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In vitro Plantlets Regeneration of Rose

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Abstract

Nodal segments and shoot tips were cultured on MS supplemented with different concentrations and combinations of BAP, NAA and zeatin for multiplying axillary shoots. Among two types of explants, the nodal explants produced the highest number of shoots when they were cultured on MS + 1.5 mg/l BAP + 0.25 mg/l zeatin + l00 mg/l CH. The number of shoots per explant in initial cultures varied from five to seven. But after three weeks when they were subcultured in the same medium, the number of shoots increased with an average of 15 per culture. Shoot tip explants also produced multiple shoots, but their performance was not as good as nodal explants. For best rooting, 34 cm long shoots were excised and implanted individually onto the rooting medium containing half strength MS fortified with 1.0 mg/l IBA and 0.5 mg/l IAA. Within four weeks of transfer to the rooting medium, 85% of the microcuttings produced seven - ten roots. The regenerated plantlets were successfully transferred to soil and the percentage of survivability under *ex vitro* condition was 80.

Introduction

The genus *Rosa* is well associated with the culture, religion and socio-economic aspects of life in Bangladesh. Rose came to us early as a source of fragrance, as a cure for illness and as a symbol of beauty but not as a popular bush for the garden until recently. It is important not only for its outstanding aesthetic beauty and but also for its potentiality marketing as cut flowers and potted plants to many countries of the world. Roses are generally multiplied vegetatively by grafting buds on stem of wild rose and by cuttings. This conventional method of propagation is very slow. Moreover, disease and

environmental hazards make the cultivar degenerate gradually. So, this conventional process is not satisfactory in multiplication of *Rosa* spp. Micropropagation method is specially applicable to species in which clonal propagation is needed (Gamborg and Phillips 1995). Since the rapid clonal multiplication of plants *in vitro* is quicker and cheaper than *in vivo,* the tissue culture technique has become the first major attraction to the scientists around the world. By applying tissue culture techniques we can earn a substantial amount of foreign currency like many other neighboring countries by exporting tissue culture derived plantlets and cut flowers. The objective of this paper was to establish an efficient and reproducible method for rapid and large scale propagation of *Rosa* spp.

Materials and Methods

Shoot tips and nodal segments were taken from garden grown rose plants in the Institute of Food & Radiation Biology, Atomic Energy Research Establishment, Savar, Dhaka. These were washed thoroughly with running tap water for one h; thereafter they were kept in a beaker for 30 min in order to remove dirt on the stem surface. Next, these were cut into small pieces and surface sterilized with aqueous solution of 0.1 % $HgCl_2$ for six min under aseptic condition and rinsed five times with autoclaved distilled water to wash away the traces of $HgCl_2$ completely. Shoot tips and nodal segments, approximately 2.0 cm in length were cut from sterilized stem for explants. MS basal medium was used for regeneration of plantlets through *in vitro* culture. Shoot tips and nodal segment explants were cultured on MS supplemented with different concentrations of cytokinin (BAP, Kn, zeatin) and auxin (NAA) singly or in combination for shoot regeneration. Half strength MS supplemented with auxin such as IBA, IAA and NAA were used for root formation. Casein hydrolysate (CH) was used in the media for shoot multiplication. The pH of the media was adjusted to 5.8 before adding agar. All media were gelled with 0.6% agar. The cultures were incubated at 26 ± 1°C under cool white fluorescent light for a daily 16 h photoperiod. Subculturing was done every three weeks. *In vitro* rooted plantlets were taken out from the test tubes and gently washed to free agar from surface. *In vitro* rooted plantlets were then transferred to small earthen pots containing a mixture of soil, sand and compost (2 : 1 : 1) and covered with transparent polyethylene bag.

Results and Discussion

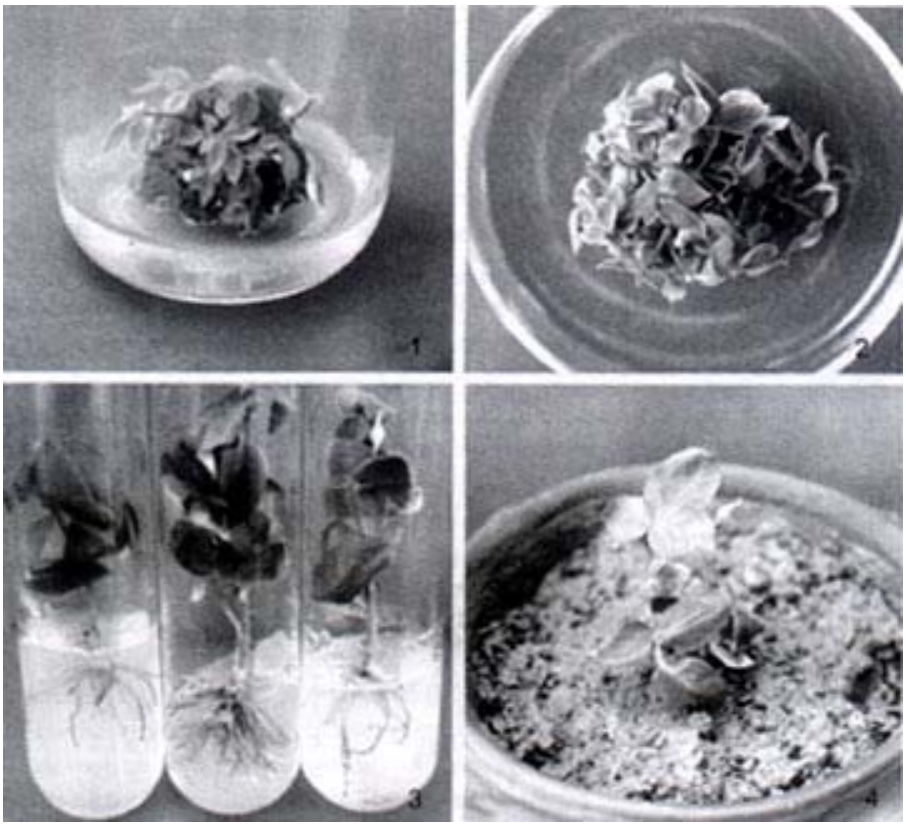
Different explants were cultured on MS supplemented with different concentrations of BAP, Kn, zeatin and NAA alone or in different combinations for multiple shoot regeneration. All explants comprising shoot tips and nodal segments were cultured for direct multiple shoot regeneration. Initiation of multiple shoots in most of the explants was observed within four weeks of culture. In both shoot tips and nodal segment explants, the highest percentage of shoot induction was observed in MS + 1.5 mg/l BAP + 0.25 mg/l zeatin. In the case of nodal segments, 85% of cultures were found to regenerate shoots and the number of regenerated shoots per explant was 10 ± 0.2 on the above medium (Table 1, Fig. 1). In the same medium, multiple shoot induction from the shoot tip explants was low. In media containing BAP with a high concentration of

Table 1. Response of different concentrations of growth regulators on shoot proliferation in *Rosa* sp. Data were taken after four weeks of culture.

Growth regulators (mg/l)	Percentage of shoot regenerants per explant	Average number of shoots/explant	
		Shoot tip	Nodal segment
1.0 BAP	48	2 ± 0.3	3 ± 0.2
1.5 BAP	80	5 ± 0.3	8 ± 0.2
2.0 BAP	70	4 ± 0.2	6 ± 0.5
1.0 BAP + 2.0 Kn	42	2 ± 0.2	2 ± 0.2
1.0 BAP + 0.5 Kn	38	3 ± 0.3	4 ± 0.3
1.0 BAP + 1.0 Kn	35	2 ± 0.5	3 ± 0.3
1.5 BAP + 0.2 Kn	60	2 ± 0.5	3 ± 1.2
1.5 BAP + 0.5 Kn	35	5 ± 0.5	6 ± 0.5
1.5 BAP + 1.0 Kn	30	2 ± 0.2	2 ± 1.5
1.5 BAP + 0.2 NAA	40	3 ± 0.8	4 ± 0.4
1.5 BAP + 0.5 NAA	33	4 ± 0.2	5 ± 1.2
1.5 BAP + 1.0 NAA	25	3 ± 0.2	3 ± 0.2
1.5 BAP + 0.2 zeatin	78	5 ± 0.4	8 ± 0.5
1.5 BAP + 0.25 zeatin	85	6 ± 0.2	10 ± 0.2
1.5 BAP + 0.5 zeatin	65	5 ± 0.3	9 ± 0.2
1.5 BAP + 1.0 zeatin	58	3 ± 0.4	5 ± 1.5
1.5 BAP + 0.2 NAA+ 0.25 zeatin	45	4 ± 0.2	7 ± 0.3
1.5 BAP + 0.5 NAA + 0.5 zeatin	40	3 ± 0.4	4 ± 0.4

NAA or Kn the frequency of new shoot regenerants was very poor. Habib et al. (1996) reported that the highest number of multiple shoot regeneration was possible from both shoot tips and nodal segments on 2. 0 mg/l BAP supplemented medium. The best response in shoot regeneration and multiplication was observed, when the culture was initiated during the winter season. Seasonal variation of *in vitro* shoot induction was also reported in many

other plant materials (Bonga 1987; Zaman et al. 1996). For further development of the medium and enhanced shoot proliferation, casein hydrolysate (CH) was added (75 - 150 mg/l) to the medium. Addition of l00 mg/l CH to the medium increased the number of shoots (15 and 10 in case of nodal segments and shoot tips, respectively) per culture



Figs. 1 - 4 : Regeneration of plantlets from nodal segments. 1. Development of multiple shoots on MS + BAP (1.5 mg/l) + zeatin (0.25 mg/l). 2. Positive effect of casein hydrolysate (100 mg/l) on the increment of number of shoots per explant. 3. Development of roots at the base of microcuttings cultured on half strength MS + IBA (1.0 mg/l) + IAA (0.5 mg/l). 4. Growth of seedlings on soil four weeks after transfer under *ex vitro* condition.

(Figs. 2 and 5). Thus the more effective medium determined for rapid high frequency regeneration of multiple shoots with suitable length was MS + 1.5 mg/l BAP + 0.5 mg/l zeatin + 100 mg/l CH. The commercial production of micro-propagated plants is often limited by poor survival rates when shoot or plantlets are transferred to greenhouse or field conditions (Korban and Donnelly 1994). For root induction, *in vitro* regenerated well-developed and

Table 2. Effect of IBA, IAA and NAA in half strength MS on root formation from regenerated shoots of *Rosa* sp. Data were taken four weeks after culture.

Growth regulators (mg/l)	Rooted shoot (%)	No. of roots/ culture
0.5 IBA	45 ± 0.5	2 - 3
1.0 IBA	70 ± 1.2	5 - 7
1.5 IBA	55 ± 1.5	5 - 6
0.5 IAA	35 ± 0.8	2 - 3
1.0 IAA	48 ± 0.2	2 - 3
1. 5 IAA	42 ± 1.2	2 - 3
0.5 IBA+ 0.5 IAA	47 ± 0.2	3 - 4
1.0 IBA + 0.5 IAA	85 ± 0.5	7 - 10
1.5 IBA+ 0.5 IAA	50 ± 1.2	4 - 5
1.0 IBA+ 0.5 lAA+ 0.5 NAA	52 ± 0.3	4 - 5
1.0 IBA+ 1.0 lAA + 0.5 NAA	35 ± 0.3	4 - 5
1.0 IBA + 1.0 IAA + 1.0 NAA	28 ± 0.5	2 - 3
1.5 IBA + 1.5 IAA + 1.0 NAA	30 ± 0.8	2 - 3
1.5 IBA+ 1.5 IAA+ 1.5 NAA	27 ± 1.2	2 - 3

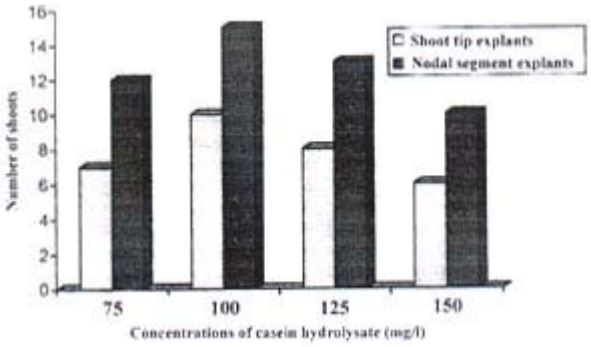


Fig. 5. Influence of different concentrations of casein hydrolysate (75 - 150 mg/l) in MS + 1.5 mg/l BAP + 0.25 mg/l zeatin on increasing number of shoots.

elongated shoots were excised and cultured on root induction media. Different concentrations of IBA, IAA and NAA were used in half strength MS for root induction. Best response was observed when 1.0 mg/l IBA and 0.5 mg/l IAA were added to half strength MS (Table 2). In this combination, it was observed that 85% shoots rooted within four weeks of culture and each microcutting produced

seven - ten roots (Fig. 3). Faisal and Al-Amin (2000) reported that 0.2 mg/l IBA and 0.2 mg/l IAA was best combination with half strength MS for root formation in *Chrysanthemum morifolium*. For hardening and plant establishment under the natural conditions, the well rooted plantlets were transferred to small earthen pots containing soil, sand and compost (2 : 1 : 1) (Fig. 4). During hardening 80% plantlets survived and those were subsequently transferred to field. The results of the present investigation demonstrate a reproducible and efficient regeneration protocol of *in vitro* micropropgation of *Rosa* spp.

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