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In vitro propagation of rose—a review

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Abstract

In vitro propagation of rose has played a very important role in rapid multiplication of cultivars with desirable traits and production of healthy and disease-free plants. During the last several years, different approaches have been made for in vitro propagation of rose. Micropropagation using apical buds or nodal segments and understanding the specific requirements at different stages has been comprehensively covered in literature. New challenges for refinements of protocols for high rate of shoot multiplication and development of cost effective methods has gained importance in the recent past. Importance of liquid static culture for shoot proliferation and root induction for rose is also discussed in the present review. Further, the development of protocol for in vitro plant regeneration which is considered as most important step for successful implementation of various biotechnological techniques used for plant improvement programmes has been adequately addressed in literature. In rose, there are several reports which indicate rapid regeneration and multiplication through organogenesis or somatic embryogenesis. On the whole, the present review gives a consolidated account of in vitro propagation in rose.

Keywords: Rose; In vitro; Liquid medium; Shoot multiplication; Root induction; Rooting vessel; Micropropagation; Regeneration; Somatic embryogenesis

Contents

Abbreviations: BAP, 6-benzyl aminopurine; IBA, indole-3-butyric-acid; NAA, α-naphthalene acetic acid; TDZ, Thidiazuron; IAA, indole-3-acetic acid.

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1. Introduction

Rose is one of the most important commercial crops. It is generally propagated by vegetative methods like cutting, layering, budding and grafting. Seeds are used for propagation of species, new cultivars and for production of rootstocks (Horn, 1992). Although propagation by vegetative means is a predominant technique in roses, yet it does not ensure healthy and disease-free plants. Moreover, dependence on season and slow multiplication rates are some of the other major limiting factors in conventional propagation.

In the last few years, in vitro propagation has revolutionized commercial nursery business (Pierik, 1991). Significant features of in vitro propagation procedure are its enormous multiplicative capacity in a relatively short span of time; production of healthy and disease-free plants; and its ability to generate propagules around the year (Dhawan and Bhojwani, 1986). Martin (1985) demonstrated that, using this technology, up to 400,000 plants could be cloned, from a single rose on annual basis. Such a method has considerable implications for the rose breeder as it allows rapid multiplication of new varieties. Micropropagated plants are well suited for cut flower production as they are more compact (Onesto et al., 1985), branch better and sometimes yield more flowers (Reist, 1985). In addition, tissue culture derived dwarf roses used for pot plant production have a faster rate of growth, early flowering, and exhibit shorter shoots and more laterals than conventionally produced plants (Dubois et al., 1988).

The history of rose tissue culture dates back to 1945, when Nobecourt and Kofler succeeded in obtaining callus and roots on the explanted buds. In the year 1946, Lamments for the first time reported the use of embryo culture in rose breeding. Studies were initiated by Nickell and Tulecke (1959) and Weinstein et al. (1962) to culture cells, cell suspension and calli with a view to understand differentiation and regeneration. The first shoot organogenesis from callus tissue was reported by Hill (1967) in a climbing Hybrid Tea rose 'The Doctor.' The earliest references of rose micropropagation were those of Jacob et al. (1969, 1970a,b) and Elliott (1970) in *R. hybrida* cv. Superstar and *R. multiflora*, respectively.

Since these pioneering efforts, a lot of data were generated and a number of papers have been published on different aspects of in vitro studies of rose with a greater emphasis on micropropagation. A consolidated account of tissue culture studies on rose is dealt with in the present review.

2. Micropropagation

The most important technique in micropropagation is meristem proliferation wherein apical buds or nodal segments harbouring an axillary bud are cultured to regenerate multiple shoots without any intervening callus phase. Work on micropropagation of rose is summarized in Table 1.

2.1. Stages involved in micropropagation

A successful micropropagation protocol proceeds through a series of stages, each with a specific set of requirements. These are (i) initiation of aseptic cultures, (ii) shoot multiplication, (iii) rooting of microshoots, and (iv) hardening and field transfer of tissue culture raised plants.

2.1.1. Initiation of aseptic cultures

2.1.1.1. Choice of explant. The choice of explant for initiation of culture is largely dictated by the method to be adopted for in vitro propagation. Explants with vegetative meristems are often suitable for enhanced axillary branching (Table 1). The most commonly used explant is a nodal stem segment, wherein the axillary bud is made to proliferate to form multiple shoots (Fig. 1a). The performance of nodal segments is much better than the shoot tips (Horn, 1992).

Different parameters influence the initial stage of micropropagation. Mederos and Enriquez (1987) found that buds taken from softwood stem were more responsive than those from hardwood. Rout et al. (1989a) and Bressan et al. (1982) observed significant differences

Table 1
In vitro propagation of rose using different explants

Species/cultivars	Explant	Response	Reference
Rosa multiflora	apm	Shoot multiplication	Elliott, 1970
Rosa multiflora, *Rosa indica*	st	Shoot multiplication	Graifenberg et al., 1975
Rosa hybrida cv. Forever Yours	st	Shoot multiplication, rooting	Skirvin and Chu, 1979
Rosa hybrida cvs. King's Ransom, Plentiful Parade, Fragrant Cloud, Lili Marlene, Garnet Yellow, Paul's Lemon Pillar	am	Shoot multiplication, rooting	Davies, 1980
Rosa hybrida cv. Improved Blaze and Gold Glow	am	Shoot multiplication, rooting	Hasegawa, 1980
Rosa hybrida cv. Improved Blaze	st	Shoot multiplication	Bressan et al., 1982
	st	Rooting	Hyndman et al., 1982a
Rosa hybrida cvs. Tropicana, Bridal Pink, *Rosa damascena*, *Rosa canina*	st, am	Shoot multiplication, rooting	Khosh-Khui and Sink, 1982a
Rosa hybrida cvs. Tropicana, Bridal Pink	st	Shoot multiplication, rooting	Khosh-Khui and Sink, 1982b
Rosa hybrida cvs. Crimson Glory, Glenfiditch	am	Shoot multiplication, rooting	Barve et al., 1984
Rosa hybrida	lb	Shoot multiplication, rooting	Sauer et al., 1985
Rosa hybrida cv. Queen Elizabeth	apm	Shoot multiplication, rooting	Douglas et al., 1989
Rosa hybrida cvs. Landora, Virgo, Sea Pearl, Super Star, Happiness, Queen Elizabeth	am	Shoot multiplication, rooting	Rout et al., 1990
Miniature rose 'Rosamini'	apm, am	Shoot multiplication, rooting	Campos and Pais, 1990
Rosa chinensis cv. Minima	st	Shoot multiplication, rooting	Chu et al., 1993
Hybrid Tea cv. Dr. Verhage	apm, am	Shoot multiplication, rooting	Voviatzi et al., 1995
Rosa hybrida cvs. Sweet Promise, Motrea	am	Shoot multiplication, rooting	Marcelis van Acker and Scholten, 1995
Rosa banksiae, *Rosa laevigata*, *Rosa odorata*, *Rosa rugosa*, *Rosa roxburghii*, *Rosa setigera*, *Rosa wichuraina*	st, lb	Shoot multiplication, rooting	Yan et al., 1996
Hybrid Tea rose cv. Peace	st, am	Shoot multiplication, rooting	Ara et al., 1997
Miniature rose 'The Fairy'	am	Shoot multiplication, rooting	Sahoo and Debata, 1997
Rosa centifolia	am	Shoot multiplication	Ganga et al., 1998
Rosa × *hybrida* cv. Sonia	am	Shoot multiplication, rooting	Singh and Syamal, 1999
Rosa damascena var. *Jwala*	am	Shoot multiplication, rooting	Kumar et al., 2001
Rosa hybrida cv. Baronesse	am	Shoot multiplication	Carelli and Echeverrigaray, 2002
Rosa damascena and *Rosa bourboniana*	am	Shoot multiplication, rooting	Pati et al., in press

am—axillary meristems; apm—apical meristems; lb—lateral bud; st—shoot tip.

in the rate of shoot multiplication depending upon position of node on the stem in different cultivars of *R. hybrida*. The buds nearest to the apex and closest to the base of the stem exhibited the slowest rate of development, but those from the mid-stem region grew very rapidly. The potential of axillary bud outgrowth, which is related to position on the main axis, appears to be determined by a balance among several hormones (Sato and Mori, 2001). Further, the axillary buds in the mid-region of plants have the potential to grow; however, they cannot grow on intact plants due to apical dominance. Removing apical dominance could change this balance. The finding of Rout et al. (1989a) and Bressan et al. (1982) on the differences in the rate of shoot multiplication depending upon position of node on the stem could be understood in this context.

Mederos and Enriquez (1987) have reported that the presence of petiole fragments in the nodal explants had inhibitory effect on shoot growth. This report was further endorsed by the work of Marcelis van Acker and Scholten (1995) in *R. hybrida*. Salehi and Khosh-Khui (1997) found that the explant length and diameter played significant roles in proliferation and shoot growth of miniature roses (*R. chinensis* cv. Minima), 'Little Buckaroo', 'Baby Masquerado' and 'Sourati'. They also reported that the best rates of shoot growth and proliferation were obtained in explants with a length of about 9.0–10.0 mm and diameter of about 3.0–3.5 mm. No such correlation was observed in *Rosa damascena* and *R. bourboniana* (Pati, 2002). Alekhno and Vysotskii (1986), while working with Hybrid Tea, Floribunda and miniature rose cultivars, reported that growing shoots in horizontal position during proliferation almost doubled the axillary branching as compared with growing shoots in vertical position. The increased axillary shoot proliferation by placing the explant in horizontal position could be attributed to greater uptake of the medium constituents due to increased contact with the medium (Mackay and Kitto,

Fig. 1. (a–d) Micropropagation of *Rosa damascena*. (a) Axillary bud proliferation. (b) Shoot proliferation in liquid and agar-gelled medium, necrosis of shoots in gelled medium. (c) Rooting in liquid medium. (d) Hardened plants in pots.

1988). Similar results were also reported in pears (Lane, 1979) and liliac (Hildebrandt and Harney, 1983).

2.1.1.2. Sterilization. For initiation of aseptic cultures, a thorough knowledge of the physiological status and the susceptibility of the plant species to different pathological contaminants is required. In most of the explants, the commonly adopted procedure involves surface sterilization of initial explants with 70% (v/v) ethanol for 20–30 s followed by 0.1% $HgCl_2$ for 5–7 min and rinsing in sterile distilled water (Rout et al., 1989a,b, 1990; Skirvin et al., 1990). However, Khosh-Khui and Sink (1982a,b), Skirvin and Chu (1979) and Hasegawa (1979) sterilized the shoot tips using sodium hypochlorite (5.25%) and "Tween 20" or Triton X (0.1%) for 5–10 min followed by washing in sterile distilled water. Salehi and Khosh-Khui (1997) used sterile solution of different antibiotics (gentamycin, ampicillin, tetracycline or amoxicillin) at different concentrations and duration for disinfection from internal contaminants. They noticed that use of an antibiotic solution before surface sterilization was unsuccessful. However, dipping in 100 mg/l solution of gentamycin or ampicillin after surface sterilization resulted in the highest percentage of disinfected explants. Such difference could be understood as during surface sterilization, the exposed ends of the explants are removed leaving the fresh conducting tissue, which allows the antibiotic solutions to penetrate deep inside the tissue resulting in higher rates of disinfection.

2.1.1.3. Browning of the medium. Browning of the medium is the result of oxidation of polyphenols exuded from the cut surface of the explants which could be overcome by adding substances such as PVP (polyvinyl pyrrolidone), citric acid or ascorbic acid or resorting to frequent subculturing (Rout et al., 1999) or incubating cultures for a day or two in total darkness after inoculation as polyphenol oxidase activity was found to be induced by light (Pittet and Moncousin, 1981). Curir et al. (1986) stated that 3 days of culture on the medium containing charcoal followed by transfer to a fresh medium was highly effective in enhancing the growth of primary explants.

2.1.2. Shoot multiplication

This is the most crucial stage of micropropagation. The success of a micropropagation protocol, to a large extent, depends on the rate and mode of shoot multiplication. Various factors that influence in vitro shoot multiplication in rose are listed below.

2.1.2.1. Species/genotypes/cultivars. Horn (1992) marked a clear effect of genotypes on in vitro propagation in different cultivars of Floribunda and Hybrid Tea rose. He observed that it was easy to propagate cultivars Kardinal and Lilli Marleen, whereas it was very difficult to propagate Anthena, Mercedes, Pasadena and Golden times. Khosh-Khui and Sink (1982a,b) observed that the rate of shoot multiplication of *R. hybrida* cvs. Tropicana and Bridal Pink, *R. damascena* and *R. canina* varied significantly in different subculture periods. While, cultivar Bridal Pink showed the highest number of shoots (5.5 ± 0.2), *R. canina* had only 3.6 ± 0.15 shoots. In *R. damascena* 5.1 ± 0.18, and in Tropicana 4.4 ± 0.27 shoots were produced per culture in a 4-week subculture period. Bressan et al. (1982) reported varied responses of two different cultivars of *R. hybrida* to BAP. At low concentrations of BAP (0.03–0.3 mg/l), the development of axillary buds was stimulated in cv. Gold Glow but not in cv. Improved Blaze. The influence of genotype on shoot proliferation could easily be interpreted by linking it with the recent progress in functional genomics of plants. Current studies indicate that there are genes responsible for increased number of bud initials and shoot proliferation. Moreover, the possible involvement of the gene in modulating hormone levels has also been reported (Tantikanjana et al., 2001).

2.1.2.2. Media. Murashige and Skoog's (1962) medium (MS) was found to be the most commonly used for rose propagation. Davies (1980) reported that the standard MS medium induced the best rates of shoot proliferation in different rose cultivars. However, the use of other media has also been reported. Pittet and Moncousin (1982) used Linsmair and Skoog's medium supplemented with BAP (0.5 mg/l) and IBA (0.1 mg/l) for shoot initiation. Norton and Boe (1982) also used Linsmair and Skoog's medium and obtained faster rates of proliferation with BAP (0.1–2.5 mg/l). Other media like Gamborg's and Lee and de Fossard's were used by Alekhno and Vysotskii (1986). Quorine Lepoivre (QL) and woody plant medium (WPM) were used for micropropagation of *R. hybrida* cv. Moneyway (van der Salm et al., 1994).

2.1.2.3. Inorganic salts and organic compounds. Avramis et al. (1982), Curir et al. (1986) and Valles and Boxus (1987) found higher multiplication rates in different species when the ammonium content of the medium was reduced. Davies (1980) investigated the effect of different nutrient salt concentrations on the multiplication of seven cultivars in *R. hybrida*. He also reduced $(NH_4)_2\ SO_4$ but found that unchanged MS salts proved to be the best. Bressan et al. (1982), however, mentioned a negative influence of low salt concentrations on vigor and viability of shoots in *R. hybrida* cvs. Improved Blaze and Gold Glow. The leaf yellowing and shoot tip necrosis could be controlled by doubling Ca concentration in the medium (Podwyszynska and Olszewski, 1995). Moreover, increasing the concentration of Ca, Mg, Fe and Mn in the medium markedly improved the quality of microshoots. The better performance of FeEDDHA, as compared to FeEDTA, was observed in *R. hybrida* (van der Salm et al., 1994). Martin et al. (1981) and Skirvin and Chu (1979) recommended the use of organic compounds of MS medium or with modifications, especially with respect to the vitamin concentration.

2.1.2.4. Carbohydrates. Sucrose (3%) was commonly used as a source of carbohydrate. In general for tissue culture, Murashige and Skoog (1962) also stated that the use of 3% sucrose is better than 2% or 4%. However, there are many accounts on the use of higher concentrations of sucrose both for shoot initiation and proliferation. Davies (1980) had used a range of sucrose concentration from 2 to 8% and indicated that 4 to 5% was the best for culture shoot tips of seven rose varieties. Choudhary (1993), on the other hand, indicated that there was not much difference in the proliferation of shoots in the variety Priyadarshini while using 3% or 4% sucrose. The use of glucose in the culture medium has also been reported in one case (Marcelis van Acker and Scholten, 1995).

2.1.2.5. Growth regulators. In vitro shoot proliferation and multiplication are largely based on media formulations containing cytokinins as a major PGR, whereas, in some cases, low concentrations of auxins and GA_3 were also used (Table 2). Inclusion of BAP (1.0–10.0 mg/l) in the culture medium was essential for bud break and shoot multiplication of *R. hybrida* (Hasegawa, 1980; Wulster and Sacalis, 1980). Bressan et al. (1982) reported maximum promotive effects with BAP as compared to 2-isopentyladenine (2-ip). Addition of IAA neither enhanced nor repressed shoot multiplication regardless of the BAP concentration. Barna and

Table 2
Commonly used growth regulators for in vitro shoot proliferation in rose

Species/Cultivars	Growth regulators (mg/l)						Reference
	BAP	TDZ	NAA	IAA	IBA	GA_3	
Rosa hybrida cv. Improved Blaze	3.0			0.3			Hasegawa, 1979
Rosa hybrida cv. Forever Yours	2.0		0.1				Skirvin and Chu, 1979
Rosa hybrida	2.0		0.004			0.1	Davies, 1980
Rosa hybrida cvs. Improved Blaze	1.0–10.0			0.3			Hasegawa, 1980
Rosa canina	0.1–2.0			0.1–1.0			Martin et al., 1981
Rosa hybrida cvs. Improved Blaze, Gold Glow	1.0			0.3			Bressan et al., 1982
Rosa indica cvs. Major, Lusambo	2.0						Avramis et al., 1982
Rosa hybrida cvs. Tropicana, Bridal Pink, *Rosa damascena*, *Rosa canina*	1.0–2.0		0.1				Khosh-Khui and Sink, 1982a
Rosa hybrida cvs. Tropicana, Bridal Pink	1.0–2.0		0.05–0.15				Khosh-Khui and Sink, 1982b
Rosa persica × *xanthina*	1.0–2.0		0.005				Tweddle et al., 1984
Rosa hybrida cvs. Duftwolke, Iseta	2.0		0.1				Sauer et al., 1985
Rosa hybrida		0.22–2.20					Barna and Wakhlu, 1995
Cut rose cv. Samantha	2.0				0.1		He et al., 1996
Rosa hybrida cv. Crimson Glory	2.0			0.02		0.01	Syamal and Singh, 1996
Miniature rose 'The Fairy'	0.25			0.1–0.25		0.25	Sahoo and Debata, 1997
Rosa hybrida cv. Sonia	2.0		0.1			0.01	Singh and Syamal, 1999
Rosa damascena var. *Jwala*		0.22 or 0.55					Kumar et al., 2001
Rosa hybrida cv. Baronesse	3.0		0.5				Carelli and Echeverrigaray, 2002
Rosa damascena and *Rosa bourboniana*	1.12						Pati et al., in press

Wakhlu (1995) and Kumar et al. (2001) have reported the use of thidiazuron (TDZ) in the micropropagation of *R. hybrida* and *R. damascena* var. *Jwala*, respectively. Another report indicated that the presence of cytokinin in the culture medium helped in the year round multiplication of shoots in hybrid roses (Rout et al., 1990). They observed a high percentage of bud break in a hormone-free medium within 10–12 days but the rate of growth was found to be very poor. Media supplemented with BAP or BAP + GA_3, however, induced early bud break within 6–8 days of culture with enhanced rates of shoot multiplication. Addition of BAP (2.0–3.0 mg/l) as the only growth regulator in the culture medium resulted in feeble callusing at the cut ends of the explants and the shoot elongation was considerably slow with 4.26 ± 0.23 shoots per explant in a 60-day culture period and the explant response varied from 63 to 80%. Incorporation of GA_3 at low concentrations (0.1–0.25 mg/l) in the BAP supplemented medium improved explant response up to 95% with more than seven shoots per explant. Vijaya et al. (1991) reported that BAP was the most effective growth regulator in stimulating shoot proliferation. Further they investigated the use of 3 auxins in combination with BAP and found that NAA was more effective than IAA or IBA in the production of multiple shoots. Pati et al. (2001) optimized the BAP concentration at 5 μM for shoot proliferation in *R. damascena* and *R. bourboniana*.

Micropropagation of rose has also been reported to be influenced by ethylene concentration. Kevers et al. (1992) reported that pulse treatments of ethylene enhanced shoot proliferation with 5 ppm concentration being the most favourable. Ethylene also inhibited shoot elongation. Similarly, Horn (1992) reported that an increased ethylene level markedly increased senescence while reducing shoot growth but enhanced shoot number and fresh weight. Use of inhibitors of ethylene biosynthesis, such as AVG (Aminoethoxy vinyl glycine) and $CoCl_2$, increased multiplication rate by inducing more number of axillary shoots (Kevers et al., 1992). Gaspar et al. (1989), however, did not find any effect of AVG or $CoCl_2$ on shoot proliferation.

2.1.2.6. Status of the medium. For plant tissue culture media, both agar and phytagel, which are natural polysaccharides with higher capabilities of gelation are commonly used as gelling agents. Their gels combine with water and absorb other compounds. Agar is most frequently used because of its desirable characteristics such as clarity, stability and its inertness (Ibrahim, 1994). However, phytagel, the alternative gelling agent, is increasingly being used because it forms a relatively clear gel and contains no contaminants (Pierik, 1987). Marginally higher multiplication rates in phytagel medium in *R. damascena* and *R. bourboniana* (Pati et al., in press) have been attributed to the

above difference between the two gelling agents. A similar study in *R. hybrida* was carried out by Podwyszynska and Olszewski (1995), where increased shoot length and fresh weight were observed in phytagel medium but phytagel was not recommended for use because of the poor quality of shoots. No such adverse effect of phytagel was observed by Pati et al. (in press), while working on micropropagation of *R. damascena* and *R. bourboniana*.

While studying water status and growth of rose plants cultured in vitro, Ghashghaie et al. (1991) reported that vitrification impaired shoot proliferation in liquid medium and that the shoot multiplication was correlated with agar concentration. Chu et al. (1993) reported that the growth of miniature roses in liquid medium was greater as compared to those cultured in two-phase (solid–liquid) medium or solid medium alone. However, in our experiments with *R. damascena* and *R. bourboniana* (Pati et al., in press), the quantity of liquid medium was found to play an important role in shoot growth and multiplication which completely eliminated the possibility of vitrification and facilitated better quality shoots in terms of shoot length and thickness as compared to gelled medium (Fig. 1b). The lowest multiplication rate was recorded in 40 ml medium, which could be due to hyperhydricity as a result of lower availability of oxygen and submergence of shoots. Alternatively, 20 ml medium was found to be most suitable with respect to optimal shoot proliferation as well as sustenance for up to 6-week subculture period (Pati et al., in press). A similar response was also observed in case of a miniature rose (Chu et al., 1993), bamboo (Godbole et al., 2002) and in tea (Sandal et al., 2001). Further, elimination of agar in the liquid medium reduces the cost (Sandal et al., 2001). Therefore, our effort in the complete elimination of agar in the multiplication medium, the use of lower volume of liquid medium, prolong the culture period and increased rate of multiplication of shoots in liquid medium all accounted for substantial cost reduction (Pati et al., in press). Moreover, better response in static liquid cultures could be ascribed to (1) a better contact between explants and the liquid medium which increases the availability of cytokinins and other nutrients in liquid state (Debergh, 1983), (2) dilution of any exudates from the explants in liquid medium (Ziv and Halevy, 1983), and (3) adequate aeration in liquid media which ultimately enhances growth and multiplication (Ibrahim, 1994). Further, the successful establishment of cultures in liquid medium has several other advantages (Smith and Spomer, 1995; Chu et al., 1993) and is an important step towards automation (Aitken-Christie et al., 1995).

2.1.2.7. Physical factors.

2.1.2.7.1. Light. Perusal of literature indicates that light intensity plays an important role for satisfactory shoot growth and 1 to 3 klux (ca. 16–46 μmol m^{-2} s^{-1}) is reported to be sufficient for shoot proliferation in rose (Horn, 1992). Bressan et al. (1982) described that 17 μmol m^{-2} s^{-1} for 12–14 h daily was optimal for shoot multiplication. Capellades et al. (1990), however, opined that cultured plantlets could resemble greenhouse grown plants if these were cultured under high light intensity (80 μmol m^{-2} s^{-1}) as compared to the light intensity that is normally available inside the culture vessels (25 μmol m^{-2} s^{-1}).

Usually 16-h light duration from Gro-lux type white fluorescent lamps or a cool source of white light is provided in culture conditions. Davies (1980) observed that 16-h illumination showed better growth and multiplication than 8 h and this fact is further corroborated by the results of Bressan et al. (1982) which showed improvements following an increase in light period between 4 and 16 h.

2.1.2.7.2. Temperature. Leyhe and Horn (1994) recorded optimal shoot formation at 21 °C for different cultivars of *R. hybrida*. A similar observation was also made by Rout et al. (1999) where they noticed reduced shoot multiplication above 21 °C. However, many researchers have used a temperature of around 25 °C for shoot multiplication (Horn, 1992; van der Salm et al., 1996; Carelli and Echeverrigaray, 2002).

2.1.2.7.3. Growth room and vessel humidity. Begin-Sallanon and Maziere (1992) reported that an increase of the vapour pressure deficit (VPD) of the growth room from 600 to 2000 Pa increased the air VPD inside the culture vessels. Under high evaporating conditions, the multiplication rate was significantly higher, leaves were small, light green and slightly epinastic. Shoots were long (1.5 ± 0.2 cm) and pink–green, due to the presence of anthocyanin pigments, thereby indicating a juvenile characteristic of the plants in vitro.

2.1.2.7.4. CO_2. Woltering (1990) reported that CO_2 had a significant effect on the growth of in vitro rose shoots. Higher concentration of CO_2 (5%), coupled with low light, had a positive effect on plant growth.

2.1.3. Rooting of microshoots

For any micropropagation protocol, successful rooting of microshoots is a pre-requisite to facilitate their establishment in soil. Considerable work has been done

to enhance rooting efficiency in different rose varieties. Rooting of microshoots can be accomplished both under in vitro and ex vitro conditions.

2.1.3.1. In vitro rooting of microshoots. The in vitro rooting capacity depends on the interaction of internal and external factors (Hyndman et al., 1982a). These are described as follows.

2.1.3.1.1. Species/cultivars. According to Horn (1992), rooting response in rose was cultivar dependent and in certain species up to 100% success could be achieved. Kirichenko et al. (1991), while optimizing factors for in vitro multiplication of ornamental and essential oil bearing roses, reported that rooting of microshoots of the essential oil bearing roses was difficult as compared to the ornamental varieties. A similar kind of observation was also made by Khosh-Khui and Sink (1982a) where they reported a lower rooting ability in old world spp. (*R. canina* and *R. damascena*) as compared to *R. hybrida*.

2.1.3.1.2. Age and size of microshoots. Rooting also depended on the age and size of the microshoots. Rout (1991) compared the performance of microshoots 0.5–1.0 cm (4 weeks old); 1.5–2.0 cm (6 weeks old) and 2.0–2.5 cm (8 weeks old) of six cultivars of hybrid roses for their rooting response. Better rooting (92–98%) was achieved when 6-week-old shoots (1.5–2.0 cm) were dipped in IBA (1.0 mg/l) solution for 15–20 min before being placed in a sterile medium containing sand–FYM–soil mixture in the ratio of 2:1:1.

2.1.3.1.3. Media. Badzian et al. (1991) reported the use of MS medium with major elements reduced to one quarter to one third strength for root induction. Khosh-Khui and Sink (1982c) reported that half-strength MS medium supplemented with NAA (0.54 μM) was adequate for inducing rooting in cv. Bridal Veil of hybrid rose. Sauer et al. (1985) reported that 10 different rose cultivars rooted within 10–14 days on one third strength of MS medium supplemented with IAA at 5.7 μM. Subsequently, Douglas et al. (1989) achieved very high percentages of rooting in cv. Queen Elizabeth using long shoots (>2 mm) and by dilution of MS medium to one fourth strength without a growth regulator.

2.1.3.1.4. Inorganic salts. Relatively low salt concentrations in the medium are known to enhance rooting of microshoots (Murashige, 1979). Hyndman et al. (1982a) succeeded in enhancing root number and length of in vitro grown shoots of *R. hybrida* cv. Improved Blaze by lowering total nitrogen concentration of MS salts (6.0 to 7.5 mM) in the culture medium keeping other salt concentrations constant. Further, when the NO_3/NH_4 ratio increased from 0.1 to 3.0, there was an increase in the number of roots/explant. Hyndman et al. (1982a) demonstrated that a decrease in KNO_3 and NH_4NO_3 concentration was the decisive factor for improving the rooting percentage.

2.1.3.1.5. Carbohydrates. Sucrose concentration acts as an enhancer of osmotic potential and also plays a vital role in root induction. Lakes and Zimmerman (1990) showed highest rooting percentage in apple on a medium with high osmolarity. Rout et al. (1990) reported that rooting of microshoots was better on a solid medium as compared to the liquid medium. Damiano et al. (1989) observed enhancement in in vitro rooting by using a double layered medium, i.e., agar-gelled medium with an upper layer of liquid medium. Lloyd et al. (1988) reported that excised microshoots rooted well on sorbarod plugs (Cellulose support plugs; Sorbarod, Baumgartner Papiers SA, Switzerland) soaked in liquid medium. On an average, about 45% of shoots were rooted within 2 weeks of culture. It was further observed that root length was short in solid medium as compared to liquid medium (Ebrahim and Ibrahim, 2000). The retardation in root length in the solidified medium may be attributed to the relatively lower aeration in agar-gelled medium for good root development as indicated earlier by Davies (1980). However, Davies (1980) achieved 100% rooting in several cultivars of rose by using MS medium devoid of growth regulators but supplemented with 40 g/l sucrose. Hyndman et al. (1982b) also observed more and larger roots with an increase in sucrose concentration from 87.64 to 262.93 mM.

Suharsono (1995) observed that in different rose cultivars viz., Eterna G, Lancome T10 and La Jocande G2, root colour was influenced by the sugar used in the medium. It was white when the medium contained glucose and black when sucrose was used. In *R. damascena* and *R. bourboniana*, it was observed that besides the influence of PGRs, an increase in sucrose concentration resulted in a corresponding increase in the root length rather than root number (Pati et al., in press). Moreover, beyond 3% sucrose, browning of root tips occurred. Although, at this point of time, it is difficult to establish the reason, yet one of the reports in maize plant suggested that certain pigment synthesizing genes were switched on in the presence of sucrose (Jeannette et al., 2000) However, the same report indicated that no pigmentation was observed in the presence of sorbitol which was used as an osmoticum. This indicates that the darkening of the roots is due the presence of sucrose as carbohydrate not as osmoticum. Perhaps this explanation could also be extrapolated for browning of root tips in the presence of higher concentration of sucrose in *R. damas-*

cena and *R. bourboniana*. Further, experimental evidence is required to establish this. When a comparison was made on rooting response in both agar-gelled and liquid media, there was only 5% rooting in the former and 85% response in liquid culture medium. Addition of mannitol (0.5 M) to an agar-gelled medium led to a marked increase in percent rooting up to 81%. A gradual decline of root induction beyond this concentration of mannitol indicated a clear correlation of the osmotic potential of the medium to root induction. Critical analysis of the liquid and agar-gelled medium with 0.5 M mannitol revealed that they had a similar osmotic potential. This distinct role of the osmotic potential in root induction clearly throws light on subtle differences in induction of roots between varieties that can now be easily managed by shifts in osmotic potential of culture media. This also reflects that agar-gelled media do not have an adverse effect on the cultures or inhibit rooting but they are at a lower osmotic potential than the liquid cultures.

2.1.3.1.6. Activated charcoal. Activated charcoal when added to the culture medium was found to have a remarkable positive influence on the rooting efficiency of cultured rose microshoots in cv. Folklore, wherein better rooting in terms of number of roots/shoot and root length was obtained (Wilson and Nayar, 1995). Moreover, addition of activated charcoal was found to reduce the days required for root initiation from 8.5 days to 7.5 days.

2.1.3.1.7. Growth regulators. In most of the earlier reports, varying concentrations of different auxins were used for root induction (Table 3). However, Skirvin and Chu (1979) induced rooting of microshoots on growth regulators free solidified medium. The roots were easily induced from excised mature microshoots on MS medium supplemented with low concentrations of auxins (IAA or IBA or NAA) in the range of 0.1–0.5 mg/l (Hasegawa, 1979, 1980). Rooting of microshoots was also achieved by dipping the cut ends of shoots for a few hours in an aqueous solution of 1 mM of IAA instead of being continuously cultured on auxin containing medium (Collet, 1985). Khosh-Khui and Sink (1982c) reported the effect of different concentrations and combinations of auxins on root formation in *R. hybrida* cv. Bridal Pink. They also indicated that a combination of IAA (0.0–0.1 mg/l) and NAA (0.0–0.1 mg/l) was quite effective in induction of roots. Arnold et al. (1995) reported that microshoots of *R. kordesii* cvs. John Franklin and Champlain rooted well in MS medium with low or no auxin. Optimum rooting in cv. Champlain was achieved at a high concentration of IAA with low concentrations of salts or intermediate concentrations of IBA and NAA with low to medium concentrations of salts. Excised shoots of *R. hybrida* cv. Peace rooted well on ½ MS supplemented with 0.5 mg/l IBA. A close scrutiny of literature indicates that rooting response with different auxins is also cultivar dependent and it is rather diffi-

Table 3
Commonly used growth regulators for in vitro rooting in rose

Species/cultivars	Growth regulators (mg/l)					Reference
	NAA	IAA	IBA	2,4-D	GA_3	
Rosa hybrida cv. Improved Blaze		0.3				Hasegawa, 1979
	Either 0.03–0.1 or 1.0					Hasegawa, 1980
Rosa hybrida	0.05–0.1					Davies, 1980
Rosa canina		0.1–1.0			0.1–1.0	Martin et al., 1981
Rosa indica	0.1					Avramis et al., 1982
Rosa hybrida cvs. Improved Blaze, Gold Glow		1.0				Bressan et al., 1982
Rosa hybrida cvs. Tropicana, Bridal Pink, *Rosa damascena*, *Rosa canina*	0.1		0.05			Khosh-Khui and Sink, 1982a
Rosa hybrida cvs. Tropicana, Bridal Pink	0.1					Khosh-Khui and Sink, 1982b
Rosa persica × *xanthina*	0–0.3					Tweddle et al., 1984
Rosa hybrida cvs. Duftwolke, Iseta		2.0				Sauer et al., 1985
Rose cv. Folklore	1.0	1.0				Wilson and Nayar, 1995
Cut rose cv. Samantha	0.5	1.0	0.5			He et al., 1996
Rosa hybrida cv. Crimson Glory	0.2		0.5			Syamal and Singh, 1996
Miniature rose 'The Fairy'	0.25			0.1		Sahoo and Debata, 1997
Rosa hybrida cv. Sonia	0.1		0.2			Singh and Syamal, 1999
Rosa damascena var. *Jwala*						Kumar et al., 2001
Rosa damascena and *Rosa bourboniana*			2.0			Pati et al., in press

cult to induce rooting in oil bearing rose cultivars (Kirichenko et al., 1991). In *R. damascena* and *R. bourboniana* rooting was initiated as a two-step procedure where IBA (10 μM) was used in MS medium and in the second step the shoots were transferred to PGR free medium containing half strength of MS (Pati et al., in press). Chu et al. (1993) reported that microshoots of *R. chinensis* cultured in liquid medium with BAP were difficult to root and only shoots that were maintained on a medium without BAP developed roots. However, we observed that there was no difficulty in rooting of microshoots that are cultured in liquid medium supplemented with BAP (Fig. 1c).

2.1.3.1.8. Culture vessel. The importance of culture vessel in plant tissue culture has been well established. McClelland and Smith (1990) showed that woody plant explants routinely produced denser shoot cultures when grown in larger vessels. The quality of individual shoots was significantly better, shoot length in many species was enhanced, and size of individual leaves also increased with increase in the size of vessel. The rooting potential for microshoots produced in large sized vessels was also substantially improved, probably in part due to enhanced leaf area and rooting co-factors present in these leaves as per their hypothesis. However, Mackay and Kitto (1988) found that culture vessels that were excessively large also inhibited shoot length compared to medium sized vessels. Campos and Pais (1990) emphasized the choice of a container to be fundamental in achieving a good rate of success on rooting of dwarf rose cultivars 'Rosamani.' They observed that the microshoots rooted in tubes were more vigorous and with better roots with higher survival percentage than those rooted in flasks. Kozai et al. (2000) suggested that the use of large culture vessels for commercial scale propagation could reduce production costs significantly

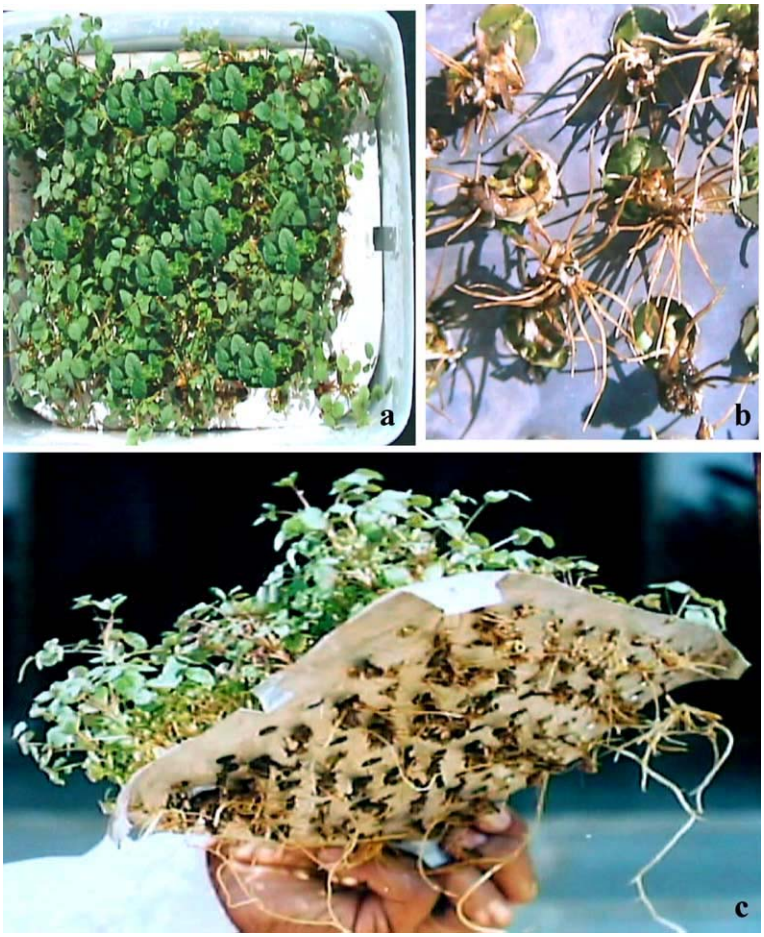

Fig. 2. (a–c) Culture vessel for rooting of microshoots of *R. damascena*. (a) Polypropylene box with bunches of shoots inserted in perforated platform with their cut ends touching the medium. (b) Perforated platform turned upside down to show profuse and healthy rooting of microshoots. (c) Rooted microshoots after 42 days of incubation in rooting vessel.

by reducing the labour cost nearly half that of conventional system.

Considering the importance of culture vessel for efficient rooting of microshoots, a rooting vessel was designed and developed in our laboratory (Pati et al., in press; Patent File No. 0427 DEL2001, 23/03/01). Such unique microchambers with platforms had several advantages: (1) a large number of shoots could be rooted concurrently at any given time (Fig. 2a–c), (2) elimination of agar resulted in substantial savings in cost (6-fold cost reduction), (3) the use of liquid culture medium in the rooting vessel provided a suitable microenvironment for rooting of bunches of microshoots, (4) ease of operation because of having a large sized container with a perforated platform, (5) damage to roots was minimized as rooted plantlets could be easily pulled out, and (6) the shape of the vessel in the form of a box facilitates stacking of these boxes in a culture shelf and hence, saves on space, thereby reducing the cost of production.

It has also been demonstrated that the period of incubation of microshoots in rooting vessel influenced the survival percentage of *R. damascena* plantlets under greenhouse conditions. Significantly higher survival percentage (96.66%) was observed after 6 weeks of incubation compared to the lowest (3.3%) after 1 week, suggesting a clear correlation of incubation period during root induction upon survival of plantlets during hardening or acclimatization (Pati et al., in press).

2.1.3.1.9. Physical factors.

2.1.3.1.9.1. Light. There are only a few reports on the role of culture environment such as light on rooting. Khosh-Khui and Sink (1982b,c) achieved about 84% rooting in cv. Bridal Pink rose with an illuminance of about 1.0 klux. They observed that higher illuminances (3.0 klux) inhibited rooting. Bressan et al.

Table 4
Summary of work on indirect organogenesis in rose

Species/cultivars	Explant	Response	PGRs (mg/l)	Media	Reference
Hybrid Tea rose 'The Doctor'	ss	c	2,4-D (0.05–5.0) and coconut milk	Unspecified synthetic medium	Hill, 1967
		sp	NAA (0.5), KN (0.2), GA_3 (20.0)		
Rosa hybrida cv. Super Star	ps	c	IBA	KB	Jacob et al., 1969
		ads	IBA (0.1), Kn (5.0)		
Rosa persica × *xanthiana*	ss	c	BAP (1.0–2.0), NAA (0.1–0.3)	MS	Lloyd et al., 1988
	c	ads	BAP (3.0), NAA (0.1–0.3)		
Rosa hybrida cv. Bridal Pink	em	c	BAP (0.22), NAA (0.05)	Modified ½ MS	Burger et al., 1990
		ads	BAP (1.0), NAA (0.01)		
Rosa damascena	ss	c	NAA (1.86)	MS gelrite 2.5 g/l	Ishioka and Tanimoto, 1990
	c	ads	BAP (2.25), IAA (0.17)	MS–NH_4NO_3	
Rosa hybrida cv. Landora	ls	c	BAP (0.5), NAA (0.1)	Modified ½ MS	Rout et al., 1992
	ss		BAP (2.0), NAA (0.5)		
	c	ads	BAP (0.2), NAA (0.01), GA_3 (10–20)	Modified ½ MS, Addl. 600 mg/l proline/glutamine	
Rosa hybrida cv. Melrutral	l, r	c	BAP (3.0), NAA (0.2)	MS	Arene et al., 1993
		ads			
Rosa multiflora 'Thornless'	Is	c	TDZ (1.0)	MS	Rosu et al., 1995
	c	ads			
Rosa hybrida cv. Carefree Beauty and *Rosa chinensis* cv. Red 'Sunblaze' and 'Baby Katie'	ls, ss	c	2,4-D (2.2–22.1) or NAA (2.0–20.11)	MS	Hsia and Korban, 1996
	c	ads	TDZ (5.0), GA_3 (1.0)	½ MS	

ads—adventitious shoot; c—callus; em—excised embryo; l—leaf; ls—leaf segment; ps—pith segment; r—root; ss—shoot primordial; ss—stem segment.

(1982), however, found that 66 μmol m^{-2} s^{-1} for 12–14 h proved to be the best for rooting. Further, they observed better rooting in the microshoots by shading the lower portion of the culture vessels. Rooting enhancement was also noticed in cultures kept in total darkness (Norton and Boe, 1982). In *R. damascena* and *R. bourboniana*, root initiation decreased by 20% under light as compared to those in dark. Skirvin and Chu (1984) found that red light had a beneficial effect on the rooting of miniature roses.

2.1.3.1.9.2. Temperature. The effect of temperature on root emergence and final rooting percentage was assessed by Alderson et al. (1988). In cv. 'Dainty Dinah' on the IBA medium, roots emerged first at 25 °C and approximately 2 and 4 days later, at 20 °C and 15 °C, respectively. Horn (1992) obtained best rooting response at 21 °C and plants cultured at this temperature were also the most successful in the weaning stage. However, Rahman et al. (1992) stated that a temperature of 28 °C was optimum for in vitro rooting.

2.1.3.2. Ex vitro rooting

Considerable attempts have also been made for root induction under ex vitro conditions. Pittet and Moncousin (1982) obtained 100% rooting of cultured shoots dipped in NAA (1.0 mg/l) for 1 h before planting these in perlite. Avramis et al. (1982) obtained 100% rooting in *R. indica* in peat vermiculite mixture after treating shoots for 5 days in a shaken mineral solution supplemented with NAA (5.5 mg/l) and sucrose (60 g/l). While treatment of shoots with IBA (50–100 mg/l) resulted in good rooting (Bini et al., 1983). Collet and Le (1987) also studied root induction in microshoots and found that a brief treatment of 16-h dip in 1 mM IAA gave good results as compared to long treatment of 7 days.

2.1.3.3. Comparison of in vitro and ex vitro rooting

A comparison of in vitro and ex vitro rooting has been made by Rogers and Smith (1992) in miniature roses (*R. chinensis* cv. Minima). They indicated that after 18 days of treatment, either in vitro (MS medium +0.5 μM NAA) or ex vitro (dipping in 0.1% IBA in a 1:1 ∷ peat:sand mixture) resulted in 95–100% rooting in three cultivars (cvs. Ginny, Red Ace and Tipper). In contrast, in cultivar Debut, 73% of shoots rooted ex vitro as compared to 93% microshoots. However, ex vitro rooting produced longer and more number of roots with increased root area. Ex vitro roots were flexible, branched and had root hairs, whereas in vitro roots were brittle, unbranched and without root hairs.

Table 5
Summary of work on direct organogenesis in rose

Species/cultivars	Explant source	Induction	Medium (PGRs mg/l) Regeneration	Additional information	Reference
Rosa persica × *xanthiana*	r	–	MS+BAP (2.0)	Shoot bud arose from the region of pericycle of the root segment	Lloyd et al., 1988
Rosa persica × *xanthiana*	l		MS+BAP (0.5l)	Shoots formed directly on the petiole and mid rib of leaflets	
Rosa laevigata					
Rosa hybrida	l	Following unpublished protocol		–	Leffering and Kok, 1990
Rosa persica × *xanthiana*	r	–	MS+BAP (2.0)	Shoot bud arose from the region of pericycle of the root segment	Lloyd et al., 1988
Rosa hybrida cv. Melrutral	l r	–	MS+BAP (2.2 μM) MS+BAP (8.9 μM)	–	Arene et al., 1993
Rosa hybrida cvs. Madelon, Only Love, Presto, Sonia, Tineke	l, pt	Modified MS+TDZ (1.5)+NAA (0.05)+casein hydrlysate (100)	MS+BAP (0.5)+NAA (0.01)+FeEDDAH (100)	Early and higher shoot bud emergence in the induction medium with $AgNO_3$	Dubois and de Vries, 1995
Rosa hybrida	lt	MS+TDZ (1.5)+IBA (0.1)	MS+BAP (0.5)+IBA (0.01)	Addition of $AgNO_3$ enhanced regeneration	Ibrahim and Debergh, 1999
Rosa damascena	pt	½ MS+TDZ (1.5)+NAA (0.05)	MS+BAP (0.5)+NAA (0.01).	Addition of $AgNO_3$ enhanced regeneration	Pati et al., 2004

l—leaf; lt—leaflet; pt—petiole; r—root.

Table 6
In vitro somatic embryogenesis in rose

Species/cultivars	Explant	Response	PGRs (mg/l)	Media	Reference
Rosa hybrida cvs. Domingo, Vicket Brown, Tanja, Azteca	l, pt	Embryogenic callus Embryo development	KN (0.1), NAA (0.05) or, KN (0.1), +NOA (0.1)	½ MS	de Wit et al., 1990
Rosa hybrida	f	Callus induction	2,4-D (2.0), Zeatin (1.5)	Modified B5	Noriega and Sondahl, 1991
		Embryogenic callus	NAA (0.25), Zeatin (1.5), GA_3 (1.5)	Modified MS	
		Embryo development	GA_3 (1.0), ABA (2.0)		
		Somatic embryo maturation	GA_3 (1.0), ABA (0.2)		
		Somatic embryo germination	–	Modified ½ N_6	
Rosa hybrida cv. Landora	ss, l	Embryogenic callus	BAP (0.5), NAA (1.0), 2,4-D (0.5–2.0)	½ MS	Rout et al., 1991
		Embryo development	BAP (0.5), NAA (0.01), GA_3 (0.1)	½ MS+L-proline (200–800 mg/l)	
		Somatic embryo germination	BAP (0.5), GA_3 (0.1)	½ MS+Adenine sulphate (24.7 μM)	
Rosa hybrida cv. Melrutral	l, r	Embryogenic callus Embryo development	BAP (3.0), NAA (0.2)	MS	Arene et al., 1993
Rosa rugosa	Ims	Embryogenic callus	No PGRs	MS	Kunitake et al., 1993
		Embryo development	No PGRs	MS, Fructose (0.1M)	
		Embryo germination	No PGRs	MS, sorbitol (0.1 M)	
Rosa hybrida cvs. Glad Tidings, Trumpeter	pt, r	Embryogenic callus Embryo development	2,4-D (1–4)	MS or SH, L-proline (300–1200 mg/l)	Marchant et al., 1996
		Embryo maturation	2,4-D (1), ABA (1), GA_3 (0.3)	MS or SH, L-proline (300–600 mg/l)	
		Embryo germination	BAP (0.5–1.0), IBA (0.1)	MS or SH, Maltose (30 g/l), Agarose (4 g/l)	

Rosa hybrida cv. Carefree Beauty and *Rosa chinensis* cv. Red 'Sunblaze' and 'Baby Katie'	l, ss	Embryogenic callus	2,4-D (2.2–22.1) or NAA (2.0–20.11)	MS	Hsia and Korban, 1996
		Embryo development	TDZ (5.0), GA_3 (1.0)	½ MS	
Rosa hybrida cv. Arizona	pt	Embryogenic callus	2,4-D (0.022), 2-iP (2.45)	Modified MS	Murali et al., 1996
		Embryo maturation	ABA (0.25)	Modified ½ MS	
		Embryo germination	NAA (0.5), Phloroglucinol (100)	½ MS	
Rosa hybrida cv. Moneyway	adr	Embryogenic callus	2,4-D (11.0)	SH	van der Salm et al., 1996
		Embryo development	No PGR		
		Embryo germination	BAP (4.44–6.75), IBA (0–0.1)		
Rosa hybrida cv. Soraya, Baccara, Mercedes and Ronto	l	Embryogenic callus	p-CPA (10.0), KN (1.0)	MS	Kintzios et al., 1999
		Embryo develoment			
		Embryo maturation	BAP (1.17), IAA (1.0)		
		Embryo germination			
Rosa hybrida cv. Soraya	l	Embryogenic callus	p-CPA(10.0), KN(1.0)	MS+Nicotinic acid (0.1)/Cystein (10)	Kintzios et al., 2000
		Embryo development			
		Embryo maturation	BAP (1.17), IAA (1.0)		
		Embryo germination			
50 different rose cultivars of Hybrid Teas, Floribundas, Miniature roses, shrubs, ramblers, rootstocks and wild species	l	Embyogenic callus	2,4-D or NAA	MS	Dohm et al., 2001
		Embryo development	Zeatin or TDZ		
		Embryo germination	IBA (0.1), BAP (2.0), GA_3 (0.1)		
Rosa hybrida L. 4th of July	l	Embryogenic callus	2,4-D (1.0) or NAA(1.0)	MS	Kim et al., 2003
		Embryo development	Zeatin (4.0)		
		Embryo germination	BAP (1.0)		

adr—adventitious roots; f—filament; ims—immature seed; l—leaf; ls—leaf segment; pt—petiole; r—root; ss—stem segment.

Moreover, ex vitro rooting is a one-step procedure comprising of both rooting and hardening (Taslim and Patel, 1995), facilitating early transplantation to soil.

2.1.4. Acclimatization and field establishment

The successful acclimatization of micropropagated plants and their subsequent transfer to the field is a crucial step for commercial exploitation of in vitro technology. However, the acclimatization of micropropagated roses was reported to be a difficult procedure because of rapid desiccation of plantlets or their susceptibility to diseases due to high humidity (Messeguer and Mele, 1986). Preece and Sutter (1991) and Sutter et al. (1992) have reviewed acclimatization of micropropagated plants in the greenhouse and in the open field. Smith et al. (1992) developed a new method of in vitro hardening of micropropagated rose plants. They developed a commercial kit, which consisted of cellulose plugs (sorbarods), for support and protecting the roots during transfer to soil, and ventilated culture vessel to improve the resistance of the plantlets to desiccation. The plantlets thus grown showed better survival when transferred to ex vitro conditions.

Douglas et al. (1989) reported that the rooting of micropropagated plants improved significantly in the compost before they were precultured for 2 weeks in vitro on sorbarods soaked in liquid medium containing IAA. Sorbarods provided the necessary protection and facility for proper handling of the microshoots and helped in the production of larger roots, which facilitated 100% successful establishment. Roberts et al. (1992) reported that the use of sorbarod transplantation plugs and growth retardants in the culture medium reduced endogenous gibberellins and prevented wilting of in vitro grown rose plantlets when transferred to soil, resulting in their better survival.

Davies (1980) achieved 15–85% rooting in a mixture of coarse perlite, peat and loam (2:2:1) depending upon the cultivar. Campos and Pais (1990) reported that about 83–100% of plantlets of dwarf rose cultivar Rosamini established well in soil within 45 days of transfer. The survival rate was higher than that reported in many other rose species (Khosh-Khui and Sink, 1982a,b; Dubois et al., 1988).

Relative humidity (RH) plays a crucial role in acclimatization and survival of tissue culture raised plants. The percentage survival of the micropropagated hybrid roses cvs. Landora, Queen Elizabeth, Virgo, Sea pearl and Happiness varied from 92 to 98% when transferred to the greenhouse at 80–85% RH and planted in earthen pots containing sand: soil:FYM::2:1:1 (Rout et al., 1989a). On the other hand, Bhat (1992) described a procedure where plantlets were acclimatized by incubating initially at 70–80% RH for 3 days before planting in vermiculite, in which 95% survival was recorded. Plants were transferred to earthen pots containing a 1:1::soil:leaf mould mixture and kept outdoors with 75.6% survival.

CO_2 enriched environment and high photosynthetic photon flux density (PPFD) can increase photosynthetic capacity and improve the acclimatization of plantlets under ex vitro conditions, even if they have no roots (Gourichon et al., 1996). In our study (Pati, 2002), these limitations were overcome by formulating a strategy for the transfer of plantlets to the hardening chamber. Large numbers of plantlets are normally transferred during the month of October to March when the ambient RH recorded to be 52% (Palampur conditions; 1300 m above msl, 32°6′N; 76°33′E), and hence, chances of disease infestations are less. Further, keeping the transplanted microshoots in a specially designed hardening chambers enriched with CO_2 ($20/11 \times 10^{-5}$ mol l^{-1} to $80/13 \times 10^{-7}$ mol l^{-1}) favoured survival up to 96.66%.

Age of the microshoots was also found to be important for the establishment of rooted plantlets (Horan et al., 1995). Our study (Pati et al., in press) indicates that the period of incubation of microshoots in rooting vessel also influenced the survival percentage of *R. damascena* plantlets under greenhouse conditions (Fig. 1d). Significantly higher survival percentage (96.66%) was observed after 6 weeks of incubation compared to the lowest (3.3%) after 1 week, suggesting a clear correlation of incubation period during root induction upon survival of plantlets during hardening or acclimatization.

3. Plant regeneration

In vitro plant regeneration is often the most important step for successful implementation of various biotechnological techniques used for plant improvement programmes. Induction of adventitious shoots and regeneration from callus cultures are of importance for somaclonal variation and, therefore, for breeding. Further, explants can also be treated with mutagens, after or prior to explanting, in order to obtain new genotypes by mutation (Horn, 1992). In rose, there are many reports which indicate rapid regeneration and multiplication through organogenesis or somatic embryogenesis (Tables 4–6).

3.1. Organogenesis

3.1.1. Indirect organogenesis

Hill (1967) reported the formation of shoot primordia on calli derived from stem explants of Hybrid Tea rose cv. The Doctor Since then, many workers have successfully induced shoot bud regeneration in rose by employing appropriate combinations of auxins and cytokinins. Summary of research work pertaining to indirect organogenesis with a variety of species/cultivars and different explants is presented in Table 2.

3.1.2. Direct organogenesis

There are few reports on direct regeneration of adventitious buds in *Rosa* (Table 3). These reports indicate that shoot regeneration response in the genus *Rosa* has been obtained from roots, leaves, leaflets and petioles (Lloyd et al., 1988; Leffering and Kok, 1990; Arene et

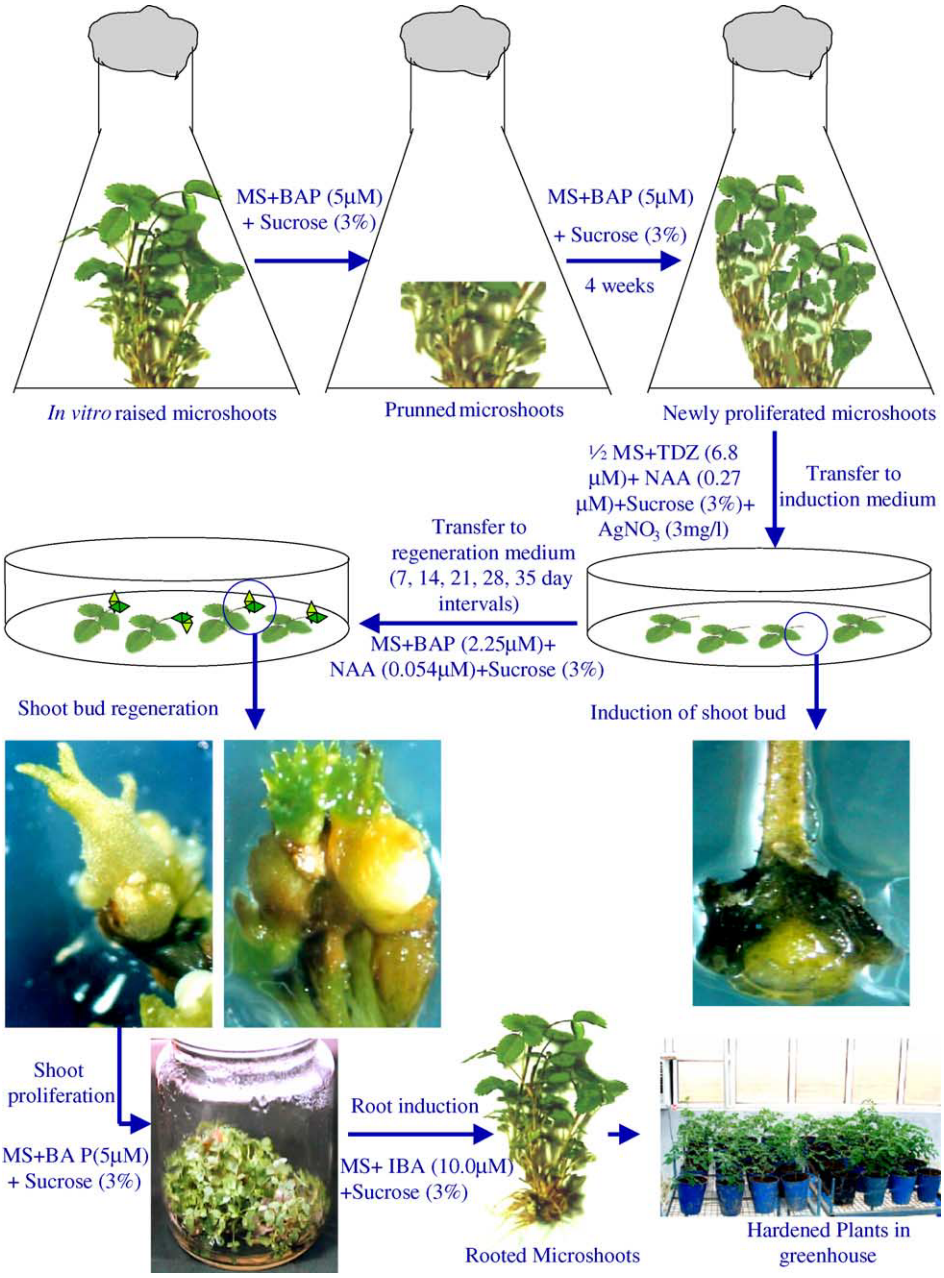

Fig. 3. Direct regeneration protocol from leaf explants of *R. damascena*.

al., 1993; Dubois and de Vries, 1995; Ibrahim and Debergh, 1999). Further, studies on direct organogenesis in the genus *Rosa* show the preponderance of use of TDZ in inducing shoot morphogenesis on leaf explants. On perusal of literature, it is also deduced that $AgNO_3$ has enhanced the regenerative potential of the leaf explants (Dubois and de Vries, 1995). However, all these observations have been on ornamental roses. We report that similar regeneration response can be evinced on leaf petioles for *R. damascena* wherein TDZ was effective in inducing shoot morphogenesis and $AgNO_3$ hastened the regeneration response by at least a week. It also highlights the importance of in vitro pruning for obtaining uniform explants after 4 weeks of pruning. Another feature of this study is the determination of hypodermal origin of the regeneration response from histological sections (Pati et al., 2004).

In our study (Pati et al., 2004), the regeneration response was evinced from leaf petioles of *R. damascena* (Fig. 3). Base of the petiole was recognized as the regeneration site in *R. hybrida* (Dubois and de Vries, 1995) and many other members of the family Rosaceae viz. *Prunus canescens* (Antonelli and Druart, 1990), *Rubus idaues* (Cousineau and Donelly, 1991), *Prunus armeniaca* and *Prunus domestica* (Escalettes and Dosba, 1993). The regenerative capacity at the base of petiole, however, could be ascribed to (a) basipetal transport of endogenous auxins and/or carbohydrates (Dubois and de Vries, 1995) and (b) position of the regenerative target cells (Margara, 1982). It was also found that addition of $AgNO_3$ to the induction medium advanced the emergence of shoot buds by 1 week. The above response could be attributed to the inhibition of ethylene synthesis by $AgNO_3$. Moreover, ethylene plays an important role in in vitro regeneration response of many crops (Cambecedes et al., 1991; Escalettes and Dosba, 1993; Table 5).

3.2. Somatic embryogenesis

Different explants such as leaf, petiole, stem and even filaments have been used for somatic embryo induction in scented roses and the medium was supplemented with varied concentrations of PGRs (Table 6). However, in *R. rugosa*, where immature seeds were used as explants, no PGRs were used in the medium for embryo induction and embryo germination (Kunitake et al., 1993). However, in *R. bourboniana*, somatic embryogenesis was induced from zygotic embryos in MS medium supplemented with 2,4-D (5–15 μM) (Fig. 4; Pati, 2002). Somatic embryos thus formed germinated to form plantlets when transferred to MS medium supplemented with BAP (5 μM) (Table 6).

4. Conclusions

Rapid multiplication of elite clones, production of healthy and disease-free plants and faster introduction of novel cultivars with desirable traits are of urgent need in rose improvement programme. In this regard, in vitro propagation techniques are likely to play a vital role. At present, there are many reproducible protocols for in vitro propagation of rose. However, the new challenges that are faced today by the tissue culture industry include cost efficiency, automation, control and optimization of the microenvironment, etc. It is, therefore, important to bring about further improvements in the existing tissue culture protocols. The recent trend in shift of the status of the medium from agar-gelled to liquid medium is a strategic step in this direction. The effective use of liquid medium during shoot multiplication and as rooting as well is a cost effective proposition and a step towards automation and commercialization. In vitro propagation of

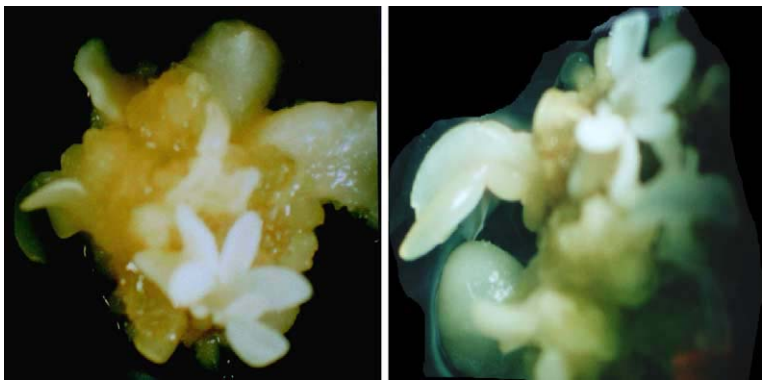

Fig. 4. Somatic embryogenesis from zygotic embryos in *R. bourboniana*.

rose via somatic embryogenesis offers a great potential for rapid propagation and improvement, and direct regeneration protocols using leaf explants from in vitro raised shoots could be effectively used in maintaining the clonal fidelity of elites and in genetic transformation programmes.

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