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Propagation practices in pomegranate: A review

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Abstract

Pomegranate (*Punica granatum* L.) is mainly propagated by vegetative means. Sexual propagation is not practiced commercially. Stem cutting is one of the most suitable methods for multiplication of planting material which is popular method 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. In the recent past attempts have been made on micro-propagation, stenting, grafting and budding. However, both sexual and asexual methods of pomegranate propagation are reviewed in this paper

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Key words: *Propagation, pomegranate, Punica granatum, stenting grafting, budding, micropropagation*

Pomegranate (*Punica granatum* L.) is an ancient edible fruit and its commercial cultivation is mainly confined in tropical and subtropical regions (Levin, 2006; Chandra *et. al.* 2010). It is native to Iran and its domestication started in the Middle East about 5000 years ago. The scientific name *Punica granatum* is derived from the name 'Pomum' (apple) 'granatus' (grainy) or seeded apple. The Romans first called this species "malum punicum" (punic apple or apple of Carthage) that evolved to "Punicum granatum". Punicaceae contains a single genus Punica with two species viz. *Punica granatum* L .and *P. protopunica* Balf. f., (syn Socotria protopunica) of which the latter is endemic to Socotra Island (Yemen). Pomegranate is a large shrub or small tree and has tendency to develop multiple trunks with bushy appearance. Pomegranate is an economically important plant and has been used by mankind since the dawn of civilization. The edible part of the fruit contains considerable amounts of acids, sugars, vitamins, polysaccharides, polyphenols and important minerals. Pomegranate has several health benefits to treat many human diseases like coronary heart diseases, cancer (skin, breast, prostate and colon), inflammation, hyperlipidemia, diabetes, cardiac disorders, hypoxia, ischemia, aging, brain disorders and AIDS with biologically active ingredients isolated from different parts of the plant (Seeram *et. al.* 2006).However, pomegranate is propagated by sexual and asexual means but, latter is most common method of propagation globally.

Sexual propagation

In pomegranate, both sexual and asexual methods are practiced for multiplication of planting material. Still in India, sexual method of propagation is used on limited scale but seedlings raised from seeds show some variability in morphological and fruit characters owing to segregation phenomenon. Thus, seedlings are not used for establishment of commercial orchards. 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) and 98% (in soft - seeded ones). The time taken for germination ranges from 10 days to >100 days depending upon seed hardiness. However, viability of seeds is influenced by the period of seed storage (Levin, 2006). Very early seed germination was noted in 'Ganesh' and 'Bhagawa' cultivars. In both the cultivars germination commenced within 8-10 days after sowing and continued up to 28 days in Solapur condition during May (NRCP, 2007) and their germination percentage was higher (61.5-79.0%). In dwarf pomegranate (*P. granatum* L. Cv. 'Nana), seed germination was very low (Jalikop, 2007). Earlier, Cervelli and Belletti (1994) reported that a water soluble inhibitor was present in dwarf pomegranate seeds and they found that removal of the fleshy seed coat enhanced emergence of seedlings by 5% and a subsequent wash in water for 48 hr by a further 26-62.3%.

Asexual Propagation

(1) Stem cutting - Stem cutting is very common method for production of elite planting material in the

world. In this method, the maturity of wood plays vital role in the rooting. Hardwood (Reddy and Reddy, 1989, 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 were evaluated for propagation in pomegranate. Hardwood cutting method proved to be the most successful. Interestingly, 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. Some reports are available that girdling increased the length and number of lateral roots in stem cuttings with improved shoot growth (Yesiloglu *et. al.* 1997). Although the maturity of wood used in making cuttings had a significant role in rhizogenesis (Chadha, 2001). Hard wood cuttings respond better to the hormonal treatment as compared to semi hard-wood cuttings (Sharma *et. al.* 2009). The wood younger than 6 months and older of 18 months found unsuitable for the stem cuttings. Similarly, hardwood lateral shoots , which usually flower and fruit , are also unsuitable for propagation and such cuttings should be avoided. Usually, semi-hardwood cuttings give high sprouting but fail to root and establish subsequently (Rajan and Markose, 2007).

Generally, the length and diameter of stem cuttings have a impact on rooting rate and subsequent survival in the field after transplanting. As per reports, 6-12mm thick pomegranate stem cuttings have been found to be suitable for propagation (Reddy and Reddy, 1990; Dhillon and Sharma, 1992; Chadha, 2001; Rajan and Markose, 2007).Usually, use of plant growth regulators (PGRs) especially auxins improve rooting in stem cuttings of pomegranate. Basal cuttings with a diameter of 10-12.5mm when treated with 5000 ppm IBA showed the highest survival percentage, number and length of roots and number of shoots followed by sub-apical cuttings. A high C/N ratio and carbohydrate reserves was found to be responsible for the high success of rooting in basal cuttings (Purohit and Shekharappa, 1985). 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 second to 5 minute treatment was beneficial for inducing roots in the stem cuttings (Panwar *et. al.* 2001; Tripathi and Shukla, 2004; Saroj *et. al.* 2008). In cv. Ganesh, Indole - Butyric acid (IBA) at 5000ppm with the quick dip method (1 min dip)was optimum for getting higher rooting (73.3%) and field survival (Panwar *et. al.* 2001). Even, IBA at 3000ppm 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 5000ppm effectively induced rooting (83.33%) in stem cuttings. Treating hardwood cuttings with IBA (2500 ppm) + paclobutrazol (2500 ppm) or IBA (2500 ppm) and NAA (2500 ppm) also increased rooting success in pomegranate (Reddy and Reddy, 1989, 1990). The use of p-hydroxybenzoic acid (PHB) + NAA (Hore and Sen, 1993) or PHB + IBA (Tripathi and Shukla, 2004) had also been reported effective for inducing roots in the stem cuttings. Very high sprouting (90.5-96%) in semi-hardwood cuttings with 2500ppm IBA was noted by Saroj *et. al.* (2008) 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. Even basal wounding along with use of IBA+NAA , each at 2500 ppm , in hardwood cuttings resulted in higher rooting with better root growth (Reddy and Reddy, 1989). Though, a lower concentration of IBA (100 ppm) in the prolonged dip method (24 hrs) with hardwood cuttings was also found beneficial for more rooting success (Sandhu *et. al.* 1991).

There are reports that rooting media play important role in the root proliferation and subsequently affecting growth of plants raised by stem cuttings. 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 that IBA at 750 ppm and rooting medium consisting of soil, sand and FYM in 2: 1: 2 ratio was the most suitable combination to raise pomegranate cuttings. Very high rooting (98%) in stem cuttings with ash medium was also reported (Hu *et. al.* 1993). The time of planting of stem cuttings in nursery and field conditions affect rooting and subsequent survival. Recently, different rooting media and planting time were tested for rooting in stem cutting of pomegranate. Vermiculite and sand media gave more than 85% rooting in pomegranate when planting was done in February under mist and bottom heat system (Khalil, 2013). High rooting success was recorded when cuttings were planted in November (Dhillon and Sharma, 2002). Saroj *et. al.* (2008) noted July-August and January-March as the most congenial period for multiplication through stem cutting in pomegranate.

(2) Air Layering- Air layering is very common in Deccan Plateau of India, especially in Maharashtra and Karnataka. Use of PGRs has been reported to induce

rooting in air-layers similar to stem cuttings. Hore and Sen (1995) found the highest rooting (99.35%) in air layers using PHB (1000 ppm) + IBA (5000 ppm). Bhosale *et. al.* (2009) indicated that sphagnum moss with IBA 5000 ppm could induce early rooting (17 days after layering) with 100% survival of air-layers. Similarly, Tomar (2011) recorded highest rooting (90%) in air layers with IBA 2000 ppm. However, the type of media used for layering also played role in rooting and survival of layers. In general, sphagnum moss is used as a substrate for air-layering (NRCP 2009), 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).Under open condition June-August is optimum time for air-layering (Hegde and Sulikeri 1989; Hore and Sen 1995).However, in greenhouse with mist facilities planting can be done throughout the year. Ground layering is used for multiplication of pomegranate planting material as reported in other fruit crops.

(3) Stool layering- This technique has commercial implication in guava. Similar effort was made to propagate pomegranate and stool layering in cv. Bhagawa proved beneficial. A spacing of 0.5x0.5m or 0.75x0.5m was found to be optimum for stool layering under Solapur condition of Maharashtra (NRCP 2009). This could be one of the options for multiplication of planting material in pomegranate especially for small and marginal farmers in semiarid regions.

(4) Grafting - Grafting is not a common method of propagation in pomegranate although it had been reported earlier (Asadov 1987; Levin 2006). Though, systematic work on this aspect is very much lacking. Kar *et. al.* (1989) explored the possibility for top working in wild pomegranate by budding and grafting methods. They reported that May, June and July were optimum time for top working. Side veneer grafting gave 100% success. Hamid and Homayoun (2011) found bench grafting a suitable grafting method in pomegranate. They reported 85.83% graft success in wedge grafting. In the recent past, Chandra and Jadhav (2012) standardized grafting methods and time in pomegranate. They noted higher scion sprouting (96.67%) when wedge grafting done on 30th January at 21 days after grafting (DAG). Though, maximum graft success (85.83%) was recorded at 90 DAG with wedge grafting done on 30 January. The growth performance of wedge grafted plants was better with 15 December grafting. In general, the wedge grafted plants had better scion-rootstock compatibility than tongue grafting. Wedge grafting done on 30 January produced more shoot and root biomass owing to its better shoot and root development Rootstock concept has not yet been fully developed in pomegranate and information on rootstock is very meager. Hamid and Homayoun (2011) tested 3 rootstocks for grafting in pomegranate with Gorj-e-Dadashi and Gorj-e-Shahvar as scion varieties. The rootstocks influenced bud take, shoot fresh and dry weight with better scion and rootstock compatibility. However, looking into soil salinity, alkalinity, drought, high density planting, wilt and other biotic and abiotic problems, the significance of rootstock is the need of hour. National Research Centre on Pomegranate, Solapur has already initiated some work on rootstock but it is in preliminary stage. Wild pomegranate accessions collected from Western Himalayas are thriving very well under Solapur condition with good bearing capability. These germplasm could be of great value for rootstock in years to come.

(5) Budding- The method of budding is the most common technique for plant propagation in commercial nurseries in different fruit crops but, little work has been done in pomegranate. Recently, Chandra *et. al.* (2013) reported patch budding a successful technique for budding on wild pomegranate rootstock. They reported more than 90% success by this method. However, the performance of budded plants is yet to be evaluated.

(6) Stenting- Recently, stenting technique of propagation has been standardized in pomegranate. In this method, cutting and grafting is performed simultaneously (Hamid**,** 2011). The scion is grafted onto a nonrooted rootstock. The formation of the union and adventitious roots on the rootstock occurs simultaneously. With this method time can be minimized for grafting and such plants established well when planted into the field. In fact stenting is now being used worldwide by rose growers (Nazari *et. al.* 2009) and is also a valuable technique in propagating species of conifers and also rhododendron, apple, plum and pear (Hartman *et al.*, 2002). Hamid (2011) found that the scion length was reduced by grafting on Gorj-e-Dadashi, Gorj-e-Shahvar and Gool Safid-e-Ashk-e-Zar.

(7) Tissue culture- The demand of disease free healthy planting material is growing very fast in India as well as in the world. Conventional method of propagation may not be sufficient to satisfy the ever growing demand of healthy planting materials. Thus, mass multiplication of pomegranate through tissue culture is very much required to bridge the gap between demand and supply of planting material. For *in vitro* propagation, apparently healthy plants with proven horticultural traits are used for excising the explants (Debergh and Maene 1981). Selection of ideal explants with proper pretreatments and surface sterilization prior to inoculations are key to successful culture establishment Damiano *et.al.* (2008) could successfully able to sterilize auxillary bud segments using NaOCl and Na methiolate with 65 per cent culture establishment.

Browning of cultures is a major obstacle in establishment of explants of pomegranate owing to high phenolic contents and frequent subculturing, use of adsorbants and antioxidants are very effective in lacking browning (Murkute *et. al.* 2003). 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. They achieved 80% success in establishment of plantlets in 1: 1: 1 (v/v) peat, perlite and sand mixture. Fougat *et. al.* (1997) achieved 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 the best on MS medium supplemented with 4.0 mg/l NAA, 2.0 mg/l kinetin and 15% CW (coconut water). Kantharajah *et. al.* (1998) found lower salt concentration in culture medium had beneficial effect on *in vitro* rooting. They obtained highest rooting higher number of roots per micro shoot on WPM medium supplemented with 2 mg/l NAA. Naik *et. al.* (1999) described an efficient procedure for *in vitro* clonal propagation in 'Ganesh' using nodal stem segments. Patil *et. al.* (2011) found nodal segments of Bhagawa on MS medium supplemented with 0.2-2mg/l BA, 0.1-1.0mg/l NAA and 0.5-0.25 mg/l $AgNO_3$. Again, Naik *et. al.* (2003) observed that the addition of ethylene inhibitors like $AgNO_3$ (10-40 ìM) and amino ethoxy vinylglycine (AVG) (5-15 ìM) 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). In the recent past, attempts were made to produce synthetic seeds in pomegranate (Naik and Chand, 2006). However, they were successful in encapsulating nodal segments from *in vitro* proliferated shoot cultures or axenic cotyledonary nodes. Protocols for regeneration of pomegranate have been developed and its commercialization has already been started in Maharashtra. Recently, NRC on Pomegranate, Solapur also developed protocol and multiplication of tissue cultured plant on public private partnership has been started through private firm.

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