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Optimizing Micropropagation Protocol of Rose (*Rosa hybrida* L.) cv. 'Double Delight'

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Abstract

An experiment was conducted to develop a suitable *in vitro* plant regeneration technique for *Rosa hybrida* L. cv. 'Double Delight' using nodal segments and leaf tissues as explants through direct and indirect organogenesis. Aseptic explants were cultured onto gelled MS medium contained various strengths of plant growth regulators alone and in combinations for callus and direct shoot induction. MS medium containing 2.0 mg/l BAP was found to be the most effective for direct shoot induction from nodal explants (87.5%) as it produced the maximum number of shoots per explant (4.20 ± 0.37). The highest callus induction frequency was found to be 80% in leaf tissue, 72% in nodal segments, and 96% in nodal segments having young leaf of *in vitro* raised plantlets in MS medium containing 2.5 mg/l 2, 4-D. The best percentage of indirect shoot organogenesis (93.33%) with maximum number and length of shoot were found when the different explant-derived callus was transferred in MS medium supplimented with 3.0 mg/l BAP. The direct and indirectly induced shoots were multiplied on MS medium containing 3.0 mg/l BAP, where the average number and length of shoots per culture were 7.20 ± 0.80 and 5.22 ± 0.47 cm, respectively. Maximum rooting (80%) was observed in ½-strength of gelled MS medium containing 15.0 mg/l sucrose with 1.0 mg/l IBA. Plantlets with the proper root system were then placed in a polybag with a 1:1:1 ratio of sand, garden soil, and compost, and they had a survival rate of about 76%.

Introduction

Rose is one of the most significant commercial ornamental crops of the genus *Rosa*, which belongs to the sub-family Rosoideae under the family Rosaceae (Leus et al. 2018). *Rosa* is a genus with more than 100 species, most of which are native to Asia, although some are

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also found in North America, Northwest Africa, and Europe. It is believed that many of these species have originated by hybridization, which is typically followed by polyploidization, either naturally or through cultivation (Zhang et al. 2013 and Riek et al. 2013). More than 30,000 rose varieties have been recorded in Modern Roses 12 despite the fact that the number of commercially accessible rose variations on the market is unknown (Leus et al. 2018).

Rosa hybrida cv. 'Double Delight' is a Hybrid Tea Rose cultivar that is widely grown across the world (Quest-Ritson and Quest-Ritson 2011). It is well known for its sweet and strongly fragrant flowers, which have creamy-white petals at the center with lightly-ruffled ruby-red edging (Gardenia.net 2023). The crimson hue of 'Double Delight' is caused by UV light interacting with natural pigments in the petals. The red petals resulted from an increase in glycosylated anthocyanins, and the up-regulation of anthocyanin synthesis genes (Hechen et al. 2022). The petals will be white in color if the plant is cultivated in a greenhouse that filters UV light. However, nobody is certain, whether its name alludes to the two opposing hues in its blossoms or whether the 'Double Delight' is its color and aroma (Quest-Ritson and Quest-Ritson 2011). *Rosa* 'Double Delight' is a very popular cut flower in the market due to the color-changing properties of petals (Hechen et al. 2022). It meets the two most important requirements for a rose: an eye-catching color combination and a superb nose-pleasing aroma. They can be used as cut flower as well as garden rose (Plants Database 2019).

Roses are typically propagated through seeds, cuttings, budding or grafting of bud scion onto the rootstock of wild rose in a particular season (Nizamani et al. 2016). Seed propagation often results in variations among the genotypes while the other methods are time-consuming and tedious with a very low rate of success (Hameed et al. 2006). Additionally, it has been noted that plants grown using these techniques are susceptible to disease that affect flower production and quality, which ultimately lowers their market value (Cline 1994, Norton and Boe 1982). Other significant barriers to traditional propagation include seasonal dependency with low multiplication rates (Pati et al. 2006). Furthermore, hybrid rose cuttings are typically challenging to root (Nizamani et al. 2016). In this situation, plant tissue culture is the most effective and dependable way for large-scale production of disease-free and identical plants of roses throughout the year. Therefore, the present investigations has been undertaken to optimize an efficient and reproducible micropropagation protocol for an economically important rose cultivar *Rosa hybrida* L. cv. 'Double Delight'.

Materials and Methods

To initiate the culture, nodal segments and young leaf tissues were collected as explants from *Rosa hybrida* cv. 'Double Delight' plant. The explants were washed under running tap water for 15-20 min before being rinsed in distilled water for 15 min with a few drops of Tween-20–and carefully washed with distilled water three to four times to get rid of

Tween 20. The explants were further sterilized in a laminar airflow cabinet with Bavistin (0.5%) for 3-4 min succeeding to 3 min with 0.2% mercuric chloride ($HgCl_2$). Following each treatment, explants were washed gently with sterile distilled water for 3-4 times. Finally, before culture in the medium, the cut ends of the explant exposed to $HgCl_2$ were removed with a sterilized scalpel and forceps under aseptic condition.

Surface sterilized nodal explants were cultured individually on gelled MS medium containing different concentrations of BAP (1.0-3.5 mg/l), either alone or in combinations with 0.5 mg/l NAA for direct shoot induction. For callus induction and proliferation, several explants from *ex vitro* (leaf and nodal segments) and *in vitro* (nodal segments having young leaf) sources were cultured to gelled MS medium containing varied concentrations of 2, 4-D (1.0-4.5 mg/l). The calli were then moved to shoot initiation medium containing different concentrations of BAP (0.5-4.5 mg/l), either alone or in conjunction with 0.5 mg/l NAA or 0.5 mg/l IAA. Directly and indirectly induced shoots were excised aseptically and cultured on gelled MS medium containing different concentrations of BAP (1.0-4.0 mg/l) alone or in combinations with NAA (0.2-0.5 mg/l) and Kn (0.5-1.0 mg/l) for shoot multiplication. The cultures were subjected to a 16-hour period of light at a constant temperature of 25 ± 2°C, and the growth chamber's light intensity ranged from 2000 to 3000 lux.

Single shoots with 5-6 cm in length were planted in full and half-strengths of gelled MS medium containing 15 g/l or 30 g/l sucrose as well as various strengths and combinations of IBA (1.0-2.0 mg/l) and NAA (0.5-2.0 mg/l) for root induction. The freshly transferred cultures were kept in dim light for three days before being incubated in light for root induction. The rooted plantlets were then transferred in a small polybag containing sun sterilized sand and soil mixed with compost in the ratio of 1:1:1. After 3-4 weeks, when the regenerated plants were firmly established in the soil, they were moved to bigger containers for continued growth and development before being planted in the field. The means and standard errors of all dependent variables, including shoot number and length, root number, and days required for root initiation under various concentrations of plant growth regulators, were analyzed by ANOVA using SPSS software version 16.0 and the means were compared using DMRT at 5% significance.

Results and Discussion

In order to regenerate shoots directly, nodal segments were cultured in the gelled MS medium with twelve different combinations of growth regulators (Table 1). Direct shoot induction was observed in all of the combinations, however MS medium fortified with 2.0 mg/l BAP demonstrated the highest frequency (87.5%) of shoot induction with the maximum number of shoots (4.20 ± 0.37) and shoot length (4.58 ± 0.30 cm) per explant. Similarly to this, Kajla et al. (2018) and Brunda et al. (2017) showed that MS medium containing 2.0 mg/l BAP was sufficient for direct shoot induction in the case of different cultivars of *R. hybrid.* Oo et al. (2021), Aggarwal et al. (2020) and Tirkey et al. (2019) also

found that BAP was the best cytokinin type growth regulator for direct shoot induction in different cultivars of *R. hybrida*. In this study, the interactive effect of BAP and NAA was determined to be less favorable than the single effect of BAP (Table 1). Meanwhile, Ali and Mangrio (2020) and Nizamani et al. 2016 in the case of *R. hybrid* reported BAP in combination with NAA were more efficient than using BAP alone to induce shoots directly from the nodal explants, which contradicts the present study.

Several explants from *in vitro* and *ex vitro* sources were transferred onto gelled MS media containing 8 different concentrations of 2, 4-D for the goal of callus induction (Table 2). However, 2.5 mg/l 2, 4-D showed the highest frequency of callus induction in all three types of explants (80% in leaf tissue; 72% in nodal segment; 96% in *in vitro* derived nodal segments having young leaf). Moreover, the nature of callus obtained from different sources differed as well, as seen in Table 2. It was supported by the work of Harmon et al. (2022) in the case of three different cultivars of *R. hybrida*; Afrin et al. (2022) in the case of *Rosa* sp.; Pourhosseini et al. (2013) in the case of *R. hybrida* cv. Apollo. Whereas, Liu et al. (2018) showed that explant tissue of *R. hybrida* could produce a 100% callus induction ratio when MS medium was supplemented with 3.0 mg/l 2, 4-D and 1.0 mg/l BAP.

Table 1. The impact of varying concentrations of BAP in MS medium, either alone or in combination with NAA on direct shoot induction.

Growth regulators (mg/l)		% of responding explant	No. of shoots produced/explant ($\bar{x}$ ± S.E.*)	Length of shoot (cm) ($\bar{x}$ ± S.E.*)
BAP	NAA			
1.0	-	48.00	1.60^{cd} 0.40	2.50^{de} ± 0.34
1.5	-	64.00	2.80^{bc} ± 0.58	3.50^{bc} ± 0.27
2.0	**-**	**87.50**	**4.20^{a} ± 0.37**	**4.58^{a} ± 0.30**
2.5	-	72.50	3.40^{ab} ± 0.51	3.76^{b} ± 0.29
3.0	-	53.33	2.60^{bcd} ± 0.40	3.44^{bc} ± 0.34
3.5	-	36.67	1.80^{cd} ± 0.49	2.98^{bcde} ± 0.28
1.0	0.5	20.00	1.20^{d} ± 0.20	2.12^{e} ± 0.06
1.5	0.5	36.00	1.40^{cd} ± 0.40	2.94^{bcde} ± 0.32
2.0	0.5	52.50	2.20^{bcd} ± 0.58	3.38^{bc} ± 0.14
2.5	0.5	40.00	1.60^{cd} ± 0.40	3.06^{bcd} ± 0.32
3.0	0.5	33.33	1.40^{cd} ± 0.40	2.76^{cde} ± 0.29
3.5	0.5	23.33	1.20^{d} ± 0.20	2.16^{e} ± 0.17

Culture duration 35 days; at least 25 explants were cultured in each concentration; values are means (± S.E.* = Standard error of mean) derived from 5-separate treatments; according to DMRT, values with different letters within a column represent a significant difference ($p < 0.05$).

Table 2. The impact of varying concentrations of 2, 4-D in MS medium on callus induction from various explants of *Rosa hybrida* L. cv. 'Double Delight'.

2, 4-D (mg/l)	Callus induction frequency (C.I.F. %)					
	Leaf tissue	Callus formation intensity	Nodal segment	Callus formation intensity	*In vitro* derived nodal segments having young leaf	Callus formation intensity
1.0	40.00	+	16.00	+	44.00	+
1.5	52.00	+	32.00	+	64.00	++
2.0	76.00	++	56.00	+	88.00	+++
2.5	**80.00**	**+++**	**72.00**	**++**	**96.00**	**+++**
3.0	68.00	++	60.00	++	80.00	++
3.5	56.00	++	48.00	+	60.00	+
4.0	36.00	+	24.00	+	40.00	+
4.5	28.00	+	20.00	+	32.00	+
Nature of callus	Light green in color; Granular in texture		Light brown in color; Nodular and compact in texture		Whitish in color; Friable in texture	

25 explants were cultured in each concentration; Intensity of callus formation: Little callus (+); Moderate callus (++); Excellent callus (+++).

The resulting calli were then separated and grown for multiplication on the same medium mixture. After multiplication, various explant-derived calli were cut to a size of 1.5 cm^2 and then cultured on MS medium containing either BAP alone or in combination with NAA or IAA in order to encourage shoot regeneration (Table 3). The medium containing only 3.0 mg/l BAP resulted in the highest percentage of responding callus (93.33 %) out of all the different concentrations and combinations tested, with an average 6.40 ± 0.51 shoots per culture and an average of 5.48 ± 0.36 cm shoot length. Similarly, Afrin et al. (2022) and Liu et al. (2018) also demonstrated that BAP was crucial for the development of adventitious buds from callus in different species of *Rosa*. When BAP concentrations were raised or lowered after 3.0 mg/l, responsive callus was also generated, but the number of shoots/culture and their length were also reduced. Additionally, it was shown that the combined impact of BAP with NAA or IAA was not as good as the single effect of BAP. Whereas, Aminsalehi et al. (2019) in the case of *R. hybrida* L. cv. Maurossia found the optimum percentage of indirect shoot induction in MS medium supplemented with BAP and NAA.

Table 3. The effects of varying concentrations of BAP, either alone or in combination with NAA or IAA on shoot morphogenesis from induced callus.

Growth regulators (mg/l)			% of responsive callus	No. of shoots produced/culture ($\bar{X}$ ± S.E.*)	Length of shoot (cm) ($\bar{X}$ ± S.E.*)
BAP					
0.5			0	No adventitious buds; calli continued to develop	
1.0			0	No adventitious buds; calli became compact green in color	
1.5			26.67	1.20^{d} ± 0.20	2.10^{d} ± 0.29
2.0			33.33	1.60^{d} ± 0.24	2.54^{cd} ± 0.56
2.5			56.67	3.20^{c} ± 0.37	3.84^{bc} ± 0.58
3.0			**93.33**	**6.40^{a} ± 0.51**	**5.48^{a} ± 0.36**
3.5			70.00	4.60^{b} ± 0.68	4.16^{b} ± 0.46
4.0			40.00	2.40^{cd} ± 0.51	2.98^{bcd} ± 0.40
4.5			26.67	A few adventitious buds with abnormal shape	
BAP	NAA	IAA			
1.0	0.5	-	16.00	1.40^{b} ± 0.40	2.02^{c} ± 0.29
2.0	0.5	-	68.00	3.80^{a} ± 0.58	4.02^{a} ± 0.84
3.0	0.5	-	48.00	2.60^{ab} ± 0.51	3.60^{ab} ± 0.81
4.0	0.5	-	24.00	2.00^{ab} ± 0.55	2.22^{c} ± 0.58
1.0	-	0.5	0	No adventitious buds; calli developed slowly and turned brown	
2.0	-	0.5	0	No adventitious buds; calli continued to develop	
3.0	-	0.5	40.00	2.20^{ab} 0.80	2.90^{bc} ± 0.67
4.0	-	0.5	28.00	A few adventitious buds with unusual shapes and turned brown	

For every experiment, at least 25 cultures were maintained; culture duration 40 days; values are means (± SE* = Standard error of mean) derived from 5-separate treatments; according to DMRT, values with different letters within a column represent a significant difference ($p < 0.05$).

For rapid shoot multiplication, induced shoots were removed from the node and callus and grown on gelled MS medium that contained various concentrations of BAP either alone or in combination with NAA and KN. It was found that increasing BAP concentrations up to 3.0 mg/l improved the percentage of shoot proliferation (88%), with an average of 7.20 ± 0.80 regenerated shoots per culture and a mean length of 5.22 ± 0.47 cm (Table 4). These findings are consistent with the findings of Oo et al. (2021) and Thi

Table 4. The impacts of varying concentrations of BAP, either alone or in combinations with NAA or Kn on shoot proliferation.

Growth regulators (mg/l)		% of culture showed shoot multiplication	No. of shoots produced/culture ($\bar{X}$ ± S.E.*)	Length of shoot (cm) ($\bar{X}$ ± S.E.*)
BAP				
1.0		24.00	1.80^{d} ± 0.37	2.66^{c} ± 0.12
1.5		40.00	2.60^{d} ± 0.40	2.78^{c} ± 0.35
2.0		56.00	3.00^{d} ± 0.55	3.54^{bc} ± 0.44
2.5		72.00	5.60^{ab} ± 0.51	4.38^{ab} ± 0.52
3.0		**88.00**	**7.20^{a} ± 0.80**	**5.22^{a} ± 0.47**
3.5		60.00	4.80^{bc} ± 0.73	3.86^{bc} ± 0.48
4.0		44.00	3.40^{cd} ± 0.51	2.96^{c} ± 0.23
BAP	NAA			
1.0	0.2	0	Callus induction from the shoot base; cultured shoot dies	
1.0	0.5	0	Callus induction from the shoot base; cultured shoot dies	
2.0	0.2	36.00	3.00^{ab} ± 0.55	2.68^{b} ± 0.21
2.0	0.5	32.00	2.60^{b} ± 0.40	2.98^{ab} ± 0.32
3.0	0.2	76.00	4.20^{a} ± 0.58	3.94^{a} ± 0.50
3.0	0.5	64.00	3.20^{ab} ± 0.58	3.52^{ab} ± 0.37
4.0	0.2	52.00	3.40^{ab} ± 0.75	3.58^{ab} ± 0.26
4.0	0.5	40.00	2.80^{ab} ± 0.58	3.40^{ab} ± 0.36
BAP	Kn			
1.0	0.5	28.00	2.00^{bc} ± 0.45	2.68^{ab} ± 0.50
1.0	1.0	44.00	2.40^{abc} ± 0.51	2.96^{ab} ± 0.34
2.0	0.5	68.00	3.80^{a} ± 0.58	3.74^{a} ± 0.29
2.0	1.0	56.00	2.80^{abc} ± 0.37	3.36^{ab} ± 0.48
3.0	0.5	48.00	3.00^{ab} ± 0.55	3.04^{ab} ± 0.35
3.0	1.0	40.00	2.20^{bc} ± 0.58	2.46^{ab} ± 0.47
4.0	0.5	16.00	1.40^{c} ± 0.24	2.10^{b} ± 0.39
4.0	1.0	0	Callus induction from the shoot base; cultured shoot dies	

Culture duration 40 days; for multiplication, 25 directly and indirectly induced shoots were inoculated in each combination; values are means (± SE* = Standard error of mean) derived from 5 separate treatments; according to DMRT, values with different letters within a column represent a significant difference ($p < 0.05$).

et al. (2008) in the case of *R. hybrida*. They showed that MS medium supplemented with 3.0 mg/l BAP was found to be the optimum concentration for shoot proliferation. However, a noticeable slowdown in the rate of shoot multiplication was observed when

the concentrations of BAP were raised to levels greater than 3.0 mg/l. This was in accordance with the study conducted by Attia et al. (2012) and Kim et al. (2003), which found that increased BAP concentrations prevented the multiplication of shoots in several *R. hybrida* cultivars.

Table 5. The effect of MS medium's strength, sugar levels, and growth regulators on *in vitro* rooting of *Rosa hybrida* L. cv. 'Double Delight' micro-shoots.

Strength of MS medium	Sucrose	Growth regulators (mg/l)		Days required for root initiation ($\bar{x}$ ± SE*)	% of rooting	No. of roots/culture ($\bar{x}$ ± SE*)	Root length
		IBA	NAA				
Full-MS	30 g/l	1.0	-	Callus formation at the shoot base			
½ MS	30 g/l	1.0	-	25.60^{bc} ± 1.33	60.00	3.60^{bc} ± 0.51	++
½ MS	**15 g/l**	**1.0**	**-**	**18.40^{a} ± 1.17**	**80.00**	**5.20^{a} ± 0.37**	**+++**
Full-MS	30 g/l	2.0	-	Callus formation at the shoot base			
½ MS	30 g/l	2.0	-	Callus formation at the shoot base			
½ MS	15 g/l	2.0	-	27.00^{bcd} ± 1.10	53.33	2.60^{cd} ± 0.24	++
Full-MS	30 g/l	-	1.0	Callus formation at the shoot base			
½ MS	30 g/l	-	1.0	28.80^{cd} ± 1.36	46.67	2.40^{cd} ± 0.40	+
½ MS	15 g/l	-	1.0	23.20^{b} ± 1.50	66.67	4.00^{ab} ± 0.63	+++
Full-MS	30 g/l	-	2.0	Callus formation at the shoot base			
½ MS	30 g/l	-	2.0	No root or callus formation at the shoot base			
½ MS	15 g/l	-	2.0	30.00^{cd} ± 1.82	40.00	2.00^{d} ± 0.32	+
Full-MS	30 g/l	1.0	0.5	Callus formation at the shoot base			
½ MS	30 g/l	1.0	0.5	31.20^{d} ± 1.46	26.67	1.40^{d} ± 0.40	+
½ MS	15 g/l	1.0	0.5	27.20^{bcd} ± 1.50	33.33	2.20^{d} ± 0.37	+

Culture duration 35 days; 15 individual shoots were inoculated in every combination for rooting; + = Very small; ++ = Small; +++ = Long; values are means (± SE* = Standard error of mean) derived from 5-separate treatments; according to DMRT, values with different letters within a column represent a significant difference ($p<0.05$).

On the other hand, in this experiment the interactive effect of BAP with NAA or Kn did not provide satisfactory results as compare to using BAP alone. Similarly, Hameed et al. (2006) shown through research on *R. indica* that combining NAA with BAP reduces the rate of shoot multiplication. The current results however, differed from the results obtained by Tawfik et al. (2018) in the case of *R. hybrida* cv. Eiffel Tower; Attia et al. (2012) in the case of *R. hybrida* L. cv. 'Al-Taif' and Shabbir et al. (2009) in the case of *R. indica*.

They obtained multiple shoots per culture bottle in the MS basal medium supplemented with BAP and KN. Moreover, Bharadwaj et al. (2006) in the case of *R. chinensis* found that MS medium enriched with BAP, KN and NAA yielded the highest multiplication rate.

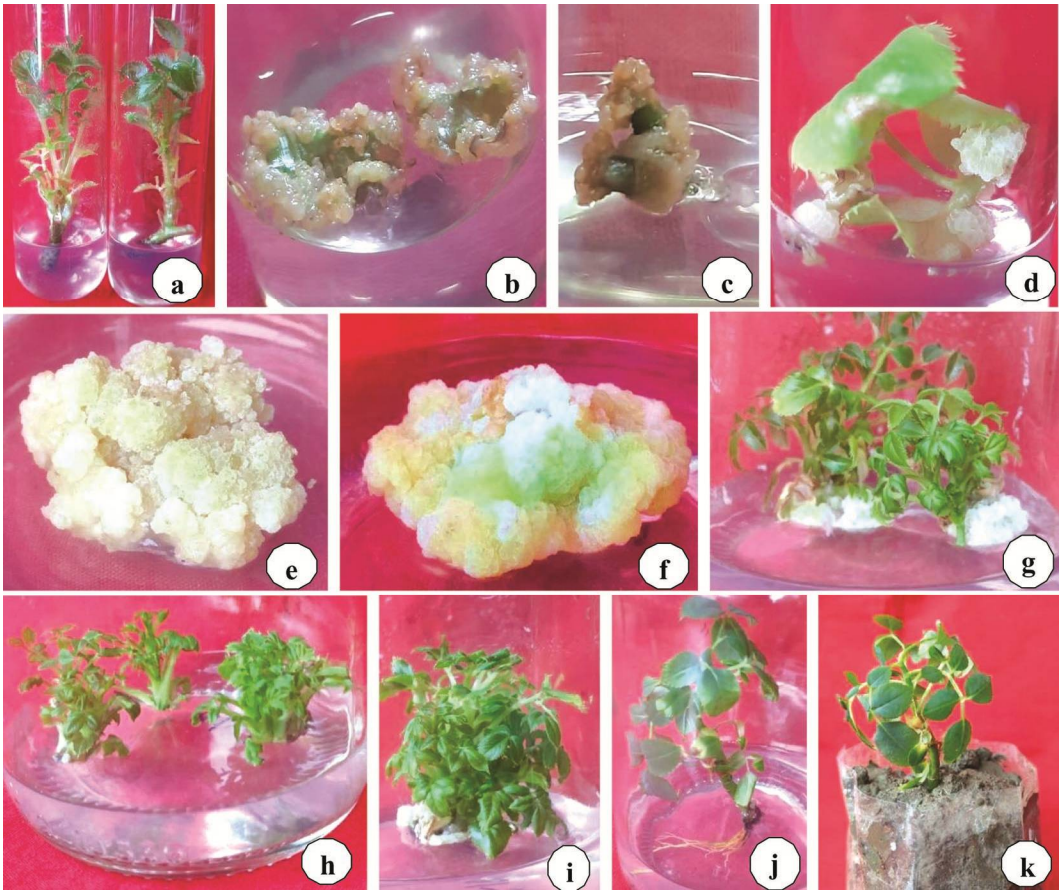

Fig. 1(a-k): Different stages of micropropagation of *R. hybrida* L. cv. 'Double Delight'. **(a)** Direct shoot induction on MS medium containing 2.0 mg/l BAP. **(b-d)** Callus induction from (b) leaf tissue, (c) nodal segment and (d) leaf and internodal segment of *in vitro* grown plantlets on MS medium containing 2.5 mg/l 2 ,4-D. **(e)** Multiplication of the callus on the same medium. **(f)** Callus turned into green color within 15-days of inoculation on MS medium containing 3.0 mg/l BAP. **(g)** Induction of multiple shoot from the callus tissues after 40-days of inoculation on the same medium. **(h)** Multiplication of shoots in MS medium containing 3.0 mg/l BAP. **(i)** Proliferation of shoots after 3rd sub-culture in the same medium. **(j)** Root induction in half-strength of gelled MS medium containing 15 g/l sucrose with 1.0 mg/l IBA. **(k)** Acclimatization of regenerated plantlets in soil conditions.

Rose shoots often proliferated easily *in vitro*, but rooted those shoots proved to be quite difficult. Moreover, concentrations of sugar and strength of basal medium salts played a significant role in rooting. In the present investigation, low level of sucrose (15

g/l) in half-strength MS medium containing 1.0 mg/l IBA gave the highest rooting percentage (80%) with maximum number of root per culture (5.20 ± 0.37) within the shortest periods (18.40 ± 1.17 days) of time (Table 5). Similar to this, Brunda et al. (2017) and Senapati and Rout (2008) also achieved rooting in several *R. hybrida* cultivars on half-strength MS medium containing 20 g/l sucrose with 0.25 mg/l IBA. Additionally, the results indicate that the single use of IBA performed significantly better than NAA and the combined effect of IBA and NAA on the rooting of micro-shoots. As opposed to this, Oo et al. (2021) demonstrated substantial rooting in hybrid *Rosa* spp. on half-strength MS medium containing 15 g/l sugar and 1.0 mg/l NAA.

In this experiment, the rooted plantlets were successfully acclimatized with a 76% survival rate in a soil combination made up of garden soil, sand, and compost mixture in a ration of 1:1:1. Utilizing almost same potting mixture Kajla et al. (2018), Maurya et al. (2013), Senapati and Rout (2008) showed 60-84% survival rate in the case of several *R. hybrida* cultivars. Whereas, Ping et al. (2019) reported a 98.33% survival rate in *in vitro* regenerated plantlets of 'Double Delight' rose after transplanting the plantlets in polybags containing a 1 : 2 : 3 : 1 ratio of fine sand, vermiculite, perlite, and coconut bran. Fig.1 depicts several phases of *R. hybrida* L. cv. 'Double Delight' *in vitro* regeneration.

The current work offers a successful and affordable micropropagation method for *Rosa hybrida* L. cv. 'Double Delight' using the nodal segments and young leaf tissues of field-grown plants. This research will aid in the conservation and large-scale propagation of hybrid tea roses for horticulture as well as for the pharmaceutical sectors.

References

Afrin S, Rahman MA, Khalekuzzaman M, Hasan MM, Fahim AHF and **Alam MA** (2022) Study on *in vitro* micropropagation of *Rosa* sp. Bangladesh J. Agri. **47**(1): 66-74.

Aggarwal D, Upadhyay SK, Kumar K, Sehrawat N, Tuli HS and **Singh R** (2020) Effects of plant growth regulators on *in vitro* propagation of economically important ornamental plant *Rosa hybrida* L. Asian J. Biol. Life Sci. **9**(2): 227-233.

Ali MA and **Mangrio GS** (2020) Effect of phytohormones and their diverse concentrations on regeneration of Rose (*Rosa Hybrida* L.). Trakia J. Sci. **18**(1): 46-51.

Aminsalehi M, Jonoubi P, Razavi K and **Zeynipour M** (2019) Micropropagation of (*Rosa hybrida* L. cv. Maurossia) and comparative anatomy of vegetative organs *in vivo* and *in vitro* plants. J. Develop. Biol. **11**(4): 1-7.

Attia AO, Dessoky ELDS and **El-Tarras AE** (2012) *In vitro* propagation of *Rosa hybrida* L. cv. Al-Taif Rose plant. African J. Biotech. **11**(48): 10888-10893.

Bharadwaj R, Singh S, Pal S and **Kumar S** (2006) An improved protocol for micro-propagation of miniature Rose (*Rosa Chinensis* Jacq. Var. Minima) cultivas. J. Orname. Hor. **9**(4): 238-242.

Brunda SM, Rani CL, Rajendran P, Smitha R and **Priya L** (2017) *In vitro* propagation of *Rosa hybrida* 'Golden Fairy' through nodal explants. Acta. Hort. **1165**: 87-90.

Cline MG (1994) The role of hormones in apical dominance. New approaches to an old problem in plant development. Physiologia plantarum. **90**(1): 230-237.

Gardenia.net (2023) *Rosa* Double Delight (Hybrid Tea Rose). From https://www.gardenia.net/plant/rosa-double-delight

Hameed N, Shabbir A, Ali A and **Bajwa R** (2006) *In vitro* micropropagation of disease free rose (*Rosa indica* L.). Mycopath. **4**(2): 35-38.

Harmon DD, Touchell DH and **Ranney TG** (2022) Tissue culture and regeneration of three rose cultivars. Hort. Sci. **57**(11): 1430-1435.

Hechen Z, Huijuan W, Yanmin L, Jie G, Xin Y, Limin W, Xiaochen W, Yinge Z and **Zhenzhu F** (2022) The chemical composition and transcriptome analysis reveal the mechanism of color formation in *Rosa hybrida* cv. 'Double delight'. Chinese Bull. Bot. **57**(5): 649-660.

Kajla S, Kala S, Kumar A, Mir H and **Singh MK** (2018) Effect of growth regulators on *in vitro* shoot multiplication and plant regeneration of *Rosa hybriad* L. from nodal explants. Int. J. Curr. Microbio. App. Sci. **7**(SI): 3804-3811.

Kim CK, Oh JY, Jee SO and **Chung JD** (2003) *In vitro* Micropropagation of *Rosa Hybrida* L. J. Plant Biotech. **5**(2): 115-119.

Leus L, Laere KV, Riek JD and **Huylenbroeck JV** (2018) Rose (Chapter 27). *In:* Ornamental Crops: Handbook of Plant Breeding, Huylenbroeck, JV (Eds.), Springer, Switzerland. **11**: 719-767.

Liu J, Feng H, Ma Y, Zhang L, Han H and **Huang X** (2018) Effects of different plant hormones on callus induction and plant regeneration of miniature roses (*Rosa hybrida* L.). Hort. Int. J. **2**(4): 201-206.

Maurya RP, Yadav RC, Godara NR and **Beniwal VS** (2013) *In vitro* plant regeneration of Rose (*Rosa hybrida* L.) cv. 'Benjamin Paul' through various explants. J. Experi. Biol. and Agri. Sci. **1**(2S): 111-119.

Nizamani F, Nizamani GS, Nizamani MR, Ahmed S and **Ahmed N** (2016) Propagation of Rose (*Rosa hybrida* L.) under tissue culture technique. Int. J. Bio. Rese. **1**(1): 23-27.

Norton ME and **Boe AA** (1982) *In vitro* propagation of ornamental Rosaceous plants. Hort. Sci. **17**(2): 190-191.

Oo KT, Lwin KM and **Khai AA** (2021) *In vitro* micropropagation of Rose (Hybrid *Rosa* spp.) through plant tissue culture technique. J. Sci. Innov. Res. **10**(1): 1-4.

Pati PK, Rath SP, Sharma M, Sood A and **Ahuja PS** (2006) *In vitro* propagation of rose: a review. Biotech. Advan. **24**(1): 94-114.

Ping LZ, Li ZC, Ying LJ, Yang YS, Sheng LH and **Feng PZ** (2019) Rapid propagation of double delight Chinese rose using tissue culture. Fujian J. Agri. Sci. **34**(1): 61-69.

Plants Database (2019) National Gardening Association Plants Database: *Rosa* 'Double Delight'. Retrieved August 4, 2019. From https://garden.org/plants/view/20/Rose-Rosa-Double-Delight/

Pourhosseini L, Kermani MJ, Habashi AA and **Khalighi A** (2013) Efficiency of direct and indirect shoot organogenesis in different genotypes of *Rosa hybrida*. Plant Cell Tiss. Org. Cult. **112**(1): 101-108.

Quest-Ritson CBC and **Quest-Ritson B** (2011) The Royal Horticultural Society: Encyclopedia of Roses, Dorling Kindersley Publishing (Reprint Eds.), London, United Kingdom. ISBN 978-0756688684.

Riek JD, Cock KD, Smulders M and **Nybom H** (2013) AFLP-based population structure analysis as a means to validate the complex taxonomy of dogroses (*Rosa* section Caninae). Mol. Phylo. Evol. **67**(3): 547-559.

Senapati SK and **Rout GR** (2008) Study of culture conditions for improved micropropagation of hybrid rose. Hort. Sci. (Prague). **35**(1): 27-34.

Shabbir A, Hameed N, Ali A and **Bajwa R** (2009) Effect of different cultural conditions on micropropagation of Rose (*Rosa indica* L.). Pakistan J. Bot. **41**(6): 2877-2882.

Tawfik AA, Ibrahim OHM, Abdul-Hafeez EY and **Ibrahim SAI** (2018) Optimizing micropropagation protocol for *Rosa hybrida* cv. Eiffel Tower with improved *in vitro* rooting ability. Egyptian J. Hort. **45**(2): 323-35.

Thi K, Khai AA and **Lwin KM** (2008) Micropropagation of Rose (Hybrid *Rosa* spp.) by *in vitro* culture technique. 3rd GMSARN International Conference on Sustainable Development: Issues and Prospects for the GMS, Kunming, China. 12-14 Nov.

Tirkey DS, Nirala DP and **Kumari P** (2019) Micropropagation from nodal explants of rose (*Rosa hybrida* L.) at different concentration of BAP (6-Benzyl Amino Purine). Int. J. Chemi. Studi. **SP. 6**: 427-430.

Zhang J, Esselink G, Che D, Fougère-Danezan M, Arens P and **Smulders M** (2013) The diploid origins of allopolyploid rose species studied using single nucleotide polymorphism haplotypes flanking a microsatellite repeat. J. Hort. Sci. & Biotech. **88**(1): 85-92.

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