial rose propagation.

Author Contributions

MAC, MTK, and GSN conducted the research work. SY and IAK supervised the experiment. MMA, AAR, and T wrote the first draft of the paper. FN and MRN helped in statistical analysis of data. RI, MJP, and MAS proofread the paper critically and revised the manuscript.

Competing Interest

All authors declare no conflicts of interest in this paper.

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