

## **Micrografting technique for cloning Hashab [*Acacia senegal* (L.) Willd]: Effect of rootstock age, scion length, scion source, sucrose and light duration**

**Ghada S. Altayeb and Mohamed A. Ali**

Agricultural Research Corporation, Tissue Culture Unit, Wad Medani, Sudan.

### **ABSTRACT**

Hashab [*Acacia senegal* (L.) Willd] is considered as an important tree species in Sudan which produces gum Arabic. It is mainly propagated by seeds, which result in variation in yield due to segregation. This study was established to develop an *in vitro* micrografting technique for cloning Hashab trees. Five experiments were conducted during 2012 to investigate the effects of age of rootstock seedling, scion length, scion source, sucrose, and light duration on the success of micrografting of Hashab in a completely randomized design with ten replicates. Two and four weeks-old seedlings were tested as rootstocks. They were grafted with scions obtained from the greenhouse. The two weeks-old seedlings had significantly higher percentage of successful grafts, and scion morphogenesis. Different scion lengths (1-1.5 cm and 2.5-3cm) were tested. Scions 1-1.5cm long had significantly higher rates of successful micrografts. Scions obtained from different sources (greenhouse, *in vitro* plantlet, and mature tree) were evaluated and those obtained from the greenhouse gave significantly higher percentages of successful grafts, number of leaves, number of nodes, and an increase in shoot length. Different concentrations of sucrose (20, 30, 40, 50, and 60 g/l) were tested and grafted plants at 40g/l sucrose gave significantly higher percentages of successful grafts. Incubation of micrografted seedlings under continuous light for two weeks gave significantly higher percentages of successful grafts.

## INTRODUCTION

Hashab [*Acacia senegal* (L.) Willd] is a tropical legume tree belonging to the family leguminosae. It is an important multipurpose tree species.

It produces gum Arabic, stabilizes sand dunes, fixes nitrogen, and is a source of fence, firewood and fodder (Katende *et al.*, 2000).

*Acacia senegal* (L.) regenerates naturally by seeds, which results in genetic variation due to cross-pollination. Vegetative propagation of most *Acacia* species by conventional means such as stem cutting was difficult and the percentage of rooting was low (Bonga, 1982). Ali (1997) reported low frequencies of rooting for old and young nodal cuttings of *A. senegal*. Vegetative propagation of *A. senegal* by grafting was reported by Palma *et al.* (1996). They grafted micro-cuttings on 3 month old stocks by combining *in vitro* and *in vivo* methods.

Micrografting of forest trees has been developed by grafting of a miniaturized scion onto *in vitro* grown rootstocks in aseptic conditions (Burger, 1985; Jonard, 1986). Micrografting of tiny shoot tips can be used as efficient means of regenerating plant material free of endogenous contaminants (Hartmann *et al.*, 1990), with enhanced potential for producing true to type clones from mature plant (Franclet, 1983). Several factors affect the success of micrografts. These include the size of the scion, as well as the cultural conditions under which the scion was grown. Navarro *et al.* (1975) found that larger scions of shoot-tip of citrus and etiolated rootstock seedlings improved the rate of successful grafting.

The objective of this study was to develop micrografting technique for Hashab. The effects of rootstock age, scion length, scion source, sucrose concentrations, and light duration on the success of grafting of Hashab were tested.

## MATERIALS AND METHODS

Seeds of Hashab were obtained from the National Seed Center of the Forestry Research Center, Soba, Khartoum. The seeds were pretreated with boiling water for ten seconds, and then soaked in 70% ethanol for 30 seconds followed by addition of 20% sodium hypochlorite (commercial bleach) with 2-3 drops of tween 20. They were sterilized for 20 minutes on a shaker and then rinsed three times with sterilized distilled water. The sterilized seeds were cultured on MS medium (Murashige and Skooge, 1962). The different experiments were conducted under aseptic condition in 2012.

Scions used in these experiments were obtained from three sources: one month - old seedlings of juvenile Hashab plants grown in the greenhouse, from which scions 1-1.5 cm, and 2.5-3 cm long nodal explants were collected. The scions were sterilized in 70% ethanol for 30 seconds and 5% bleach with 2-3 drops of tween 20 for 10 minutes and then rinsed three times with sterilized distilled water.

Mature plant material scions of Hashab were collected from tree grown outdoors. New twigs were removed from this tree after pruning. The nodal explants were sterilized as mentioned above, but with a higher concentration of bleach (15%). The third source of scion was a plantlet of Hashab grown *in vitro* from which nodal explants were used as scions.

Four and two weeks old rootstock seedlings were removed from the culture tube. Epicotyl, cotyledon leaves and axillary buds of seedlings were removed and the roots were reduced. A cleft was made using a sterilized sharp blade on the central part of the rootstock and scions were inserted into the split of rootstocks. The scion and rootstock were compatible in diameter. Then the grafts were placed in MS medium. The pH of the MS medium was adjusted to 5.7 prior to autoclaving under 1.2 bar at 121<sup>0</sup> C

for 15 minutes. Sucrose concentrations on grafted plants were 20, 30, 40, 50, and 60g/l. Light durations were one week darkness, one week light, two weeks darkness and two weeks light. All cultures were incubated in an incubation room where temperature was adjusted to  $25 \pm 2^{\circ}$  C, under light intensity of 1000 lux and a 16 hours photoperiod. Data were collected on percentage of successful grafts, number of leaves, number of nodes, and length of shoot. Rooted plants were acclimatized under a plastic house condition ( $25 \pm 5^{\circ}$  C, 60-80% RH) for three months. A completely randomized design was used in all experiments with ten replicates. Data analysis was carried out using MSTATC program. Data on percentage of successful grafts, number of leaves and number of nodes were transformed to square root before analysis. Mean separation was done using Duncan's Multiple Range Test and Student t test.

## RESULTS AND DISCUSSION

The two weeks old rootstocks significantly improved the success of grafting. There was an increase in all parameters, i.e. percentages of successful grafts, number of leaves, number of nodes, and shoot height compared to the four weeks old rootstock. The higher success of grafts of the two weeks old rootstock might be due to preservation of more food materials in cotyledons and actively growing stage of rootstock that enhanced union of the graft. The juvenile rootstocks usually have vascular cambiums with active meristematic cells which readily unite with those of the scion and result in a successful graft union. Similar observations were reported by Sanjaya *et al.* (2006) for sandalwood. Khalafalla and Daffalla (2008) found that a 14 days old rootstock of Hashab had a high percentage of successful grafts (100%), while only 83% was obtained from 7 days old rootstocks. Results were in agreement with those of Ali *et al.* (2004) who reported a high percentage of successful grafts and good vegetative growth for the two weeks old rootstock of grapefruits (Table 1).

Table 1. Effect of rootstock age of Hashab on successful grafts after eight weeks after grafting.

Age of rootstock (weeks)	Successful grafts (%)	No. of leaves per shoot	No. of nodes per shoot	Increase in shoot height (mm)
Two weeks	100.0 (9.9)	2.3(1.5)	2.0(1.4)	10.0
Four weeks	80.0 (8.8)	1.4(1.1)	1.1(1.0)	8.3
CV%	11.4	19.10	15.6	12.6
SE±	0.78	0.07	0.06	0.09

Transformed data between brackets using square root.

The scion length of 1-1.5 cm gave a high percentage of successful grafts and number of leaves, while the taller scion of 2.5-3 cm gave significantly low percentage of successful grafts and number of leaves. Results concluded that scion length range of 1-1.5 cm was suitable for successful grafts. These results are inconsistent with the results of Amiri (2005) who found that when the size of *Prunus avium* meristems increased from 5 to 10 mm, the rate of successful grafts increased from 32 to 45%. This might be due to genotypic differences (Table 2).

Table 2. Effect of length of scions grafted on two weeks old rootstocks of Hashab on successful grafts after eight weeks after grafting.

Scion length (cm)	Successful grafts (%)	No. of leaves per shoot	No. of nodes per shoot	Increase in shoot height (mm)
1 -1.5	95.0(9.7)	2.2(1.4)	1.3(1.1)	9.7
2.5-3	70.0(8.2)	1.0( 1.0)	1.0(1.0)	7.5
CV%	13.9	30.1	22.0	17.1
SE±	0.3	0.09	NS	NS

Transformed data between brackets using square root.

NS= not significantly different.

The effect of scion source on successful grafts gave significant higher percentages of successful grafts, number of leaves, number of nodes and length of shoot were obtained by scions from seedlings grown under greenhouse conditions compared with those from *in vitro* plantlets and mature trees. This might be due to the high level of relative humidity maintained in the greenhouse which reduced water loss from scion and hence resulted in a successful graft union and the regular pruning of these plants resulted in juvenile scions. Similar results were obtained on *A. mangium* by Monteuis (1995), who reported that grafts with young scions elongated faster than adult scions. Ali *et al.* (2004) found that a high percentage of successful grafts was obtained when shoot tips of grapefruit were obtained from seedlings grown under greenhouse conditions than mature trees. No successful grafts were obtained when scions were obtained from mature trees of Hashab due to the aging of the whole donor tree, however, the successfully established micrografts from mature trees were more recalcitrant to elongate than those from juvenile seedlings. This might cause physiological and biochemical differences between the two origins. This was probably caused by physiological aging (Hackett, 1983; Monteuis, 1989) (Table 3).

Addition of 40 g/l of sucrose gave the highest percentage of successful grafts, compared to other concentrations. It might be attributed to the need of micrograft seedlings to higher sucrose concentrations. High sucrose concentrations give high energy which was required for rapid rate of

Table 3. Effect of source of Hashab scions on successful grafts after eight weeks after grafting. Means having the same letters in each column are not significantly different according to

Source of scion	Successful graft (%)	No. of leaves per shoot	No. of nodes per shoot	Increase in shoot height (mm)
Greenhouse	100.0 (9.9) a	2.0 ( 1.4) a	1.5 (1.2) a	12.0 a
<i>In vitro</i> plantlets	55.0 (7.4) b	1.0 ( 0.9) b	0.7 ( 0.8) b	4.0 b
Mature tree	0.0 (0.17) c	0.0 ( 0.0) c	0.0 ( 0.0) c	0.0 c
CV%	9.1	35.1	21.0	32.1
SE±	0.12	0.12	0.1	0.28

Duncan's Multiple Range test at 5% level of probability.

Transformed data between brackets using square root.

growth and hence the formation of the graft union. Similar results were obtained by Ali *et al.* (2004) who reported that the highest percentage of successful grafts was induced on MS medium supplemented with 75g /l sucrose (Table 4).

Table 4. Effect of sucrose concentrations in MS medium on percentage of successful grafts of Hashab after eight weeks after grafting.

Sucrose concentration (g/l)	Successful graft (%)	No. of leaves per shoot	No. of nodes per shoot	Increase in shoot height (mm)
20	65.0 ( 7.9)	1.4 (1.2)	1.1 (1.0)	7.5
30	80.0 ( 8.8)	1.5 (1.2)	1.2 (1.1)	8.5
40	100.0 (10.0)	1.9 (1.4)	1.3 (1.1)	8.5
50	60.0 (7.7)	1.4 (1.2)	1.4 (1.1)	8.5
60	75.0 (8.5)	1.6 (1.2)	1.1 (1.0)	6.5
CV%	14.9	21.8	25.6	16.4
SE±	0.2	NS	NS	NS

Transformed data between brackets using square root.

NS= not significantly different.

Exposure of micrografted seedlings to light had significant effect on percentage of successful grafts and number of leaves. Micrografted seedlings subjected to continuous light for two weeks gave significantly higher percentages of successful grafts and number of leaves, which might be due to the need of micrografted seedlings to a long period of light. The lowest percentage of successful grafts was obtained when Hashab seedlings were incubated for two weeks in darkness. These results are in agreement with those reported by Monteuis (1996) who found that placing the micrografts for two weeks in darkness just after grafting resulted in fewer successful grafts of *A. mangium* than those under

standard lighting conditions. This procedure, which also inhibited scion elongation, is in contrast to that reported for *Picea abies* (Monteuuis, 1994) (Table 5).

Table 5. Effect of light durations on successful grafts of Hashab after eight weeks after grafting.

Light duration	Successful graft(%)	No. of leaves per shoot	No. of nodes per shoot	Increase in shoot height (mm)
Continuous darkness for one week	65.0 (7.95)	1.50 (1.2)	0.95 (0.9)	7.2
Continuous light for one week	55.0 (7.4)	0.75 (0.9)	0.80 (0.9)	6.0
Continuous darkness for two weeks	50.0 (7.1)	0.70 (0.8)	0.95 (0.9)	6.0
Continuous light for two weeks	70.0 (8.2)	1.75 (1.2)	1.30 (1.04)	7.3
CV%	14.8	35.4	32.5	17.7
SE±	0.19	0.06	NS	NS

Transformed data between brackets using square root.

NS= not significantly different.

In conclusion, cleft micrografting technique for Hashab was successfully established. This was accomplished by using two weeks old rootstocks, grafted with scions of 1-1.5 cm length, obtained from the greenhouse and cultured on MS medium supplemented with 40g/l sucrose and incubated under continuous light for two weeks.

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## أثر عمر الأصل وطول الطعم ومصدره وتركيز السكر (*Acacia senegal* (L)) للهشاب تقنية التطعيم الدقيق وفترة الإضاءة

غادة صالح الطيب ومحمد أحمد علي

هيئة البحوث الزراعية، وحدة زراعة الأنسجة، واد مدني، السودان.

### الخلاصة

تعتبر شجرة الهشاب من الأنواع الشجرية المهمة في السودان لإنتاج الصمغ العربي. يتم عادة إكثار الهشاب عن طريق البذور مما ينتج عنه اختلاف في الإنتاجية نتيجة للتلقيح الخلطي. تهدف هذه الدراسة لتطوير تقنية التطعيم الدقيق لشجرة الهشاب. نفذت خمس تجارب لاختبار أثر عمر أصل شتلات الهشاب و طول الطعم و مصدر الطعم و تركيز السكر في الوسط الغذائي وفترة الإضاءة في إنجاح التطعيم الدقيق في تصميم كامل العشوائية بعشرة مكررات. أظهرت النتائج أن الأصل الذي عمره أسبوعين من شتلات الهشاب أعطى أعلى نسبة نجاح للطعم الدقيق للهشاب. وجد أن الطعم الذي طوله 1.5 - 1 سم أعطى أعلى نسبة نجاح معنوي للطعم الدقيق للهشاب. النباتات النامية في البيوت المحمية هي أفضل مصدر للطعم حيث أعطت أعلى نسبة نجاح للطعم الدقيق و عدد الأوراق و عدد العقد و زيادة ارتفاع السوق الخضرية للهشاب. زادت نسبة نجاح الطعم الدقيق بتركيز السكر 40 جراما للتر في الوسط الغذائي. يمكن الحصول على نسبة نجاح أعلى للطعم الدقيق عند وضع النباتات في حضانة تحت ظروف الإضاءة المستمرة لمدة أسبوعين.