Recent trends in pomegranate propagation: A review

Mounika Gadapa and Gurpreet Singh

Abstract
The present review focuses on and enlists all the various methods of pomegranate propagation. Pomegranate is mainly propagated through asexual (vegetative) means of propagation since sexual propagation is limited in pomegranate. Seed (sexual) propagation is only limited to be used in breeding programs where higher genetic variability is needed. Stem cutting is the simplest and most common, mostly hardwood cuttings are used for commercial purposes which would result in higher rooting and survival. Another important asexual method was Air layering (gottee) which is very popular that has been adopted by many foreign countries and India also practiced majorly in agricultural universities, research areas, and commercial orchards. Mound layering has also been practiced in the seasons when elite mother stocks or parent plants are in the resting phase. Different methods of grating like stenting, cleft, and wedge grafting were also in practice for clonal propagation of desired parent cultivars. Nowadays many researchers take complete advantage and utilization of advanced biotechnology like micropropagation (in-vitro culture) which involves the use of various techniques like somatic embryogenesis, synthetic seeds, organogenesis, embryo culture, molecular markers, primers, etc., for the quick mass clonal propagation of elite pomegranate cultivars. The main theme of this particular review article is, it comprises numerous newly adopted approaches, current innovative progress, and drifts related to pomegranate propagation that are used commercially.

Keywords: Air layering, cuttings, grafting, micropropagation, tissue culture, vegetative propagation

1. Introduction
Pomegranate (Punica granatum L) is one of the major important fruit crops in the world, which belongs to the family ‘Punicaceae’, that consists of only one genus of plants, related to the family Lythraceae, in which leaves are spiral, simple, and without stipules, flowers are perigynous (5-8 sepals and petals), numerous stamens and carpels, which adnate to the receptacle. Pomegranate has been growing since ancient days. The fruit consists of an external leathery pericarp that contains many juicy arils. It is native to Northern Africa and Caucasian. The name ‘pomegranate’ comes from the Latin word ‘pomum’ - which means apple and ‘granatus’ - which means full of seeds. It is the most suitable crop for arid and semiarid regions throughout the world and gaining enormous popularity in sub marginal and marginal lands of tropical and subtropical regions of India (Mahishi et al. 2012) [1]. The current status and future challenges which we are going to face in terms of commercial cultivation of pomegranate are clearly explained here (Pal et al. 2014) [2].

Pomegranate is both a self-pollinated and cross-pollinated crop, the main pollinators are honey bees. Seed propagation results in high genetic heterozygosity which is a most useful aspect of various breeding programs of pomegranate to develop resistant cultivars and rootstocks against various diseases and pests of pomegranate. The practice of various asexual (clonal) propagation methods of pomegranate also considers a hi-tech structure of propagation, how expensive newly advanced technologies are also must be taken into consideration. Various recent advancements in the propagation of pomegranate concerning India were discussed here (Chandra et al. 2017) [3]. Many important advanced technologies of pomegranate propagation in various aspects of its cultivation were discussed here (Saroj et al. 2019) [4]. In addition to this, it’s important to know, how to practice the major approaches of cutting and grafting to achieve high productivity more efficiently.

Major six types of pomegranate propagation (Singh et al. 2020) [5] and advanced agrotechnological approaches for the cultivation of pomegranate were briefly discussed here (Sau et al. 2021) [6]. Pomegranate can be propagated by air layering (Tayade 2017a and 2018a) [7,8], Cuttings- Hardwood cuttings (Chater 2017a; Rathwa et al. 2016) [9, 10], Semi-hardwood cuttings (Kahramanoglu et al. 2018) [11], stooling-mound layering (Singh et al. 2017a) [12], grafting (Karimi 2011a; Valizadehkaji et al. 2020) [13,14] and through micropropagation also.
These are commercially possible due to diverse success rates, effortless processes. The major foremost specific objective of this review is to deliberate the various common and commercial methods of pomegranate propagation that are used formerly on large-scale production of elite plantlets and progressive technologies which have been made recently.

2. Propagation of pomegranate by seed
2.1. Sexual propagation
There are many countries still using a traditional method of pomegranate propagation i.e., through ‘seed’. It is the simplest, common method for propagating pomegranate trees but takes longer periods nearly 4-5 years to reach the fruiting stage but the main disadvantage is that seed propagation results in higher genetic variability in their nature. It is the main reason for unprofitable orchards (or) farms, as it gives out poor fruit quality and lowers yield. Another disadvantage of seed propagation it requires more time for the production of seedlings (Rawat et al. 2008) [13]. And also, the seedlings have an extensive juvenile (vegetative) phase, which delays the reproductive phase occurs. Consequently, seed propagation is commercially followed only in hybrid breeding programs where the higher genetic variability among plants is essential along with desired important characteristics. Many research areas of pomegranate production use air layering and grafting technology on a large scale to gain enormous profits in pomegranate orchards.

For propagating the pomegranate through seeds, mature fruits of selected desired plants are selected and harvested, then after the seeds are removed and clean up the fleshy arils surrounding and these should be dried in the shade for a few seconds. Later on, seeds sown in polybags or loose soil, which contain a growing media which is well-drained, heat is the second factor that should be provided to the seed to ensure good germination. Germination can exceed 92% and generally occurs in about 30-45 days.

Another method that should be discussed here is how to plant a pomegranate seed it is called the ‘Baggie method’, many scientists who are immensely involved in breeding programs curse this method for growing pomegranate through seeds. Seed germination can be improved to nearly 92% by using various pre-sowing seed treatment methods, the efficiency of various seed treatments on germination and seedling emergence of pomegranate seeds were studied (Kumar et al. 2014) [16]. The storage period is also an important factor that influences seed germination as it is correlated to the viability of the seed. Germination in ‘Ganesh’ and ‘Bhagwa’ started within the initial 8-10 days after sowing and continued up to 28 days during summer (May). Higher germination was recorded and achieved in the ‘Bhagwa’ variety (75-79%) than the Ganesh cultivar (61.5-67.5%). However average germination was observed in various pomegranate cultivars ranging from 60-75% in India. A popular dwarf variety (Punica granatum. L) cv. ‘Phule Arakta’ cuttings under arid conditions are undertaken. Many research areas of pomegranate production use air layering and grafting technology on a large scale to gain enormous profits in pomegranate orchards.

In several transplantation methods, air layering and hardwood cuttings have achieved a high success rate in the propagation of different pomegranate varieties. These two methods are predominantly important for developing various soil-borne disease-resistant rootstocks and used for the cloning of elite cultivars. The major asexual (vegetative) methods of propagation practiced in pomegranate are briefly described below:

2.2. Asexual propagation
The asexual propagation techniques have been practiced more traditionally in pomegranate, it is majorly considered as a difficult-to-root plant by stem cuttings, as they resulted in a very low success rate in the rooting process (Singh 2017 and 2018) [20, 21]. Vegetative propagation is the most convenient and cheapest method for obtaining true-to-type, fully matured plants. Considerably in very less time, improve rooting and reduced the mortality rate of stem cuttings. Air layering, hardwood cuttings, and stool (mound) layering are mainly used for the rapid mass multiplication of pomegranate in many countries.

In India nowadays mostly used commercial method of propagation of pomegranate is ‘Air Layering’ in various research centers, orchards, and agricultural universities. The cuttings are taken from the stem that lacks root-promoting co-factors i.e., phenolic compounds, low sugar content, and C/N (carbon, nitrogen) ratio. Pre-conditioning of cuttings (shoots) like girdling and etiolation increases the accumulation of root promoting co-factors at the point of girdling and initiates rhizogenesis (Batista et al. 2011) [22]. The use of different rooting media on a more scientific basis mainly in (Punica granatum. L) cv. ‘Phule Arakta’ cuttings various arid conditions are undertaken. Many research areas of pomegranate production use air layering and grafting technology on a large scale to gain enormous profits in pomegranate orchards.

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2.2.1. Stem cutting: Hardwood and semi-hardwood cuttings
The propagation of pomegranate via stem cuttings of pomegranate is a very common and simplest method but the mortality rate is high through this method of propagation. Recently, air layering and hardwood cuttings methods have become popular according to data given by NHB-2019. Timing and efficient use of PGRs along with certain chemical combinations has been standardized for obtaining maximum
rhizogenesis in cuttings. The maximum rooting, root number, high root length is observed by using IBA (Indole 3 butyric acid) @500ppm + Borax 1% in both semi-hardwood and hardwood cuttings (Sharma et al. 2009) [23]. An increased dose of IBA has a direct effect on the rooting behavior of cuttings and also rooting progress majorly depends according on various cultivars of pomegranate (Owasis 2010) [24].

Especially in hardwood cuttings which are treated with IBA and p-hydroxybenzoic acid are rooted easily and give rise to plants, in difficult-to-root cuttings varieties this combination works well (Tripathi et al. 2004) [25]. The length and diameter of stem cuttings have also an impact on rhizogenesis, normally 6-12mm diameter shoots roots successfully (Rajan and Markose 2007) [26]. High percentage rooting was recorded in 31-N-01 type when treated with IBA of concentration 1000ppm. Increased dose of IBA upsurges the chance of rooting more effectively (Ayetkin Polat et al. 2006) [28]. Kumar et al. (2021) [29] reported that combined rooting media which consists of Soil: Sand: Vermicompost showed good results in the performance of stem cuttings and earliest sprouting, high shoot-root growth, more survival capacity have been recorded when stem cuttings are treated with IBA 2000 ppm + Boric acid 1% + Wounding (Kumar et al. 2021 and 2020). Singh (2014) [29, 32] reported in his experiment that with different IBA concentrations through the Quick dip method in Punica granatum L. cv. ‘GANESH’ variety under mist under conditions have given high success rooting. Basal cutting should have a diameter 1.0-1.25mm treated with 5000ppm of IBA gave highest survival percent, Sub-apical cuttings with 5000ppm IBA were next best, whereas apical cuttings failed to sprout and root at all any conc. of IBA (Purohit et al. 1985) [33]. However, without treatment with IBA-Indole-Butyric acid, there would be no induction of rooting in cuttings. New pomegranate cultivar like ‘Emek’, a red and early-ripening variety is mainly propagated by rooting of their cuttings (Poland et al. 2014).

Cuttings planted in January month and those treated with 5000ppm of IBA showed high rooting percentage, increased number of sprouts per cutting, and also shown very effective results in various aspects of successful cuttings (Mehta et al. 2018) [35] and rooting media (Alikhani et al. 2011) [36]. However, the main reasons for the behavior of rooting and sprouting in stem cuttings are the presence of humidity at the time of harvest, age of the plant, and moisture content of cutting, its rooting behavior, etc., are to be taken into consideration. Kaur et al. (2016) [37] revealed that different combinations of plant growth regulators IBA 1000ppm and PHB (p-hydroxybenzoic acid) 750ppm enhanced higher rooting, sprouting, and suggested August as the best month for planting. Bisen (2010) discovered that mechanical efforts like ringing, girdling of shoots also increased rooting to 78 percent. Various combinations of indole-3butyric acid (IBA) +GA3+Melatonin (MEL) +Hydrogen peroxide (H2O2) and Ascorbic acid (ASC) influence rooting, longest root length was obtained in combination 17mg of H2O2+1000mg IBA. The human hormone Melatonin (MEL) 1.16mg also showed a positive response on rooting along with a combination of ascorbic acid (ASC) 352.24mg or alone (Sarrou et al. 2014) [39]. The use of different rooting media also affects the rooting rate (Deol and Uppal 1990) [40] and vegetative growth in Punica granatum L cv. ‘Kandhari’ (Manjula et al. 2017; Tanuja et al. 2017) and cv. ‘Bhagwa’ (Kumari 2014; Patel et al. 2020) and in cv. ‘Ganesha’ (Deshmukh et al. 2019) [41, 42, 45]. Rajakumar et al. (2016) revealed that in Punica granatum Lcv. ‘Phule Arkta’ the rooting, survival, and establishment of cuttings which dipped in IBA 2500ppm and planted in vermiculite as rooting medium found to be most effective. The influence of propagation media in semi-hardwood cuttings (Rathwa et al. 2017) [47]. Different growing media effects on rooting and survival of cuttings of Punica granatum. L cv. Super ‘Bhagwa’ discussed here (Netam et al. 2020; Raut et al. 2015) [48] and effects of growth regulators (Seiar et al. 2016) [50].

2.2.2. Layering

Success in layering majorly depends on the early commencement and the formation of enough adventitious roots for its survival. There are two types of layering is commonly implemented for the propagation of superior parental plants i.e., Air layering (gottée) and Mound layering in pomegranate.

2.2.2.1. Air layering

Air layering is the most commercial method of pomegranate propagation, which is carried out in May/June. Healthy, vigorous 1–2-year-old mature shoots of 45-50cm are selected and a circular bark strip just below bud is removed, later on, rooting hormone IBA and NAA 50mg in lanoline paste are applied over girdled portion to promote early rooting. Adventitious roots will form in 50-60days i.e., in July/Sep. To prevent loss of moisture moist sphagnum moss is packed around this portion and tied with polythene cover. Adventitious roots will form in 50-60 days i.e., in July/Sep. The rooted shoot should be slowly detached from the main shoot by giving 2-3 periodical successive cuts and the separated rooted shoot is transplantated in September/October. Various exterior and interior factors are affecting the regeneration of roots, whereas the etiolated part excites rooting and it has enhanced rooting in various fruit plants. Air Layering is a rapid, effective & most profitable way to clone elite and superior parental plants, it is the most inexpensive method. Growth regulator IBA with 2000ppm is best for high rooting and enhanced successful propagation of pomegranate air layers than NAA (Tomar 2011).

Patel et al. (2012) revealed that sphagnum moss as rooting media and IBA 5000ppm as growth regulator was found to be the best combination for the occurrence of a greater number of adventitious roots and high survival percentage in air layers at a high economical rate. Similar combination in Punica granatum L. cv. ‘Sindhu’ has also resulted in early root initiation and achieved a high survival rate, response of different rooting media, and PGR combinations (Bhosale et al. 2014) [57].

The propagation of pomegranate is majorly influenced by the month in which we are going to perform propagation. The highest survival percentage was obtained in air layered roots of Punica granatum Lcv. ‘Bhagwa’ those propagated in August month showed promising results (Tayade 2017b and 2018b) [8] and the effect of IBA along with different chemicals for earlier rhizogenesis, showed the high rooting percentage (98.33) and percent survival success rate (98.33)
in cv. ‘Bhagwa’ (Munde et al. 2016) [58]. Some recent studies showed that pattern root distribution in 6(six) year old pomegranate tree of cultivar Ganesh was observed shallow root system under semi-arid regions (Hiwale et al. 2011) [59]. For quick propagation of miniature pomegranate trees with fruits is done through air layering (Jingyun 2007) [60]. Air layers of cv. Bhagwa were treated with IBA 5000ppm was found to be significantly superior in early and profuse rooting and also good in its survival percent (Thoke) and treatment of air layers with a combination of IBA 2000ppm + Salicylic acid 2500ppm was found to be superior in better rooting and high degree survival of air layers (Pawar). The usage of the black poly wrapper while wrapping air layers resulted in better success and survival rate of air layers in pomegranate (Punica granatum L.) cv. Bhagwa (Hade).

A combination of sphagnum moss + coco peat in the ratio of (1:1) was identified as the best substrate rooting medium for good rooting and survival capacity of pomegranate air layers (Kisan). In air-layered plants, the level of light or dark and yellowness or blueness values for fresh fruits are 60.76 and 31.65, respectively (Singh 2020). In the G-137 variety, rooting media sphagnum moss + IBA 500ppm combination was found to be best as it resulted in a greater number of roots, high survival percentage, and high economical rate for preparing air layers of pomegranate (Manubhai).

2.2.2.2. Mound Layering/Stooling:
In Mound Layering/Stooling, a well-developed mature enough plant shoot is suppressed onto the ground in the resting season and enclosed with enough soil. After some days, those shoots start rooting, later on, they are detached and taken as new individual plants. Chadha (2001) has suggested that ground layering exploitation is an alternative method in the propagation of pomegranate. Singh et al. (2017b) [62, 67] reported that the performance of stooled shoot was pointedly influenced by spacing between mother plant stocks. Rooting of stool shoots was found to be increased enormously with an increase in higher spacing from 0.5 x 0.5 m to 1 x 1 m in cv. Bhagwa. The production of rooted ground layers from well-established orchards per unit (m2) is very high, therefore this method of propagation is enough possible to produce a greater number of saplings, and also it is an alternative option for marginal and small farmers efficiently.

2.2.3. Grafting
Karimi et al. (2001a) performed the “stenting” (cutting and grafting) method which resulted in high bud-take percentage was achieved through the use of rootstocks ‘Gorj-e-Dadashi’, ‘Gorj-e-Shahvar’, and ‘Gool Safid-eAshk-e-Zar’, and the scions were of the variety Malas-e-Yazdi and treated with IBA 500mg/l and also achieved successful callus formation near graft union, good bud takes percentage through Bench-grafting (Karimi 2011b). Effects of various rootstock and scion on vegetative parameters and success of grafting are discussed here with detailed information (Karimi 2017). Recent studies on graft compatibility of various rootstocks in Punica granatum L. cv. ‘Phule Bhagwa’ by using wedge grafting method of propagation, highest bud sprout (80%), maximum percent survival (76.6%), and more vegetative growth was recorded in rootstock ‘Bedana Suri’ in about 30 days after grafting (Ahire et al. 2016). Effect of a particular time and choosing of specific grafting method for graft success in Punica granatum L. cv. ‘Bhagwa’ (Chandhra et al. 2011).

Nowrozi et al. (2016) reported that rootstocks and scions of various genotypes have no major effect on the success of grafting and also proved that cleft grafting has shown that, this is the best successful propagation method for graft success in the case of vegetative parameters and also for changing scion cultivar in grafting. Rootstocks of various cultivars like Bedana Suri, Jallore Seedless, Ganesh, Kandhari, and Andali were found to have better graft compatibility with cv. ‘Phule Bhagwa’ by wedge grafting (Ranpise and Ahire 2016). The technique of ‘omega grafting’ is applied in pomegranate as stenting grafting, but the type of rootstock used in grafting has a typical effect on graft compatibility and graft success. However, more graft compatibility has been obtained by grafting the ‘Ranab-e-Neyriz’ scion on the ‘Gorj-e-Dadashi’ rootstock (Karimi 2001b).

3. Micropropagation
The extension of clonal propagation of pomegranate through in-vitro conditions has enabled quick mass multiplication of clonal propagation of superior parent plant material. The ultimate goal is to achieve increased pomegranate cultivation area around the whole world, mass multiplication of superior genotypes through tissue culture is necessary. This comprises and had the major key role of biotechnological tools, including cell, tissue culture, and micropropagation (i.e., somatic embryogenesis, organogenesis, synthetic seeds, somaclonal variation, mutagenesis, haploidy, in-vitro conservation), genetic transformation, molecular markers these techniques are used to improve pomegranate germplasm in all aspects (da saliva et al. 2013).

Gorad et al. (2018) reported that the highest root length (3.20 cm) was found on a medium which is having ½ MS + IBA mg/l, and during acclimatization in various hardening media, it was observed that higher survival percentage (71.87%) of plantlets from shoot tip explants. In cultivar, Punica granatum L. cv ‘Bhagwa’, nodal segments are taken as explants for culturing (Usharani et al. 2014). Double nodal segments in cv ‘Bhagwa’ (Prabhuiling and Huchesh 2018) taken as explants and cultured mainly on two different media MS (Murashige and Skoog) and Woody Plant Medium (WPM) which prepared as basal medium accompanied with 0.2-2mg/l BAP(6-benzyl amino purine), 0.1-1mg/l 1-naphthalacetic acid(NAA), 0.5-2.6 mg/l silver nitrate (AgNO3), 15-50mg/l adenosine sulfate, and for the stage of establishment and proliferation stage, 0.1-0.5mg/l IBA and NAA were used and for rooting stage 0.25 and 0.5mg/l IBA, NAA on MS and WPM medium were used and the plantlets raised in MS media were found to have highest survival percentage, thicker roots are formed by the usage of IBA than NAA (Patil et al. 2017) and efficient auxillary protocol for shoot proliferation and rooting in cv. ‘Bhagwa’ (Desai et al. 2018; Bachake et al. 2019) and in-vitro culture establishment. Prajwala et al. (2021) showed good results in the regeneration of plantlets in in-vitro culture and followed various micropropagation techniques like the preservation of phenol exudation for good culture establishment and shoot proliferation by using growth regulators and effective photoperiod. Interestingly by the use of different PGRs for achieving optimum regeneration in cv. ‘Bhagwa’ (Vala et al. 2021). Current biotechnological advances in pomegranate were discussed here in detail (Chauhan and Kanwar 2012). Use of nodal stem segments from a mature tree through
axillary shoot proliferation (Naik et al.1999; Murkute et al. 2004) and in-vitro mass multiplication from cotyledonary explants in cv. ‘Ganesh’ (Singh et al. 2013). Hardening of in-vitro propagated seedlings/plantlets of pomegranate done by using four strains of arbucular mycorrhizal fungi (AMF) bio hardening agent’s induction (Singh et al. 2012). Cotyledon explants when excised from germinated in-vitro seedlings were incubated in MS medium which is supplemented with different PGRs, resulting in 80% of explants developed callus and high-frequency in-vitro rooting (Kanwar et al. 2010). In Iranian cultivar Punica granatum L.cv. ‘Males Yazdi’ efficient in-vitro propagation with various combinations of IBA, NAA, Silver nitrate (AgNO3), etc using shoot tips and nodal explants (Kaji et al. 2013). A high success rate of auxiliary shoot formation and efficient plant regeneration from cotyledonary nodes was achieved (Naik 2000 and 2011). Use of multiple somatic embryogenesis, regeneration of plantlets has been achieved from somatic embryos which have their origin from cotyledonary tissues, with regular sub-culturing embryogenic cell clusters proliferated strongly after 20 days on RBF medium containing BAP, kinetin, and 2, 4-D. Early embryogenic initial cells contained dense cytoplasm and were attached to non-embryogenic cells (Bhansali 1990). Micropropagation protocol in cultivar ‘Mridula’ (Chaugule et al. 2007) and the fertigation effect on economics & yield in tissue culture discussed here (Shanmugasundaram and Balakrishnamurthy 2015). In-vitro regeneration of plantlets from juvenile explants (Deepika and Kanwar 2010) and induction of multiple shoots (Devidas et al. 2017). Apical and axillary vegetative buds as explants, best growth and multiplication was achieved on media having NAA (0.1mg/l) and higher concentration of BAP(0.5mg/l), whereas shoots have rooted with high degree adaptation easily with IBA (0.1 mg/l concentration) (Drazeta 1997). Interestingly in-vitro propagation in two Iranian commercial pomegranate cultivars “Malas Sevah” and “Yusef Khani” (Valizadekaji et al. 2013) and induction of callus, plant regeneration from leaf explants in Punica granatum L. cv. ‘Nana’ (Bonyanpour and Khosh-Khui 2013). Advanced study on in-vitro propagation (Kalabandi et al. 2014) and aseptic culture establishment and indirect organogenesis along with valuation of genetic fidelity using molecular Random Amplified Polymporphic DNA (RAPD) markers in var ‘Bhagwa’ (Guranna and Hoolageri 2017) and in vitro regeneration through organogenesis and embryogenesis in cv ‘Bhagwa’ (AA et al. 2018). Indirect organogenesis from leaf explants is influenced by rejuvenation in Punica granatum L. cv. ‘Kandhari Kabuli’ (Soni and Kanwar 2016). To develop sterile explants, from stem cuttings of cv. ‘Shishe Cap Ferdos’ by treating with two types of chelating agents (EDDHA and EDTA), different media, PGRs were evaluated to reduce subsequent browning (Eshaghi et al. 2020). Browning of cultures is the major obstacle in tissue culture due to the presence of high phenolic contents this can be reduced by treatment with activated charcoal (adsorbent) and ascorbic acid-antioxidant (Murkute et al. 2003). In in-vitro conditions, the nodal segments of pomegranate are used for distribution and exchanging germlasm through encapsulation of nutrient-alginante (Naik et al. 2006). Recent advancement in the cloning of matured pomegranate cv. “Jalore seedless” in the in-vitro technique of ex-vitro rooting and shoot production (Dinesh et al. 2019). Golozan and Shekafabdeh (2010) suggested that a combination of alcohol, lukewarm water, and NaClO (Sodium Hypochlorite) was found to be very effective to control fungal and bacterial contaminations in both pomegranate cultivars like cv. ‘Rabrab’ and in cv. ‘Rabab Neyriz’ by using nodal segments as explants (Valizadekaji et al. 2014). Axillary buds were used as explants, the shoots of both cvs. “Malas Yazdi” and “Shirine Shahvar”, were multiplied in in-vitro conditions and the maximum shoot height was reported on Murashige and Skoog (MS) medium having 2 mg/L BA (benzy1 adenine) (Mulaei et al. 2020). Apical shoots as explants have increased scope for evolving disease-resistant varieties mainly pomegranate bacterial blight-free plantlets by standardizing/sterilizing explants in suitable media, also enhanced callus induction up to 92% growth through indirect organogenesis (Satheesh and Sridharan 2014). Acclimatization practices (GACP-Good Agricultural and Collection Practices) have shown multiple benefits in cv. ‘Mridula’ (Vasane et al. 2011). Nodal segments as explants were taken from cv. ‘Phule Arakta’, the clonal fidelity of the in-vitro grown cultures were evaluated using RAPD and ISSR markers for mass production of true-to-type plantlets (Raman et al. 2019). Effect of different cytokinin types and their concentration on shoot proliferation, BA (Benzy1 adenine) and Kinetin showed highest shoot proliferation than Zeatin in cv ‘Khadouri’ (Bensaad et al. 2013). The RAPD-PCR and ISSR techniques generated a higher number of 79-94 and 57-72 fragments of DNA, with the help of primers mostly for Taify genotype OPG08 primer, and for Yemeni genotype OPA04 and OPD07 are used as primers (Alquarashi and Sadik 2020). More advanced techniques in commercialization, medicinal status, and in-vitro propagation applications enhanced the exporting of some important pomegranate cultivars (Shukla et al. 2015), mainly ‘Maniflouty’ and ‘Nab El-Gamal cultivars (El-Agamy et al. 2009). A high number of proliferated shoots are rooted highly in auxin NAA 1.0mg/L, the full strength of Murashige and Skoog (MS) media combining 2% of each sucrose and mannitol as carbon sources shown effective results (Dessoky et al. 2017). Maximum growth and multiplication have resulted on a medium containing 0.5 mg/L BAP and 0.1 mg/L NAA, the average rooting was induced in the MS medium half-strength comprising of 1.0 mg/L IBA. This has been a procedure that serves to establish an in-vitro genetic bank achieved in some cultivars of pomegranate through interspecific variability of RAPDs (Random Amplified Polymorphic DNA) (Bacu et al. 2009 and 2013). Genetic similarity among mother plant regenerated shoots, and callus in in-vitro conditions was evaluated using 8 Simple Sequence Repeat markers, among all those the highest number of shoots and leaves were obtained on MS media having complete strength with 6.9 µM kinetin, and the longest root was reported on MS media having 5.3 µM IAA (Indole-3-acetic acid). High somaclonal variation was observed more in regenerated shoots than in callus (Stimela et al. 2019). Browning in vitro-regeneration is increased with an increase in length and the position of explants, it was controlled by sub-culturing nodal ex-plants twice (Singh and Patel 2016). The highest number of shoots (4.80) and shoot length (3.50 cm) resulted under high light intensity (3000 lux), maximum shoot proliferation also observed on MS medium supplemented with 1. 0 m g/L B A P + 1. 0 mg/L kinetin along with 40 mg/L adenine sulfate and with 3% sucrose in the medium (Singh et al. 2014). In pomegranate cv.
Malassaveh, the peak shoot length (1.67 cm) was obtained in a liquid medium which is supplemented with 13µM BA (Benzyl adenine) + 5.5 µM NAA (Naphthalene acetic acid) and also maximum rooting was achieved in MS media containing 2 mg/L IBA i.e., 3 roots per explant (Soukhak et al. 2012). Medium containing 1 mg/l BA with 0.1 mg/l GA3 produces the best length (2.45cm) and also highest rooting was obtained with 2 mg/l IBA with 4.8 root/explant, this medium is considered as the best medium for a high success rate (Soukhak et al. 2009), Mercuric Chloride (0.1%) disinfectant was considered to be the best for disinfecting explants (Zareian et al. 2020). Nodal segments were proven to be superior for good callus induction and among all the PGRs BA (Benzyl adenine) 5 mg/L and NAA 0.40 mg/L resulted in the induction of quantitatively and qualitatively excellent callus (Prabhuling et al. 2017). Activated charcoal (200 mg/l) resulted to be the best antioxidant for controlling phenolic compounds accumulation in culture media and maximum shoot bud induction in the nodal segment, also in shoot apex explant (Kumar et al. 2018).

4. Conclusion
Many of the recently published work on the propagation of pomegranate achieved good progress in stem cuttings, layering, and micropropagation. But only limited work was done on grafting, rooting media, sexual propagation, and micropropagation. Some of the future recommendations in which future research has to be done are like more emphasis is to be given for the study of root physiology in layering and cuttings, also for the mass multiplication of elite varieties through micropropagation by adopting and effective utilization of advanced biotechnological tools. Works of excellence are reported mainly in micropropagation which involves hi-tech technology for the evolution of desired varieties which gives us enormous benefits in terms of quality and quantity aspects respectively. Recently, IIHR at Bangalore has also recommended that propagation of pomegranate through Air-layering is an effective method and declared it can also be used by marginal farmers and small commercial farms. Therefore, all the above-mentioned various methods of propagation are quite good to perform in diverse weather conditions and timings throughout the world for the successful multiplication and effective propagation of pomegranate.

5. References


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