

SEED PROPAGATION INCREASES GENETIC VARIATION AND MICROPROPAGATION TO MULTIPLY SELECTED SHRUB WITH DESIRABLE CHARACTERS

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Abstract

This work was done to explain the high genetic variation resulted from seed propagation of dioecious jojoba plant; and how a shrub with desirable characteristics can be selected and multiplied using in vitro techniques. Since the studied shrubs were obtained from seed propagation, they showed variations in seed characters, yield, shrub height, diameter of shrub canopy, stem contour, leaf area, nodal distance, number of flowers, flower density, and seed germination under NaCl stress. Some of these characters including leaf area, nodal distance, flower density, and seed weight and size were in correlation with yield. The shrub with high elite characters could be selected and efficiently multiplied using in vitro culture technique; it ensured availability of the plant materials throughout the year without negative effect on the vegetative growth of the mother plant. Effective protocol for in vitro multiplication was established on MS medium supplemented with 3 mg/l BAP, 0.01 mg/l NAA and 2 gm/l NaCl where one nodal segment resulted in 182 shoots in 6.5 months. Fifty percent of these shoots formed roots on MS medium supplemented with 2 mg/l IBA, 1.3 mg/l NAA and 1.25 mg/l BAP.

Keywords: Genetic variation, growth regulators, jojoba, micropropagation, morphology

INTRODUCTION

Jojoba (*Simmondsia chinensis* (Link) Schneider) is a new industrial crop that is attracting attention in many semiarid parts of the world for its ability to withstand water shortage. When irrigated by flooding, a plant would need watering 12-15 times per year but it can survive for more than 6 months without irrigation. In Egypt, one Feddan with 800 jojoba shrubs needs only about 1600 m³/year (EL MOGY 1999). This low need for water resulted from low transpiration rate and high water use efficiency. In addition, jojoba tolerates salinity up to 3,000 ppm NaCl (BENZIONI et al. 1999; MILLS et al. 1997) without any impact on the yield. Jojoba tolerate relatively high temperature since it originated in Sonoran Desert and thus may be suitable for cultivation in north of Africa and Arab countries. Jojoba can be planted commercially for its seed-oil as replacement for sperm whale oil, and as additive for lubricants, cosmetics, pharmaceuticals, inks manufacture, varnishes, waxes, detergents, resins and plastics (BOTTI et al. 1998; BAGBY 1988, TYAGI 2004). Jojoba has other characteristics of favorite crops such as: (1) resistance to major pathogens or insects, (2) withstanding many chemical sprays needed for control, (3) requirement of low labor as it is easy to grow, (4) being a long-lived perennial, evergreen, nonpoisonous plant, it requires low-maintenance, (5) its deep root system, make it suitable for plantings on highways and roadsides, landscape in gardens and parks, for soil conservation and as green belt around cities suffering from particulate air pollution.

Since jojoba is dioecious shrub, it is an obligatory cross-pollinated species (GENTRY 1958), therefore the obtained plants from seed planting are genetically variable in growth uniformity, physiological characteristics, yield and early bearing (VERBISCAR, BANIGAN 1978; MILLS et al. 1997, KUMAR et al. 2012). In spite of plant variability within and among natural populations, several traits such as wax composition, is extremely uniform in spite of broad botanical variability and differences in geographic origin (YERMANOS 1982). Before flowering, it is not possible to distinguish male from female shrubs, but has been reported that female shrubs on average have a large leaves and more open canopies than males. Generally, the male plants outnumber the female plants when raised from seeds (HARSH et al. 1987). This would seem to create a problem when the field is established through seed

sowing. These difficulties are circumvented by overplanting extra females using vegetative as well as in vitro propagations. On the other side, seed sowing increase the genetic variation within shrubs of the farm which can be used as a matter for selection of desirable individual.

Rooted cuttings are used widely to establish commercial fields but it depends on the size of the mother plant and requires considerable controlled greenhouse facilities (BASHIR et al. 2001; 2007). Root formation on shoot cuttings is a difficult process but it is still the most used asexual method (PALZKILL, FELDMAN 1993; ZHOU 2002). Micropropagation of elite individuals offers a promising means of commercial mass production of pathogen-free superior clones. In vitro- derived jojoba plants grow more vigorously than both seedlings and rooted cuttings, and are significantly larger after the first year of growth (REDDY, CHIKARA 2010; CHATURVEDI, SHARMA 1989).

The high variation which results from obligatory cross-pollination exerts some limitation on the application of seeds as effective material for commercial plantation, since only a less than 1% of the plant population originating from seeds of native plants has the potential to produce valuable yields (PURCELL 1988; RAMONET-RAZON 1988; BENZONI et al. 1992, 1999). Establishment a plantation with asexual propagules is more expensive than with seed but it saves time in replanting plants as well as crop produced of known sex and lineage. For selection of elite clones, seed propagation can be used to establish high variation between the obtained shrubs in the field and vegetative or micro-propagation techniques can be applied to multiply the selected clone with desirable characters. In the current old shrubs obtained from seeds in Ismailia Plantation were used to describe variation of plants obtained from seeds. In addition, rapid and efficient protocol was described to propagate a selected shrub of valuable characteristics using in vitro multiplication techniques.

MATERIALS AND METHODS

Jojoba (*Simmondsia chinensis* [link] Schneider) seeds were obtained from the Egyptian Natural Oil Company (S.A.E.) farm in Al-Salam District, of Ismailia, Egypt. The farm (88,200 sq.m) was grown from seeds obtained from the USA in Ismailia in 1991 and had used for research and production. Drip irrigation system was applied in the orchard and weed and pest control, and fertilization conducted following the standard agro-management practices. All the studied shrubs were subjected to the same condition including irrigation, farm practices and etc.

Morphological parameters. Measurements were done in two successive years (2012-2013), three times in March to determine flowering, June to make green fruits measurements and in September to determine the yield measurements. Since shrubs of the studied field were obtained from seed propagation, they showed high variation in their growth rates and growth habits. To get shrub samples, the farm were classified into nine sectors. The sample is the shrub in the center of each sector. Consequently, nine shrubs were selected for data collection, three shrubs beside each side and three in the middle of the farm. For each plant, height of the shrub, diameter of the vegetative growth and stem contour were measured. The seed yield of successive two years was estimated.

Three representative branches of each studied shrub were selected from the mid-level of the plants for detailed analysis. Number of nodes, the distance between nodes, number of leaves, number of flower, flower density and number of green fruits were estimated in 0.5 meter lateral branch. Ten leaves of each branch were used to estimate leaf area, fresh and dry weight, and leaf water content.

Seed characteristics were estimated using 100 seeds of each shrub, both seed weight and seed volume were determined. In addition, thirty green fruits were used to estimate fresh and dry weight of the green fruits as well as the water content.

Seed germination parameters. Jojoba seeds were washed with tap water for 10 min. and surface-sterilized in commercial bleach solution for 7 min, followed by 3 min treatment in 70% (v/v) ethanol. After three successive rinses in sterile distilled water for 10 min each, seeds were placed on autoclaved three cotton layers wetted by 65 ml of distilled water in glass jars. For seed germination, jars were incubated at 30°C under dark condition for 35 days. Germination percentage was determined upon the

emergence of seed radicle. On the other side, some seeds formed radicle without from plumule formation. Consequently, germination resulted in the formation of seedling with radicle and plumule was referred to as percentage of seedling. Germination/seedling ratio was estimated as the ratio of the number of seeds formed radicles and plumules to the number of germinated seeds with radicles only. Fresh and dry weight, water content and other parameters were estimated for plumules, cotyledons and radicles.

Germination under salinity stress. Thirty seeds for each of nine shrubs were disinfected and germinated on three successive cotton layers in glass jars wetted by 65 ml of 6000 ppm NaCl solution. The concentration of NaCl was determined according to the preliminary experiment, it resulted in significant reduction of germination of jojoba seed lot. Fresh and dry weight, water content and other parameters were estimated for each for plumules, cotyledons and radicles of germinating seedlings.

In vitro propagation of elite shrub. Shrubs were compared depending on seed size and yield and a shrub named G5 was selected for in vitro propagation. Shoot culture was established using nodal segments of 22 years old shrub G5. Node segments of G5 shrubs were washed with tap water for 30 min. Then, explants were surface sterilized in 0.2% mercuric chloride solution for 10 min, followed by 10 min in commercial bleach and 70% (v/v) ethanol for 2 min. After three successive rinses in sterile dist. water, the explants were prepared for sprouting and multiplication.

Explants were sprouted on MS medium (MURASHIGE, SKOOG 1962) supplemented with 3 mg/l BAP and 0.1 mg/l NAA. In vitro obtained shoots were sub-cultured on MS medium supplemented with 3mg/l BAP, 0.1 mg/l NAA and 2 g/l NaCl. The medium was solidified with 8 g/l agar at pH 5.8. Vitamins (mg/l) were: myoinositol (100), vitamin B1-hydrochloride (4), nicotinic acid (4), pyridoxal hydrochloride (0.7), biotin (0.04) and folic acid (0.5). Cultures were sub-cultured on the same medium under tissue culture conditions (16 h daily light at $100 \mu\text{mol m}^{-2}\text{s}^{-1}$) at $25 \pm 2^\circ\text{C}$. After establishment of shoot culture, shoot multiplication was fulfilled by subculturing shoot cutting on the same medium for each 65 days each. After three subcultured, the number of obtained shoots was estimated.

Induction of adventitious root formation. To obtain roots, shoot cuttings (4-5 mm radius $\times$ 5-10 mm long) were obtained from established shoot culture, and vertically cultured on MS medium containing 1.5% sucrose, and several growth regulators combination as included in Table 8. Six replicates of each treatment were incubated, each containing 5 segments at $25 \pm 2^\circ\text{C}$. After 8 weeks, the rooted plantlets were transferred to soil for adaptation.

RESULTS

The jojoba shrubs obtained from seed propagation showed high variation in their growth rates and growth habits. They have pale green leaves and stems, and they have been continuing growth depending on the availability of low water supply and relatively high temperature (reaches 45°C in shade in summer) in Ismailia region. After 22 years, jojoba shrubs reached relatively high height with big contour of their stems and produced valuable yields. Height at the age of 22 years ranged between 112 and 354 cm, the diameter of vegetative canopy ranged from 92 to 215 cm, and stem contour ranged between 56 and 120 cm (Table1). In addition, there was a clear relation between shrub high and seed yield (Table 1) where G5 and G6 were taller than other studied shrubs and they expressed higher yield. The value of plant stem contour tended to be in parallel relation with both shrub high and diameter of the shrub growth. Seed yield of shrubs ranged from 4.315 Kg to 1.49 Kg, The G5 shrub produced the highest yield.

High variation among shrubs was observed for almost all of the measured parameters (Table 2) particularly in the number of nodes, distance between nodes, number of leaves, number of flowers and number of fresh fruits. The variation in number of nodes was in parallel with the number of leaves in all studied shrubs. Number of nodes estimated for the 0.5 meter lateral branches ranged between 55 and 144, the number of leaves ranged from 82 to 282, and the number of fruits ranged between 9 and 40 (Table2). There was positive correlation between some of the measured parameters and yield, for example, G5 expressed high seed yield and that was proportional to nodal distance, number of flower

and flower density (Table 2). Shrub G3 had the lowest fruit number and yield. Most of the studied shrubs gave flower density ratio of 0.4. Three shrubs had flower density ratio more than 0.4, they were G4, G5 and G6; these shrubs also expressed high yield. On the other hand, G3 had the lowest flower density ratio and the lower seed yield compared to the other shrubs.

Variation in leaf parameters were detected between studied jojoba shrubs (Table 3). Leaf area ranged between 1.46 cm² and 2.07 cm². Leaf area strongly affected fresh and dry weight; consequently, great correlation was detected between these three parameters. In addition, shrubs expressing high leaf area (G1, G4, G5, G6 and G9) also expressed high yields. No relation could be detected between water content of the leaves and any other parameters given in Table 3.

Many parameters were estimated for seeds of the studied shrubs, variation in these parameters was detected (Table 4 and Fig.1). With respect to dry seeds, some shrubs have a low seed weight and therefore a low yield potential e.g. shrubs G2, G3 and G7. Shrub G3 expressed the lowest seed weight and seed yield (Tables 1 and 4). On the other hand, G5 showed the highest seed weight, seed volume and seed yield. Also positive correlation was detected between the yield and flower density ratio as well as both dry weight of the green fruits and weight of the dry seed.

No correlation was detected between germination efficiency, expressed as percentage of germination (Table 5), and any other seed parameters e.g. seed weight (Table 4) or shape (Fig.1). Germination/seedling ratio was estimated as the ratio of the number of seeds forming roots and plumules to the number of germinated seeds with radicles only. Germination/seedling ratio did not depend on the percentage of seed germination and differed from one shrub to another, it ranged between 0.4 to 0.8 (Table 5). Some parameters of seedlings including fresh weight, dry weight and water content were estimated for each shrub (Table 5) and they were showed different values. Shrub G5 expressed the highest values of seedling fresh and dry weight as well as seedling water content.

Seed germination differed greatly in response to stress imposed by 6000 ppm NaCl (Table 6). The shrub-G1 was the most tolerant followed by G7 and G2 compared to other shrubs, as the used concentration of NaCl improved their seed germinations. These three shrubs were characterized by high percentage of seeds that formed plumules and consequently expressed high value of germination/seedling ratio. The germination of other shrubs was negatively influenced by supplementing the germination medium with 6000 ppm NaCl. Seedlings of different shrubs expressed different values of growth parameters and water content.

To verify genetic variation between the studied shrubs, the RAPD technique was used (data not shown). Three (OPA7, OPA-11 and OPA-15) of 15 primer detected genetic variation between the studied shrubs. The percentage of polymorphic bands (number of polymorphic amplicons / total number of amplicons) was 100% when OPA-7 or OPA 15 was used.

Depending on the previous studied parameters, the shrub with desirable characteristics (G5) was selected and propagated using in vitro culture techniques. Aseptic nodal cutting of this shrub was obtained, disinfected and subjected for sprouting on medium containing 3 mg/l BAP and 0.01 mg/l NAA. The obtained shoots were sub-cultured on MS medium with the same growth regulators and 2 mg/l NaCl (Fig.2). While, medium containing BAP and NAA resulted in sprouting and multiplication of jojoba shoots valuable improvement (25%) was obtained when the MS medium containing both of them and 2 mg/l NaCl.

The efficiency of in vitro multiplication in jojoba could be deduced by studying the offspring of one node cutting after three successive subcultures. One nodal segment resulted in production of 180 shoots in 6.5 months (Table 7). The effect of two types of explants was also studied, nodal segment expressed higher multiplication value (Table 7) than that obtained by cotyledonary segment, Fig. 2 and 3, respectively.

Induction of root formation was established on MS medium supplemented with selected concentrations of growth regulators (Table 8). MS medium supplemented with 2.0 mg/l IBA, 1.25 mg/l BAP and 1.3 mg/l NAA produced extensive root system (Fig. 4) in relatively high frequency (50%). Medium containing high concentration of IBA and NAA (5.0 mg/l) with low concentration of BAP (1.25 mg/l)

resulted in reduction of root frequency but increase the number of roots per explant. When the concentration of BAP was increased in rooting medium, it resulted in low rooting data. The rooted plantlets were transferred to soil after adaptation for at least 6 weeks.

DISCUSSION

The pale green color of jojoba leaves and stems provide the plant the ability to reflect light radiation and consequently reduce the amount of intercepted light, and reduce evapotranspiration, lower leaf temperature and consequently improve water use efficiency (OSMAN, ABOHASSAN 1998). The wild nature of jojoba makes it one of the best crops which can be planted where temperature during summer days reaches 46 degree in shadow (El MOGY 1999). Consequently, jojoba shrubs are suitable plant to cultivate in the Ismailia region where high temperature and low water availability. The optimal conditions for jojoba seed germination are darkness, constant moisture, and temperatures of 26 °C to 30 °C (YERMANOS 1982). Also, it can grow and reproduce depending on the availability of low water content in the soil (AL-ANI et al. 1972; YERMANOS 1982).

The 22 years old shrubs showed great variation in growth parameters (Table 1) including shrub height, stem contour and diameter of tree canopy; they were proportional to the yield, where shrubs had higher growth parameters expressed higher seed yield. As was reported by LIBBY (1987) that most tree species have a high degree of fecundity and due to wild pollinated out crossing mating system, large amount of heterozygosity and considerable genetic variability were detected in this species (KOHORN 1994).

High variation was reported in lateral branches traits (Table 2) including number of nodes, distance between nodes, number of leaves, number of flowers and number of fresh fruits. While node density was assumed to be relevant to yield (BENZIONI 1995), in our work, there is no relation between these two parameters and seed yield. BOTTI et al. (1998) reported significant differences between jojoba clones in the majority of such parameters and corroborated large amount of variability to genes existing in jojoba. In addition, variation in leaves parameters were detected (Table 3).

In general, shrubs with high flower density expressed high yields and vice versa (Tables 1 & 2); shrub with the lowest flower density had the lowest seed yield. Since plant leaves are the manufacturer of the organic matters and flowers give rise to the seeds, these two organs control plant yield. Consequently, vegetative growth, flowering and fruiting parameters (Table 2), the extent of fruit set and seed weight (Table 4) directly affect yield potential. The majority of studied jojoba shrubs carried one flower bud on every second node, then the shrubs have flower density index of about 0.5 (Table 2) as was reported by GENTRY (1958), but some clones with a higher flower bud density have a flower index of about 0.6 or more (BENZIONI et al. 1999) but they were not indexed in Ismailia farm.

A positive relationship between seed weight and seed volume was strongly established (Table 4 & Fig. 1) as well as seed yield where shrub G5 was scored as the highest seed yield (Table 1). Also, positive correlation was detected between the yield and dry weight of the green fruits (Table 4). Similar findings were reported by BOTTI et al. (1998) who reported that the number of flower buds produced per node, the extent of fruit set, and seed weight, directly affect yield potential. Variation in seed morphology between the studied shrubs was clearly observed. GINWAL et al. (2005) also scored a significant variation in seed morphology (color, size and weight), seed germination parameters and seedling growth parameters (survival percentage, seedling height, collar diameter, number of leaves/plant, and seedling biomass).

Percentage of seedling did not depend on the percentage of seed germination and differed from one shrub to another (Table 5). High percentage of heritability coupled with moderate intensity of genetic gain, was observed for seed germination traits, which indicates that germination is under strong genetic control and can be exploited for improvement of plant species (GINWAL et al. 2005; GHOSH, SINGH 2011). While, the efficiency of seed germination is governed by seed size (GHOSH, SINGH 2011), in our work, there was no correlation between variation in germination efficiency, expressed as percentage of seed germination, and any other parameters of seed.

While germination of seeds of certain shrub was improved under salt stress up to 6 gm/l NaCl, others were negatively influenced (Table 6). Germination of jojoba seeds was negatively affected by relatively high concentration of NaCl (3-7 g/l) but low concentration (1 mg/l) stimulated germination (BERRICHI et al., 2010). BENZIONI et al. (1992) reported that jojoba responded to soil salinity by reducing its vegetative growth. In addition, the rate of the plumule emergence of jojoba seeds decreased by 50% when 3% NaCl was applied in germination medium and was completely inhibited at 5g/l (BERRICHI et al. 2010). The effect of salinity on length of seedlings resulted in considerable reduction in the number of leaves and internodes (BERRICHI et al. 2010).

The RAPD technique is a simple identifier of polymorphism in several plants, i.e. jojoba, where the polymorphism is very high (GABER et al. 2006). Three (OPA7, OPA-11 and OPA-15) of 15 primer were used to detect genetic variation between the studied shrubs (GABOOR 2014). Application of one primer could be used to detect not only variation between studied female shrubs but also to detect sex in jojoba (AGRAWAL et al. 2007). It is concluded that jojoba plants in the natural habitat of Egypt may belong to different genotypes (GABER et al. 2006). Both RAPD and ISSR analysis showed high level of genetic diversity (96 and 91.4%, respectively) among the 39 jojoba genotypes studied (SHARMA et al. 2009). Also, our results are in agreement with other workers detected high variation among jojoba individuals grown from seeds (AMARGER, MERCIER 1996).

The studied shrubs were obtained from seed propagation, consequently, variations in seed characters including yield, shrub height, diameter of shrub canopy, stem contour, leaf area, nodal distance, number of flowers, flower density, and seed germination under NaCl stress were reported. Some of these characters (leaf area, nodal distance, flower density, and seed weight and size) were in strong correlation with yield. In this work, shrub G5 was selected for multiplication. In vitro plant regeneration from nodal explants of mature jojoba shrubs was described but the multiplication rate was low (MILLS et al. 1997). As was described in our protocol, successful and reliable regeneration protocol was described to offer opportunities for mass multiplication and the improvement of jojoba (Fig. 2). In this protocol, nodal segments from selected shrub were cut, disinfected and used to establish shoot culture, the in vitro obtained plant materials were subcultured for fast multiplication. Our results are generally congruent with the report by Kumar et al. (2012). As was in our work, the efficiency shoot multiplication increased significantly when in vitro-raised shoots were used as the source of explants (AGRAWAL et al. 2002). Our work indicated that nodal explants were not better than cotyledonary node cuttings but nodal explants were recommended as a guarantee of true-to-type (Table 7).

In our work, BAP (3 mg/l BAP) in combination with low concentration of NAA (0.01 mg/l NAA) was used to stimulate sprouting and multiplication from nodal segment obtained from 22 year old shrub (Fig.2) or cotyledonary node of disinfected seed (Fig. 3). Combination between BAP and NAA or IAA was found to be very effective for the establishment and multiplication of jojoba cultures (KUMAR et al. 2009; AGRAWAL et al. 2002; BASHIR et al. 2007). In general, the superiority of application of BAP for shoot induction and multiplication maybe due to the ability of plant tissues to metabolize BAP more readily than other synthetic growth regulators, or the ability of BAP to induce natural hormones synthesis such as zeatin (SINGH et al. 2008). BASHIR et al. (2007, 2008) showed that BAP alone was better than kinetin alone or BAP and kinetin for in vitro shoot initiation in jojoba.

While sprouting and shoot multiplication could be obtained using 3 mg/l BAP and 0.01 mg/l NAA, addition of 2 mg/l NaCl improved jojoba multiplication. The ability of jojoba to resist stress condition was reported previously (MILLS, BENZIONI 1992; BOTTI et al. 1998; MILLS et al. 2001). Jojoba has been characterized as an include where NaCl was accumulated in high levels within the plant cells, probably in vacuole solutions of mature leaves, resulting in an effective osmotic adjustment up to a non-toxic salinity level (MILLS, BENZIONI 1992; BENZIONI et al. 1992; MILLS et al. 2001; HASSANEIN et al. 2014). In addition, jojoba plants grown under salt stress did not show great difference from those grown under non-saline conditions for most of the morphological and anatomical parameters (BOTTI et al.1998). Therefore, medium with growth regulators and low concentration of NaCl was used to verify superiority of in vitro multiplication as propagation procedure in jojoba. Using one nodal segment, great number of shoots (180) could be obtained in 6.5

months; fifty percent of them formed roots (Table 7). Then, 90 rooted shoots (Fig. 4) could be obtained within 8.5 months. In traditional vegetative propagation, three or four nodal cuttings were used to obtain one rooted plant and it depends on the size of the mother plant (BASHIR et al. 2001). Consequently, micropropagation is strongly recommended because it can be used for mass production of an elite individual at any time of the year and without effect on the vegetative growth of the mother shrub.

In our work, two auxins in combination with BAP were used to get good combination for induction of adventitious root formation in jojoba (Table 8 & Fig. 4). In this concern, several experiments were done using auxin alone or in combination with other hormones and significant differences were found (CHATURVED, SHARMA 1989; TYAGI, PRAKASH 2004; BASHIR et al. 2008; SINGH et al. 2008). In jojoba, rooting percentage ranged between 20 and 95% depending on the clone and the used chemical and physical conditions (MILLS et al. 1997). Relatively low salt concentrations in half-strength medium are known to enhance rooting of micro-shoots (LLORENTE, APOSTOLO 1998; KUMAR et al. 2009).

CONCLUSION

Since jojoba is dioecious plant species, great variation between 22 years old shrubs of the studied orchard was reported. They varied in several growth and seed characters. Positive correlation was detected between the yield and several studied parameters such as leaf area, nodal distance, number of flowers, flower density, and seed weight and size. The results confirmed that the previous reported parameters could be used to select a elite shrub. The selected shrub was efficiently multiplied using in vitro culture technique; it ensured availability of the plant materials throughout the year without negative effect on the vegetative growth of the mother plant. Consequently, effective protocol for in vitro multiplication was established on MS medium supplemented with 3 mg/l BAP, 0.01 mg/l NAA and 2 gm/l NaCl.

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Table 1. Some measured traits (tree height, stem diameter, diameter of vegetative growth canopy and seed yield/tree/year) of 22 years old - nine jojoba shrubs (G1- G9).

Shrub Code	Shrub height (Cm)	Stem contour (Cm)	Diameter of shrub canopy (Cm)	Seed yield (Kg)
G1	230	95	140	3.80
G2	262	88	121	2.62
G3	112	56	92	1.49
G4	224	72	111	3.23
G5	354	120	170	4.30
G6	246	86	130	4.22
G7	239	96	108	2.25
G8	174	76	89	3.05
G9	305	118	215	3.91

Table 2. Some measured parameters (number of nodes, distance between internodes, number of leaves, number of flowers and number of fruits) of 0.5 meter of lateral branch of 22 old - nine jojoba shrubs (G1- G9).

Shrub Code	Number of nodes	Node length (cm)	Number of leaves	Number of flowers	Flower density	Number of fruits
G1	60±3	3.1±0.3	120±5	23±8.9	0.38±0.1	16±7.0
G2	66±16	3.2±0.1	131±32	25±2.0	0.37±0.2	40±4.5
G3	83±2.50	3.46±0.3	166±5	31±7.5	0.37±0.1	9±1.5
G4	55±12.0	4.1±0.2	110±24	32±3.0	0.58±0.2	18±3.5
G5	73±2.5	4.46±0.33	147±5	37±1.0	0.50±0.01	12±2.5
G6	58±4.0	4.4±0.26	117±8	31±9.8	0.53±0.2	17±4.1
G7	144±8.0	2.5±0.2	282±32	35±3.0	0.24±0.2	25±6.1
G8	96±7.0	4.39±0.12	191±14	37±9.3	0.38±0.07	15±6.5
G9	82±13.0	3.86±0.1	82±13	33±4.1	0.4±0.05	20±4.7

Table 3. Some measured parameters (leaf area, fresh weight/leaf (FW), dry weight/leaf (DW) and leaves water content) of leaves of 22 old - nine jojoba shrubs (G1- G9).

Shrubs	leaf area (cm ²)	Leaf FW (gm.)	Leaf DW (gm.)	Water constant (%)
G1	2.07±0.11	0.37±0.03	0.16±0.03	56
G2	1.46±0.24	0.21±0.01	0.11±0.01	47
G3	1.55±0.20	0.25±0.02	0.14±0.01	44
G4	1.81±0.35	0.35±0.03	0.16±0.02	54
G5	1.85±0.3	0.39±0.03	0.22±0.02	43
G6	2.03±0.1	0.27±0.02	0.16±0.01	40
G7	1.3±0.12	0.5±0.03	0.3±0.03	42
G8	1.6±0.14	0.5±0.02	0.3±0.01	36
G9	1.5±0.3	0.5±0.05	0.3±0.02	40

Table 4. Some measured parameters (weight/100 seeds, volume/100 seeds, fresh weight (FW) / green seed, dry weight (DW)/green seed and percentage of water content) of seeds of 22 old - nine jojoba shrubs (G1- G9).

Shrubs	Weight/ 100 seed (gm)	Volume/ 100 seed (cm ³)	Green Fruit FW (gm)	Green fruit DW (gm)	Water cotenant %
G1	120±2.9	131±2.1	1.6±0.06	0.6±0.03	61.9
G2	71±1.2	76±1.3	2.1±0.01	0.9±0.01	59.2
G3	69±1.3	71±1.1	2.5±0.06	1.0±0.02	60.9
G4	113±1.2	148±1.1	3.5±0.03	1.1±0.07	69.2
G5	158±3.6	165±1.9	4.3±0.03	1.6±0.04	61.9
G6	99±1.0	106±1.4	4.0±0.09	1.4±0.03	65.3
G7	61± 2.6	60±4.3	2.7±0.04	1.1±0.05	59.1
G8	75±1.2	75±2.4	2.4±0.03	1.1±0.02	55.1
G9	77±1.7	78±2.0	3.9±0.09	1.6±0.04	59.3

Table 5. Some measured parameters [percentage of seed germination (PSG), percentage of seedling formation (PSF), germination/seedling ratio (GSR), germination period day (GPD), fresh weight (FW- and dry weight (DW)/seedling and percentage of water content] of seed of nine jojoba shrubs; seeds were germinated in glass jars containing cotton layers wetted by dist. water for five week under room condition. Values are mean $\pm$ SD.

Studied shrubs	PSG	PSF	GSR	GPD	FW/ seedling gm	DW/ seedling gm	Water content (%)
G1	49 $\pm$ 12.5	25 $\pm$ 12.5	0.5	14 $\pm$ 1	2.74 $\pm$ 1.08	0.67 $\pm$ 0.11	76 $\pm$ 6.7
G2	66 $\pm$ 7.5	41 $\pm$ 19	0.6	15 $\pm$ 2	2.98 $\pm$ 0.30	0.69 $\pm$ 0.06	73 $\pm$ 2.4
G3	79 $\pm$ 7.5	66 $\pm$ 5.8	0.8	13.1	2.70 $\pm$ 0.36	0.60 $\pm$ 0.02	73 $\pm$ 3.4
G4	58 $\pm$ 6.9	33 $\pm$ 19	0.6	14 $\pm$ 2	3.1 $\pm$ 0.88	0.97 $\pm$ 0.07	70 $\pm$ 6.5
G5	66 $\pm$ 14	41 $\pm$ 14	0.6	14 $\pm$ 1	5.98 $\pm$ 0.84	1.36 $\pm$ 0.16	80 $\pm$ 2.2
G6	83 $\pm$ 6.9	45 $\pm$ 19	0.5	15 $\pm$ 2	3.08 $\pm$ 0.64	0.79 $\pm$ 0.12	67 $\pm$ 3.8
G7	87 $\pm$ 12.5	58 $\pm$ 7.2	0.7	16 $\pm$ 1	2.02 $\pm$ 0.50	0.48 $\pm$ 0.03	76 $\pm$ 5
G8	45 $\pm$ 7.5	20 $\pm$ 7	0.4	14 $\pm$ 1	2.46 $\pm$ 0.88	0.67 $\pm$ 0.12	76 $\pm$ 6.7
G9	83 $\pm$ 14	41 $\pm$ 19	0.5	13 $\pm$ 1	3.19 $\pm$ 0.62	0.66 $\pm$ 0.08	75 $\pm$ 3.1

Table 6. Some measured parameters [percentage of seed germination (PSG), percentage of seedling formation (PSF), germination/seedling ratio (GSR), germination period day (GPD), fresh weight (FW- and dry weight (DW)/seedling and percentage of water content] of seed of nine jojoba shrubs; seeds were germinated under the influence of 6000 ppm NaCl for 5 weeks. Values are mean $\pm$ SD.

Shrubs	PSG	PSF	GSR	GPD	FW/ Seedling gm	DW/ seedling gm	Water content (%)
G1	87 $\pm$ 16	66 $\pm$ 13	0.75	10 $\pm$ 0.8	2.85 $\pm$ 0.29	0.78 $\pm$ 0.08	69.57 $\pm$ 6.82
G2	83 $\pm$ 14	58 $\pm$ 9.2	0.69	12 $\pm$ 1.8	2.62 $\pm$ 0.18	0.66 $\pm$ 0.12	74.71 $\pm$ 4.62
G3	62 $\pm$ 8.0	41 $\pm$ 10	0.66	13 $\pm$ 0.8	2.78 $\pm$ 0.77	0.50 $\pm$ 0.34	79.43 $\pm$ 3.23
G4	20 $\pm$ 8.0	8 $\pm$ 9.3	0.4	14 $\pm$ 3	3.62 $\pm$ 0.27	0.96 $\pm$ 0.07	80.87 $\pm$ 1.59
G5	58 $\pm$ 9	29 $\pm$ 16	0.5	11 $\pm$ 1.8	3.87 $\pm$ 1.79	1.18 $\pm$ 0.28	73.94 $\pm$ 4.35
G6	50 $\pm$ 0.0	12 $\pm$ 8.0	0.24	11 $\pm$ 0.8	2.09 $\pm$ 0.20	1.06 $\pm$ 0.08	61.77 $\pm$ 4.97
G7	83 $\pm$ 13.8	54 $\pm$ 0.93	0.65	10.8 $\pm$ 1.7	2.09 $\pm$ 0.41	0.47 $\pm$ 0.14	62.94 $\pm$ 24.97
G8	58 $\pm$ 9	33 $\pm$ 13.8	0.56	10 $\pm$ 0.8	2.16 $\pm$ 0.34	0.60 $\pm$ 0.08	75.84 $\pm$ 7.93
G9	62 $\pm$ 8.0	37 $\pm$ 16	0.59	12 $\pm$ 1.8	2.45 $\pm$ 0.19	0.81 $\pm$ 0.18	72.23 $\pm$ 7.35

Table 7. Comparison between the shoot multiplication of two explants after three successive subcultures, 65 days each. Values are mean $\pm$ SD.

Subculture	Type of nodal explant			
	Cotyledon		Nodal	
	Shoot number	Frequency (%)	Shoot number	Frequency (%)
First subculture	17 $\pm$ 3	80	6 $\pm$ 2	34
Second subculture	85 $\pm$ 7	70	33 $\pm$ 5	89
Third subculture three	410 $\pm$ 22	80	182 $\pm$ 11	90

Table 8. Effect of different concentration of IBA, BAB and NAA on rooting of the excised shoots jojoba cultured on MS solid medium. Values are mean $\pm$ SD.

Concentration hormones/mg/l			Shoot length(cm)	Leaf number /shoot	node number /shoot	Shoots number /explant	Length of root (cm)	Root number /explant	Root frequency %
IBA	BAB	NAA							
2.0	1.25	1.3	3.0 $\pm$ 0.2	6.0 $\pm$ 1.0	3.7 $\pm$ 0.6	1.3 $\pm$ 0.6	4.2 $\pm$ 0.4	2.3 $\pm$ 0.6	50.0
5.0	1.25	5.0	4.0 $\pm$ 0.5	8.0 $\pm$ 2.0	4.0 $\pm$ 1.0	2.3 $\pm$ 0.6	2.8 $\pm$ 0.4	8.7 $\pm$ 0.6	23.3
2.0	4.0	1.3	3.5 $\pm$ 0.3	7.0 $\pm$ 1.0	3.7 $\pm$ 0.6	3.3 $\pm$ 0.6	2.4 $\pm$ 0.7	1.0 $\pm$ 0.0	13.4
1.4	1.25	1.3	2.3 $\pm$ 0.2	6.0 $\pm$ 1.0	3.0 $\pm$ 0.0	1.3 $\pm$ 0.6	1.9 $\pm$ 0.5	4.3 $\pm$ 1.5	20.0

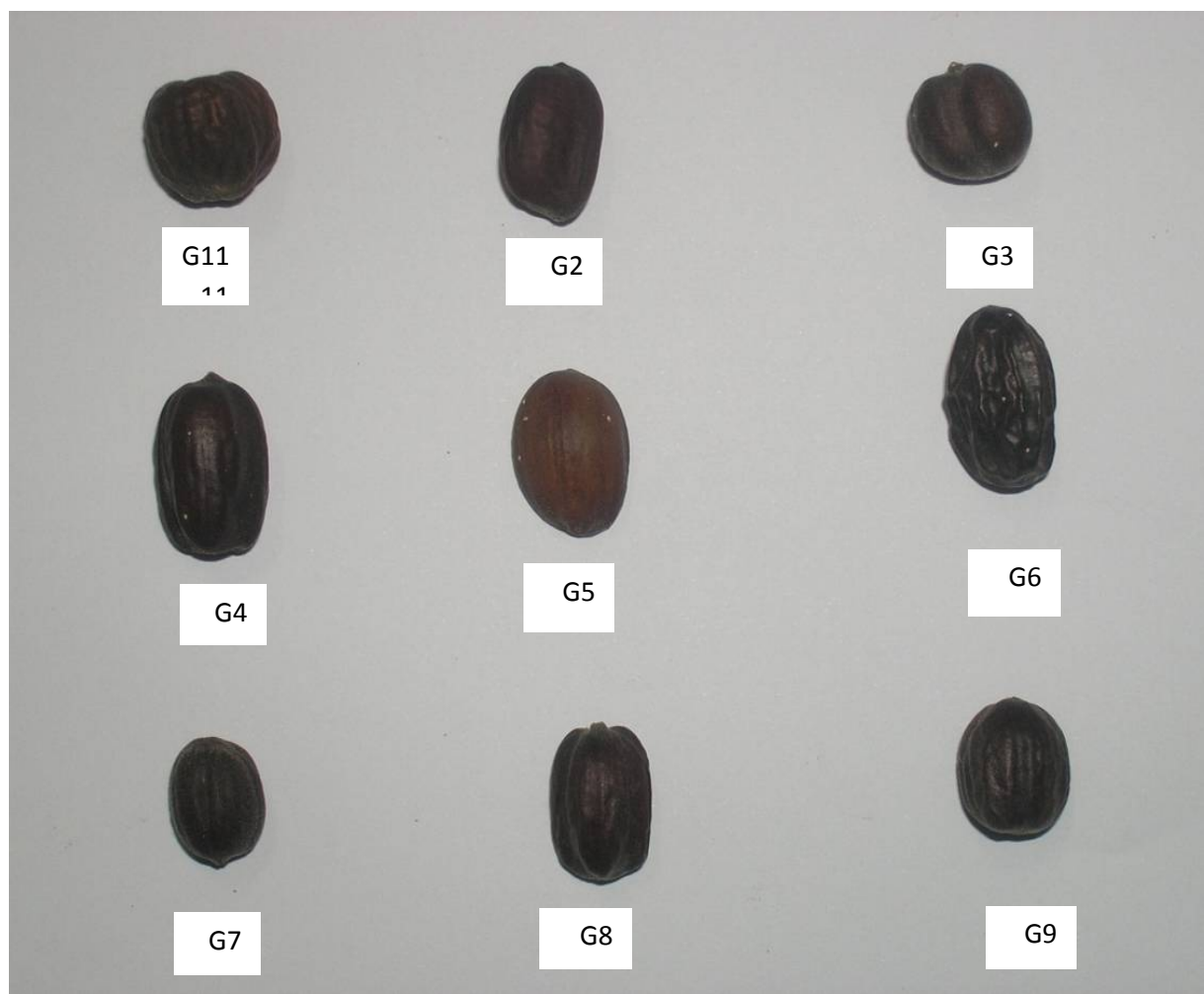


Fig. 1. Photo showed variation in seed morphology of nine jojoba shrubs (G1- G9) obtained from seed sowing since 22 years ago.

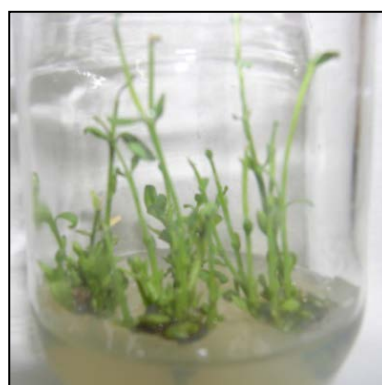


Fig. 2. Photograph shows shoot multiplication on shoot cuttings obtained from in vitro grown plant materials and cultured on MS medium supplemented with 3 mg/l BAP, 0.1 mg/l NAA and 2 gm/l NaCl.



Fig. 3. Photograph shows multiplication on cotyledonary node segment cultured on MS medium with 3mg/l BAP, 0.01 mg/l NAA and 2 mg/l NaCl and transferred for further multiplication on the same medium.



Fig. 4. Photograph shows shoot cuttings with formed roots; the cuttings were cultured on MS medium supplemented with 2 mg/l IBA, 1.25 mg/l BAP and 1.3 mg/l NAA).