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Recent trends in pomegranate propagation: A review

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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 '*Puniaceae*', 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 agro-technological 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, effortlessness 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) [15]. 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. 'Nana' of pomegranate has a very low germination percentage due to the presence of water-soluble germination inhibitor in its seeds and which can be deactivated at very low temperatures to commence germination early (Cervelli *et al*. 1994) [17]. He has also proven that the best results have been obtained by removing fleshy pulp three months before sowing, seed harvest, and stratification is done at 3degree centigrade for 30days in case of intact seeds increased both emergence rate when compared to unstratified seeds or stratified to 15°C/30 days.

Falcinelli *et al*. (2017) [18] observed that the genotype nature of the seed affects the seed sprouting in pomegranate. As there is very limited information and knowledge of sexual propagation in pomegranate, due to its greater variability in their progeny, long juvenile period to reach fruiting stage, season reliance, large quantities of plant material needed in commercial nurseries for propagation, etc these reasons at a standstill the usage of sexual propagation effectively but limited only to breeding programs and for raising rootstocks of superior cultivars.

Taheri *et al*. (2014) [19] revealed that hydropriming is an efficient method for the mass production of pomegranate seedlings. Therefore, propagation through seed in pomegranate results in higher genetic variability. Hence, sexual (seed) propagation of pomegranate is very much limited for raising only commercial rootstocks varieties in nurseries and it is only confined to breeding planning programs (or) mass multiplication of superior cultivar rootstocks for desired (scion) elite varieties.

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 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:

2.2.1. 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 (Aytekin 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-Indole3-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 500ppm 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 H_2O_2+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. 'Ganesh' (Deshmukh *et al*. 2019) [41, 42, 45].
Rajakumar *et al*. (2016) revealed that in *Punica granatum L*.cv. '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].
Chater *et al*. (2017b) [51] on 12 pomegranate varieties among those the cuttings of cv. Ambrosia has rooted best at 3gm/lit IBA. Excellent performance of hardwood cuttings was reported when cuttings were treated with 2500 ppm IBA for all root and shoot growth parameters (Tahwar 2018; Kharat) [53].

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 (gottee) 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 transplanted 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 L*.cv. '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 Super' 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 30days 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 Alandi 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' (Prabhuling 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-napthalacetic acid(NAA), 0.5-2.6 mg/l silver nitrate (AgNO3), 15-50mg/l adenine 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 axillary 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 arbuscular 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 RBM 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" (Valizadehkaji *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 Polymorphic 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 germplasm through encapsulation of nutrient-alginate (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. 'Rabbab' 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 (benzyl 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 (Benzyl 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 'Manfalouty' and 'Nab EI-Gamal cultivars (EI-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

1. Mahishi D, Muralikrishna A, Shivashankar G, Kulkarni R. (2012, December). Pomegranate (*Punica granatum* L.). In Horticulture-New Technologies and Applications: Proceedings of the International Seminar on New Frontiers in Horticulture, organized by Indo-American Hybrid Seeds, Bangalore, India, November 25-28, 1990;12:215. Springer Science & Business Media.
2. Pal RK, Babu KD, Singh NV, Maity A, Gaikwad N. Pomegranate Research in India-Status and future challenges. Progressive Horticulture. 2014;46(2):184-201.
3. Chandra R, Jadhav VT, Sharma J. Global scenario of pomegranate (*Punica granatum* L.) culture with special reference to India. Fruit Veg. Cereal Sci. Biotech. 2017;4(2):7-18.
4. Saroj PL, Kumar R. Recent advances in pomegranate production in India-a review. Annals of Horticulture. 2019;12(1):1-10.
5. Singh NV, Parashuram S, Roopasowjanya P, Babu KD, Mundewadikar DM, Sangnure V, et al. Performance evaluation of plants raised through different propagation methods in pomegranate (*Punica granatum*). Indian Journal of Agricultural Sciences. 2020;90(4):685-8.
6. Sau S, Sarkar S, Mitra M, Gantait S. Recent trends in agro-technology, post-harvest management, and molecular characterization of pomegranate. The Journal of Horticultural Science and Biotechnology, 2021, 1-19pp.
7. Tayade SA, Joshi PS, Raut HS, Shete MB. Effect of time and air layer per shoot on rooting and survival of air layers in pomegranate cv. Bhagwa. International Journal of Minor Fruits, Medicinal and Aromatic Plants. 2017;3(1):20-24.
8. Tayade SA, Joshi PS, Shete MB, Nagre PK. Response of several air layers per shoot in pomegranate (*Punica granatum* L.). Society for Minor Fruits, Medicinal and Aromatic Plants, 2018, 30pp.
9. Chater JM, Merhaut DJ, Preece JE, Blythe EK. Rooting and vegetative growth of hardwood cuttings of 12 pomegranate (*Punica granatum* L.) cultivars. 2017.
10. Rathwa AD. Influence of Propagation Media on Rooting and Growth of Hardwood and Semi-Hardwood Cuttings of Pomegranate Cv. Bhagwa (Doctoral dissertation, Department of Fruit Science Aspen College Of Horticulture And Forestry Navsari Agricultural University Navsari). 2016.
11. Kahramanoglu I, Umar K. Effects of cutting length, time, and growing media on the sprouting of dormant semi-hardwood cuttings of pomegranate cv. Wonderful. African Journal of Agricultural Research. 2018;13(45):2587-2590.
12. Singh NV, Chandra R, Awachare CM, Babu KD, Pal RK. A novel method of propagation in pomegranate: Mound layering. Progressive Horticulture. 2017;49(1):92-94.
13. Karimi HR, Farahmand H. Study of pomegranate (*Punica granatum* L.) propagation using bench grafting. Journal of Fruit and Ornamental Plant Research. 2011;19(2):67-72.
14. ValizadehKaji B, Abbasifar A, Bagheri H, Zandievakili G, Daryabeigi A. First report: Grafting of Three Iranian Commercial Pomegranate Cultivars on Drought Tolerant Rootstocks. International Journal of Horticultural Science and Technology. 2020;7(1):9-79.
15. Rawat JMS, Tomar YK, Rawat SS. Effect of stratification on seed germination and seedling performance of wild pomegranate. Progressive Agriculture. 2008;8(1):15-16.
16. Kumar S, Singh K, Prasad R. Effect of pre-sowing seed treatment on germination behavior of pomegranate (*Punica granatum* L.) cv Bhagwa. Indian Journal of Agroforestry. 2014;16(1):37-44.
17. Cervelli C. Effect of thermic treatments and seed manipulation on the emergence of dwarf pomegranate (*Punica granatum* L. 'Nana'). In International Symposium on Agrotechnics and Storage of Vegetable and Ornamental Seeds. 1994 June;362:189-196.
18. Falcinelli B, Marconi O, Maranghi S, Lutts S, Rosati A, Famiani F, Benincasa P. Effect of genotype on the sprouting of pomegranate (*Punica granatum* L.) seeds as a source of phenolic compounds from juice industry by-products. Plant Foods for Human Nutrition. 2017;72(4):432-438.

19. Taheri MN, Gholami M, Baninasab B, Mobli M, Moradi S. Pomegranate seed germination and dormancy breaking techniques. Seed Technology, 2014, 139-149.
20. Singh KK. Vegetative propagation of pomegranate (*Punica granatum* L.) through cutting-A Review. International Journal of Current Microbiology and Applied Sciences. 2017;6(10):4887-4893.
21. Singh N, Parashuram S, Roopasowjanya P, Babu KD, Mundewadikar DM, Sangnure V, Pal RK. 2020.
22. Batista PF, Maia SSS, Coelho M, Benedito CP, Guimarães IP. Vegetative propagation of pomegranate in different substrates. Revista Verde de Agroecologia e Desenvolvimento Sustentável, 2011, 6(4).
23. Sharma N, Anand R, Kumar D. Standardization of pomegranate (*Punica granatum* L.) propagation through cuttings. In Biological Forum-An International Journal. 2009;1(1):75-80.
24. Owais SJ. Rooting response of five pomegranate varieties to indole butyric acid concentration and cuttings age. Pakistan journal of biological sciences: PJBS. 2010;13(2):51-58.
25. Tripathi SN, Shukla HS. Propagation of pomegranate (*Punica granatum* L.) cultivars by stem cuttings with indole butyric acid and p-hydroxybenzoic acid. Indian Journal of Horticulture. 2004;61(4):362-365.
26. Rajan S, Markose BL. Horticultural science series-6. Propagation of horticultural crops. New Delhi, India: New India Publishing Agency, 2007, 110-113.
27. AAC, Bhande V. *In vitro* Regeneration through Organogenesis and Embryogenesis in Pomegranate Cultivar Bhagawa. 2018.
28. Aytekin Polat A, Caliskan O. Effect of indolebutyric acid (IBA) on rooting of cutting in various pomegranate genotypes. In I International Symposium on Pomegranate and Minor Mediterranean Fruits. 2006 Oct;818:187-192.
29. Kumar S, Prakash S, Malik S, Dhyani BP. Effect of different rooting media treatments on rooting and growth of stem cuttings in pomegranate (*Punica granatum* L.). 2021.
30. Kumar S, Prakash S, Prasad Y, Kumar A, Chand P, Dhyani BP. Effect of IBA and boric acid treatments on rooting and growth of stem cutting in pomegranate (*Punica granatum* L.). Journal of Pharmacognosy and Phytochemistry. 2020;9(5):2714-2716.
31. Kumar S, Prakash S, Kumar A, Dhyani BP. Effect of IBA, boric acid, and wounding treatments on rooting and growth of stem cuttings in pomegranate (*Punica granatum* L.). 2021.
32. Singh KK. Effect of IBA concentrations on the rooting of pomegranate (*Punica granatum* L.) cv. Ganesh hardwood cuttings under mist house condition. Plant Archives. 2014;14(2):1111-1114.
33. Purohit AG, Shekharappa KE. Effect of type of cutting and indole butyric acid on rooting of hardwood cuttings of pomegranate (*Punica granatum* L.). Indian Journal of Horticulture. 1985;42(1-2):30-36.
34. Holland D, Bar-Ya'akov I, Hatib K. 'Emek', a Red and Very Early-ripening New Pomegranate Cultivar. Hort Science. 2014;49(7):968-970.
35. Mehta SK, Singh KK, Harsana AS. Effect of IBA concentration and time of planting on rooting in pomegranate (*Punica granatum*) cuttings, Journal of Medicinal Plants Studies. 2018;6(1):250-253.
36. Alikhani L, Ansari K, Jamnejad M, Tabatabaie Z. The effect of different mediums and cuttings on growth and rooting of pomegranate cuttings. 2011.
37. Kaur S, Kaur A, Kaur G. Effect of IBA, PHB, and time of planting on rooting of pomegranate (*Punica granatum* L.) cuttings cv. Ganesh. Asian Journal of Science and Technology. 2016;7(11):3757-3761.
38. Bisen A. Effect of mechanical treatments on rooting in cuttings of guava, lemon, and pomegranate. Journal of Horticulture and Forestry. 2010;2(5):95-98.
39. Sarrou E, Therios I, Dimassi-Theriou K. Melatonin and other factors that promote rooting and sprouting of shoot cuttings in *Punica granatum* cv. Wonderful. Turkish Journal of Botany. 2014;38(2):293-301.
40. Deol IS, Uppal DK. Effect of different rooting media on rooting and growth of hardwood and semi-hardwood cuttings of pomegranate (*Punica granatum* L.). Punjab Horticultural Journal. 1990;30(1-4):140-144.
41. Manila T, Rana DK, Naithani DC. Effect of different growing media on vegetative growth and rooting in pomegranate (*Punica granatum* L.) cv. "Kandhari" hardwood stem cuttings under mist. Plant Archives. 2017;17(1):391-394.
42. Tanuja M, Rana DK, Naithani DC. Effect of different growing media on vegetative growth and rooting in pomegranate (*Punica granatum* L.) cv." Kandhari" hardwood stem cuttings under mist. Plant Archives. 2017;17(1):391-394.
43. Kumari KR. Studies on the effect of IBA and rooting media on rhizogenesis of cuttings of pomegranate (*Punica granatum* L.) cv. Bhagwa under shade net conditions (Doctoral dissertation, Dr. Ysr Horticultural University). 2014.
44. Patel KD, Butani AM, Thummar BV, Purohit HP, Trambadiya RD. Response of different media and IBA on rooting and survival percentage of hardwood cutting in pomegranate (PunicagranatumL.) CV. Bhagwa. Journal of Pharmacognosy and Phytochemistry. 2020;9(5):322-329.
45. Deshmukh S, Bharad SG, Deshmukh PL, Gholap SV. Effect of Different Media and Time of Cutting on Rooting and Growth of Pomegranate Cuttings. SS Narkhede, AD Rane, GD Shirke, VG More and MC Kasture. 2019;43:97.
46. Rajkumar JSG, Kumar R, Singh A, Kumar A. Establishment, survival, and growth of pomegranate cuttings with different concentrations of indole butyric acid and rooting substrates. 2016.
47. Rathwa A, Singh V, Patel D, Patel A. Influence of Propagation Media on Growth of Hardwood and Semi-Hardwood Cuttings of Pomegranate cv. Bhagwa. The trend in Biosciences. 2017;10(28):6023-6027.
48. Netam SR, Sahu GD, Markam PS, Minz AP. Effect of different growing media on rooting and survival percentage of pomegranate (*Punica granatum* L.) cuttings cv. super bhagwa under Chhattisgarh plains condition. IJCS. 2020;8(5):1517-1519.
49. Raut UA, Avachat SA, Bhogave AF. Effect of IBA and different rooting media on pomegranate cuttings. Trends in Biosciences. 2015;8(9):2227-2233.
50. Seiar YA. Effect of Growth Regulators on Rooting of Cuttings in Pomegranate (*Punica granatum* L.) Cv.' Bhagwa'. Journal of Horticultural Sciences. 2016;11(2):156-160.
51. Chater JM, Merhaut DJ, Preece JE, Blythe EK. Rooting

and vegetative growth of hardwood cuttings of 12 pomegranate (*Punica granatum* L.) cultivars. 2017.
52. Tanwar DR. Effect of IBA and Rooting Media on Hardwood Cuttings of Pomegranate (*Punica granatum* L.) cv. Bhagwa (Doctoral dissertation, MPUT, Udaipur).
53. Kharat BD. Effect of IBA and Rooting media on the growth of pomegranate cuttings (*Punica granatum* L) (Doctoral dissertation, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani).
54. Tomar KS. Effect of different concentrations of growth regulators on rooting and survival percentage of pomegranate air layers. Progressive Agriculture. 2011;11(2):431-433.
55. Patel DM, Nehete DS, Jadav RG, Satodiya BN. Effect of PGR's and rooting media on-air layering of different pomegranate (*Punica granatum* L.) cultivars. Asian Journal of Horticulture. 2012;7(1):89-93.
56. Alqurashi M, Sadik AS. DNA Fingerprinting of *in vitro* Micropropagated Pomegranate Genotypes. Pakistan journal of biological sciences: PJBS. 2020;23(5):619-627.
57. Bhosale VP, Shinde SM, Turkhade PD, Deshmukh SB, & Sawant SN. Response of different media and PGRs on rooting and survival of air layers in pomegranate (*Punica granatum* L.) cv. Sindhuri. Annals of Horticulture. 2014;7(1):73-77.
58. Munde GR, Nainwad RV, Mane SS, Gajbhaye RP, Pawar JV. Effect of IBA and Chemicals on Root Characters of Pomegranate CV. Bhagwa. BIOINFOLET-A Quarterly Journal of Life Sciences. 2016;13(2a):286-288.
59. Hiwale SS, More TA, Bagle BG. Root distribution pattern in pomegranate' Ganesh'(*Punica granatum* L.). Acta horticulturae. 2011;890:323.
60. Jingyun L. Rapid Propagation of Miniature Pomegranate with Fruits by Air Layering. Journal of Anhui Agricultural Sciences. 2007;35(20):6080.
61. Thoke NU. Effect of different growth regulators on air layering in pomegranate (*Punica granatum*) cv. bhagwa (Doctoral dissertation, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani).
62. Pawar JV. Response of different chemicals and IBA for Rooting to air layering in pomegranate (*Punica granatum* L.) Cv. Bhagwa (Doctoral dissertation, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani).
63. Hade SM. Studies on the effect of different poly wrappers on success and survival of air layering in pomegranate (*Punica granatum* L.) Cv. Bhagwa (Doctoral dissertation, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani).
64. Kisan TS. Effect of Different Substrates on Rooting and Survival of Air Layers in Pomegranate. (*Punica granatum* L.) (Doctoral dissertation, Mahatma Phule Krishi Vidyapeeth, Rahuri, District-Ahmednagar, (Maharashtra)).
65. Singh N, Parashuram S, Roopasowjanya P, Babu KD, Mundewadikar DM, Sangnure V, Pal RK. 2020.
66. Manubhai PD. Effect of Pgr's and Rooting Medias on Air Layering of Different Pomegranate (*Punica granatum* L.) Cultivars (Doctoral dissertation, Anand Agricultural University, Anand).
67. Chadha K. Pomegranate. Handbook of horticulture, (Eds.): Shashi, A. Verma, and Somdutt, 2001, 297.
68. Karimi HR. Stenting (cutting and grafting) is a technique for the propagation of pomegranate (*Punica granatum* L.). Journal of Fruit and Ornamental Plant Research. 2011;19(2):73-79.
69. Karimi HR, Nowrozy M. Effects of rootstock and scion on graft success and vegetative parameters of pomegranate. Scientia Horticulturae. 2017;214:280-287.
70. Ahire DB, Ranpise SA, Shirsath H. Study of Pomegranate (*Punica granatum* L.) Propagation Using Wedge Grafting. Advances in Life Sciences. 2016;5:8823-8826.
71. Chandra R, Jadhav VT, Sharma J, Marathe RA. Effect of grafting methods and time on scion sprouting, graft success, and subsequent growth of grafted plants of pomegranate (*Punica granatum* L.) 'Bhagawa'. Acta horticulturae, 2011;890:83.
72. Nowrozi M, Karimi HR, Mirdehghan SH. Effect of rootstock, scion, and grafting method on vegetative propagation of pomegranate. Iranian Journal of Horticultural Science, 2016, 47(2).
73. Ranpise SA, Ahire DB. Effect of different grafting methods and rootstocks on growth and survival of pomegranate (*Punica granatum* L.). International Journal of Minor Fruits, Medicinal and Aromatic Plants. 2016;2(2):22-28.
74. Da Silva JAT, Rana TS, Narzary D, Verma N, Meshram DT, Ranade SA. Pomegranate biology and biotechnology: A review. Scientia Horticulturae. 2013;160:85-107.
75. Gorad DS, Shaikh SA, Kallalbandi BM, Mali AS. *In vitro* propagation of pomegranate. 2018.
76. Usharani TR, Meenakshi S, Sunisha C, Mohandas S. *In vitro* shoot regeneration from nodal explants of pomegranate (*Punica granatum* L.) cv Bhagwa. Advances in Applied Research. 2014;6(1):66-71.
77. Prabhuling G, Huchesh H. *In vitro* Regeneration in Pomegranate (*Punica granatum* L.) cv. Bhagwa using Double Nodal Segments. Research Journal of Biotechnology. 2018;13:8.
78. Patil VM, Dhande GA, Thigale DM, Rajput JC. Micropropagation of pomegranate (*Punica granatum* L.) 'Bhagava'cultivar from the nodal explant. African Journal of Biotechnology. 2011;10(79):18130-18136.
79. Desai P, Patil G, Dholiya B, Desai S, Patel F, Narayanan S. Development of an efficient micropropagation protocol through axillary shoot proliferation for pomegranate variety 'Bhagwa'. Annals of Agrarian Science. 2018;16(4):444-450.
80. Bachake SS, Jadhav VB, Deshpande PP, Tele AA, Banda MA, Adki VS, Singh NV. Standardization of *in vitro* propagation protocol for pomegranate cv. Super Bhagwa. Journal of Pharmacognosy and Phytochemistry. 2019;8(3):2548-2553.
81. Prajwala KA, Subbaramamma P, Viswanath M. *In vitro* propagation techniques in pomegranate (*Punica granatum* L.): A review. pomegranate (Kanwar *et al*., 2008; Kanwar *et al*., 2010). 2021;19:18.
82. Vala AG, Tejas B, Kalathiya IU, Gajera SB, Parakhia MV, Rathod PJ, Golakiya BA. 2020.
83. Chauhan RD, Kanwar K. Biotechnological advances in pomegranate (*Punica granatum* L.). *In vitro* Cellular & Developmental Biology-Plant. 2012;48(6):579-594.
84. Naik SK, Pattnaik S, Chand PK. *In vitro* propagation of pomegranate (*Punica granatum* L. cv. Ganesh) through axillary shoot proliferation from nodal segments of the mature tree. Scientia Horticulturae. 1999;79(3-4):175-

183.
85. Murkute AA, Patil S, Singh SK. *In vitro* regeneration in pomegranate cv. Ganesh from mature trees. Indian Journal of Horticulture. 2004;61(3):206-208.
86. Singh P, Patel RM, Kadam S. *In vitro* mass multiplication of pomegranate from cotyledonary nodal explants cv. Ganesh. African Journal of Biotechnology, 2013, 12(20).
87. Singh NV, Singh SK, Singh AK, Meshram DT, Suroshe SS, Mishra DC. Arbuscular mycorrhizal fungi (AMF) induced hardening of micro propagated pomegranate (*Punica granatum* L.) plantlets. Scientia horticulturae. 2012;136:122-127.
88. Kanwar K, Joseph J, Deepika R. Comparison of *in vitro* regeneration pathways in *Punica granatum* L. Plant Cell, Tissue and Organ Culture (PCTOC). 2010;100(2):199-207.
89. Kaji BV, Ershadi A, Tohidfar M. *In vitro* propagation of pomegranate (*Punica granatum* 1.) Cv.'Males Yazdi'. Albanian Journal of Agricultural Sciences. 2013;12(1):43.
90. Naik SK, Pattnaik S, Chand PK. High-frequency axillary shoot proliferation and plant regeneration from cotyledonary nodes of pomegranate (*Punica granatum* L.). Scientia Horticulturae. 2000;85(4):261-270.
91. Naik SK, Chand PK. Tissue culture-mediated biotechnological intervention in pomegranate: a review. Plant cell reports. 2011;30(5):707-721.
92. Bhansali RR. Somatic embryogenesis and regeneration of in plantlets in pomegranate. Annals of Botany. 1990;66(3):249-253.
93. Chaugule RR, More TA, Kamble AB, Karale AR. (2007). Studies on micropropagation in pomegranate (*Punica granatum* L.). In Recent trends in horticultural biotechnology, Vol. I and II. ICAE national symposium on biotechnological interventions for improvement of horticultural crops: issues and strategies, Vellanikkara, Kerala, India, 2005 Jan;10-12:195-199. New India Publishing Agency.
94. Shanmugasundaram T, Balakrishnamurthy G. Effect of fertigation on yield and economics of tissue culture pomegranate (*Punica granatum* L.) cv. Mridula. 2015.
95. Deepika R, Kanwar K. *In vitro* regeneration of *Punica granatum* L. plants from different juvenile explants. J Fruit Ornamental Plant Res. 2010;18(1):5-22.
96. Devidas T, Sharad T, Nagesh D. Multiple Shoot Induction of Pomegranate (*Punica granatum* L.) Through Different Juvenile Explants. Bulletin of Environment, Pharmacology and Life Sciences, 2017, 17(1).
97. Drazeta L. Pomegranate (*Punica granatum* L.) propagation by *in vitro* method of tissue culture. [A short version of master thesis]. Review of Research Work at the faculty of Agriculture (Yugoslavia). 1997.
98. ValizadehKaji B, Ershadi A, Tohidfar M. *In vitro* propagation of two Iranian commercial pomegranates (*Punica granatum* L.) CVs. 'Malas Saveh'and 'Yusef Khani'. Physiology and Molecular Biology of Plants. 2013;19(4):597-603.
99. Bonyanpour A, Khosh-Khui M. Callus Induction and Plant Regeneration in *Punica granatum* L.? Nana'from Leaf Explants. Journal of Central European Agriculture. 2013;14(3):0-0.
100. Kalalbandi BM, Waskar DP, Khandare VS, Gorad DS. Micropropagation studies on pomegranate var. Bhagwa. Indian Journal of Horticulture. 2014;71(4):564-566.
101. Guranna P, Hoolageri HC. Studies on establishment of aseptic culture in pomegranate cv. Bhagwa. Annual Research & Review in Biology, 2017, 1-7.
102. AAC, Bhande V. *In vitro* Regeneration through Organogenesis and Embryogenesis in Pomegranate Cultivar Bhagawa. 2018.
103. Soni M, Kanwar K. Rejuvenation influences indirect organogenesis from leaf explants of Pomegranate (*Punica granatum* L.) 'Kandhari Kabuli'. The Journal of Horticultural Science and Biotechnology. 2016;91(1):93-99.
104. Eshaghi Sanayi T, Zare Mehrjerdi M, Sharifi A. Effect of Medium, Iron-Chelating Agent and Plant Growth Regulator on Micropropagation of Pomegranate (*Punica granatum* L.). Journal of Plant Productions (Agronomy, Breeding and Horticulture). 2020;43(2):309-322.
105. Murkute AA, Patil S. Exudation and browning in tissue culture of pomegranate. Agricultural Science Digest. 2003;23(1):29-31.
106. Naik SK, Chand PK. Nutrient-alginate encapsulation of *in vitro* nodal segments of pomegranate (*Punica granatum* L.) for germplasm distribution and exchange. Scientia Horticulturae. 2006;108(3):247-252.
107. Dinesh RM, Patel AK, Vibha JB, Shekhawat S, Shekhawat NS. Cloning of mature pomegranate (*Punica granatum*) cv. Jalore seedless via *in vitro* shoot production and ex vitro rooting. Vegetos. 2019;32(2):181-189.
108. Golozan AB, Shekafandeh A. Effects of plant growth regulators on pomegranate (*Punica granatum* L. cv. Rabbab) shoot proliferation and rooting. Advances in Horticultural Science, 2010, 207-211.
109. Valizade Kaji B, Ershadi A, Tohidfar M. *In vitro* Propagation of Pomegranate (*Punica granatum*) cv. Rabab Neyriz Using Nodal Segments. 2014.
110. Mulaei S, Jafari A, Shirmardi M, Kamali K. Micropropagation of Arid Zone Fruit Tree, Pomegranate, CVs. 'Malase Yazdi'and 'Shirine Shahvar'. International Journal of Fruit Science. 2020;20(4):825-836.
111. Satheesh M, Sridharan T. Standardization of *Punica granatum* explant and callus induction through micropropagation-indirect organogenesis. Int J Renew Energy Technol. 2014;3:19-27.
112. Vasane SR, Patil JL. Multiplying Benefits with Acclimatization Practices (Gacp) In Tissue Culture Propagated¿ Mrudula¿ Pomegranate. In V International Symposium on Acclimatization and Establishment of Micropropagated Plants 2011 Oct; 988:77-84.
113. Raman T, Gupta VS, Shukla S. A robust micropropagation protocol for genetically true-to-type plants of Phule Arakta pomegranate. The Horticultural Society of India (Regd.). 2019;76(1):23-31.
114. Bensaad ZM, Milad KM. Influence of Type of Explant, Salt Composition of Basal Medium, And Cytokinins on Micropropagation of Pomegranate (*Punica granatum*'khadouri'). In III International Symposium on Pomegranate and Minor Mediterranean Fruits. 2013 September;1089:91-96.
115. Alqurashi M, Sadik AS. DNA Fingerprinting of *in vitro* Micropropagated Pomegranate Genotypes. Pakistan journal of biological sciences: PJBS. 2020;23(5):619-627.

116. Shukla Susmita, Raman T, Shukla SK. A review on the scope for increasing *in vitro* production of pomegranate (*Punica granatum* L.) cultivars and its application in the human health sector with an emphasis on the Indian industry. Plant Cell Biotechnology and Molecular Biology. 2015;16(1-2):60-73.
117. El-Agamy SZ, Mostafa RA, Shaaban MM, El-Mahdy MT. *In vitro* propagation of Manfalouty and Nab El-Gamal pomegranate cultivars. Res J Agric Biol Sci. 2009;5:1169-1175.
118. Dessoky ED, Ismail IA, Attia AO, Sadik AS. Genetic studies, *in vitro* propagation, and conservation of some pomegranate cultivars grown in Taif province, Kingdom of Saudi Arabia. Rev Kasmera. 2017;45:69-86.
119. Bacu A, Mata V, Kongjika E, Kukali E, Damiano C. Interspecific variability of rapds and micropropagation of some pomegranate varieties (*Punica granatum* l.) From central and north-western albania. Albanian journal of Natural & Technical Sciences, 2009, 26(2).
120. Bacu A, Mata V, Kongjika E. Pomegranades of Albania, The Molecular Evaluation of Their Genetic Diversity and Possible *in vitro* Propagation of Best Varieties. Poljoprivreda i Sumarstvo. 2013;59(2):157.
121. Stimela T, Kasili RW, Mamati EG. Pomegranate Micropropagation, Callogenesis and Genetic Integrity Assessment Using Simple Sequence Repeat Markers. Journal of Agricultural Science, 2019, 11(1).
122. Singh P, Patel RM. Factors affecting *in vitro* degree of browning and culture establishment of pomegranate. African Journal of Plant Science. 2016;10(2):43-49.
123. Singh Pushpraj, Patel RM. Factors influencing *in vitro* growth and shoot multiplication of pomegranate. The bioscan. 2014;9(3):1031-1035.
124. Bhansali RR. Somatic embryogenesis and regeneration of in plantlets in pomegranate. Annals of Botany. 1990;66(3):249-253.
125. Soukhak F, Khalighi A, Ghaemmaghami SA. Study of Direct Adventitious Shoot Regeneration in Pomegranate (Punicagranatum cv. MalasSaveh) through Cotyledonary Explants. International Journal of Agricultural Science and Research. 2012;2(3):19-26.
126. Soukhak F, Vaez Livari B, Khalighi A, Ghaemmaghami SA. Study of *in-vitro* propagation on pomegranate (*Punica granatum* cv. Malas Saveh) through axillary shoot proliferation from single nodes. In Agronomy and Horticulture. 2009.
127. Zareian Baghdad, Abadi Z, Kamali Ali, Abad K, Tabandeh Saravi A. Comparison of Different Culture Media and Hormonal Concentrations for *In-vitro* Propagation of Pomegranate. International Journal of Fruit Science. 2020;20(3):S1721-S1728.
128. Prabhuling G, Iranna H, Huchesh CH. Studies on callus induction in Pomegranate cv. Bhagwa. International Journal of Tropical Agriculture. 2017;35(4):819-827.
129. Kumar R, Jakhar ML, Sekhawat K, Kumawat S, Burdak A. Effect of Antioxidants on *in vitro* culture of Pomegranate cv. Sindhuri. The Journal of Plant Science Research. 2018;34(2):311-318.