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Propagation of Pomegranate – A Review

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ABSTRACT

Pomegranate (*Punica granatum* L.) is mainly propagated by vegetative (clonal) means. However, sexual propagation is not a commercial venture. Stem cutting is the most important method of propagation in major parts of the world, excluding India, where air-layering (*gootee*) is prevalent. Generally, hardwood and semi-hardwood stem cuttings show high rooting success and survival. Certain efforts on micropropagation and grafting have been made, although these techniques will take some time for commercial implementation. Both sexual and asexual methods of pomegranate propagation are reviewed in this paper.

Keywords: air-layering, grafting, micro-propagation, stem cutting

CONTENTS

INTRODUCTION

Pomegranate (*Punica granatum* L.) is one of the most suitable fruit crops for arid and semi-arid regions of the world, including India. It is gaining popularity on marginal and sub-marginal lands of tropical and subtropical regions, especially in the Deccan Plateau of India. For the last decade, its area, production and export have increased tremendously in India (Chandra *et al.* 2006; Jadhav and Sharma 2007) and the country has occupied prime position globally. The demand for planting material is also increasing in major pomegranate-growing parts of the world. In general, it is propagated by stem cuttings. However, in some parts of India seedling plants are still used, but such seedlings show wide variation in growth and yield attributes (Patil *et al.* 2002). At present, commercial orchards in the world are established by stem cuttings (Levin 2006a; Day and Wilkins 2009; Finetto 2009). Exceptionally, air-layered plants are commonly used for the culture of pomegranate in the Deccan Plateau of India (Chandra *et al.* 2008). Although stem cuttings are difficult to root, the use of plant growth regulators (PGRs) helps to induce roots in cuttings (Panwar *et al.* 2001; Saroj *et al.* 2008; Reddy 2009) and also in air-layers (Hore and Sen 1995; Bhosale *et al.* 2009). Certain microorganisms like *Azotobacter*, *Azospirillium*, *Trichoderma harzianum* and *Pseudomonas* have been reported to induce rooting in stem cuttings. Besides, significant achievements were made in the past with regard to mass multiplication of pomegranate through tissue culture. Murashige and Skoog basal medium supplemented with 4 mg/l α-naphthaleneacetic acid (NAA), 2 mg/l kinetin and 15% coconut water was used for callus induction in pomegranate cv. 'Kandhari' from different explants (roots, hypocotyls, cotyledons, stems, shoot-tips, leaves and embryos). A high frequency of direct regeneration of roots, shoots and whole plants, without callus formation, occurred with cotyledon, leaf and stem explants (Jaidka and Mehra 1986). A protocol for *in vitro* regeneration of pomegranate using cotyledonary nodes derived from axenic seedlings has been developed (Naik *et al.* 1999, 2000; Naik and Chand 2003). Even, they standardized techniques for acclimatization and establishment of the plantlets in soil. Recently, Singh *et al*. (2007) achieved very high rate of survival (89%) of *in vitro* raised plantlets using glass jar with polypropylene cap. However, systematic efforts are needed on grafting and standardization of rootstock to overcome wilt and abiotic stresses.

SEXUAL PROPAGATION

There is little significance of sexual propagation in pomegranate culture as seedlings raised from seeds show variability in morphological and yield attributes although a number of seedling origin varieties were selected earlier and are still popular in the world. While performing self pollination studies, an interesting phenomenon was noted by Levin (2006b), who inbred a generation of seedlings that yielded fruits identical to the parents; a similar trend was recorded in the 2nd inbred generation, too. However, he suggested further studies in this regard. In general, seed germination in pomegranate depends on seed hardiness, variety and sowing season. The germination percentage varied between 7% in varieties with the hardest seeds to 98% in soft-seeded ones. Interestingly, the time taken for germination varied from 21 days to more than 100 days depending upon seed

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Invited Mini-Review

Table 1 Rooting in pomegranate stem cuttings with PGRs , other chemicals and microbial formulation.

Cultivar	Type of stem cuttings	Method of treatment	Optimum concentration (mg/l)	Reference
Bassein Seedless	Hardwood	Quick dip	2500 IBA + 2500 Paclobutrazol	Reddy and Reddy 1989
Bassein Seedless	Hardwood	Quick dip	2500 IBA + 2500 NAA	Reddy and Reddy 1990
Bedana	Hardwood	Quick dip	1000 PHB + 2500 NAA	Hore and Sen 1993
Jyoti, RCR-1	Hardwood	Prolonged dip	*Trichoderma harzianum*	Patil 2001
Ganesh, Dholka, Kandhari	Hardwood	Quick dip	1000 PHB + 5000 IBA	Tripathi and Shukla 2004
Jalore Seedless	Hardwood and semi-hardwood	Quick dip	2500 IBA	Saroj *et al.* 2008
Ganesh	Hardwood	Quick dip	5000 IBA	Barche *et al.* 2009
Ganesh	Hardwood	Quick dip or prolonged dip	2000 IBA or 100 IBA	Singh *et al.* 2009

IBA = indole-3-butyric acid; NAA= α-naphthaleneacetic acid; PHB= *p*-hydroxybenzoic acid

hardiness. The period of storage also influenced seed germinability (Levin 2006a). In India, germination in 'Ganesh' and 'Bhagawa' commenced within 8-10 days after sowing and continued up to 28 days during May (NRCP 2007), but germination percentage was higher in 'Bhagawa' (75.5-79.0%) than 'Ganesh' (61.5-67.5%). In general, 60-75% seed germination has been observed in most cultivated varieties. Interestingly, the germination percentage differed significantly in F_1 and F_2 hybrid seeds (Jalikop 2003). Jalikop also noted low seed germination (0.12-38.82%) in F_2 and as high as 88.6% in F_1 hybrid seeds. However, in dwarf pomegranate (*P. granatum* L. cv. 'Nana') seed germination was very low (Jalikop 2007). Cervelli and Belletti (1994) reported the presence of a water-soluble inhibitor in dwarf pomegranate seeds and they tried to improve the seed germination by water and stratification treatments. However, removal of the fleshy seed coat improved emergence by 5% and a subsequent wash in water for 48 h by a further 26-62.3%. In fact, information on sexual propagation of pomegranate is very limited and systematic research on its various aspects is needed.

CLONAL PROPAGATION

Cutting

Pomegranate is considered to be difficult-to-root by stem cuttings. In fact, the maturity of wood used in making cuttings plays an important role in rooting of cuttings. Propagation by stem cuttings is common practice in major pomegranate-growing regions of the world (Mendilcioglu 1968; Hu *et al.* 1993; Levin 2006a; Barche *et al.* 2009; Day and Wilkins 2009; Reddy 2009). Although several groups have tried hardwood (Reddy and Reddy 1990; Sandhu *et al.* 1991; Panwar *et al.* 2001), semi-hardwood (Deol and Uppal 1990; Panda and Das 1990) and softwood (Ghosh *et al.* 1988; Patil *et al.* 2002) stem cuttings to perpetuate pomegranate, hardwood cuttings were reported to give better rooting success than semi-hardwood and softwood ones. In pomegranate, stem cuttings lack root-promoting cofactors i.e. low sugar content, phenolic compounds and C/N ratio. Pre-conditioning of its shoots during June-July by girdling and etiolation increases the level of root-promoting cofactors considerably although the maturity of wood used in making cuttings plays a significant role in rhizogenesis (Chadha 2001). Chadha reported that wood younger than 6 months and older than 18 months is unsuitable for the stem cuttings and that hardwood lateral shoots, which usually flower and fruit, are also unsuitable for propagation. Semi-hardwood cuttings usually result in high sprouting but fail to root and establish (Rajan and Markose 2007). Girdling increased the length and number of lateral roots in stem cuttings and the number of shoots and their length and diameter were also improved significantly (Yesiloglu *et al.* 1997). The length and diameter of stem cuttings have an impact on rooting rate and subsequent survival in the field after transplanting.

Generally, 6-12 mm thick pomegranate stem cuttings induce optimum rooting (Reddy and Reddy 1990; Dhillon and Sharma 1992; Chadha 2001; Rajan and Markose 2007). Even the type of stem cutting collected from shoots has been reported to influence rooting success. Basal cuttings with a diameter of 10-12.5 mm, when treated with 5000 mg/l IBA showed the highest survival percentage, number and length of roots and number of shoots followed by sub-apical cuttings although apical cuttings failed to sprout or root. In this study, a high C/N ratio and carbohydrate reserves were responsible for the high success of rooting of basal cuttings (Purohit and Shekharappa 1985). To induce rooting in pomegranate stem cuttings, different PGRs, other chemicals and microbial formulation were tested (**Table 1**). In fact, the method of chemical treatment influences rooting success in cuttings. The quick dip method (Ghosh *et al.* 1988; Hore and Sen 1993) is mostly preferred over the prolonged dip method (Panda and Das 1990; Sandhu *et al.* 1991; Dhillon and Sharma 2002) for the treatment of stem cuttings. In the quick deep method, 30 sec to 5 min treatment was beneficial for inducing roots in pomegranate stem cuttings (Panwar *et al.* 2001; Tripathi and Shukla 2004; Saroj *et al.* 2008). In 'Ganesh', indole-3-butyric acid (IBA) at 5000 mg/l with the quick dip method (1 min dip) was optimum for higher rooting (73.3%) and field survival (Panwar *et al.* 2001). Interestingly, IBA at 3000 mg/l in talc under mist gave 80-100% rooting and survival rate in pomegranate (Rajan and Markose 2007). Earlier, Ghosh *et al.* (1988) tested the efficacy of IBA and NAA in pomegranate and found that IBA at 5000 mg/l effectively induced rooting (83.33%) in stem cuttings; a similar result was also reported by Panda and Das (1990). Even a lower concentration of IBA (100 mg/l) in the prolonged dip method (24 hrs) with hardwood cuttings enhanced rooting success under Punjab conditions (Sandhu *et al.* 1991). The treatment of pomegranate hardwood cuttings with IBA (2500 mg/l) + paclobutrazol (2500 mg/l) or IBA (2500 mg/l) and NAA (2500 mg/l) increased rooting success (Reddy and Reddy 1989, 1990). Similarly, the use of *p*-hydroxybenzoic acid (PHB) + NAA (Hore and Sen 1993) or PHB + IBA (Tripathi and Shukla 2004) was also quite effective in inducing roots in stem cuttings. Saroj *et al.* (2008) reported that semi-hardwood cuttings treated with 2500 mg/l IBA recorded 90.5-96% sprouting under controlled environmental conditions; they reported that in general, phenol, protein and carbohydrates and the C/N ratio were higher in hardwood cuttings, but N content was higher in semi-hardwood cuttings. However, they were of the opinion that in semi-hardwood cuttings, endogenous protein levels and conducive microclimatic conditions might have favoured higher rooting of cuttings. Even basal wounding along with use of IBA + NAA, each at 2500 mg/l, in hardwood cuttings resulted in higher rooting with better root growth (Reddy and Reddy 1989). In addition to PGRs, especially auxins, certain microorganisms like *Azospirillium*, *Trichoderma*, *Azotobacter*, *Pseudomonas*, etc. were also found to induce rooting in pomegranate stem cuttings. Patil *et al.* (2001) tried 4 micro-organisms viz., *Azospirillum lipoferum*, *A. brasilense*, *Trichoderma harzianum* and *Azotobacter* sp. for rhizogenesis in stem cuttings of pomegranate. Among them, *T. harzianum* resulted in higher rooting and survival of cuttings. Jaganath *et al.* (2009a) noted the positive response of microbial inoculants on rooting of hardwood cuttings in 'Bhagawa' and 'Ganesh'. Inoculation of potting mixture with *Pseudomonas fluorescence* + *Azotobacter choococcum* + *T.*

Table 2 Growing condition, type of stem cuttings and rooting percentage in pomegranate.

Cultivar	Growing condition	Type of stem cutting	Rooting percentage	Reference
Ganesh	Intermittent misting	Hardwood	-	Bose and Mandal 1972
Ganesh	Intermittent misting	Hardwood, semi-hardwood and soft wood	83.33	Ghosh *et al.* 1988
Bedana	Intermittent misting	Hardwood	92.75	Hore and Sen 1993
-	Intermittent misting with full illumination	Softwood	100	Hu *et al.* 1993
Jalore Seedless	Intermittent misting	Hardwood and semi-hardwood	90.5-96*	Saroj *et al.* 2008

*Sprouting %

harazianum and subsequently planting of stem cuttings in medium recorded highest rooting percentage (70.56%) with better root parameters (number of roots per cutting and root length and girth) under open and low-cost greenhouse conditions. Similarly, use of 2000 mg/l IBA + *Trichoderma* inoculum with potting mixture improved rooting in stem cuttings of 'Ruby' and 'Mridula' (Jaganath *et al.* 2009b).

Rooting medium also plays an important role in the root proliferation and further growth of plants raised by stem cuttings, although information on this aspect is very limited in pomegranate. Earlier, different rooting media were tested in pomegranate and diverse results with respect to rooting success were recorded. River silt medium showed quite encouraging result in response of rooting success, especially in hardwood cuttings (Baghel and Saraswat 1989; Deol and Uppal 1990). Bahadur *et al.* (2009) found IBA at 750 mg/l and rooting medium consisting of soil, sand and FYM in a 2: 1: 2 ratio to be the most suitable combination to raise pomegranate cuttings. Interestingly, the use of ash as rooting medium also induced 98% rooting in cuttings (Hu *et al.* 1993). Raising stem cuttings under controlled environmental conditions (**Table 2**) was more suitable than in open-air conditions.

In pomegranate, the time of planting of stem cuttings in nursery and field conditions affects rooting and subsequent survival. In California some farmers establish new orchards with freshly harvested cuttings from dormant mature trees, but difficulty in managing these widely spaced cuttings to mature size can lead to higher initial production costs and less favourable tree development (La Rue 1977). However, some growers produce their own nursery stock for crop establishment (Day and Wilkins 2009). Under nursery condition, 20cm long 3-4 budded cuttings after treatment with IBA 100 mg/l (prolonged dip) and 2000 mg/l (quick dip) planted in the month of January exerted positive effect with regard to sprouting and rooting percentage, number of roots per cutting, longest root, root weight, plant height and shoot girth (Singh *et al.* 2009). However, high rooting success was recorded when cuttings were planted in November (Dhillon and Sharma 2002). Recently, Saroj *et al.* (2008) noted July-August and January-March as the most congenial period to multiply hard- and semi-hardwood cuttings of pomegranate under arid conditions of Rajasthan in India.

Although propagation by root cutting is not a common method in pomegranate and very scanty literature is available in this regard. Long back it was reported that root cuttings from young trees (<4 yrs old) gave better rooting in the cuttings than those taken from older ones. The best time for preparing root cuttings is February rather than December (Mendilcioglu 1968). However, the effect of IBA on rooting percentage and growth depends on the concentration used. In fact, sprouting of shoots from roots is a common phenomenon in pomegranate (Levin 2006a).

Air-layering

In major pomegranate-growing areas of the Deccan Plateau of India, air-layered plants are used for culture (Chandra *et al.* 2008; NRCP 2009b). Use of PGRs and bio-agents has been reported to induce rooting in air-layers similar to stem cuttings. In the past, Hegde and Sulikeri (1989) tested different concentrations of IBA (250-1000 mg/l) to induce rooting in air-layers of pomegranate. Hegde and Sulikeri noted that rooting was increased with increasing levels of IBA. However, preparation of air-layers from ringed and etiolated shoots after treating with ethrel (1000 mg/l) + NAA (5000 mg/l) produced most and longest roots per layer (Hore and Sen 1994). Interestingly, the highest survival percentage was observed in air-layers when it was treated with ferulic acid (1000 mg/l) + NAA (2500 mg/l) or PHB (1000 mg/l) + NAA (10000 mg/l). Subsequently, Hore and Sen (1995) found the highest rooting (99.35%) when shoots (branches) were treated with PHB (1000 mg/l) + IBA (5000 mg/l). Bhosale *et al.* (2009) indicated that sphagnum moss with IBA 5000 mg/l could induce early rooting (17 days after layering) with 100% survival of air-layers. Instead of IBA, application of a formulation of *Pseudomonas fluorescence* having 10^9 cfu/g at the girdled portion of air-layers could induce roots. The type of media used for layering plays a vital role in rooting and survival of layers in a nursery. In general, sphagnum moss is used as a substrate for air-layering in India (NRCP 2009b), but soil, sand and cow dung manure in a 2: 1: 1 proportion was also reported as a suitable media for preparation of air-layers (Hore and Sen 1994). Even sawdust and fly ash were tested in pomegranate but the former was most ideal for rooting in air-layers (Alloli *et al.* 2001). However, June-August is optimum for air-layering with regard to rooting and survival percentage (Hegade and Sulikeri 1989; Hore and Sen 1995). Generally, air-layering is most successful when relative humidity is high (58-90%) in the atmosphere.

Stool layering

Ground layering is also an option for multiplication of pomegranate planting material (Chadha 2001). No efforts were made to propagate this crop by stool layering. Recently, stool layering in pomegranate cv. 'Bhagawa' was initiated in India. Shoot production per plot was higher (50.54 per m^2) with 0.5 × 0.5 m spacing followed by 0.75 × 0.5 m (NRCP 2009b). Indeed, more trials under different agro-climatic conditions are needed to popularize this technique among farmers and also for fast multiplication of quality planting material.

Grafting

No doubt, the grafting method of propagation in pomegranate has been reported earlier (Asadov 1987; Levin 2006), but systematic work on this aspect is meager. However, Kar *et al.* (1989) explored the possibility for topworking in wild pomegranate by budding and grafting methods. They reported that May, June and July were optimum for topworking. Side veneer grafting gave 100% success. Presently, wilt is an emerging threat to the pomegranate industry in India (Sharma *et al.* 2006) and without the availability of a standard grafting technique and tolerant rootstocks, this problem cannot be solved. Taking this into consideration, an attempt was recently made to standardize the grafting method and time in pomegranate. Wedge grafting on the 30th January gave 85% graft success with one-year-old seedlings of cv. 'Phule Arakata' as rootstock. In general, scion sprouting started between 8 and 12 days after grafting. The grafted plants during January had a perfect union, indicating better scion and rootstock compatibility (Chandra *et al.* 2009; NRCP 2009a).

MICROPROPAGATION

In view of increasing pomegranate cultivation area around the world, mass multiplication through tissue culture is necessary. Various factors such as genotype ('Ganesh' and 'Mridula'), explant type (axillary bud, cotyledon, shoot tip, etc.), season, media (MS and WPM) and PGRs (BA, IAA, IBA, NAA, kinetin, etc.) influence the micropropagation of pomegranate (Moriguchi *et al.* 1987; Omura *et al.* 1987; Kantharajah *et al.* 1998; Patil *et al.* 2002; Naik *et al.* 2003; Murkute *et al.* 2004; Chaugule *et al.* 2005). Earlier, Jaidka and Mehra (1986) demonstrated a high frequency of direct regeneration of roots, shoots and whole plants, without callus formation, with cotyledon, leaf and stem explants and Omura *et al.* (1987) could achieve organogenesis using leaf explants. They noted that shoot elongation was stimulated most efficiently when the initial calli were transferred from the shoot induction medium to half-strength MS supplemented with 2.0 µM benzyladenine (BA), elongated shoots rooted easily on a medium of one-half MS and 0.1 µM NAA. Similarly, Moriguchi *et al.* (1987) explored the possibility of anther culture in pomegranate using MS medium containing BA 5 or 10 µM and NAA 1 or 5 µM for callus production and the callus produced were transferred to MS medium containing 2.0 µM of BA and 0.5 µM of NAA for shoot regeneration. Later on, Bhansali (1990) regenerated plantlets successfully from somatic embryos originating from cotyledonary tissues of pomegranate through multiple somatic embryogenesis. In fact, Bhansali tried different growth regulators supplemented MS media for regeneration of plantlets. Yang *et al.* (1991) reported regeneration of plantlets from dormant bud segments cultured *in vitro* on MS medium supplemented with IBA at 0.1-1.0 mg/l, at 25-27°C. The regenerated plantlets had a rooting rate of >90%, which, when transplanted to the field during April-June, had a survival rate of 12-98% and grew normally. Mahishni *et al.* (1991) cultured shoot tip explants on MS medium and subsequently transferred them to Lloyd and McCrown woody plant medium for rapid growth and elongation of shoots, which were rooted on modified MS medium before transfer to potting mixture. They achieved 80% success in establishment of plantlets in 1: 1: 1 (v/v) peat, perlite and sand mixture. Even, Nataraja and Neelambika (1996) induced plantlets from petal explants on MS medium supplemented with various PGRs. Numerous embryoids formed from friable callus on MS medium with 5 mg/l IAA, IBA or NAA; after 8 weeks they had only developed roots, but those formed on MS medium with 5 mg BAP (6-benzylamino purine) or kinetin/l developed shoots. Explants cultured on MS medium supplemented with 5 mg/l IAA and 5 mg/l BAP differentiated numerous embryoids but they developed multilobed or fused cotyledons. However, callus induced on MS medium with 1 mg/l IAA or 1 mg/l IBA on subculturing in the same medium with half-strength salts and sucrose (4%) produced both roots and shoots, resulting in plantlets. Fougat *et al.* (1997) observed that axillary branching of nodal segments and proliferation of shoot tip meristems was best on MS medium supplemented with 0.5 mg/l kinetin, 1.0 mg/l BA and 500 mg/l CH (cycloheximide), although rooting was best on MS medium supplemented with 4.0 mg/l NAA, 2.0 mg/l kinetin and 15% CW (coconut water). Further, Kantharajah *et al.* (1998) tried to standardize tissue culture for multiplication of pomegranate plantlets. Naik *et al.* (1999) described an efficient procedure for *in vitro* clonal propagation in 'Ganesh' using nodal stem segments of a mature tree. Subsequently, a complete protocol was developed for its *in vitro* regeneration, using cotyledonary nodes derived from axenic seedlings (Naik *et al.* 2000). Again, Naik *et al.* (2003) observed that the addition of ethylene inhibitors like $AgNO_3$ (10-40 µM) and aminoethoxyvinylglycine (AVG) (5-15 µM) to MS medium containing BA and NAA markedly enhanced the regeneration frequency as well as the number of shoots per explant of pomegranate. Among different strategies adopted for enhancing hardening of *in vitro*-raised plantlets, maximum success (89%) was achieved by the use of glass jars with polypropylene caps (Singh *et al*. 2007). Browning of cultures is a major obstacle due to high phenolic contents that hamper establishment of explants of pomegranate (Murkute *et al.* 2003). Murkute *et al.* (2003) tested several treatments, including the use of adsorbents and antioxidants, with different explants *viz.*, leaf segment, cotyledon, nodal segment and shoot tip. The sub culturing of explants three times, at a 24-h interval controlled browning in all explants used, except for cotyledon. An attempt was also made to produce synthetic seeds in this crop (Naik and Chand 2006). They were successful in encapsulating nodal segments from *in vitro* proliferated shoot cultures or axenic cotyledonary nodes. These tissues were encapsulated in calcium alginate hydrogel containing MS medium supplemented with 4.44 µM BAP and 0.54 µM NAA. However, a combination of 3% sodium alginate and 100 mM calcium chloride was most suitable for formation of synthetic seed. Encapsulated nodal segments could be stored up to 30 days at 4°C were capable of sprouting. No doubt, protocols for regeneration of pomegranate have been developed in different countries but commercialization of these techniques is yet to be taken up in years to come.

CONCLUDING REMARKS

Impressive advances in propagation of pomegranate have been made in the past. Most of the current published work on pomegranate is pertaining to stem cuttings and micro propagation with only a little fraction focused on grafting, layering, sexual propagation, rooting media and bio-agents. Emphasis on physiology of rooting in cutting and layering, mass multiplication through tissue culture, exploitation of bio-agents for propagation and nutrition, scion and rootstock compatibility and rootstock adoptability in different climatic and soil conditions need special attention.

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