Bangladesh J. Agri. 2022, 47(1): 66-74 *DOI: https://doi.org/10.3329/bjagri.v47i1.60593*

STUDY ON IN VITRO MICROPROPAGATION OF *ROSA SP.*

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Abstract

Tissue culture has long been recognized as an ideal experimental paradigm for studying the mechanisms governing plant cell growth, division, and physiological and biochemical processes. The present investigation was undertaken to establish a standard method for callus induction, shoot and root regeneration of rose at Genetic Engineering and Biotechnology Laboratory, University of Rajshahi, Bangladesh in 2010. Shoot tip and nodal segments were used as experimental materials excised from field grown plants for callus induction. The direct shoot was observed from both shoot tip and nodal segments for shoot regeneration. *In vitro* regenerated shoot cuttings were used for root regeneration. Proper manipulation of auxin (2, 4 -D and NAA) was used to induce callus from different explants. Different growth regulators (BAP, KIN and GA_3) were casted-off in combination for shoot regeneration and proliferation. Again, different concentrations of auxins (IBA, NAA, and IAA) were applied for root initiation. Among the hormonal supplements used, 2, 4 -D was found best in all respect of callusing response for all types of explants. The highest percentage (90%) of callus induction was observed in media having MS (Murashige and Skoog) + 4.0 mg/L 2, 4 -D. In case of shoot regeneration, 100% of cultured explants regenerated shoot in media with MS + 2.0 mg/L BAP + 0.5 mg/L KIN. While, 2.0 mg/L BAP + 0.5 mg/L KIN + 0.1 mg/L GA_3 induced 100% shoot proliferation. Moreover, for root induction, ½ MS + 1.0 mg/L IBA + 1.0 mg/L NAA proved to be the best (80%) from *in vitro* regenerated shoot cuttings, and the highest mean number of roots was 5.0. Rooted shoots were acclimatized and successfully established in a natural condition where 60% of the transplanted plants survived.

Keywords: Callus induction, Hormones, Micro propagation, Regeneration, Rose root induction, Shoot proliferation

Introduction

Rosa is a genus of approximately 100 species that can be found throughout the Northern Hemisphere's temperate and subtropical climates (Rehder, 1960). The number of chromosomes ranges from 2n = 2x = 14 to 2n = 8x = 56. (Darlington and Wylie, 1955). The rose genome has some of the lowest DNA content of any Angiospermae with *R. wichuraiana* (2n = 14) having a 4C value of only 0.45-0.48 pg (Lloyd, 1986). Hybrid

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Tea (big flowered), Floribunda (cluster flowered), and tiny roses are cultivars that are currently most widely grown in gardens as aesthetic plants (Short and Roberts, 1991). Hybrid Tea and miniature roses are also produced under glass to sell cut flowers and pot plants. Each of these rose classes can bloom at any time during the growing season. The rose, on the other hand, is connected with beauty and special occasions all across the world, and it is the most popular decorative plant in many nations (Loo, 1982). Every year, more than 200 million rose bushes are planted in gardens around the world, representing US$720 million retail market (Short and Roberts, 1991). Sales of almost four billion blossoms with an estimated annual retail value of US$11 billion demonstrate the rose's prominence as a cut flower. Approximately 30 million field-grown plants and 0.5 million cut flowers are marketed in the United Kingdom each year. Cut flowers are in higher demand in continental Europe with 900 million sold annually in just one market at Aalsmere (Holland). On the other hand, rose oil is in high demand in the perfume industry (Krussmann, 1981; Kukreja *et al.*, 1989; Chomchalow and Sahavacharin, 1982). A recessive gene is responsible for this "perpetual blossoming" trait (Hurst, 1941). Wylie (1954) highlighted the modern garden rose's limited genetic foundation claiming that only eight species contributed considerably to their gene pool. Introgressive hybridization attempts to introduce novel genes into current garden roses are hampered by F_1 sterility, which might result from variations in the parents' ploidy levels or chromosomal incompatibility (Short and Roberts, 1991). *R. rugosa* cultivars are commonly used in amenity horticulture, especially for roadside verges and urban environments. They have a longer flowering season than other "species" of roses, despite not having the trait for eternal flowering. The majority of cultivars are very heterozygous and do not produce true to type offspring. As a result, they are vegetatively propagated (Short and Roberts, 1991). Other classes, such as *R. canina* 'Inermis,' *R. multiflora* 'Simplex,' and *R. dumentorum* 'Laxa,' are propagated by budding or grafting onto root stocks of species like *R. canina* 'Inermis,' *R. multiflora* 'Simplex,' and *R. dumentorum* 'Laxa' (Short and Roberts, 1991). The benefits and drawbacks of "own-rooted" plants are significant factors to consider while evaluating the market for micro-propagated roses. Micropropagation is a technique for multiplying significant cultivars using aseptic tissue culture procedures (Short and Roberts, 1991).

Micro-propagation has numerous advantages and applications in commercial nursery operations (Chu, 1986; McCown, 1986; Pierik, 1987; Stimart, 1986). New plantlet within a short period after utilizing a small amount of initial plant tissue, viruses and diseases free plantlet, season independent supply of seedlings throughout the year after utilizing ten times lower space than the conventional technique supplying new subspecies and variety and looking to cultivate challenging plants, such as specific breeds of roses to find more success with the tissue culture process than traditional soil can be ensured by exploiting micropropagation/tissue culture technique (Plant Cell Technology, 2022) in this era of climate change, especially in Bangladesh. In recent years, worldwide research activity on the *in vitro* method of micropropagation has expanded substantially (Murashige, 1977). Bangladesh is no exception when it comes to efforts in poor countries. As a result, this research was carried out to develop a standard method of *in vitro* micropropagation of *Rosa sp.* to encourage commercial production of the plant in Bangladesh.

Materials and Methods

In the present investigation, *Rosa sp.* (L.) was used as experimental material. For *in vitro* culture, shoot tips, nodal segments, leaves, and internodes were used as explants. Explants were collected from the Botanical garden of Rajshahi University. MS (Murashige and Skoog, 1962) media were used for primary culture and their subsequent subculture, callus induction, shoot differentiation and root induction. In all cases, sucrose was added at the rate of 30 g/ml. Growth regulators were added separately to different media according to requirements. Agar was added at the rate of 7 g/ml. Different constituents of MS including growth regulators of the culture media were separated into stock solutions for ready use during the preparation of culture media. Shoots of *Rosa sp.* (L.) were cut into pieces, namely shoot tips, nodal segments, leaves, and internode segments carefully with the help of a forceps and dissecting blade. These explants were taken into a conical flask and thoroughly washed under running tap water for 30 minutes to remove loose contaminates attached to explants. Then these explants were washed with distilled water containing 1% sterilant, namely savlon (v/v) and 2 drops of Tween-80 for 20 minutes to remove gummy substance. This was followed by three successive washing with distilled water to make the material free from savlon. Subsequently, the materials were transferred to running laminar air flow hood. The shoot apices were taken into three sterile conical flasks and suspended in different concentrations of sodium hypochlorite for different periods of 1-10 minutes to ensure a contamination free culture. To remove every trace of the strident, the materials were then washed at least six times with sterile distilled water. The pH was adjusted to 5.8 by using KOH or HCl. The inoculated culture vessels were incubated in a growth chamber containing a special culture environment. The vessels were placed on the shelves of a cupboard in the growth chamber. Unless mentioned specially, cultures were grown in the growth chamber illuminated by 40 watts white fluorescent tubes fitted at a distance of 30-40 cm from the culture shelves. The cultures were maintained at 26 ± 1°C under the warm fluorescent light intensity varied from 2000 to 3000 lux. Generally, the photo-period was maintained as 16 hours light and 8 hours dark. The vessels were checked daily to observe the response.

Results and Discussion

Callus induction is the prerequisite on the way to generate somaclonal variation in plants. MS medium was employed with various concentrations of auxin alone or in combination of auxin and cytokinin to see the callus induction efficiency of different explants. Among them, the highest percentage (90%) of very good callus (+++) induction was observed in MS + 4.0 mg/L 2,4-D, it took 15-20 days to form a complete callus, and the minimum percentage was 40% in MS + 2.0 mg/L 2,4-D after 28-30 days (Fig. 1A). It was white in color, but no regeneration was found when they were sub cultured in a new medium. When the explants were treated with NAA alone, the highest percentage of callus induction was 80% with MS + 4.0 mg/L NAA after 18-20 days and the lowest was 60% with MS + 2.5 mg/L NAA after 28-30 days (Table 1).

In this experiment, 10 different concentrations of BAP + KIN were used to observe their effects on multiple shoot induction from nodal segments. Among those concentrations, 2.0 mg/L BAP+ 0.5 mg/L KIN showed the highest (100%) shoot induction, the maximum number of shoots per plant was 6 and mean length was 4.0 cm approximately after 7-8 days (Table 2). Minimum shoot induction was observed (55%) in 1.0 mg/L BAP + 0.1 mg/L KIN and the number of shoots per plant were 3 and mean length was 1.8 cm approximately after 26-28 days (Table 2).

Table 1. Effects of different concentrations of Auxin in MS medium for callus induction from nodal segments.

Hormonal supplements mg/L	No. of explants inoculated	% of explants produced callus	Mean days to Callusing	Degree of formation of Callus
2,4-D				
2.0	20	40	29.00	+
2.5	20	50	26.00	+
3.0	20	75	19.00	++
4.0	20	90	17.50	+++
4.5	20	70	20.00	++
Mean	20	65	22.30	-
SE	0.00	8.94	2.21	-
NAA				
2.5	20	60	29.00	+
3.0	20	75	21.00	++
4.0	20	80	19.00	+++
5.0	20	70	23.00	++
5.5	20	70	26.00	++
Mean	20	71	23.60	-
SE	0.00	3.32	1.78	-

2, 4 D = Auxin; NAA = Naphthalene Acetic Acid; SE = Standard Error; + = Good; ++ = Better and +++ = Best.

For shoot proliferation growth regulators, especially Cytokinins are one of the most important factors affecting the response (Lane, 1979; Bhojwani, 1980). GA_3 helps in increasing shoot length, MS medium supplemented with different combinations and concentrations of BAP, KIN, and GA_3 were used to observe the growth of *in vitro* cultured shootlets. Results are shown in Table 3. Among all the combinations, 2.0 mg/l BAP + 0.5 mg/L KIN + 0.1 mg/L GA_3 showed very significant (100%) proliferation of shoot (Fig. 1B). Number of shoots per explant was 4 and mean length of shoot was 2.50 cm after 28 days.

Media with different hormonal combinations and concentrations, such as (IBA + NAA, IBA + IAA, IBA + IAA + NAA) were used. Root induction was observed significantly in some media. Medium supplemented with different concentrations (0.2 + 0.2, 0.2 + 0.5, 0.5 + 0.5, 1.0 + 1.0, and 1.0 + 1.5 mg/L) of IBA + NAA were tested to observe the effect of this auxin on root induction with different concentrations. The highest percentage of root induction was found supplemented with 1.0 mg/L IBA + 1.0

Table 2. Effects of different concentrations and combinations of BAP and KIN in MS medium for shoot induction from nodal segments of mature plants.

Hormonal supplements mg/L	No. of explants inoculated	% of explants in which shoot regenerated	Highest no. of shoot produced	Mean length (cm) of longest shoot	Days to shoot regeneration	Degree of shoot growth
BAP+ KIN						
1.0+0.1	20	55	3	1.8	27.00	+
1.5+0.1	20	60	4	2	22.00	+
2.0+0.1	20	65	4	2.5	22.00	++
2.0+0.2	20	88	5	3.5	11.00	+++
1.0+0.2	20	70	4	2.0	19.00	++
2.0+0.4	20	75	4	3.0	18.00	++
2.0+0.5	20	100	6	4.0	7.50	+++
2.5+0.5	20	75	4	3.20	13.50	++
2.5+1.0	20	65	3	3.10	22.50	+
2.5+1.5	20	60	4	2.50	24.00	+
Mean	20	71.30	4.10	2.76	18.65	-
SE	0.00	4.39	0.28	0.23	1.96	-

BAP = Benzyl Amino Purine; KIN = Kinetine; SE = Standard Error; + = Good; ++ = Better and +++ = Best.

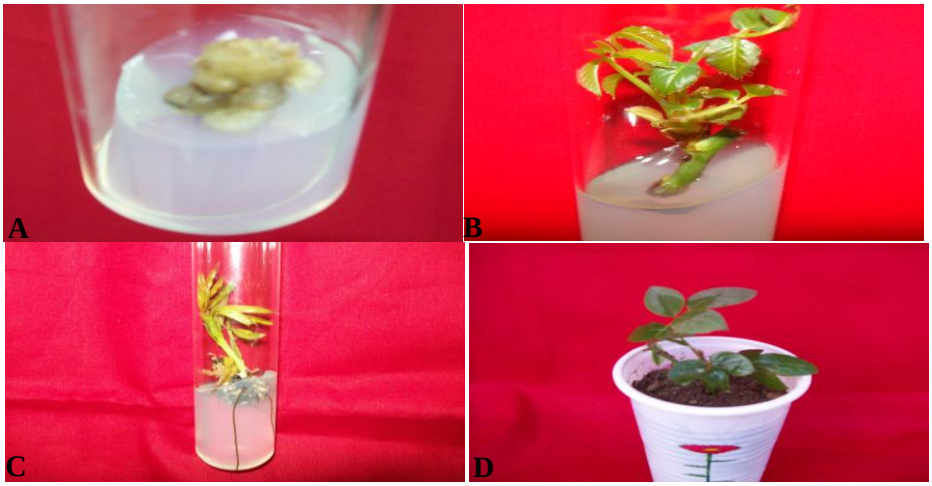

Fig. 1. A-D Plant regeneration in *Rosa sp.* (L.). A. Callus induction with 4.0 mg/L 2, 4-D. B. Multiple shoot regeneration and elongation with MS+2.0 mg/L BAP+0.5 mg/L KIN C. Root induction with ½ MS+1.0 mg/L IBA+1.0 mg/L NAA. D. Potting of rose plant

mg/L NAA. In this case, 80% of the explants produced root within 15-20 days (Fig. 1C). Mean number of roots per explant was 4 and mean length of the longest root was 6.3 cm.

Different concentrations (0.5 + 0.5, 0.5 + 1.0, 0.5 + 1.5, 1.0 + 0.2 and 1.0 + 0.5 mg/L) of IBA + IAA were applied to observe the effect of this auxin on root induction with different concentrations. The highest percentage of root induction was found supplemented with 1.0 mg/L IBA + 0.5 mg/L IAA. In this case, 70% of the explants produced root within 15-18 days.

Table 3. Effects of different concentrations and combinations of BAP, KIN and GA_3 in MS medium for shoot proliferation from explants of *in vitro* cultured plants (Results after 28 days).

Hormonal supplements mg/L	No. of explants inoculated	% of explants proliferated shoot	Mean no. of shoot per explant	Mean length of shoot
BAP+KIN+GA_3				
1.0+0.1+0.01	15	75	1.70	1.34
1.5+0.1+0.05	16	80	2.15	1.50
1.5+0.2+0.07	16	80	2.28	1.26
1.5+0.5+0.10	18	90	3.61	2.44
1.0+0.2+0.10	17	85	2.50	2.00
2.0+0.2+0.10	17	85	2.42	1.98
2.0+0.5+0.10	20	100	4.00	2.50
2.5+0.5+0.10	18	90	3.55	2.00
2.5+1.0+0.15	17	85	2.71	1.90
2.0+1.0+0.15	16	80	1.80	1.16
Mean	17	85	2.67	1.81
SE	0.45	2.24	0.25	0.15

BAP = Benzyl Amino Purine; KIN = Kinetine; GA3 = Gibberellic Acid; SE = Standard Error.

Mean number of roots per explant was 5, and mean length of the longest root was 6.3 cm IBA + IAA + NAA with different concentrations (ranges from (0.5 + 0.2 + 0.2 to 1.0 + 1.0 + 1.0)) were used and 60% explants produced root with the combinations of 0.5 mg/l IBA + 0.5 mg/L IAA + 0.5 mg/L NAA within 18-22 days, mean number of roots were 3.2, and mean length was 3.1 cm (Table 4). About 50% of plant survived following a hardening stage. Micro-propagated *Rosa sp.* (L.) once established in soil, showed vigorous and uniform growth (Fig. 1D). No morphological abnormalities have been observed.

The present study provides a method that ensures micropropagation of *Rosa sp.* (L.). Auxin is carefully required for the induction of callus from variety of tissue explants except cambial tissue that proliferate without an exogenous supply of Auxin. Many researchers observed, 2, 4-D as the best Auxin for callus induction as common as even in dicot (Nadel *et al.,* 1989). Similar effects were seen during using 2, 4 -D for callus induction. Duran villa *et al.,* (1989) reported that nodal segments of citrus species (seedling) showed good shoot in MS medium supplemented with 3.0 mg/L BAP.

Hasegawa (1980) reported that nodal explants of rose responded better in BAP-KIN combination with BAP. The findings of the present investigation showed full similarities with the reports of Hasegawa (1980). The best rooting (up to 100%) was obtained by culture on half strength MS medium with 0.15 mg/L NAA (Ognjanov *et al.*, 1989). In some cases, more than one auxin acted synergistically for the induction of roots e.g., IBA + NAA (0.2 mg/L each) for guava (Duran villa *et al.*, 1989) were used for successful root induction. In the present investigation also show the similarities with the previous experiments. This technique described here seemed to be adaptable for large micropropagation on *Rosa sp.* (L.) and can be applied for economic purposes.

Table 4. Effects of different concentration and combination of auxin in ½ strength MS medium for root induction from *in vitro* regenerated shoot cuttings.

Hormonal supplements for rooting	No. of shoots sub cultured	Shoots derived from the explants of mature plants.			
		% of explant produced root	Days to root regeneration	Mean no. of roots	Mean length of roots (cm)
IBA + NAA					
0.2 + 0.2	18	10	27.50	2.7	3.0
0.2 + 0.5	18	40	21.00	2.9	4.0
0.5 + 0.5	20	60	19.00	3.2	4.2
1.0 + 1.0	20	80	17.50	5.0	6.3
1.0 + 1.5	20	50	20.00	3.9	5.0
Mean	19.20	48	21.00	3.54	4.50
SE	0.49	11.58	1.72	0.42	0.54
IBA + IAA					
0.5 + 0.5	15	40	22.50	2.5	2.0
0.5 + 1.0	15	50	22.50	3.6	2.5
0.5 + 1.5	18	50	22.50	2.3	3.0
1.0 + 0.2	18	60	19.00	3.9	4.5
1.0 + 0.5	18	70	16.50	4.0	6.0
Mean	16.80	54	20.60	3.26	3.60
SE	0.73	5.10	1.23	0.36	0.73
IBA + IAA + NAA					
0.5 + 0.2 + 0.2	15	30	27.50	2.8	2.5
0.5 + 0.5 + 0.2	15	40	27.50	2.7	3.0
0.5 + 0.5 + 0.5	15	60	20.00	3.2	3.1
1.0 + 0.5 + 0.5	15	50	21.00	2.1	2.5
1.0 + 1.0 + 1.0	15	50	21.00	2.4	3.5
Mean	15	46	23.40	2.64	2.92
SE	0.00	5.10	1.68	0.19	0.19

IBA = Indole Butyric Acid; NAA = Naphthalene Acetic Acid; IAA = Indole Acetic Acid; SE = Standard Error.

Conclusion

For year-round supply of rose and rose products need to have a reliable and disease-free propagule by adopting a sound method where micropropagation would be a panacea. Application of different hormones in different combinations or alone in diverse concentrations for callus formation, shoot regeneration, and root initiation is an effective technique of vegetative propagation of rose. In this study, MS + 4.0 mg/L 2,4-D combination for callusing, MS + 2.0 mg/L BAP + 0.5 mg/L KIN treatment for shoot regeneration, 2.0 mg/L BAP + 0.5 mg/l KIN + 0.1 mg/L GA_3 for shoot proliferation and ½ MS + 1.0 mg/L IBA + 1.0 mg/L NAA for root initiation were observed better for micropropagation of *Rosa sp.* (L.). Rooted shoots after applying the aforesaid method could easily be acclimatized and transplanted in the natural condition for ensuring quality supply of seedlings of commercial cultivars of roses within a short time. This study has provided a technique of micropropagation of *Rosa sp.* (L.) which could effectively be used by progressive gardeners as well as in rose industry.

Acknowledgement

This study was approved and funded by the Genetic Engineering and Biotechnology Department of the University of Rajshahi, Bangladesh.

Conflicts of Interest

The authors declare no conflicts of interest regarding publication of this paper.

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