

## Propagation of Jojoba, *Simmondsia chinensis* (Link) Schneider: Constraints and Prospects

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### ABSTRACT

Jojoba being a dioecious plant has separate male and female plants. Propagation through sexual means i.e., from seed gives more than 50% male plants is a major constraint to being unproductive in future production. Plant sex needs 3-4 years to be identifiable when starts bearing fruit and delays fruiting. 90% of female plants are required when grown on a commercial scale to get a high yield. Being an allogamous species, plants originating from seeds showed huge variation among morphological and yield-related parameters is another constraint that can affect the commercial yield of jojoba. The success of growers and the industry of jojoba is directly dependent on the planting of genotypes that confer high yield and can be multiplied by asexual means via air layering, grafting, cuttings, and tissue culture to yield true-to-type plants is future prospect in jojoba productivity. Different methods of vegetative propagation and their efficiencies concerning jojoba are discussed and reviewed.

**Keywords:** Air layering, Grafting, Micropropagation, Stem Cuttings.

### INTRODUCTION

Jojoba [*Simmondsia chinensis* (Link) Schneider] is a multi-stemmed evergreen woody plant commonly grows between 0.6 and 3 meters in height [1,2]. Flowers are unisexual, dioecious, apetalous and wind pollinated [3]. Jojoba is a tap-rooted plant. Jojoba plant has extensive root system that can reach 15-25 meter with significant parallel primary and secondary roots to absorb moisture and nutrition from a considerable volume of soil [4, 5], thus permitting jojoba plants to flourish under drought conditions where a number of plants unable to survive are grouped as a true xerophyte [6, 7].

Jojoba seed resembles a peanut or acorn in shape, commonly plain, with black to brown colour. The seed consists of allied tissues of the cotyledon and doesn't contain endosperm or some time a little bit [8, 9]. Wild type jojoba genotypes can yield 40-60%

oil contents. Beyond the geographical diversity of plant material, oil quality exhibited a minute variation in respect of quality related parameters [10, 11, 12]. The use of jojoba beans has been reported for food and oil as a medicine for cramping, chronic renal disorders, cancer, easing childbirth and in healing wounds. Pertaining high economic and commercial value, jojoba is gaining popularity among farmers. Currently it is cultivated on an area of 8,500 hectares around the globe. Annual world demand is estimated about 64000-200000 tonnes per annum [13]. Currently, 3500 metric tonnes of Jojoba is annually produced in the world and the product in the global market is traded as "Jojoba oil" [14].

### Importance of Jojoba

Most jojoba seeds contain 40-60% oil [15]. The makeup industry is the prime user of the jojoba oil by utilizing a big hand of world production amounting to about 2000 tonnes per annum. Medicine sector is on the second rank in the demanding cue of jojoba production [16]. Lubricant applications also utilize a big share of 100 tonnes of jojoba oil annually [17, 18]. Jojoba oil is also serving as a prime quality alternate for sperm oil (from the sperm whale), which is limitedly available as restrictions are imposed on whaling [19]. Aside from the oil, the jojoba meal after oil extraction contains high level of protein and fiber content, but a pre-treatment for

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detoxification requires to make it consumable under live-stock sector [20].

### **Propagation**

Plantlets produced through tissue culture techniques, seeds and seedlings are used to develop new plantation sites [21, 22]. However, the species can also be propagated by layering and grafting [23]. During the early growth periods, it is a very challenging task to identify the exact sex of the plants that are produced by using sexual propagation methods. Sexually produced plants showed genetic variation that led to markable differences in the growth and yield attribute [24, 25]. During the beginning years of growth, sex determination in dioecious species by utilizing molecular marker technique is highly preferred [26]. For commercial planting male to female plant ratio must be 5: 1 that's why it is very important to determine sex of the plant during early stages of growth [27]. Asexual means of propagation yields true to type planting material for commercial level cultivation [28, 29].

### **Seed Propagation**

Jojoba seeds don't face dormancy and it can be sown for nursery soon after harvesting [30]. Mean daily temperature of 27 to 38°C along with moist conditions and alkaline sandy nature of potting media boosts the germination rate [8, 31]. Jojoba seeds can stay viable for years as compared to other oil seed crops. These may either be sown directly in the field or first grown as potted nursery stock and then transplanted. However, the nursery stock does not transplant well [31]. The easiest and most used method is planting seeds directly in the field. The percentage of germination from fresh seeds is 95-100%, and seeds stored for 4 or 5 years may still give as high as 90%. About 38% germination has been recorded in seeds stored for 11 years in an open shed in California [8]. Maximum seed germination of 80.0 to 83.3 % was recorded when seeds were sown on 16th September for three years [32]. Seed soaking prior to sowing improves seed germination and reduces time to emergence. One hour soaking of seeds prior to sowing gave maximum germination (89 %), followed by 8 hours soaking (85 %). Unsoaked seed took maximum time to germinate (25 days). In general, plants from seeds or sexual means gives a greater number of males as compared to female [16, 33]. Further, sexually propagated plants showed genetic diversity which resulted in decreased average yield [34, 35].

### **Vegetative Propagation**

Genetic variability in morphology, anatomy and physiology within species is very large when raised through seed and enables selection of individual plants for high yield and other agricultural attributes [36]. Direct selection based on identifying potentially

promising genotypes and their testing after vegetative propagation has enabled improvement in crop performance. Vegetative propagation enables the establishment of plantations with the desired proportion of male to female plants of pre-selected superior clones [34, 37, 38]. Vegetative propagation has four main advantages over seed propagation of jojoba; (a) higher and more uniform yield, (b) early bearing of fruit, (c) reduced costs of later cultural and harvesting operations and (d) evolution of true to type uniform, desirable clonal variety for future multiplication [37]. Different methods of asexual propagation can be used to produce jojoba plants, and one can use a method of his choice. The asexual method can yield air-layered plants [23,28], grafted plants, stem cuttings [21, 39], and micro propagated plants [22, 40].

### **Propagation by Air Layering**

Centuries ago, Chinese developed the technique of rooting of arial branches when they are still attached to their mother plant and named this methodology as "Air layering". Many plant species showed poor success in rooting but can effectively propagated by utilizing the air layering technique for extensive multiplication of planting material [41]. The wounded area of the arial branch subjected to rooting is first covered with the moist media in ball shape that favors rooting and then wrapped with polyethylene and tied tightly on both ends of the ball. The branch should be removed when roots come out of media and then planted in the pot in nursery for future care. [42]. In female jojoba plants 91% rooting was obtained by treating their air layers with 406.4 mg/L IBA + 3724 mg/L Naphthalene Acetic Acid (NAA), and 90% rooting in male jojoba plants when treated with 4064 mg/L Indole Butyric (IBA) + 3724 mg/L NAA [28]. Air layering technique with 500-1000 mg/L of IBA for vegetative propagation of jojoba as well as root initiation by air layering in jojoba are very successful [43, 44, 45]. Air layering from third week of January to second week of March and in mid-August by treating ringed shoots (a ring of 2.5 cm after removing the bark) with 500 mg l-1 IBA, covering the point with gunny bag or saw dust and wrapping with polyethylene sheet resulted in root development in the branches and the layered plants after detachment well survived [46]. Layering on year old branches from 3.5-year-old jojoba shrubs from June to August (rainy months) in Hyderabad, India with auxin treatment resulted in rooting of air and ground layered branches. Indole-butyric-acid among all the auxins treatments at 6000mg/L concentration gives notable outcomes as compared to others. IBA and NAA reciprocity was noticed on multiple roots at each layer of rooting. A direct relation in rooting associated parameters and

increasing concentrations of hormones was observed. Success of 68.1 % and 72.7 % of rooting of air and ground layers was achieved when IBA was used at 6000 mg/L [47]. Plants and plant parts, auxin induces and promotes the synthesis of RNA and proteins. Such synthesis may be the pre-requisite for auxin induced growth. Several asexual methods of propagation have been applied to propagate jojoba including air layering [23, 28, 48] and ground layering [23]. Although air layering and ground layering are successful methods for vegetative propagation of jojoba, yet these cannot be applied at large scale because of a smaller number of layered plants to fulfil the requirements of female plants per unit area for new plantation of jojoba.

#### **Propagation by Grafting**

Grafting is a technique of plant propagation in which the top and root systems of the new plant come from separate species or kinds [48]. In grafting, scion must consist of two or more buds and then unite to rootstock in a way that the cambium tissues of both come in firm contact, so the cell division in cambial section of scion-stock is closely knitted [49]. The effects of several edaphic, biotic, and genetically unfavorable conditions can be minimized by using the grafting technique along with remarkable advantages [50]. The imbalance of males to females could be met with hormonal treatment and by grafting males with females and vice versa [43, 51]. One to two years old mature brown jojoba branches used for scion selection by using splice or whip method in spring (mid-February to mid-April) with 0.5 to 1.25 cm long scion with single bud from mature branch showed best outcomes as compared to immature [2, 8]. About 20% of three-year-old male plants of jojoba in the field turned efficiently in to female one by grafting of 200 plants and majority of plants yield nuts in just two years [52, 53]. Grafting of jojoba has many advantages as keeping the deep tap root of the rootstock which helps to survive in drought and salinity conditions, propagating plants with previously known sex, propagating unique and desirable shrubs which will allow predictable plant growth and yield, also shortening juvenile phase of jojoba. Also, they have shorter juvenile period than those grown by the seed and can be applied at somewhat large scale for in-situ propagation of jojoba.

#### **Propagation By Cuttings**

Jojoba is a hard to root plant, however, semi-hard cuttings can give success in rooting and allow to multiply favorable genotypes [54]. Propagation through stem cuttings is the most commonly used vegetative method for jojoba multiplication [55, 56]. Several cases of rooting the jojoba cuttings were reviewed and concluded that fresh shoot growth

treated with root induction hormone i.e., IAA, had given the best result. However, the superiority of stem cuttings in propagation of favourable selections were manifested [31]. The genotype and mother plant being used greatly influence the rooting success in jojoba [8, 57, 58]. Success of rooting is directly affected by time of the year, length, and juvenile stage of the cutting. Cutting taken from present year growth and of semi hard nature proven best for rooting. Cuttings in a state of active growth (softwood cuttings) also root well [57]. Heating of substrate apparently increases the success rate in clones with least rooting percentage [58]. Under uniform conditions, rooting success may vary from 0 to almost 100% depending on the clone being used. Most clones have intermediate to high rooting percentages (50-90%), if cutting material is in good condition [59]. It was reported by the National Research Council (1930) that cuttings from some shrubs demonstrated rooting rates up to 95 percent. Males and female seem to root equally well [60]. However, marked differences were observed for rooting capability among the 12 clones tested [61]. These differences for rooting among varieties with different genetic makeup can be eliminated by applying specific rooting treatments, notably with different concentrations of IBA [62]. Four node terminal and middle cuttings rooted best compared to 4-node basal ones [59]. The cuttings with 5-nodes yield extensive rooting system as compared to those with single node cuttings, and double-eye cuttings produced apparently more roots than single-eye [61]. The use of 10 to 12.5 cm long terminal cuttings [8], about 15 cm long cuttings with seven pairs of leaves [62], six-node cuttings [63], tip cuttings of jojoba have also been successfully rooted. Cuttings from new and old shoots of female jojoba plants given the rooting success of 60-64 and 3-5%, respectively [39]. The rooting ability of different cuttings sizes (single node, double node, and 3-nodes) from different individuals of jojoba showed that young plants rooting extensively as compared to the matured [29]. Several scientists have observed changes in rooting at different times of the year. In Israel, rooting percentage increased progressively from fall to winter to spring to summer. Spring cuttings had the longest roots. However, winter cuttings which rooted had the most numerous roots [59]. In California, from the end of March to the end of August is the best time, after that the percentage drops rapidly. In Arizona, the percentage of rooting of cuttings taken in July or August ranged from 30 to 70% [8, 64]. The root system of cuttings planted in July remained significantly better than those of cuttings planted in August, even though rooting was 100% at both times [57, 65]. In Pakistan, the cuttings taken in October

rooted well. NAA and IBA was used for rooting hormone separately and with the combination of Triiodo benzoic Acid (TIBA) to treat jojoba stem cuttings with leaves in April, July and November and dipped in IBA 3000 mg/L + TIBA100 mg/L and planted in different media. They observed that treatment during the month of April with IBA 3000 mg/L+TIBA 100 mg/L solutions apparently increased all rooting and growth parameters [66] used.

For many species propagated through semi-hardwood cuttings, auxins are applied to the base of the cutting prior to planting to stimulate rooting. Several researchers have reported on the use of such material for successful rooting of jojoba cuttings using IAA as a rooting stimulant. 50-60% of the cuttings produced adventitious roots, but cuttings continued beyond a few weeks never ever observed [31]. Since then, many others have relied upon the use of auxins for rooting jojoba cuttings. Most researchers have used synthetic auxins such as IBA the water-soluble potassium salt of IBA, NAA or commercial preparations containing one or more of these materials [29, 67]. Synthetic materials have often been found to be more effective root stimulant when tested on other species and in general, they are more stable than the naturally occurring IAA]. It was concluded that treated cuttings with IBA gives success of 56% as compared to NAA treatment that gives only 26% rooting success [59].

Concentrations of auxins also determine the success of rooting. Concentrations of IBA in water solution were evaluated for effects on rooting percentage, root length and root number [47]. Success of rooting is directly dependent on the increasing concentration from 0-15000mg/L. A very minute effect of concentration on root length was observed. However, the number of roots unexpectedly jumped at concentrations of 15000 and 20000 mg/L [38, 59]. The studies conducted in Israel on stem cuttings of jojoba revealed that treatments with 50% Ethylenediaminetetraacetic Acid (EDTA), and IBA @ 1000, 4000, 8000, 12000, 16000 and 20000 mg/L resulted in 1.7, 25.0, 50.0, 61.7, 53.3 and 56.7 percent rooting, respectively while untreated cuttings did not root at all [43]. Stem cuttings of jojoba treated with IBA @ 500, 1000, 2500, 3000, 4000, 5000, 6000, 8000, 10000 or 15000 mg/L plus 31 mg/L boric acid with speedy dip (20-30 seconds) in IBA (5000 mg l-1) markedly induced rooting [21]. Terminal cuttings [8] or five-node cuttings [68,69] treated with 4000 mg l-1 IBA also rooted successfully. Double eye (DE) and single eye (SE) single node cuttings at semi-hardwood stage gives best rooting success when treated with 2000mg/L [61, 70]. However, semi-hardwood cuttings rooted

not more than 55% when treated with this concentration of IBA. Rooting of cuttings treated with 1000 mg l-1 IBA on a heated propagation bench [55,71]. Application of IBA @ 1000, 2000, 3000 and NAA @ 1000, 2000 mg l-1 to jojoba cuttings and was found that IBA (1000 mg/L) was good to increase rooting in cuttings at semi-hardwood stage [29]. NAA, IBA and IAA all at concentration of 100ml/L when applied to cuttings gave 80%, 82% and 76% success for rooting respectively, and 125-261 days to root cuttings [39].

When leafy cuttings of jojoba are planted, the primary consideration during rooting is reduction of water loss through transpiration. Several methods are available to reduce water loss, including intermittent misting of the cuttings, fogging, use of anti-transpirants, and enclosing cuttings in some type of protected structure to maintain high humidity [59]. Moderate shaded growth chamber with extensive humidity increase the rooting success rate of jojoba cuttings [8, 61], in a ventilated high-humidity fogging greenhouse [62], in mist propagation chambers [72], on a humidified heated growth beds [55] and under moderately shaded plastic sheet tunnel having humidity  $\geq 90\%$  Maximum success for rooting and hardening attained by providing 100% moist conditions unless of the saturation of growing media in jojoba cuttings [73]. It is reported that 80 - 85% relative humidity is essential for rooting of jojoba cuttings [39].

Rooting, growth and success rate of cuttings are directly affected by potting media used for planting. The use of perlite-vermiculite (1:1) medium for IBA treated cuttings have been reported by [8, 61, 63, 74]. IBA (1000 mg l-1) treated cuttings resulted in significantly less rooting (64%) in peat-perlite medium than did in other two media i.e. peat-perlite-vermiculite (78%) and perlite-vermiculite (74%), respectively [55]. Peat-polystyrene gave the best results [59]. However, after rooting, it was suggested to transplant the rooted cuttings in poly containers consisting of soil, sand, peat and polystyrene (3:3:3:1) for 4 to 6 weeks [43]. Tissue nutrient level and growth-related parameters improved by application of fertilizer but, rooting percentage remained unattended [75]. Root development was retarded when phosphorus applied at higher dose with irrigation, while growth of shoot not effected by low dose of P [76]. A relation was observed in varying levels of growing media temperature, 20 - 25 and 27 - 30 °C, and jojoba clones for induction of rooting in cuttings [58, 66]. It is reported that perlite along with vermiculite proven best media for jojoba cuttings as, growth related parameters such as length, rate of survival, roots number per cutting, diameter, and number of leaves per cutting observed higher as

compared to other growing medias [34]. Jojoba cuttings treated with different concentrations i.e., 1500, 2500 and 3000 mg/L of IBA for propagating different genotypes of jojoba utilizing growth media of equal composition of vermiculite, perlite, and peat moss and recorded success to initiate roots from only 3000 mg/L [77].

It remained a concern that saplings planted in field conditions would not perform better than seedlings. Tap roots were developed in plants that were propagated through seeds to extract moisture and nutrition from depth as compared to saplings that yields more fibrous roots than tap roots which would fails to survive under stressed environment [59], but such type of root system might be beneficial under irrigated conditions [31]. Plantation from saplings bear earlier than those established from seedlings. Plantation of selected jojoba plants was established near Bakersfield (USA) in 1979 from saplings and harvests were made in 1981 from 2.5-year-old plants and in 1982 from 3.5-year-old plants [37]. Saplings should be transplanted to the field 8 - 10 months after their transfer to the bags. The survival rate of jojoba cuttings after transplanting ranges from 75 to 95% [39]. Normally one- and two-year-old saplings are considered as a suitable material for planting in the field when the soil humidity is adequate [78].

### **Micropropagation**

Although the use of jojoba cuttings with uni-node, bi-node, and tri-nod by treating with various Phyto hormones will enhance the number of plantlets attained by plant stock [29], yet the highest propagules quantity is checked by size of the plant and time of the year [59]. A vast number of quality plants can be produced from mother/ stock plant by applying micropropagation technology [79] in a short possible time independent of the season. In latest years, under in-vitro conditions growing plants by utilizing micropropagation techniques in a few plant species has been proven successful [80]. Plantlets can produce a few shoots when propagated in vitro by producing roots. Thus, tip of shoot, segment of node can be used as explant to develop a number of identical plants each year. Tissue culture originated plants showed higher growth and vigor even after first year of transplanting as compared to both propagated by seeds or cuttings [81]. Production for commercial level plantation can be gained by producing micro-propagated identical plants of highly productive genotypes observed during trials [82].

During the year 1973-74, an attempt was made to produce in-vitro meristematic tissue cultured plants of jojoba. Either leaves were produced from the callus using certain chemicals but no roots, or roots were produced but no leaves. Rootless plantlets

(shoots) were removed from the culture medium and rooted like cuttings. A very low percentage of these shoots rooted and smaller number of these survived when transplanted to soil mixture. A breakthrough was achieved during 1979-80 when both leaves and roots were produced simultaneously. By 1981, a large number of tissues cultured plants was available from about 50 different female and male clones. A large shipment was sent to southern California where it was grown under greenhouse conditions in small paper containers to prepare them for later field planting. The results were disastrous. Nearly 10% survived in the field and few made significant growth during one and half years later [8].

For in-vitro culture standards are known for jojoba, but still some difficulties are there that hinder efficacy during micropropagation. For in vitro production of liquid wax, studies are available for somatic embryogenesis of jojoba [83, 84, 85, 86] and cultures from both axillary and terminal buds for in vitro multiplication [81, 87, 88, 89].

Varied reciprocation is given by the genotypes when propagated in-vitro. Marked able changes in clones have been reported at all steps when cultured in-vitro in response to growth regulators. Nourished media may serve as an initiating step for many genotypes although, there is a need for its standardization for every clone to refine its respond during in-vitro culture [90]. The prominent relation of growing media composition and genotypic characteristics effect was reported [91]. Distinct physical or morphological response shown by female and male In-vitro cultured shoots of jojoba under the effect of different supportive [92]. At multiplication various changes to growth and division rate of clones of jojoba was reported [93]. Shoot regeneration required various notable levels of Benzyladenine (BA) for explants of nodal origin of various genotypes [40]. Manyresearch used various levels of plant growth regulators for culturing shoots of jojoba under in-vitro conditions. For successful culturing different combinations of cytokinins and auxins or GA3 cytokinins have been used [89]. different levels of BA, Gibberellic Acid (GA3, and NAA in combination or alone were used in MS media, and significant outcomes were obtained from the media containing 2mg/L BA. Except BA no other cytokinin apparently favors physical growth [94]. As compared to kinetin treated plants the growth of shoot was markedly higher in those that were treated with BA [95]. New shoots bearing 15 nodes were obtained after six weeks cultured on BA contained MS media [96]. MS media with added BA 1 mg/L resulted in 4.6 times, a greater number of shoot at 30th day of culturing [90]. BA @ 2.25 and 4.5 mg/L gives 3.5 and 4.7 shots/ explant in Male and female,

respectively when added to B5 and MS media. Segment of shoot having one node cultured on MS media containing BA 4.0 mg/L resulted in better shoot proliferation [97]. A mean number of shoots 2.7 per explant were found in female clone EC 33198 on BA 4.5 mg/L added MS media [98]. 2.25 and 4.50 mg/L BA showed the best results for shoot demarcation in all types of explants [92]. MS media supplemented with BA 2.25 mg/L yields higher number of shoots (10) in EC 99692 and lower (9.3) in EC 171282 female and male genotypes, respectively when cultured by using nodal shoots [40]. Driver Kuniyuki media with added variable levels and combinations of BA and silver nitrate or separately, resulted in significant shoot proliferation [99]. Enhanced multiplication of shoot was observed in female when treated with TIBA combined with BA [92]. Meristematic action showed significant outcomes by using 1 mg/L Zeatin and 0.1 mg/L GA3 added MS media [100]. MS medium containing 0.01 NAA, 0.5 6-BA and 0.4 mg/L GA3 gave 60% sprouting of explants [101]. Shoot formation was best when the MS media added with 10 mg/L BA + 5 mg/L IAA [102]. Axillary shoots were produced on modified medium contain MS media along with 0.5 and 1.0 mg/L Kinetin and 6-Benzyl Adenine, respectively at 30th day of culture [103]. Least success was gained in multiplication when nodal segments from mature plant of jojoba cultured under In vitro conditions [25]. Complete plantlets of jojoba were taken when 1.0 mg/L 6-Benzyl Adenine and 1-naphthaleneacetic acid respectively added to MS media after 35-40 days of incubation, the length of shoot was observed maximum in higher concentrations of 6-Benzyl Adenine [104]. 6-Benzyl Adenine, adenine sulfate, and indole-3-acetic acid @ 1, 40, and 3.0 mg/L, respectively added to MS media yields significant development of shoot of buds at encapsulated stage. MS media containing 6-Benzyl Adenine and 1-naphthaleneacetic acid 2 and 0.1mg/L respectively, gives 5 number of shoots at each cultured explant [22]. Established nodal segment cultures from jojoba plant having age of 5 year by using shoot from axials were regenerated on from 5-year-old jojoba tree through axillary shoot proliferation on MS media containing almost ½ inorganic salts level, multiple shoot formation on MS media +BAP (1 mg/L) + Gibberellic acid (0.5 mg/L), enhanced further strength of shoots on replanting of transplants to MS+BAP (1 mg/L) + CH (500 mg/L) and used full MS+BAP (1 mg l-1) + CH (250 mg l-1) medium for shoot division and continuation of shoot [24]. Four media viz MS, SH, DKW and Lloyd and McCown (Woody plant medium) were tested for in vitro reproduction of jojoba by culturing cuttings having terminal portion and single node and was

noted that 6-Benzyl Adenine and indole-3-acetic acid @ 1mg/L each in MS media had the best results for initiating multiple shoots in single nodal cuttings [105]. MS media containing 2.0 mg/L BAP yields maximum (9.13) number of shoots when different levels (.5, 2 and 2.5 mg/L) KIN, BAP and TDZ (0.22,0.44 and 0.88) mg/L were combined by using with NAA, IBA and IAA @ 1.25 mg/L) [27]. Significant results for maximum division, number of shoots, leaves and length of shoot was observed by combined application of 3 and .5 mg/L of BA and NAA, respectively on 60 days old tips of shoots and semi-soft wood cuttings from a 6-month-old plant of 5 genotypes of jojoba when cultured on various levels of plant growth regulators [77]. Axillary bud of a nodal segment taken from a male or female plant to proliferate in a modified Schenk and Hildebrandt's (SH) medium supplemented with 1 mg /L each of BAP and IAA and obtained 5–8 shoots per explant of single-nodes in initial cultures were induced, but the number of shoots in subsequent cultures increased substantially to about 15 in low BAP (0.5 mg/L) containing medium [106, 107, 108, 109, 110]. Shoots treated with IBA by placing for seven days in media and replanted in basic media gives success of 67%. Treatment for 14 days was required for NAA as compared to IBA for same results [96]. Root formation was best when MS media was added with 10 mg/L IBA + 1 mg/L IAA [102]. Shoots were transferred to liquid ½ strength MS media having NAA 10.0 mg/L for 72 hours and incubated in the dark (for prompt initiation of root) and then shifted to ½ strength MS rooting medium containing activated charcoal @ 2500 mg/L gives initiation of root in just a week and a month is required for development of shoot ≥ 80% [103]. Jojoba explants only rooted in Bourgoin and Nitsch media containing 1-naphthaleneacetic acid @ 0.93mg/L without any effect of putrescine of rooting [106]. Micro propagated explants of shoot origin yield 31.08% success of rooting on ½ strength MS media enriched with 3 mg/L IBA after 70 days [107]. 20-95% success depending upon specification of genotype and trial methodology was attained [25]. Agrobacterium rhizogenes alone or in combination with 100 mg/L IBA was observed to enhance the induction of root after two months of culturing. 250 mg/L Cefotaxime significantly enhanced the percentage of rooting, number of roots, and length of root. Combined A. rhizogenes-IBA-cefotaxime treatments significantly augmented all studied parameters [108]. Certain efforts were made for induction of rooting in micro propagates of jojoba on different medias such as MS media in combination with indole-3-acetic acid, indole-3-dutyric acid and 1-naphthaleneacetic acid and maximum rooting was observed in media that

was added with IBA [91]. Shoots propagated on modified MS medium nourished with 3.0 mg/L IBA developed roots (25%) after 15 days. Apparently varied response by clones was observed [90]. A 20 and 40 minutes pulsed-treatment of 10 mg/L indole-3-butyric acid resulted in approximately 80% success in rooting of male and female shoots cultured in-vitro [94]. 6-Benzyl Adenine, Indole-3-butyric acid, and activated charcoal @ 0.2-2.0, 2.0 and 5000 mg/L, respectively added in MS media resulted in effective root induction from shoots [97]. Rhizogenesis induction was observed in shoots generated at proliferation stage when IAA, IBA, and NAA treatment was applied. Development of roots in shoots was observed at 64% when treated with 10mg/L of both Indole-3-butyric acid and 1-naphthaleneacetic acid, respectively [99]. MS media at half strength, IBA @ 1.0 mg/L and IAA @ 1.0 mg/L reported efficient for induction of rooting [29]. Approximately 85% production of root was observed in 10mg/L IBA treated shoots before shifting to MS medium containing 6-Benzyl Adenine, Indole-3-butyric acid, and activated charcoal @ 0.23, 2.0, and 5000 mg/L, respectively [98]. Shoots to culture in-vitro treated for 24 h with indole-3-butyric acid @ 120mg/L gives maximum rooting before shifting to MS media having no PGRs as compared to that growing media containing IBA 4 mg/L and NAA 2mg/L. Significant rooting was observed on growing media having combination of IBA and NAA as compared to that one having both PGRs separately [22]. [24] achieved rooting in shoots as high as 82% on 1/4 MS+IBA (0.5  $\mu$ M) medium. IBA @ 5 mg/L in combination with 1.0 and 2.0 mg/L AC added MS media observed beneficial for root induction from shoots of jojoba of shoot tip and nodal segment origin. [109] achieved rooting on MS media with mineral salts of half strength enriched with Vitamin B5 and IBA @ 14.7  $\mu$ M used to treat for a period of 7 days and shifted to media un-supplemented with auxin for induction of roots. [105] recorded high frequency of rooting on full MS +7 mg l-1 IBA. Maximum value (50%) of rooting was attained on MS media (half strength) having 1.5% sucrose, 1 mg/L IBA and 0.5% activated charcoal, followed by various levels of NAA, IBA, and IAA containing MS media. 30% success was gained by using 1.0mg/L IBA and 40% by utilization of 3.0 mg/L in combination with MS media [27]. However, failure was recorded in five jojoba genotypes in initiation of roots as well as in proliferated plants when various levels 0.5, 1 and 2mg/L of BAP, NAA and TDZ were used as treatment [77].

Difficulties observed for acclimatization when micro propagates of jojoba are required to transplant in field [56, 79, 110], significant mortality of rooting

system was also reported when provided with high humidity and reduction in roots aeration [110], as small sized leaves generated during culture [79]. The Agrosoil<sup>TM</sup> planting medium yields significant outcomes for acclimatization, perhaps because its porous nature favored good aeration. The prompt growth of shoot resulted by the presence of O.M. in growth media [90]. Hardening-off standards and tools for in-vitro generated propagules are developed now. One part peat and perlite or one part peat and two parts perlites identified as best medium for jojoba plantlets as potted media [22]. Micro propagated plantlets transplanted in soil survived more than 70% [24]. Plantlets of selected jojoba genotypes were acclimatized by applying reasonable moisture with (5:1:1) peat: perlite: soil (1:1:5) media [109]. Regenerated plantlets transferred successfully to media containing same ratio of peat moss, vermiculite and sand resulted in 90% success [27]. Acclimatized rooted shoots successfully survived with 75% survival rate [105].

### CONCLUSION

Being an allogamous species and dioecious nature, jojoba plants propagated through seeds have large variation in morphology and production that affect the commercial farming of jojoba. These constraints could be removed by following the innovation and techniques in asexual propagation of this precious plants. Jojoba plants raised through seeds, and this way is genetically variable, differing in growth and yield characteristics are major constraints in jojoba production. However, applying asexual propagation efficient methods i.e., air layering, grafting, cuttings, tissue culture are prospects to overcome the constraints in jojoba growth and productivity.

### FUTURE PERSPECTIVE

- Genetically uniform plants can be produced through various methods of asexual propagation including air-layering, grafting, stem cuttings and micropropagation with varying rates of success.
- Selection of high yielding genotypes and their multiplication through vegetative means.
- Sex determination of plants in early stages of growth via latest biotechnological tools.

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