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IN VITRO PROPAGATION OF *ROSA HYBRIDA* CV. QUEEN ELIZABETH

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Internodal segments of rose cv. Queen Elizabeth (3-5 mm) thick were established in modified MS medium with different additives for callogenesis. The cultures were incubated at 25 ± 2^0 and exposed to light of 28 μmol sec^{-1} $m1^{-2}$ for 16 hr a day. The best callus initiation was noted when culture medium was added with 2.5 mgL^{-1} 2,4-D, 0.25 mgL^1 kinetin and 2 mg L^{-1} casein hydrolysate. Callus index in dark condition was about 400 compared with 300 in light. The developed calli showed optimum shoot initiation when BA was employed @ 2.5 mgL^{-1}, but in some cultures, green nodular areas and rudimentary root formation was also observed. Axillary buds placed in the MS-media containing 3.5 mgL^{-1} BA were found to be the best as they produced new plants in 12- 14 weeks of time. For root regeneration 2.5 mg L^{-1} NAA was found the best. Plants, thus, obtained were planted in a compost having clay, sand and leaf mold (1:1:1), the survival was about 80 %.

Key words: *In vitro*, *Rosa hybrida*, Tissue culture.

Introduction

Hybrid roses are difficult to vegetatively propagate from cuttings, and are therefore perpetuated by asexual methods, which have their own limitations. Moreover, certain viral and bacterial diseases cause clonal degradation. Multiple shoot proliferation method has been used successfully in a number of rose varieties, by using different culture media, [2,3,6,9,18,19]. In this method sterilized shoot tips or nodal portions are explanted onto agar medium, proliferated shoots are transferred to fresh medium for root regeneration. This process takes about 8-12 weeks to produce new clones.

Another method is callus culture that has been extensively used in various physiological and related studies in many families and even in genus Rosa, [1,2,14]. Callus culture, though undesirable for commercial propagation but has advantages for specialized purposes; in breeding, Behnke [4]; elimination of chimeras and virus, Lane *et al.* [13]. Free living plants have been produced from genus Prunus, e.g., in apple, [5,15,17] ; in pear, Yehia [22]; and in peach Hammerschlag *et al.* [8]. Khosh-Khui *et al.* [11] used two cultivars of roses to get shoots from callus. They were able to get green callus areas and/or green calli but were unable to regenerate root or shoot.

To date there has been no or very little successful attempt of plant regeneration from callus in roses. Keeping in view the commercial application of *in vitro* techniques for roses, a research project was initiated to find the best combination of growth hormones for callus induction, and then root and shoot initiation in the rose cv. Queen Elizabeth. In this study, the growth hormones were used in different combinations as Jacobs *et al.* [6] and Gladysheva and Kosheler [4] recommended that although auxin is responsible for callus initiation but it needs the addition of cytokinins and in some cases organic complexes. An attempt was also made to compare this procedure with shoot proliferation method from nodal region.

Materials and Methods

Internodal segments of field grown rose cv. Queen Elizabeth were obtained from the horticultural garden of the University of Agriculture, Faisalabad and were surface sterilized with chlorox (1.0% w/v) added with 0.01% tween-20, for 10 min.. The explants, 3-5 mm thick and 5-7 mm in diameter, were given two rinses with sterilized distilled water, and inoculated in culture tubes having autoclaved-MS salts [16] with the following combinations in mgL^{-1}.

T1 = Control
T2 = MS + 0.1 NAA + 0.25 K
T3 = MS + 0.5 NAA + 0.25 K
T4 = MS + 1.0 NAA + 0.25 K
T5 = MS + 1.0 2,4-D + 0.25 K + 2.0 CH
T6 = MS + 2.0 2,4-D + 0.25 K + 2.0 CH
T7 = MS + 2.5 2,4-D + 0.25 K + 2.0 CH

NOTE : MS =Murashige and Skoog salt, NAA = α-Naphthalene acetic acid; and K =Kinetin; CH=Casein hydrolysate.

The medium was autoclaved at 1.05 kg cm^{-2} at 121° for 15 min. The cultures were incubated at $25 \pm 2°$ with a 16 hrs. photoperiod provided by cool white fluorescent lamps. The photoperiodism was kept at approximately 28 μ mol sec^{-1} m^2 irradiance. All the treatments were repeated four times. Callus index was calculated for light and dark conditions by using the following formula:

$$\text{Callus index} = \frac{100\ n \times G}{N}$$

where: n = number of explants initiating callus, G = average

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callus rating of initiated explants, N = total number of explants plated.

The calli obtained were placed in the MS-media containing 0.5 - 4.5 mgL^{-1} BA (Benzyl adenine) for shoot initiation and the initiated shoots were again placed in the freshly prepared MS- media supplemented with 0.5 - 4.5 mgL^{-1} BA for shoot proliferation. While for root initiation of the proliferated shoots NAA was used ranging from 0.5 to 4.5 mgL^{-1}.

Results and Discussion

The results of the experiments are shown in Tables 1 and 2 and are discussed in the following paragraphs.

Callus formation. In T1 (control) no callus initiation was noticed. When NAA was used @ 0.1 (T2) and 0.5 (T3), calli initiated but were not of good quality as they were pale in colour and compact in texture. The better callus formation was found in T4 where NAA was used @ 1.0 mgL^{-1}.

In T5 (1.0 2,4-D) and T6 (2.0 2,4-D) the callus initiation was less and slow. The best (upto 100 %) and the fastest (within four weeks) response to callus initiation was observed in a combination of 2.5 mgL^{-1} 2,4-D, 0.25 mgL^{-1}K and 2 mgL^{-1} casein hydrolysate, i.e., T7 (Table 1). These results are in conformity with the findings of Jacobs *et al.* [10] and Gladysheva and Koshelev [7].

The comparison of light and dark photoperiodism showed that the callus initiation was faster in the dark than in the light (Table 1) and the optimum callus initiation was found in T6 where the callus index was found to be 402 in the dark and 301 in the light. The treatment T7 was ranked as second followed by T5 and T4. While the combination T3 and T2 were observed to be the least effective. The Control (T1) showed no response towards callus initiation.

Shoot Initiation. The concentration of 0.5 and 1.0 mg L^{-1} BA showed negative results for shoot initiation. The optimum initiation was found in case of 2.5 mgL^{-1} BA, whereas 3.5 and 4.0 mgL^{-1} BA showed lesser shoot initiation (Table 2). Two to three weeks after transfer, some localized granular green areas appeared and in some tubes all the callus turned green. In some of the cultures, after 4 to 5 weeks, meristemoides were seen which took nodular appearance later on. Some of the cultures showed ill-defined and the other well-defined shoots, a few root primodia also developed, but none could be transplanted to soil successfully. These results are indicative of the regeneration potential of callus of the variety under study. Such a shoot initiation was also reported by Lakshami *et al.* [12].

Hammerschlag *et al.* [8] were able to get shoot regeneration from callus in peach plants when they increased BA level, two to five folds in culture medium, regardless of NAA concentration, than the callus medium consisting of 0.27 μM NAA and 2.2 μM BA. They were more successful when they used smooth-white nodular callus instead of green nodules. Khosh-Khui *et el.* [11] obtained green callus areas and subsequently all the callus turned green on subculturing without getting any shoot or root on rose callus. They have enlisted a number of factors responsible for callus deterioration upon subculturing, like species, culture medium, callus size and friability, culture conditions, and transfer time.

Shoot and root proliferation. It is obvious from Table 2 that the more shoots were proliferated, from the axillary buds, when cultured in MS-medium with 3.5 mgL^{-1} BA. Below (2.5 mgL^{-1} BA) and above (4.5 mgL^{-1} BA) this level less shoots

Table 1. Effect of Dark and Light on the Callus Index and of Different Hormones on the Callus Initiation of *Rosa hybrida* cv. Queen Elizabeth (Average of Four Replications).

Code treatments (mgL^{-1})	Callus index		Callus initiation
	Dark	Light	
T1 Control	0	0	–
T2 MS + 0.1 NAA + 0.25 K	188 ± 5.00	155 ±15.43	+
T3 MS + 0.5 NAA + 0.25 K	189± 20.31	93± 10.70	+
T4 MS + 1.0 NAA + 0.25 K	214 ± 14.37	208 ± 11.58	++
T5 MS + 1.0 2,4-D + 0.25 K + 2.0CH	298 ± 18.55	276 ± 10.78	++
T6 MS + 2.0 2,4-D + 0.25 K + 2.0CH	402 ± 9.91	301 ± 22.82	++
T7 MS + 2.5 2,4-D + 0.25 K + 2.0CH	338 ± 11.11	299 ± 14.72	+++

(+) little, (++) moderate, (+++) profuse, (-) no response.

Table 2. Effect of Different Concentrations of BA on Shoot Initiation and Proliferation.

BA (mgL^{-1})	Shoot initiation	Shoot proliferation
0.5	-	-
1.5	-	-
2.5	++	+
3.5	+	++
4.5	+	+

(+) little, (++) moderate, (+++) profuse, (-) no response.

were produced, while 0.5 and 1.5 mgL^{-1} BA showed no response for shoot proliferation.

Separated shoots, thus, produced 100% roots in MS- medium with 2.5 mgL^{-1} NAA. Rooted shoots were transplanted after 4 to 5 weeks in soil medium containing clay, sand and leaf mold (1:1:1) with 80% survival. This part of the study was carried out to standardize a culture medium for micropropagation of this commercially important cultivar. Our results are in conformity to those found by Skirvin *et el.* [18] and Hasegawa [9]. However, it took about two weeks more in our observations as compared to those authors who were able to produce free living plants within 8 to 12 weeks by using shoot meristems.

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