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Healthy Saplings – Key to Sustainable Pomegranate Production

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ABSTRACT: Pomegranate is a commercial fruit crop of semi-arid regions of the world. It is an ancient fruit crop in modern horticulture catering nutritional, nutraceutical and livelihood needs of its stakeholders. During recent years, the crop has gained tremendous popularity due to its versatile adaptability, hardy nature, nutritional and industrial values. The many-folds increment in the pomegranate acreage globally demands large scale availability of elite planting material. India alone needs about 15 million healthy saplings annually to match the pace of pomegranate expansion in the country. The challenges like pomegranate bacterial blight and wilt, which are many-a-times transmitted to distant places through infected saplings or potting mixture (wilt complex) causing severe crop losses thus are major threats for the sustainable pomegranate production. The situation warrants large scale production of QPM to avoid spread of these diseases and ensure sustained profit in pomegranate cultivation. To meet the progressively increasing healthy sapling requirement in pomegranate, the conventional commercial propagation methods like stem cuttings and air layering must be upgraded with standard sanitation nursery management protocols to ensure healthy sapling production and the emerging commercial propagation methods like micropropagation needs to be promoted. The research on screening of wild pomegranate germplasm and the utilization of promising ones as roots tocks should be intensified and larger scale field trials on grafted plants should be taken up under different pomegranate growing regions to meet the vision of climate smart pomegranate production system in near future.

Abbreviation: QPM-Quality planting material,
Keywords: Pomegranate Propagation, Air layering, Stem Cuttings, Grafting and Budding.

INTRODUCTION

Pomegranate (*Punica granatum* L.) is predominant suitable fruit crop in dry land regions of the world, which belongs to the family Lythraceae (Singh *et al.*, 2020). It has played significant role in shaping the economy of the farmers in arid and semi-arid regions of India. During recent years, popularity of the pomegranate has spread vastly due to its health benefits, hardy nature, nutraceutical and therapeutic values (Ahire *et al*., 2017; Marathe *et al*., 2010; Singh *et al*., 2021). Globally India is the largest pomegranate producer with almost fifty percent area and production of the globe. Pomegranate cultivation has witnessed constant increase in area, production and productivity during last 15 years. In India the pomegranate crop occupies an all-time high area of 2.83 lakh ha, with production of 31.86 lakh MT during recent times. Today, more than 2.5 lakh families, mainly in climatically and edaphically challenged semi-arid regions, are dependent on this crop for their livelihood in India. More than three folds increase in acreage of pomegranate during last two decades in India has warranted the propagation of more than 15 million QPM annually. Currently, plants raised through air layering, stem cuttings and micropropagation are used to establish commercial orchards around the world (Levin, 2006; Finetto, 2009; Singh *et al*., 2021). In the Deccan Plateau region of India, air-layered plants are commonly used for establishment of new pomegranate orchards (Singh *et al*., 2021). Healthy sapling is the foundation stairway toward successful crop production, and this is especially important in perennial horticultural crop like pomegranate where diseases like bacterial blight and wilt are serious concerns and can be spread to uninfected plants/saplings through infected saplings and/or potting mixtures. These diseases will develop in 5-12 months after orchard planting thus infected sapling to new locations can cause heavy economic losses to the pomegranate farmers. So, to meet the progressively increasing healthy sapling requirement in pomegranate, the conventional commercial propagation methods like stem cuttings and air layering must be clubbed with a standard sanitation and nursery management protocol to produce elite healthy saplings. The emerging commercial method of propagation *i.e.*, micropropagation including biopriming need to be promoted more aggressively particularly in new areas of pomegranate plantation where bacterial blight incidence has not yet been reported. Large scale screening of wild pomegranate germplasm and utilization of biotic and abiotic stress tolerant germplasm as rootstocks is the need of the hour to have climate resilient pomegranate production system in coming future (Singh *et al*., 2021).

AIR LAYERING (POT LAYERING/ CIRCUMPOSITION / MAR COTTAGE)

Air layering is the most commercial method of pomegranate propagation in the Deccan Plateau region of India for establishing pomegranate orchards,for air layering, upright branches of 8 mm to 15 mm in diameter from healthy tree are selected and girdled 2 to 3 cm in length during rainy season. Rooting hormones like IBA at concentration of 2000 to 3000 ppm is used on upper part of the cut (Singh *et al*, 2021; Chandra and Jadhav 2012; Sharma *et al*., 2014; Tomar, 2011). The stem is wrapped with moist rooting medium having enough water holding capacity such as sphagnum moss (peat moss) and covered with the help of small black or white polythene strip having 200 to 300 gauze and both the sides of covered strip are tied with coir/ jute thread or string (Singh *et al*., 2021). Girdling blocks the carbohydrates and hormones downward movement, thus, accumulating at the upper part of the cut. While etiolation reduces the production of lignins, thus, inlieu of forming lignins, phenolic metabolites may be channeled to enhance root initiation (Hudson *et al*., 2004). In general, sphagnum moss is utilized as a substrate for covering the girdled part of air layers, while IBA, alone or in combination with p-hydroxybenzoic acid, is often used to stimulate early roots in air layers for high success (Bhosale *et al*., 2009; Hore and Sen 1995; Tomar, 2011). Use of formulation of *Pseudomonas fluorescens* having 10^9cfu/g at gridled portion of air-layers proved beneficial for inducing roots in air layers of pomegranate. The type of media used for layering has vital role in rooting and survival of layers. Generally, water-soaked sphagnum moss is used as substrate for air-layering, but soil, sand and cow dung manure in 2:1:1 proportion was also reported as a media for preparation of air-layers (Hore and Sen 1994). Coconut coir, fly ash, saw dust and pond soil have also been used as rooting substrates in pomegranate air layering (Allioli *et al*., 2001). A minimum of 30 to 40 days required for the rooting and well rooted layered plants are separated from the mother plant about 60 days after layering and can be planted in polythene bag or in nursery which contain potting mixture of sand, soil and well-rotten FYM with ratio 1:1:1 for couple of months before planting in field (Chandra *et al*., 2014; Tayade *et al*., 2017).

STEM CUTTING

Commercially, Pomegranate orchard in the worldwide are propagated through saplings from stem cuttings (Levin, 2006). Initial sprouting in pomegranate cuttings can be seen in excess of 85% of the time, but due to the failure of some cuttings to create an adventitious root system, the end cutting success drops significantly (Singh *et al*., 2015). Rooting success of pomegranate propagation through stem cuttings is mostly affected by maturity of wood (Chandra and Babu 2010). During or immediately after dormancy or rest period of the orchard 6 to 18 months old stem can be used for the propagation of pomegranate through hardwood cuttings. The cuttings with 20 to 25 cm length having 4 nodes and 6 to 12 mm thickness result in high cuttings success in pomegranate (Chandra and Babu, 2010; Chandra *et al*., 2014; Purohit and Shekharappa, 1985; Rajan and Markose 2007; Saroj *et al*., 2008) (Table 1). Singh *et al.*, (2019) has standardized the protocol for healthy hard wood cuttings of ‘Bhagwa’ stated as to practice pruning after rest and dormant stage, use of 6-18 months old hard-cutting followed by IBA treatment (2.5g/l) for high cutting success in ‘Bhagwa’. Sandhu *et al*. (1991) examined the rhizogenesis in pomegranate hardwood cuttings for cvs. Kandhari and Malas and found hardwood cuttings of 20 cm in length are optimum for good cutting success. Generally, stem cutting in pomegranate have low root initiation cofactor due to factors like levels of hormones in stem, nutrient reserve, genetic material, age and maturity of the stem which affect significantly on cutting success in pomegranate but preconditioning factors like girdling, ringing, etiolation and basal wounding can increase rooting success in the pomegranate propagation by stem cuttings (Ready and Keaay 1989; Singh *et al*., 2021; Yesiloglu *et al*., 1997). It can be also influenced by plant growth regulators, mostly auxins like IBA, NAA and IAA. In the presence of auxins, enhancement of callus and vascular differentiation due to hydrolytic activity that might play a vital role in cutting success. External or endogenous auxin is essential for the development of adventitious roots on stems, and divisions of the first root initial cells (Singh 2014; Frick *et al*., 2018). Adding Boron to the IBA solution had a direct effect on auxin transport or activity, which stimulated root initiation. This could be related to boron's increased mobilization of O_2-rich citric and iso-citric acids, which changes the acid metabolism of cuttings. In addition, exogenous auxin converts starch into simple sugars, and boron increases sugar mobilization, which is needed more for the development of new cells and increased respiratory activity in regenerating tissues at the commencement of new root primordia (Sharma *et al*., 2009). Combination of auxins with nutrients results in high rooting success (IBA 500 ppm + Borax 1%) in hardwood cuttings and semi- hardwood cuttings (Sharma *et al*., 2009). Reddy and Reddy (1990) used IBA and NAA at 2500 ppm to hardwood cuttings of pomegranate cv. Bassein Seedless. Kaur *et al*. (2016) reported the highest cutting success of in pomegranate var. ‘Ganesh’ with PHB 750ppm + IBA 1000ppm during August. The best vegetative metrics of pomegranate cuttings with 1000 ppm IBA treatment (Kamboj *et al*., 2017). Hakim *et al*. (2018) while working with stem cuttings of pomegranate cvs. ‘Ruby’ and ‘Bhagwa’ found that the

NAA 1500 ppm + IBA 1500 ppm + Bio mix treatment generated the best results in terms of cutting sprouting, total chlorophyll content and leaf area. The IBA 2000ppm-treated pomegranate cuttings generated the most shoots per cutting, the higher IBA concentrations might have resulted in enhanced cell division and elongation, as well as greater shoot growth activation, which presumably increased the number of nodes and led to the development of additional leaves (Damar *et al*., 2014). Wilt and bacterial blight are the serious concerns in many cases. So, before planting, it is desirable to sanitize the cuttings in a solution of 2-bromo-2-nitropropane-1, 3-diol @ 0.5 g/l + Carbendazim 50 WP @ 2.0 g/l dissolved in lukewarm water at 45°C for 15 min, dipping the cuttings in this solution may help in removing non-systemic surface pests and diseases. After that, for surface sterilization cuttings are dipped in solution of sodium hypochlorite for 15 minutes. For the induction of roots, lower part of cuttings (2-3 cm) are dipped in the 2500 ppm solution of IBA for 1.0 min and then planted in suitable media like cocopeat and sand (4:1 v/v), mixture of cocopeat, perlite vermiculite or cocopeat alone. The well rooted cuttings must be transferred to nursery bags having pre-sterilized sand: soil: FYM in 2:1:1 ratio in lower half and cocopeat in the upper half of the nursery bag (Singh *et al*., 2021). Application of pre-multiplied beneficial microflora formulation containing *Pseudomonas fluorescens*, *Trichoderma* spp. *Aspergillus niger*, AMF, *Penicillium pinophilum* in the root zone should be done at the time of transfer of rooted cuttings to nursery bags. There are reports on the use of plant beneficial micro-organisms like *Trichoderma harzianum*, *Pseudomonas flourescence*, *Azosprilium* sp., *Azotobacter* sp, etc. to improve rooting of cuttings in pomegranate due to their growth promoting properties (Patil *et al*., 2001; Jaganath *et al.*, 2009). After 45 days of growth in nursery bags, these cuttings can be planted in the field. Keep these cuttings in the shade for a week before transferring to the field for acclimatization (Singh *et al*., 2021). Tanwar *et al*. (2020) discovered that the minimum number of days required for first sprouting of cuttings on rooting medium Coco peat, Vermiculite, Perlite, and 2000 ppm IBA for pomegranate cv. Bhagwa. The shooting response of pomegranate cv. "Kandhari" stem cuttings was the highest on vermiculite based medium with 76.67 % sprouted cuttings. Similarly, Ratna Kamari's (2014) found vermiculite medium significantly better over other rooting medium with the highest sprouting of 'Bhagwa' cuttings.

Table 1: Various growth regulator (GR) treatments used in pomegranate for cutting success.

Variety and Reference	Type of cutting	Treatment type	Concentration of GR (ppm)
Bassein Seedless (Reddy and Reddy 1990)	Hard wood cutting	Quick dip	2500 IBA + 2500 NAA
Ganesh (Ghosh *et al*., 1988)	Hard wood, semi hardwood & soft wood cuttings	Quick dip	5000 IBA
Bedana (Hore and Sen 1995)	Hard wood cutting	Quick dip	1000 PHB + 2500 NAA
Ganesh, Dholka (Tripathi and Shukla 2004)	Hard wood cutting	Quick dip	1000 PHB + 5000 IBA
Jalore seedless (Saroj *et al*., 2008)	Hard wood & semi- hard wood cutting	Quick dip	2500 IBA
Bhagawa (Singh *et al*., 2014; Yesiloglu *et al*., 1997)	Hard wood cutting	Quick dip	2500-5000 IBA
Bhagawa (Blumenfeld *et al*., 2000; Singh, 2017)	*In situ* hard wood cutting	Quick dip	2500-5000 IBA
Wonderful (Sarrou *et al*., 2014)	Hard wood cutting	Quick dip	100 IBA +500 GA_3
Ganesh (Sharma *et al*., 2009)	Hard wood &Semi hard wood cutting	15 minutes treatment	500 IBA + Borax 1%
Pomegranate-ME12, CR02 and PT08 (Melgarejo *et al*., 2000)	Hardwood cutting	Quick dip and basal wounding	12000 IBA

MICROPROPAGATION AND BIOPRIMING

Vegetatively propagated saplings sometimes have more risk of carrying infection/latent infection as mother plants are exposed to various biotic and abiotic stresses under open field conditions where nurserymen are having limited control, however, by using micropropagation, quality planting material in pomegranate can be multiplied in bulk with very limited number of mother plants which can easily be maintained under very well monitored protected structures. There is a growing demand for blight and wilt free elite saplings of pomegranate and micropropagation ensures rapid production of a large quantity of uniform disease-free plants (Sheela and Nair 2001). The utilization of micro-propagated plants should be made mandatory for expansion of pomegranate in areas which are still free from pomegranate bacterial blight so as to avoid spread of these difficult to manage diseases to these areas (Singh *et al*., 2021). Several standardized micropropagation protocols are available in the public domain in the form of published articles but these published literatures have shown lot of variations in explants used, basal medium compositions, growth regulators and media supplements type and concentration.

Murashige and Skoog (MS) culture medium with modifications was used by most researchers but Woody Plant Medium (WPM) (Lloyd and McCown, 1980) and B5 (Gamborg *et al*., 1968), DKW/Juglans and Quorin and Lepoivre media have also beend reported for micropropagation in pomegranate. Damiano *et al*. (2008) showed good shoot multiplication onto a basal Quorin and Lepoivre medium supplemented with BA (0.4 mg/l) and IBA (0.05 mg/l). Nodal segments were important for regeneration of 'Bhagawa' on MS-based medium in the presence of 0.2–2 mg/l BA, 0.1–1 mg/l NAA and 0.5–2.5 silver nitrate ($AgNO_3$) (Patil *et al*., 2011). Woody plant medium (WPM) has been used for proliferation of pomegranate cultivars (Valizadeh *et al*., 2013). Most researchers have used Benzyl Adenine (BA) as cytokinine for pomegranate proliferation besides Zeatin, Kinetin, and Thiadiazuron (TDZ) (Naik *et al*., 1999). Singh *et al*. (2014) reported that the best result for pomegranate regeneration was obtained on MS medium culture containing 0.2-2.0 mg/l BAP and 0.1-1.0 mg/l NAA. Micropropagation of pomegranate cv. 'Robab' can be achieved with the best proliferation rate of 5.66 with the maximum length of 3.83 cm in the culture medium containing 2.0 mg/lBA and 1 mg/l NAA (Moatri, 2015). Many researchers have also reported the use of Adenine Sulphate (30-80 mg/l) and silver nitrate (1-2.5 mg/l) for good shoot proliferation in pomegranate (Patil *et al.,* 2011; Bachake *et al.,* 2019). Singh *et al*. (2013) also reported NAA as effective growth regulator to induce rooting in pomegranate at concentration of 0.5 mg/l along with 200 mg/l activated charcoal. Bachake *et al.* (2019) reported the best in vitro rooting (76.00%) with WPM medium supplemented with 1.0 mg/l (NAA). Generally, low salt medium, auxins and adsorbing agents promote *in vitro* rooting of microshoots. Kantharajah *et al.* (1998) also reported rooting of pomegranate (cv. Wonderful) on WPM medium supplemented with 2mg/lNAA. Naik and Chand (2011) reported the half strength WPM and MS medium for *in vitro* rooting of pomegranate with varying success. Abadi *et al*. (2020) reported woody plant medium with 1.4 mg/l of BAP as the best culture medium for shoot proliferation in pomegranate and woody plant medium with NAA and IBA 1.0 mg/l was detected as the most suitable for in vitro rooting.

The *ex-vitro* conditioning and survival of micropropagated plants is crucial for an effective micropropagation protocol as these plants are having certain physiological underdevelopments and exposed to changing environment. These plantlets have weekly formed and underdeveloped root system (Hazarika 2003), underdeveloped vascular connection of root and shoot (Schubert *et al*. 1990), poorly developed cuticle and sub optimally functional stomata (Hazarika, 2003). Media combination comprised of cocopeat, perlite and vermiculite in different combinations is mostly used as primary hardening medium to achieve good *ex vitro* survival (Naik *et al.*1999; Naik *et al.,* 2000; Murkute *et al.,* 2004; Singh *et al.,* 2007; Adabi *et al.,* 2020). Development of robust root system in the key element for field establishment of tissue cultured saplings and *ex-vitro* performance of tissue culture raised plantlets can effectively be improved by utilizing plant beneficial microbes or bioagents like arbuscular mycorrhizal fungi (AMF) and *Aspergillus niger* strain AN-27 in the hardening process (Rupnawar and Navale 2000; Mondal *et al*., 2000). Many fruit crops develop a symbiotic mycorrhizal relationship and exhibited a high degree of dependence on this symbiosis for normal development and improved field performance (Aseri *et al*., 2008). Furthermore, AMF can mitigate the effects of extreme variations in temperature and water stress by improving the uptake of water and nutrients through an increased exploration of rhizosphere area (Krishna *et al*., 2006). Singh *et al*. (2012) conducted a study on hardening of micropropagated pomegranate plantlets by using arbuscular mycorrhizal fungi (AMF) and found the plantlets inoculated with *G. mosseae* recorded the higher survival percentage. The micro-cloned plantlets of *Chlorophytum borivilianum* registered higher plantlet establishment when inoculated with *Glomus aggregatum, Trichoderma harazianum* and *Piriformospora indica* (Mathur *et al*., 2008). The improved performance of AMF inoculated pomegranate plants in terms of more root and shoot biomass production, enhanced photosynthesis and better nutrient uptake and water balance of the plants was observed in pomegranate and other fruit crops (Krishna *et al*. 2006; Singh *et al*., 2012 and 2016; Bachake *et al*., 2019). The bio-hardening agents infect and establish themselves in the roots or rhizosphere of *in vitro* raised plants and help in mobilizing nutrients and increasing soil exploration capacity through their mycelia for better uptake of various nutrients.

GRAFTING AND BUDDING

Grafting and budding are horticultural techniques that have been used in many regions of the world for many years. It is primarily used to obtain rootstock advantages for soil and water salinity, soil-borne pests and diseases, and benefits such as fruit tree precocity and dwarfing, as well as other biotic and abiotic stresses in the climate change scenario. Drought, salinity of water and soil, soil-borne diseases, cold, and nutritional imbalances are among the issues restricting pomegranate cultivation around the world. Screening and evaluation of wild pomegranate germplasm against various abiotic and soil borne issues and utilization of promising genotypes as rootstocks through grafting and budding may be helpful in reducing the severity of biotic and abiotic stress and promote climate resilient pomegranate production system (Singh *et al*., 2021; Singh, 2017). Farmers in some parts of Iran have recently employed this technology to graft new varieties over old varieties as rootstocks to change the cultivars of pomegranates in their farms. The grafting success, survivability and growth of the grafted plants depend on several factors like compatibility of rootstock and scion, grafting methods, scion and rootstock type, age of scion and rootstock, environmental conditions (temperature, humidity and oxygen), worker skill and available biochemical compound in rootstock and scion (Karimi, 2011). Correlation between phenolic compounds, soluble sugar of rootstock and scion with graft success percentage and mortality percentage Karimi *et al.* (2017) showed correlation between phenolic compounds, soluble sugar and scion grafting. Study showed positive correlation between soluble sugar rate of scion with graft success percentage whereas the phenolic compounds of rootstock had negative correlation with mortality percentage (Karimi *et al*., 2017). Wedge grafting, omega grafting, cleft grafting, bench grafting, patch budding, ring budding, shield budding and stenting techniques are practiced for clonal propagation of desired parent cultivars (Karimi and Nowrozy 2017; Chandra *et al*., 2012). Chandra *et al.* (2010), research showed one-year-old wild rootstock and a Bhagwa patch bud of 20 mm × 10 mm is ideal for patch budding with a success of about 90 % and highest scion-sprouting (96.67%) after wedge grafting. Wedge grafted scion-rootstock produced more shoot and root biomass owing to better shoot and root development. Ahire *et al*. (2017) conducted a large-scale experiment on wedge grafting and patch budding in pomegranate and reported highest stock/scion girth ratio (1.00) when 'Bhagwa' was grafted over 'Ganesh', 'Bedana Suri' and 'Kandhari'.

Table 2: Grafting and budding methods attempted in pomegranate.

Budding and Grafting method & References	**Ideal Time***
Wedge Grafting (Chandra and Jadhav 2012; Chandra *et al*., 2011)	January
Omega Grafting (Karimi and Nowrozy 2017)	Early March
Cleft Grafting (Nowrozy *et al*., 2014; Nowrozy *et al*., 2016)	Early March
Bench Grafting (Karimi and Farahmand 2011; Nowrozy *et al*., 2016)	Early March
Stenting (Karimi, 2011; Karimi and Nowrozy 2017)	Early March
Patch Budding (Chandra *et al*., 2014)	November to February
Chip Budding (Nowrozy *et al*., 2014; Nowrozy *et al*., 2016)	Late March
Shield Budding (Nowrozy *et al*., 2014)	Late June
Ring Budding (Nowrozy *et al*., 2014)	Late June

*Will depend on the season of the geographical location

CONCLUSION

The foundation for ensuring sustained profitable yield in pomegranate is the use of quality planting material from the certified source for establishing orchards. The acreage under pomegranate in expanding and so the disease and pests and many of them are spreading through infected planting material. Thus, there is an urgent need to multiply true-to-the type planting material following standard propagation methods, proper sanitation and nursery management protocols. The commercial propagation methods namely, air layering, stem cutting and micropropagation should be utilized to meet the increasing requirement of healthy planting material with strong regulatory framework. The research on exploitation of rootstocks for climate resilient pomegranate production system needs to be up scaled and intensified.

FUTURE SCOPE

Quality and disease-free planting material is of foremost importance for sustainable production in pomegranate particularly in the era of climate change. The propagation of disease-free elite saplings through micropropagation coupled with bio-hardening and exploration and use of rootstock through grafting and budding for climate resilient pomegranate production hold the promise for continued quality sapling production for sustained profit in pomegranate cultivation.

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Conflicts of Interest. None.

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