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BIOTECHNOLOGY OF ROSES: PROGRESS AND FUTURE PROSPECTS

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ABSTRACT

Roses (*Rosa* spp.) are one of the most important flower crops in the world and have an economic value in ornamental, pharmaceutical and cosmetic trade. Significant progress has been made in biotechnology of roses due to its many potential and practical applications in commercial production and in breeding of roses. Rapid multiplication and production of disease-free plants *in vitro* have played a vital role in propagation of commercial rose cultivars. Genetic transformation is emerged as an alternative promising tool in rose breeding since it eliminates the difficulties associated with sexual hybridization such as lengthy breeding cycles, sterility, polyploidy and high level of heterozygosity. Biotechnology also allows chimera segregation and can overcome some of the sterility problems through embryo rescue. *In vitro* seed germination protocols are ways to shorten breeding cycles and could be used to germinate the seeds that are not possible to germinate by other means. In this present review, the progress in regeneration, *in vitro* propagation, chimera segregation, callus and protoplast culture, embryo rescue, *in vitro* germination, and genetic transformation of roses were discussed and the impact of biotechnology on rose breeding was evaluated.

Keywords: Rose, tissue culture, genetic transformation, breeding.

GÜLÜN BİYOTEKNOLOJİSİ: GELİŞMELER VE EĞİLİMLER

ÖZET

Güller (*Rosa* spp.) dünyadaki en önemli çiçek ürünlerinden biri olup gerek süs bitkileri sektöründe gerekse parfümeri ve kozmetik sanayinde önemli bir yere sahiptir. Güllerin ıslahı ve ticari üretiminde çok sayıda potansiyel ve pratik uygulamalardan dolayı güllerin biyoteknolojisinde önemli gelişmeler görülmüştür. Doku kültürü yöntemi ile hastalıklardan arı ve hızlı bitki üretimi, ticari gül çeşitlerinin çoğaltılmasında önemli bir rol oynamıştır. Gen transferi, kısırılık, poliplodi, yüksek heterozigot düzeyi ve uzun ıslah süreci gibi klasik ıslah yöntemleri ile ilgili zorlukları ortadan kaldırdığından dolayı gül ıslahında alternatif bir yöntem olarak ortaya çıkmıştır. Biyoteknoloji aynı zamanda kimera dokularının ayrıştırılmasına ve kısırılıktan kaynaklanan zorlukların ıslaha verdiği sıkıntıları embriyo kurtarma ve *in vitro*'da çimlendirme olanaklarıyla ortadan kaldırılmasına katkıda bulunmuştur. *In vitro*'da tohum çimlendirme ile diğer yöntemlerle çimlendirilemeyen tohumların çimlendirilebilmesine olanak sağlanmış ve böylece ıslah süresinin kısaltılmasına önemli katkı sağlanmıştır. Bu çalışmada, gülün regenerasyonu, *in vitro* üretimi, gül kimeralarının doku kültürleri yöntemiyle ayrıştırılması, kallus ve protoplast kültürleri, embriyo kurtarma ve *in vitro* çimlendirme çalışmaları, gen transferi alanındaki gelişmeler ve bu gelişmelerin gül ıslahına etkileri üzerinde bilgiler sunulmuştur.

Anahtar kelimeler: Gül, doku kültürü, gen transformasyonu, ıslah.

1. INTRODUCTION

The roses (*Rosa* sp.), favourite ornamental plants armed with prickles, are among most important floricultural crops in the world. Most rose cultivars are traditionally propagated by cuttings or grafting onto seedling or clonal rootstocks. Rose improvement has depended on crossings followed by selection among large population. However, grafting is expensive and conventional breeding is a time consuming procedure. Biotechnology has emerged as important alternative to conventional rose propagation and breeding systems. It has found many potential and practical uses in areas associated with rose propagation and breeding such as rapid multiplication, *in vitro* mutagenesis, cultivar development via somaclonal variation and genetic transformation. Biotechnology can overcome some of the sterility problems by employing embryo rescue protocols and shorten breeding cycles through *in vitro* germination.

Tissue culture can be used as an alternative to traditional production methods. In contrast to grafting, tissue culture can yield large numbers of self-rooted plants in a very short time. However, grafting retains its importance for specific situations where clonal rootstocks are important, for instance some rootstocks give resistance to soil-borne disease and insects as well as resistance to various soil conditions. Even in this case, tissue culture can be used to propagate rootstocks (Skirvin *et al.*, 1990).

Sterility caused by embryo abscission, poor seed set and low germination rates have been obstacles for rose production and improvement via the sexual system. Embryo rescue and *in vitro* seed germination are feasible and practical procedures used to shorten the breeding cycle and to germinate seed *in vitro*.

Thornless mutants roses have been described (Morey, 1969; Nobbs, 1984; Oliver, 1986; Druit and Shoup, 1991; Canlı, 1997); unfortunately, most are chimeras consisting of mutant thornless epidermis that grows together with normal thorny internal tissues (Nobbs, 1984; Rosu *et al.*, 1995). These chimeral plants often revert to thorny state due to their unstable nature. Tissue culture protocols have been described that enabled researchers to obtain pure thornless roses.

Although important progress have been made in developing superior rose cultivars using conventional methods, breeding via conventional methods is very cumbersome procedure due to the limited gene pool, heterozygosity, polyploidy and high degree of sterility. As a result of the development of both efficient and reliable recent regeneration protocols, genetic transformation has emerged as an alternative tool for the genetic improvement of roses. The present review gives a consolidated account of progress of rose biotechnology, which became an indispensable part of commercial production and breeding programs.

2. BIOTECHNOLOGY OF ROSES

2.1. Proliferation of Roses *In Vitro*

The first report on rose shoot proliferation and rooting was made by Elliot (1970) and Jacobs *et al.* (1970). Skirvin and Chu (1979) developed protocols for proliferation and rooting of *R. hybrida*. Similar reports were made by Hasegawa

(1979). At the same time Davies (1980) published another report on rose propagation *in vitro*. Since that time there have been many reports on the proliferation of roses from shoot tips and meristems *in vitro* (Bressan *et al.*, 1982; Carelli and Echeverrigaray, 2002; Dubois *et al.*, 1988; Horn, 1992; Ganga *et al.*, 1998; Ibrahim, 1994; Pittet and Moncousin, 1982; Sato and Mori, 2001; Singh and Syamal, 1999; Skirvin and Chu, 1979; Skirvin *et al.*, 1990; van der Salm *et al.*, 1996). Dubois *et al.* (1988) published protocols for about 36 different "dwarf" roses.

Shoot tips (Khosh-Khui and Sink, 1982a) and stem segments with buds (Douglas *et al.*, 1989) are often employed to establish rose tissue cultures, but according to Mederos and Enriques (1987), the presence of a petiole may inhibit the development of axillary buds. Rose tissue cultures established from various organs are disinfected by commercial bleach (NaOCl, 10% bleach = 5.25% sodium hypochlorite) for 10 to 30 min, then rinsed with sterile distilled water 2-6 times (Skirvin *et al.*, 1990). The Murashige and Skoog medium (MS, 1962) and its modifications are commonly used to establish and maintain rose tissue cultures (Skirvin *et al.*, 1990; Vijaya and Satrayana, 1991). Temperature at 21°C was reported to be optimal for shoot formation of different cultivars of *R. hybrida* (Leyhe and Horn 1994; Rout *et al.*, 1999). However, many other researchers have successfully used a higher temperature of 25°C for shoot multiplication (Horn, 1992; Carelli and Echeverrigaray, 2002; van der Salm *et al.*, 1996). In commercial scale propagation of roses, use of large culture vessels could decrease the production costs significantly (Kozai *et al.*, 2000). Genotype was an important factor significantly effecting shoot proliferation rate in many studies (Bressan *et al.*, 1982; Horn, 1992; Khosh-Khui and Sink, 1982a).

Like many perennials, roses often contain internal contaminants which are difficult to eliminate. Such contaminants may remain unobserved for years and suddenly express themselves as milky exudate from the wounded part of plant. Such contamination is commonly known as the "white ghost". These contaminants can sometimes be eliminated by a second cycle of disinfection, but the situation can sometimes be overcome by adding an antibiotic such as Gentamicin to the medium. Other times the cultures must be eliminated and new lines established (Skirvin *et al.*, 1990).

Some roses exude phenolic substances into the medium. These compounds cause the medium to brown and can be autotoxic to the plant. The browning develops when enzymes and other compounds exuded from the cut end of the plant react with compounds in the medium. Browning can be prevented by oxidative inhibitors such as, pharmaceutical grade polyvinyl pyrrolidone (PVP) or ascorbic acid. In the case of roses, the browning is sometimes inhibited by keeping subcultured shoots in darkness for 2-3 days after subculturing (Skirvin *et al.*, 1990). Reducing the medium salt strength by half reduced the amount browning is in some apple (*Malus* sp.) cultivars (Werner and Boe, 1980). Skirvin *et al.* (1990) reported that browning is a special problem for fresh rose explants and it can be prevented by transferring explants repeatedly into fresh medium until the browning no longer occurs.

Bharadwaj et al. (2006) reported that best multiplication rate (6.9 shoots/explant) for miniature rose (*R. chinensis* Jacq. var. minima) was obtained from the MS medium fortified with 4.0 mg BAP+2.0 mg kinetin/litre and 0.1 mg NAA/litre. Minimum of 15-20 plants from a single explant of tree hybrid tea rose varieties (Christion Dior, Papa Meilland and Black Lady) were obtained within three months on MS medium if supplemented with adenine sulphate (3 mg/l) in addition to the growth regulators (Chavan et al., 2007), therefore the protocol was reported to hold promises for commercial application.

Shoot proliferation *in vitro* is largely the result of the cytokinin in the medium (Skirvin et al., 1990). Although several different cytokinins have been used in rose proliferation, best proliferation rate was obtained by using BA. According to Bressan et al. (1982), 6-benzylaminopurine (BA) at low concentrations ranging from 0.13 to 1.3 μ M resulted in good proliferation rate for 'Golden Glow', but not for 'Improved Blaze'. The authors claimed buds in the middle part of the stem gave better proliferation than the others. The presence of cytokinin in the culture medium improved year round multiplication in hybrid roses (Rout et al., 1990). High percentage of bud break in a hormone-free medium was observed within 10–12 days, but both the rate of growth and shoot multiplication were very low as compared to media supplemented with BAP or BAP+GA3. Early bud break (within 6–8 days) was also observed with the addition of BAP or BAP+GA3. Inclusion of GA3 (0.1–0.25 mg/l) in the BAP supplemented medium improved multiplication rate (more than seven shoots per explant). Pati et al. (2001) reported that the BAP concentration at 5 AM was optimum for shoot proliferation in *R. damascena* and *R. bourboniana*. BA concentrations from 5 to 20 μ M yielded the highest number of shoots and were most suitable for the *in vitro* rapid multiplication of 'Fairmount 1', a *Rosa multiflora* (Canlı, 1997). These concentrations were also reported to be also optimum for many other rose species (Compas and Pais 1990; Jabbarzadeh and Khosh-Khui 2005; Khois-Khui and Sink 1982a; Kumar et al., 2001; Skirvin et al., 1990). TDZ was also used for the micropropagation of *R. hybrida* and *R. damascena* (Kumar et al., 2001) and *Rosa multiflora* (Canlı and Skirvin, 2003). The best multiplication rates were observed between 0.9 and 5.4 μ M TDZ concentrations, however as the TDZ concentration increased, the shoot length decreased significantly and excessive callus formation was observed (Canlı and Skirvin, 2003).

2.2. Stability of Roses Derived from Tissue Culture

In vitro propagation of plants has been accepted as a rapid and reliable method to propagate many ornamental species. However, propagators and researchers have realized that clonal stability is not always the case and intra clonal variability has been observed in many crop species. Variation can be a serious problem to a propagator who requires extreme clonal stability, but such clonal variation could facilitate the selection of unique forms of standard cultivars (Skirvin et al., 1994).

Rose shoots develop *in vitro* in two different ways, from pre-formed buds (axillary) or as adventitious shoots (not pre-formed). Most tissue culturists agree that clonal stability is maximized when shoots develop from axillary bud cultures that have been proliferated at slow to moderate rates. Shoots derived adventitiously

or from rapidly proliferating axillary bud cultures are the least stable and are more likely to show tissue culture-induced (somaclonal) variation (Skirvin, 1978). Unfortunately, the clonal stability of rose cultivars derived from axillary buds *in vitro* has not been thoroughly investigated (Skirvin *et al.*, 1994).

There have been many roses harvested from *in vitro* conditions. In general, rose plants derived from the axillary buds are mostly fertile and identical to the parent (Barve *et al.*, 1984); adventitious shoots are less stable (Lloyd *et al.*, 1988, Skirvin *et al.*, 1990). According to Dubois *et al.*, (1988) no significant differences were observed among tissue culture-derived plants and plants from single node cuttings for lateral breaks, number of flower buds, and number of petals. Martin *et al.* (1981) reported no variation among 2125 rose plants growing for 3 years in a field. Somaclonal variation is most common among adventitious regenerates of most plants (Skirvin, 1978). This case also true for rose. Lloyd *et al.* (1988) reported significant variation among plants derived from callus of *R. persica* x *xanthina*. Whether the variation among these plants was somaclonal (stable) or epigenetic was not reported by the authors (Skirvin *et al.*, 1990). If stability is desired for propagation purposes, callus development should be minimized by avoiding media that induce callus (Skirvin *et al.*, 1990).

2.3. Embryo Rescue and Germination in Roses

Germination ability of rose seeds is important in production of rootstocks and developing new varieties through hybridization. Low seed set and germination rates have been obstacles for rose production and breeding. Hormonal treatments, scarification and stratification have been used to improve germination rates. However, these procedures are often ineffective and time consuming (Arunachalam and Kaicker, 1994). Embryo culture can be used to shorten the breeding cycle of roses (Lammerts, 1946; von Abraham and Hand, 1956) and accepted as a feasible and practical procedure. Lammerts (1946) stated that embryo culture in rose can be employed to obtain two generations in a year.

Burger *et al.* (1990) reported an embryo rescue system for rose, but, the frequencies of shoot growth are low and their system is not a direct germination system, that is, first embryos form callus which is forced to regenerate *in vitro*. However, embryo excision is difficult, labor intensive and contamination is a serious problem limiting the use of this method (Arunachalam and Kaicker, 1994). To overcome these difficulties an *in vitro* germination protocol was developed for roses (Canlı, 1997). In some species, the excised embryos obtained from freshly harvested seeds are not dormant and germinate quickly on sterile agar medium (Lammerts, 1946; von Abraham and Hand, 1956, Canlı, 1997). The primary dormancy of rose seeds is induced by factors in the seed coat such as abscisic acid (ABA). In this case, scarification treatments have not been very successful, because it is difficult to remove completely both the outer and inner seed coats which control dormancy (Semeniuk, 1969). Embryos taken from mature rose seeds cultured *in vitro* do not need after-ripening treatment (Semeniuk, 1969). Freshly harvested seeds of *R. multiflora* germinated successfully *in vitro* without being subjected to any stratification period and *in vitro* germination protocols can be

integrated into breeding programs to shorten the breeding cycle of roses (Canlı, 1997).

2.4. Regeneration and Somatic Embryogenesis

Availability of an efficient and reliable regeneration protocol is the first prerequisite for the development of a genetic transformation technology for any species and also for the successful implementation of many biotechnological techniques used for cultivar development such as somaclonal variation and mutagenesis.

There are several reports on adventitious shoot regeneration and somatic embryo formation from different explants of roses (Arene et al., 1993; Burger et al. 1990; Dubois and de Vries, 1995; Estabrooks et al. 2007; Firoozabady et al., 1994; Hsia and Korban 1996; Kamo et al., 2004; Lloyd et al., 1988; Noriega and Sondahl 1991; Kim et al. 2003a; Kim et al. 2004a; Kunitake et al., 1993; Pati et al., 2004; Rosu et al., 1995; Skirvin et al., 1990; Tweedle et al., 1984; Visessuwan et al. 1997), but most protocols are useful only for specific genotypes, or occur at such low frequencies making their protocols of limited value for most roses.

Lloyd et al. (1988) obtained adventitious shoots of *Rosa persica* x *xanthina*, from a callus derived from newly forming shoots which had developed after being transferred to medium containing BA (3.0 mg/l⁻¹) and α -Naphthalenacetic acid (NAA) (0.1-0.3 m/l⁻¹).

Another important protocol was developed by de Wit et al. (1990) for a "cut rose cultivar". In this study, low frequencies of somatic embryos were obtained from callus derived from leaf explants of *R. hybrida* (cvs Domingo and Vicky Brown). Rout et al. (1991) reported that they succeeded in obtaining somatic embryos from callus derived from immature leaf and stem segments of *R. hybrida* cv Landora. Noriega and Sondahl (1991) obtained somatic embryos, which gave whole plants, from callus of *R. hybrida* cv Royalty initiated from filament explants. Arene et al. (1993) reported direct adventitious shoot regeneration from leaf and root explants as well as somatic embryogenesis from callus derived from various plant (anther, petal, receptacle, leaves) of *R. hybrida* cv Meirital. *R. hybrida* leaf explants also formed adventitious shoots and the addition of AgNO₃ enhanced regeneration rate in this species (Ibrahim and Debergh, 1999).

Shoot regeneration was achieved from petiole explants of *R. hybrida* (Dubois and de Vries, 1995), *R. multiflora* (Canlı, 1997) and *R. damascena* (Pati et al., 2004). The regenerative ability of petiole base was also reported in other members of the Rosaceae and it was recognized as the regeneration site due to its high regenerative capacity (Antonelli and Druart, 1990; Cousineau and Donnelly, 1991; Escalettes and Dosba, 1993).

Rosu et al. (1995) reported a regeneration protocol for a chimeral thornless type of *R. multiflora*. They reported that shoots harvested from MS proliferation medium, supplemented with gibberellic acid (GA₃, 0.5 to 1.0 mg/l⁻¹) and silver nitrate (3.4 mg/liter), formed nodular callus and occasional putative adventitious shoots when subcultured on the same media supplemented with different levels of

thidiazuron (TDZ). The best callus and regeneration occurred on medium with 1 μ M TDZ, which produced putative adventitious shoots after a few subcultures.

Matthews *et al.* (1991) reported obtaining plantlets from callus of *R. persica* x *xanthina* which was initiated from the isolated protoplasts discussed earlier. Firoozebody *et al.* (1994) reported isolating transgenic rose plants from embryogenic callus of 'Royalty'.

Hsia and Korban (1996) reported regeneration of shoot (3.3%) and somatic embryos (6.6%) from the callus derived from stem explants of *R. hybrida* and *R. chinensis minima* on a medium containing N-phenyl-N¹,2,3-Thidiazuron (Thidiazuron) (TDZ) (23 μ M) and Gibberellic acid (GA₃) (3 μ M).

Due to its increasing importance in genetic engineering and breeding of roses, somatic embryogenesis protocols were developed from different explants of roses such as immature seed-derived calli of *R. rugosa* (Arene *et al.*, 1993; Kim *et al.*, 2003b; Kunitake *et al.*, 1993), leaf explants of *R. hybrida* (Kim *et al.*, 2003c; Kintzios *et al.*, 2000; Visessuwan *et al.* 1997), Hybrid Teas (Dohm *et al.*, 2001) and *R. canina* (Visessuwan *et al.* 1997). Both somatic embryo induction and embryo germination from immature seeds of *R. rugosa* were successfully achieved in a medium that contains no plant growth regulators (Kunitake *et al.*, 1993). However, Pati (2002) reported that plant growth regulators were necessary to induce somatic embryogenesis from zygotic embryos of *R. bourboniana* (5–15 μ M 2,4-D) and to germinate somatic embryos (5–15 μ M BAP). Somatic embryogenesis in rose was also achieved from other explants such as petioles (Marchant *et al.*, 1996; Estabrooks *et al.*, 2007), roots (Arene *et al.* 1993; Marchant *et al.*, 1996; Sarasan *et al.* 2001), filaments (Noriega and Sondahl 1991) and petals (Murali *et al.* 1996). Plant regeneration was recently being obtained through protocorm-like bodies induced from rhizoids derived from leaf explants (Tian *et al.*, 2008). The regeneration of adventitious shoots or somatic embryos is still very rare occurrences and frequencies of regeneration are low for most rose species.

2.5. Callus Culture of Rose

There are many reports of rose callus cultures (Hill, 1967; Jacobs *et al.*, 1968, 1970; Khosh-Khui and Sink, 1982b; Lloyd *et al.*, 1988; Tweddle *et al.*, 1984; Wulster and Sacalis, 1980). Rose callus cultures have been utilized to investigate physiological events and to produce secondary products including essential oils and "pharmaceutical compounds" such as ascorbic acid (Banthorpe *et al.*, 1983; Hsia, 1995; Skirvin *et al.*, 1990; Wegg and Townsley 1983). Friable callus was obtained by Khosh-Khui and Sink (1982b). The cultures could be maintained in either lighted or dark conditions. However, extensive regeneration studies with this calli failed to produce shoots (Skirvin *et al.*, 1990). Callus formation from leaf explants of *R. multiflora* was significantly increased if explants were maintained in the dark (Canlı, 2003a).

Although Hill (1967) reported obtaining "shoot primordia" from long-term callus of hybrid rose, no shoots were obtained. Tweddle *et al.* (1984) and Lloyd *et al.* (1988) reported shoot formation from callus cultures of *R. persica* x *xanthina*. They reported that callus established from newly formed shoots gave adventitious

shoots in 4 weeks. Some other rose species (*R. laevigata* and *R. wichuriiana*) failed to form shoots under these conditions (Lloyd *et al.*, 1988). The authors found that non-regenerating cultivars had large numbers of starch grains in their cells.

2.6. Suspension and Protoplast Cultures of Roses

Suspension cultures of 'Paul's Scarlet' rose have been used to investigate physiological events at the cellular level such as glutamate metabolism (Fletcher, 1974), minimal components of a tissue culture medium (Nesius *et al.*, 1972), phenol synthesis as affected by carbohydrate and nitrogen (Amorim *et al.*, 1977), carbon dioxide and pH requirements of nonphotosynthetic cells (Nesius and Fletcher, 1973). Amorim *et al.* (1977) also found that phenolic production is highest at the stationary stage of the growth cycle. According to Muhitch and Fletcher (1985) addition of sucrose and spermidine in the stationary stage cultures of 'Paul's Scarlet' rose caused an increased yield and wider range of phenols.

Suspension cultures of *R. glauca* and *R. damascena* were established and used to study the structure of primary cell wall (Joseleau and Chambat, 1984) and lignin production (Mollard and Robert, 1984). *R. damascena* cells were used to investigate the efflux of K^+ and HCO_3^- ions to the medium (Murphy *et al.*, 1983; Murphy, 1984). Pearce and Cocking (1973) isolated protoplasts of 'Paul's Scarlet' rose. Krishnamurthy *et al.* (1979) and Strauss and Potrykus (1980) later reported callus colony formation from isolated protoplasts.

Regeneration has also been achieved via protoplast cultures of roses (Kim *et al.*, 2003a; Matthews *et al.*, 1991; Schum *et al.*, 2001). Matthews *et al.* (1991) reported formation of shoots from the callus of *R. persica* x *xanthina* initiated from protoplast colonies. They first isolated protoplasts from embryogenic suspension cultures of *R. persica* x *xanthina*. After their protoplasts developed into colonies, they were transferred to Schenk and Hildebrandt's (1972) medium containing 2,4-dichlorophenoxyacetic acid (2,4-D) (3 mg/l^{-1}). In this medium, globular and later stage embryos developed into full plants on cellulose plugs soaked in MS medium containing Indole-3-butyric acid (IBA) (0.05 mg/l^{-1}) and BA (0.1 mg/l^{-1}). Schum *et al.* (2001) and Kim *et al.* (2003a) also regenerated plants efficiently from cell-derived protoplasts.

2.7. Rooting and Acclimatization of Roses

Most rose species root easily *in vitro* and some plants root spontaneously on proliferation medium (Skirvin *et al.*, 1990; Canlı and Skirvin, 2003). Most rooting media involve a modification of the MS high mineral salt medium with or without growth regulators (Douglas *et al.*, 1989; Hasegawa, 1979; Khosh-khui and Sink, 1982c; Skirvin and Chu, 1979; Skirvin *et al.*, 1990). The most common auxins used for rose root induction are NAA (naphthaleneacetic acid $0.03\text{--}0.1\text{ mg l}^{-1}$), IAA (Indole-3-acetic acid $0\text{--}1\text{ mg l}^{-1}$) and IBA (3.0 mg l^{-1} IBA indole-3-butyric acid). All are effective in rooting of rose *in vitro* (Arnold *et al.*, 1995; Hsia, 1995; Khosh-Khui and Sink, 1982a). Another factor that affects rooting of rose is the salt concentration of the nutrient medium (Douglas *et al.*, 1989; Khosh-Khui and Sink, 1982c; Skirvin and Chu, 1979). Many roses rooted well in diluted medium; half or quarter strength MS salt concentrations often promote rooting (Hasegawa, 1980;

Skirvin and Chu, 1979). The correct concentration can even eliminate the need for auxins (Skirvin *et al.*, 1990).

Arnold *et al.* (1995) reported that as the concentration of salt increased, the amount of IBA and NAA required for optimum root growth also increased in *R. kordesii* cv. Champlain. They also reported that the addition of auxin to their media reduced the average root length for all cultivars studied, but salt concentration had minimal effect on root length. They obtained 90% to 100% rooting for each cultivar on at least one combination of salt and auxins. However, contrary to earlier reports, they obtained the highest percentage of rooting when salt concentrations were high.

Environmental factors also affect the ability of roses to root. According to Khosh-Khui and Sink (1982a) rose shoots grown at low light intensity (1.0 Klux) gave a higher rooting percentage (84%) than those grown under higher light intensities (3.0 Klux). Skirvin *et al.* (1990) reported that red light can have positive effect on rooting of miniature roses (*R. chinensis*) (Skirvin and Chu, 1984). They also reported their miniature roses proliferated better under cool white fluorescent light than under warm white fluorescent. Pittet and Moncousin (1982) and Avramis *et al.* (1982) developed similar protocols for rooting roses directly in the soil. In both protocols, rooting was promoted by shaking nonrooted plants in solutions which contained low concentrations of auxin, glucose, and vitamins (Skirvin *et al.*, 1990). The polyphenol content and catechol oxidase activity were reported as important factors effecting rooting of Pingyin rose cultivars and there was negative correlation between the rooting index and these factors (XuJuan *et al.*, 2007). Khosh-Khui and Jabbarzadeh (2007) studied the rooting ability of Damask rose (*R. damascena*) and reported that 2.5 mg 2,4-D/litre for 2 weeks in MS medium following transfer of the explants to MS medium without any growth regulator was the best treatment for rooting.

2.8. Segregation of Chimeral Thornless Roses into Pure Types *in Vitro*

Thorns make roses difficult to grow and handle, therefore, thornless roses would be preferred by many growers and by the public (Nobbs, 1984; Rosu *et al.*, 1995; Canlı, 2003b). Chimeral thornless mutant roses have been described (Canlı, 2003b). Tissue culture allows us to separate the pure thornless genotype from the thorny tissue growing with it (Canlı, 2003b). In this way, pure thornless roses will pass the thornless character through a sexual cycle to the varieties of interest. Rosu *et al.* (1995) and Canlı and Skirvin (2003) modified the procedures used earlier by McPheeters and Skirvin (1983, 1989) for chimeral blackberries to obtain putative pure thornless *R. multiflora* thunb ex. J. Murr. roses.

2.9. Genetic Transformation of Roses

Successful transformation systems for a number of rose species have been reported using *Agrobacterium*-mediated protocols (Asano and Tanimoto, 2003; Condliffe, 2003; Firoozabady *et al.*, 1994; Kim *et al.*, 2004b; Li *et al.*, 2002b; Souq *et al.*, 1996; van der Salm *et al.*, 1997; van der Salm *et al.*, 1998) and particle bombardment-mediated transformation systems (Marchant *et al.*, 1998a and b).

Firoozabady et al. (1994) and Souq et al. (1996) obtained transgenic plants of *Rosa hybrida* from embryogenic tissues derived from filament cultures. Van der Salm et al. (1998) produced *Rol* gene transformed plants of *R. hybrida* L. via *Agrobacterium*-mediated transformation using strain GV3101. The grafting of untransformed scion onto the transformed rootstock resulted in axillary-bud release of the scion (van der Salm et al., 1998). Marchant et al. (1998a) first developed a biolistic bombardment-mediated transformation protocol using embryogenic callus of *R. hybrida*, then they transformed a chitinase gene into *R. hybrida*. The expression of the chitinase transgene significantly decreased the blackspot disease development (Marchant et al., 1998b). Cysteine and acetosyringone are reported to be two important factors effecting transient GUS expression in *Agrobacterium*-mediated transformation of *R. hybrida* cv. Nikita (OngChia et al. 2007). Kim et al. (2004b) successfully transformed embryogenic calluses of *Rosa hybrida* cv. Tineke using *Agrobacterium tumefaciens* strain LBA4404. *Agrobacterium tumefaciens*-mediated transformation protocol was also used to insert potentially useful transgenes into a number of rose cultivars to improve flower production, disease resistance or scent (Condliffe et al., 2003). Miniature roses have also been successfully transformed by co-cultivating embryogenic calli with *Agrobacterium*. Garden rose cultivars Heckenzauber and Pariser Charme were transformed by Dohm et al. (2002) to obtain partial resistance to fungal diseases simultaneously by overexpressing genes for particular antifungal proteins. Researchers reported transformation efficiency reached a maximum of 3 % at most.

3. CONCLUSIONS

Biotechnology has become an important and indispensable part of rose breeding and propagation programs since it can eliminate sterility problems through embryo rescue, shorten breeding cycles via *in vitro* germination, create variation by *in vitro* mutagenesis and led to cultivar development via somaclonal variation. Disease-free plant propagation via tissue culture plays a vital role in commercial production. Therefore, further optimizations of the tissue culture protocols are crucial to integrate these technologies into commercial applications.

New rose cultivars have been successfully developed through sexual hybridization; however, it is time consuming and in an effort to introduce one useful trait another may be eliminated. Genetic transformation appears to be a promising alternative tool to conventional methods since it eliminates the difficulties associated with sexual hybridization and allows improvement of a favourable variety for a single specific trait without disruption of the pre-existing characteristics. Regeneration systems have been developed for most roses and transformation systems reported for a limited number of species, however, regeneration rates and transformation frequencies are still low. Reliable regeneration systems with higher regeneration frequencies and more efficient transformation protocols need to be developed for roses so that the introduction of agronomically important genes into most rose cultivars become more of a routine procedure. With all this said, rose biotechnology offers great potential for the

genetic improvement of roses in near future especially for the traits such as pest and disease resistance, vase life and flower colour.

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