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Research Article

IN VITRO MORPHOGENESIS OF ADVENTITIOUS ROOTS FROM NODAL EXPALNTS IN LOCAL SCENTED CULTIVAR OF *ROSA INDICA* L

***Bimal R. and Kiran N.**
Department of Botany, Biotechnology Lab, University,
B.R.A. Bihar University Muzaffarpur 842001, India
**Author for Correspondence*

ABSTRACT
The success of a microproagation protocol depends on the regeneration of shoot and root which may either be completed in one or more than one steps. In rose tissue culture shoot formation is highly convenient but root formation seems to be a difficult step especially in essential oil producing species (Kirichenko *et al.*, 1991; Pati *et al*., 1995). Keeping previous works in view, a protocol for 'Quic Root' (Ashwin, Bangalore) induced *in vitro* proliferation of well developed adventitious root system was established in scented local cultivar of *Rosa indica* L. using nodal and internodal explants. Treatment of the basal ends of explants for 30-60 seconds with 'Quic Root' was followed by transfer to ½ strength liquid MS medium. All the explants showed proliferation of 3-7 roots in 3-5 days of culture. The 'Quic-Root' treated nodal and internodal explants were planted in soil for e*x vitro* rooting but showed inconspicuous and very initial stages of root induction in 25-30 days. The *in vitro* regenerated roots were stout, white, unbranched. The well developed, stout and strong root system of the *in vitro* regenerated plants may be useful in reducing the hardening period as well as early transplantation to soil for a successful and convenient rose nursery of local scented variety of *R. indica.*

Keywords: *Rosa Indica, Quic Root, Adventitious Roots, in Vitro and ex Vitro Rooting*

INTRODUCTION
Rosa indica (Rose) is one of the most important crop in floriculture industry and produces beautiful, highly prized rose flowers. The scented petals have been widely used in extraction of rose oil for use in cosmetic industry and medicine. It is propagated commercially by vegetative methods like cutting, layering, budding and grafting, however, the propagation by vegetative means does not ensure healthy and disease-free plants. The other limiting factor in rose floriculture industry is slow multiplication of plants and season dependent flowering. However, in last few years, clonal propagation through tissue culture has revolutionized commercial nursery establishment (Pierik, 1991). Onesto *et al.*, (1985) and Reist (1985) reported significant advantages of *in vitro* regenerated plants in terms of flower production, early flowering, and better branching system and round the year production. The most significant feature of micropropagation process is the production of large number of healthy and disease free plants in relatively short span of time and also has the potential of producing propagules throughout the year. The first shoot organogenesis was reported by Hill (1967) in a climbing Hybrid Tea rose 'The Doctor'. The rose micropropagation techniques were reported by Jacob *et al.*, (1969, 1970) and Elliot (1970) in *R.hybrida* var. Superstar and *R.multiflora* respectively. In rose tissue culture shoot formation is highly convenient but root formation seems to be a difficult step. There are many works reporting enhancement of rooting efficiency in different cultivars of rose. Hyndman *et al*., (1982) reported that rooting under *in vitro* conditions depends on both internal and external physico-chemical factors such as genotype, age and size, inorganic salts, carbon level, presence or absence of hormone(s) as well as light and temperature (Pati *et al.*, 2005). Rose cultivars either essential oil producing (Kirichenko *et al*., 1991) or native of old world viz. *R.canina* and *R.damascena* (Khosh-Khui and Sink, 1982) have been reported to be difficult systems for *in vitro* induction of root in micro shoots. Thus, ability of rooting in rose is dependent on cultivar. Rout (1991) reported the best rooting response (92%-98%) in about 1.5 cm-2.0cm long microshoots obtained in six weeks of culture. The plant hormone such as IBA, NAA or IAA have been

shown to induce rooting response (Hasegawa, 1979, 1980). NAA and IBA have been used for *in vitro* induction of rooting the scented varities *R.indica* and *R.damasena* (Avramis *et al.*, 1982; Kumar *et al.*, 2001; Pati *et al*., 2005; Raageeva and Niti, 2014). Murashige (1979) reported the presence of low salt concentration promoting rooting response. The rooting percentage was found to improve by lowering the concentration of KNO_3 and NH_4NO_3 in the medium (Hyndman, 1982).The role of sucrose is also significant in the *in vitro* proliferation of roots (Davies, 1980; Raageeva, 1986; Suharsono, 1995). The role of physical condition of the medium also influenced rooting in *R.damascena* and *R.bourboniana* (Jeannette *et al*., 2000; Pati *et al*., 2005). Keeping the importance of rose and difficulties faced in the rooting of scented and essential oil producing varieties in view, studies on morphogenesis of adventitious roots in tissue culture of local scented variety of rose *R.indica* has been undertaken in the present paper.

MATERIALS AND METHODS

Fresh explants of rose (*Rosa indica*) such as nodal, internodal were collected from Botany department of B.R.A. Bihar University Muzaffarpur. The explants were washed thoroughly under running tap water for half an hour followed by washing in liquid detergent for next half an hour. Later, the explants were again washed under running tap water for 10 min.

The explants were surface sterilized with 1- 2 % $HgCl_2$ solution for 5 min. and finally washed two to three times with double distilled sterile water. These explants were cultured on Murashige and Skoog's (1962) medium supplemented with Coconut Water (CW10% v/v), IAA (0.1-5 mg^{-l}), 2,4-D (0.1-5mg^{-l}), NAA(0.1-5mg^{-l}) IBA(0.1-5mg^{-l}) either singly or in various combinations. The cultures were incubated under continuous light condition at $25^0 \pm 5^0$C in culture room. The standard methods of collection and preparations of explants have been followed. The properly washed explants were treated with the commercial Rooting Chemical 'Quic Root' (Ashwin Chemical, Banglore) for 1 min, followed by the transfer of explants into ½ MS liquid medium. A set of stem cutting treated with 'Quic Root' was also planted in soil for comparison of root development in nutrient culture medium as well as in soil. The observations were taken at frequent intervals to assess the phenomenon of root development. All the experiments were repeated at least five times.

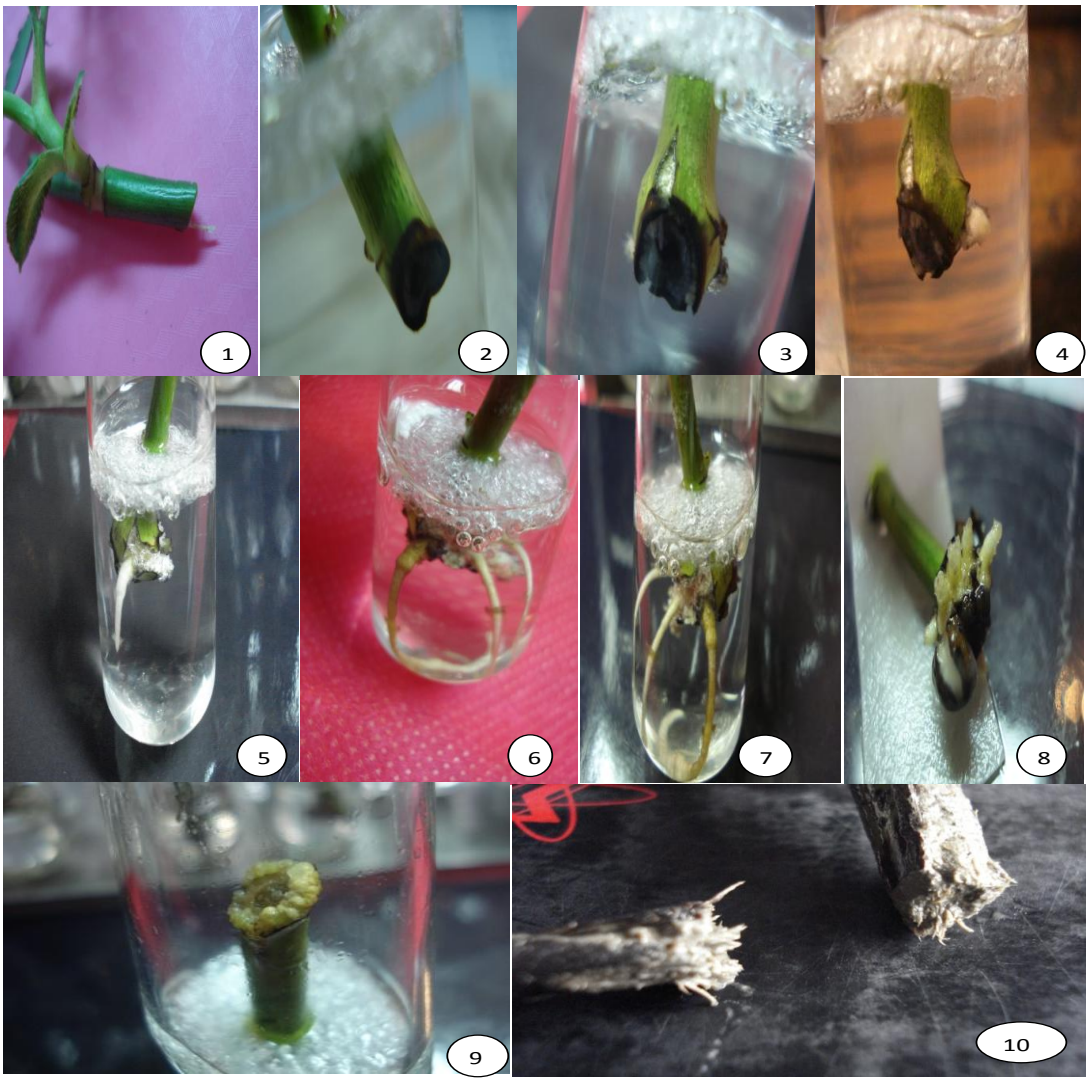
RESULTS AND DISCUSSION

Rooting potential of stem (nodal) cuttings of *R. indica* was examined in laboratory, following treatments with IBA and Rooting Chemical (Quic Root) purchased from market, both *in vitro* in liquid MS medium and *ex vitro* in soil. In the present paper, induction and development of adventitious roots in *R. indica* in the presence of rooting chemical has been studied and described (Figures 1-10). The smooth surface morphology of untreated stem piece was taken as control (Figure 1). The initial changes in the stem surface morphology after treatment with the 'Quic Root', the rooting chemical, became visible in 2-3 days of treatment followed by inoculation in ½ MS liquid medium. Figure (2) shows the blister like proliferation on the stem surface and blackening of the cut end treated with 'Quic Root' was significant. The rupturing of the surface starting from the cut end spreading upward towards node has been observed in *R. indica* (Figure 3).

The widening of the rupture exposing white crystalline callus like cells in treated nodal explant of *R. indica* cultured in ½ MS liquid medium has been shown in (Figure 4).These callus like cells later organized into definite roots. The photographs (Figures 2-4) show islands of callus like formations at the blackened cut portion as well as horizontal profusion of callus like structure from nodal region of *R. indica* cultured in ½ MS liquid medium. A little more advanced stage of development was observed as two sites of root initiation in treated *R. indica* nodal explants cultured in ½ MS liquid medium. In figure (4) a late stage of rupturing of 'Quic Root' treated end of *R. indica* developing root initiating sites has been shown. The initial bulging of first root at treated end of stem nodal explants of *R. indica* cultured in MS (½) liquid medium was visible in 5-6 days of culture (Figures 4-5). Figure (5) shows development of one properly organized root as well as the second root initial at the 'Quic Root' treated end of cultured nodal explants of *R. indica.* The initiation of other roots was also observed from treated end of stem nodal

explants (Figures 6-7). In some cases, the upper untreated end of the nodal explants showed callus like development, whereas the 'Quic Root' treated lower end of the explants produced roots (Figures 8-9). The other distinct type of morphogenetic response was also observed.

PLATE



Figures 1-10: *In vitro* morphogenesis of adventitious roots in local scented cultivar of *Rosa indica*. Figure 1: Shows the photograph of the surface morphology of untreated stem cutting taken as control. Figure 2: The blister like proliferations on the stem surface and blackening of the cut end treated with 'Quic Root'. Figure 3: Shows rupturing of the surface starting from the cut end spreading upward towards node. Figure 4: Shows the widening of the rupture exposing white crystalline callus like cells in treated nodal explant. Figure 5: Shows development of one properly organized root as well as the second root initial at the treated end of stem nodal explant cultured in ½MS medium. Figure 6: Shows differentiation of three roots. Figure 7: Shows formation of 4-5 roots. Figure 8: Shows 5-7 roots produced almost horizontally in the early stage of development. Figure 9: Shows callus like bulbous structure at the untreated end. Figure 10: Shows inconspicuous *ex vitro* root differentiation from stem pieces treated with 'Quic Root', planted in the soil and observed after 25 days

Besides, the sequential differentiation of roots, 5-7 roots of the same texture and size were produced simultaneously and were growing almost horizontaly in the early period of differentiation (Figure 8). For *ex vitro* rooting experiment, the stem pieces treated with 'Quic Root' were planted in the soil. However, even after 25 days these explants showed inconspicuous differentiation of adventitious roots (Figure 10).
A higher plant is an axially organized multicellular organism and the arrangement of cell division, elongation and differentiation is organized along the plant axis. Experiments on regeneration in plants established the concept of polarity. As early as 1878, it was observed that cutting of Willow stems form root at their physiologically basal ends irrespective of the size of the cutting. So it was concluded that polarity is fixed in the plant cell and is irreversible (Vochting, 1878). Sometimes a more readily modified polarity was observed (Leopold and Kriedemann, 1975). The pattern of regeneration of root cuttings was studied in Taraxacum (Warmke and Warmke, 1950) in which any cutting regenerates buds at the physiologically proximal end and roots at the distal end (nearest the root tips), regardless of orientation of the piece during regeneration. The regeneration of roots is regulated at least in part by auxin and the localization of the differentiation of roots at the basal end of the cuttings is due to the polar movement of auxin towards the physiologically lower end. The differentiation of roots was first reported to be a consequence of auxin stimulation (Thimann and Went, 1934) and the location of roots at the base of the cutting is presumed to be a result of the polar transport of auxin to the base of the isolated piece. It has also been reported that the number of roots intiated is related to the size of the cutting. Sinnot (1960) states that polarity is the first step in differentiation. The existence of other natural compounds which regulate rooting as an interacting set of influences were described by Hess (1964) and he also observed that certain phenolic compounds promote rooting. Brian and Halery (1973) have also shown that in addition to stimulatory factors, there can be endogenous inhibitor of rooting. Bouillenne (1964), Tomaszewski (1964) and Challenger *et al.*, (1964) found root promoting effect of o-diphenols in addition to auxin, Catechal, Caffeic acid, Chlorogenic acid are among the most effective promoters and the effect appears to be separate from the phenolic sparing action on IAA oxidase (Hess, 1964). Each of the hormone has effect on root initiation, besides the promotion effect of auxin there is an inhibition effect by gibberllin (Brian *et al.*, 1955), an even greater inhibition effect by cytokinins (Humphries, 1960) and a promotive effect by ethylene (Zimmermans and Hitchcock, 1933; Fernquist, 1966; Kawase, 1971). The induction of adventitous root formation in stem cuttings with different auxins and commercial rooting mixtures is well known (Street and Winter, 1963; Blazich, 1978; Purohit *et al.*, 2008). The effect of rooting chemical 'Quick Root' has been studied *in vitro* in *R. indica* for the first time. The efficiency of root induction and morphogenesis observed in 'Quic Root' treated *R. indica* explant was significant. Altogether 3-7 roots differentiated and attained proper growth. The rooting chemical has been reported to enhance cambial activity by mobilizing reserve food materials to the site of root formation (Gurumurithi *et al.*, 1984; Purohit *et al.*, 2008). Although spring or the season when the fresh flush starts has been found to be a favorable period for rooting experiment, in the present experiment 'Quic Root' induced rooting can be obtained round the year.

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