ISSN (Online) = 2522-6754 ISSN (Print) = 2522-6746
WORLD JOURNAL OF BIOLOGY AND BIOTECHNOLOGY
Research Manuscript www.sciplatform.com

Asexual propagation of most promising genotypes of jojoba *Simmondsia chinensis*

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Authors' Contribution	**Hafeez, Z.** developed the concepts of the study and conducted the experiments, **S. A. H. Shah, M. I. Shahid & M. A. Bashir** helped in design and performing the experiments and analysis of results
Article History	*Corresponding email address: farheenj.uok@gmail.com **Received: 15 April 2020, Revised: 26 June 2020, Accepted: 03 July 2020, Published Online: 06 July 2020**

ABSTRACT

Propagation of jojoba (*Simmondsia chinensis*) by direct seeding has genetic heterogeneity and about half of the seedlings may be males. Plantation with asexual propagules, though expensive, but it saves time to replant plants of known sex with high production. Vegetative propagation through layering, grafting, cuttings, tissue culture are even successful in jojoba. The current study was initiated to find out appropriate time of grafting and most responsive jojoba genotype for asexual propagation. The female plants of five promising jojoba genotypes i.e. BRJ-5, BRJ-10, BRJ-22, BRJ-24 and BRJ-46 propagated through grafting, in the months of September and March during the year 2015-16 and 2016-17. The pooled analysis of the data revealed that the grafting practiced in the month of September performed better than in the month of March. The September grafting resulted in longer sprouts (18.49 cm) with more grafting success (74.43%) than that practiced in March. However, sprouts from March grafting took fewer days to sprout (52.56) than that of September grafting (57.35 days). As far as the genotypes was concerned, BRJ-22 surpassed the others in taking minimum days (41.45) to sprout; attaining maximum sprout length (21.49 cm) and giving maximum success percentage (82.35). The study indicated that the optimum time of grafting for jojoba is the month of September and BRJ- 22 is the best suited strain for grafting.

Keywords: Grafting; jojoba; asexual propagation; *Simmondsia chinensis*.

INTRODUCTION: Jojoba *Simmondsia chinensis*is also pronounced as ho-ho-ba is a dioecious, long-lived perennial evergreen shrub that grows wild in the semi-arid region of the Sonora desert in Northern Mexico and Southwestern USA. Its natural lifespan appears to be between 100 and 200 years. The average seed weight varies between 0.2 and 2.2 g and the seeds contain 50% oil and 15-20% proteins (Ayanoğlu, 2000). Its importance due to its saturated seed oil waxes is well recognized for its utilization in cosmetics, lubricants and Pharmaceuticals etc.

Plantations are established by using seeds, seedlings, rooted cuttings, or plantlets produced from tissue culture. Being dioecious, the male plants outnumber the females when raised from seeds. Several asexual methods of propagation have been used to propagate jojoba; these include air-layering (Bashir *et al.*, 2005), grafting (Khattab *et al.*, 2013), cuttings (Bashir *et al.*, 2009) and tissue culture (Bashir *et al.*, 2008). In some cases, one of these might be the method of choice. Each of these a sexual method shares the major advantage over seed propagation in that these allow propagation of unique and desirable genotypes. An additional advantage of asexually propagated plants over seedlings is that they have shorter juvenile period than those grown from the seed. Thomson (1982) described that splice or whip grafting in jojoba during early spring from mid-February to mid-April, using 0.5 to 1.25cm scion of mature wood with grayish brown bark from 1 or 2 years old plants produced the best results but poor results when immature wood was used. Assaf (1990) successfully transformed 20% jojoba males into females by grafting in afield on three-years-old 200 plants. However, only 5% transformation was achieved when grafting was on one and half year old plants in the nursery. Most of the grafted males produced nuts in two years. Jojoba males or low yielding females can be converted into productive females by grafting (Thomson, 1982; Assaf, 1990). Veneer grafting is successful in jojoba (Shah and Bashir, 2000) and economical to multiply true to type elite selections from heterogeneous seedling populations. Jojoba Research Station, Bahawalpur, Pakistan has a number of selected genotypes on the basis of plant shape, flowering, fruiting, yield, seed size and oil contents etc. However, the potential of these genotypes for vegetative propagation through *in vivo* and *in vitro* techniques, growth and survival of their clones has not been determined. The clones released as cultivars, if properly evaluated by clonal propagation techniques, could cover the marginal area of Pakistan by this precious plant species.

OBJECTIVES: The present study was envisaged to find out the optimum time of grafting for five promising genotypes of jojoba. The outcomes of this study became helpful in multiplication of future varieties of jojoba for planting in arid and semi-arid areas of the country.

MATERIALS AND METHODS: The study was conducted at Jojoba Research Station, Bahawalpur, Pakistan during 2015 to 2017. The ten years old female plants of promising jojoba genotypes i.e. BRJ-5, BRJ-10, BRJ-22, BRJ-24 and BRJ-46 were propagated by grafting technique. Five years old seedlings preferably male or non-productive female were used as rootstock and they were pruned as much leaving only four branches in each seedling. These four branches of each seedling were grafted with 4 scions from mother plants of the selected genotypes. The scions were taken from 2 years old branches with a size of 20 - 25 cm in length, 0.25 - 0.50 cm in diameter and at least with 4 - 6 pairs of leaf buds. The scion and rootstock were prepared for grafting as described earlier (Shah and

Bashir, 2000). The top of rootstock was kept intact until union was established, after which it was removed. All sprouts arising from rootstock below the scion-stock combinations were removed during the experimental period. On attaining the reasonable size of the sprouts from scions, the branch of the rootstock above the scion-stock combination was cut back leaving 5 cm portion intact with the scion. A total of 320 branches of 80 seedlings were grafted during 2 years experimental period. Each year 160 branches of 40 seedlings, 80 branches of 20 seedlings each in March 2015 and September 2015 were grafted. Similarly 80 branches of 20seedlings in March 2016 and same number in September2016 were grafted. Out of these, 16 branches of 4seedlings were allocated to each strain keeping 4 grafts under one replication. During the experimental period the data regarding the following characteristics was recorded.

Number of days to sprout: The grafted plants were carefully observed during the experimental period. The days were counted from the start of treatment to the appearance of sprouts from the scion within polythene sheet covering the scion-stock combination. The days were averaged over the total sprouts in each replication of each genotype.

Sprout length: Six months after grafting and before the application of next treatment, the length attained by the sprouts of each scion was measured and averaged over the total sprouts in each replication of each genotype.

Success percentage: Six months after grafting the success percentage was estimated as:

$$\text{Success (\%)} = \frac{\text{Number of sprouted scions}}{\text{Number of grafted branches}} \times 100$$

The layout of the experiment was RCBD with 2 factors and 3 replications. The first factor was time of grafting i.e. March and September. The second factor was jojoba genotypes i.e. BRJ-5, BRJ-10, BRJ-22, BRJ-24 and BRJ-46. The data of both years was combined and analyzed for pooled analysis by using analysis of variance. The means obtained from the analysis were compared by Duncan's Multiple Range test at α = 5 % (Steel and Torrie, 1986).

RESULTS AND DISCUSSION:

Number of days to sprout: Pooled analysis data revealed that the effect of both the time of grafting and the strains on the parameter under study was statistically significant (table 1).

Genotypes	Time of grafting		
	September	March	Average
BRJ-5	62.5	53.0	57.75 b
BRJ-10	68.75	72.11	70.43 a
BRJ-22	42.75	40.15	41.45 c
BRJ-24	61.89	55.23	58.56 b
BRJ-46	50.89	42.33	46.6 c
Average	57.35 a	52.56 b	
S.E. (Genotypes)= 2.93			

Table 1: Number of days to sprout affected by time of grafting and jojoba genotypes. S.E. (Time) = 1.79 & S.E (Interaction)= 4.20.

Scions grafted in March sprouted one week earlier than those grafted in September. The earliness of sprouting from March grafting could be attributed to the active growth activity of the plants during spring season due to favorable environmental conditions. The scion uses the reserved carbohydrates and metabolites for new growth under optimum temperature and humidity. Delay in sprouting from scions of September grafting could be related to the low nutritional status of the mother plant as it used maximum of reserved carbohydrates and nutrients during fruiting stage that prevails from March to end of July depending upon the weather conditions. Genotype BRJ-22 took the minimum time (41.45 days) to sprout and stood statistically at par with BRJ-46 (46.6 days). BRJ-10 took the maximum time (70.43 days) to sprout. Thomson (1982) also found that certain scions from some bushes of jojoba take the graft more readily than others from grafting by splice or whip technique during early spring from mid-February to mid-April. The interaction between two factors remainednon-significant. It was because of the decreasing trend in number of days to sprouting of five genotypes (except BRJ-22that showed a little increasing trend) for March grafting and vice versa for September grafting (Shah and Bashir, 2000; Khattab *et al.*, 2013)

Sprout length: The time of grafting has significant effect on the sprout length (table 2).

Genotypes	Time of grafting		
	September	March	Average
BRJ-5	17.61	15.27	16.44 b
BRJ-10	14.57	12.27	13.42 c
BRJ-22	23.34	19.64	21.49 a
BRJ-24	16.12	15.60	15.86 b
BRJ-46	12.02	19.27	15.64 b
Average	18.49 a	13.70 b	
S.E. (Genotypes) = 0.8			

Table 2: Number of days to sprout affected by time of grafting and jojoba strains. S.E. (Time) = 0.45 & S.E. (Interaction)=1.11 and means sharing similar letters in a group are non-significant at α = 5% (DMR test).

September grafting gave longer sprout (18.49 cm) than that of March grafting (13.70 cm). The slower growth of the sprouts from March grafting could be due to the stress caused by the gradual increase in temperature and dry climatic conditions that started after March grafting as described above. The sprout length was also significantly affected by the genotypes. The graft from BRJ-5, BRJ-24 and BRJ-46 exhibited similar length of sprouts. The graft from the strain BRJ-10 attained the shortest sprout length (13.42 cm). There was no interaction between two factors (time of grafting x genotypes) for this parameter. This seemed due to decreasing trend in sprout length of five genotypes for March grafting and opposite trend to it for September grafting (Shah and Bashir, 2000; Khattab *et al.*, 2013).

Success percentage: The pooled analysis of the data showed that the success percentage was affected significantly both with the time of grafting and the genotype (table 3). The higher success (72.24%) was found in September than in March grafting (44.43%). A higher success in September grafting could be attributed to the optimum temperature and humidity that boosted the graft success. However, the lower success in March grafting was due to the follow of stress caused by the increase in temperature and decrease in humidity, which is in line with the findings of Shah and Bashir (2000). Regarding the genotypes, BRJ-22 led with 76.86% success followed by BRJ-5 (61.52%). Both the genotypes behaved statistically alike: BRJ-

10 revealed minimum success (44.93%). Earlier, Thomson (1982) obtained variable results with some bushes of jojoba that refused to take a graft and other taking 100%. Assaf (1990) successfully transformed 20% jojoba males into females by grafting technique on three-years-old plants.

Genotypes	Time of grafting		
	September	March	Average
BRJ-5	80.27	42.77	61.52 b
BRJ-10	55.27	34.43	44.93 c
BRJ-22	90.12	63.60	76.86 a
BRJ-24	71.93	46.93	59.43 b
BRJ-46	63.60	34.43	49.01 c
Average	72.24	44.50	
S.E. (Genotypes) = 3.41			

Table 3: Success percentage affected by time of grafting and jojoba genotypes. S.E. (Time) = 1.96 & S.E. (Interaction) = 4.82 and means sharing similar letters in a group are non-significant at $\alpha = 5\%$ (DMR test).

The interaction between times of grafting and the genotype was statistically non-significant. It happened so because all five genotypes expressed reduction in success for March grafting. It seemed that gradual decrease in temperature from 36ºC (September) to 32ºC (January) with high humidity range 76 to 86% favored the growth and success of grafts after September grafting. On the other hand gradual increase in temperature from 31ºC (March) to 42ºC (June) with low humidity range 60 to 78% disfavored the growth and success of grafts after March grafting as both high temperature and low humidity caused stress (Khattab *et al.*, 2013).

The study revealed that the time of grafting and prevailing climatic factors like temperature and humidity after grafting were important for success and growth of grafts. Plant food reserves and the stress conditions also play a role in success or failure of grafting. The clones significantly differed in parameters of study in response to grafting. BRJ-22 strain was the most successful followed byBRJ-5 compared to other genotypes for grafting. Grafting in September was better than that in March. None of the genotypes was specific to the time of grafting as all genotypes behaved alike for both time of grafting.

CONCLUSION: It is concluded from the studies that grafting practiced in the month of September performed better in the characteristics under studied than the grafting in the month of March and month of September is highly recommended for grafting of jojoba for better results. It is also revealed that BRJ-22 is well suited genotype for grafting.

CONFLICT OF INTEREST: Authors have no conflict of interest

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