MICROPROPAGATION OF *ACACIA TORTILIS* SUBSP. *RADDIANA* AND *A. NILOTICA* UNDER *IN VITRO* CONDITIONS

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AZIZ, A. A. S., OMARI, M. A. & KAFAWIN, O. M. 2002. Micropropagation of *Acacia tortilis* subsp. *raddiana* and *A. nilotica* under *in vitro* conditions. Experiments were conducted to investigate propagation of *Acacia tortilis* subsp. *raddiana* and *A. nilotica* in tissue culture. Micropropagation of *A. nilotica* from nodal segment was achieved on MS medium. The highest average number of shoots per explant (3.58) was obtained on medium containing 2.5 mg l^{-1} BA. Individual shoots, when transferred onto half strength MS medium with 4.0 mg l^{-1} IBA, formed healthy roots in 62.5% of the cultures. *Acacia tortilis* failed to produce more than one shoot per explant and did not develop roots in any media tested.

Key words: *Acacia tortilis* subsp. *raddiana* - *Acacia nilotica* - *in vitro* - micropropagation

AZIZ, A. A. S., OMARI, M. A. & KAFAWIN, O. M. 2002. Pembiakan mikro *Acacia tortilis* subsp. *raddiana* dan *A. nilotica* di bawah keadaan *in vitro*. Ujian dijalankan untuk mengkaji pembiakan *Acacia tortilis* subsp. *raddiana* dan *A. nilotica* dalam kultur tisu. Pembiakan mikro *A. nilotica* daripada bahagian buku dicapai pada media MS. Bilangan purata pucuk tertinggi setiap eksplant (3.58) diperoleh daripada media yang mengandungi 2.5 mg l^{-1} BA. Setiap pucuk yang dialih ke atas media MS separuh kekuatan dengan 4.0 mg l^{-1} IBA membentuk akar yang sihat dalam 62.5% daripada kultur tersebut. *Acacia tortilis* gagal menghasilkan lebih daripada satu pucuk setiap eksplant dan tidak membentuk akar dalam mana-mana media yang diuji.

Introduction

In dry zones *Acacia* species play an important role as fodder plants and have considerable role as emergency food. In addition, the wood is useful for fuel while the trees provide shade and shelter and are important in soil conservation (Doran *et al.* 1983). Some researchers investigate the use of *Acacia* species for medicinal purposes (Dafallah & Al Mustafa 1996, Rohner & Ward 1997). In Jordan, *A. tortilis* subsp. *raddiana* and *A. nilotica* grow naturally. However, due to various reasons, few *A. tortilis* trees remain and *A. nilotica* has become rare.

The regeneration of these trees in nature is quite low (Dewan *et al.* 1992). Seeds of *Acacia* are often infested by seed beetles, which kill between 72 and 99% of the seeds, depending on species (Halevy 1974). The vegetative propagation of acacias is less popular, less economic and less efficient in providing continuous plant stock for large-scale projects compared with a source of good seed (Al-Mudaris 1994). Therefore, micropropagation, which is often used successfully for the multiplication of herbaceous and woody plants, represents an interesting alternative for these species (Badji *et al.* 1993).

Micropropagation is the true-to-type propagation of selected genotype using *in vitro* culture technique (Debergh & Read 1991). Micropropagation of tree species offers a rapid means of producing clonal planting stock for afforestation, woody biomass as well as conservation of elite and rare germplasm (Bajaj 1986).

In vitro techniques have been employed successfully in the propagation of mature trees (Kumar 1992). However, leguminous trees are relatively recalcitrant to *in vitro* regeneration (Dewan *et al.* 1992), being comparatively slow and difficult to grow (Kyte 1987).

There are many reports that discuss tissue culture techniques for the propagation of leguminous trees like *A. mangium* (Galiana *et al.* 1991), *Prosopis tamarugo* (Nandwani & Ramawat 1992), *Bauhinia purpurea* (Kumar 1992) and *Tamarindus indica* (Jaiwal & Gulati 1991).

This study was undertaken to define a suitable methodology for rapid propagation of *A. tortilis* and *A. nilotica* through the *in vitro* culture of nodal explants.

Materials and methods

Sterilisation and shoot culture

Seeds of *A. tortilis* and *A. nilotica* were obtained from the Department of Forestry and Rangeland, Ministry of Agriculture, Jordan. The seeds which were collected in 1995 required gentle scarification to improve the germination percentage. The seeds were soaked for 15 minutes in 96% H_2SO_4. They were then rinsed three times in sterile distilled water. During rinsing, the seeds were shaken on a rotatary shaker for six minutes in a laminar air flow cabinet to completely remove H_2SO_4. The seeds were then germinated in MS medium (Murashige & Skoog 1962).

Four weeks after germination stem nodes were excised and cultured on MS basal medium supplemented with a combination of N^6-Benzyladenine (BA) (0.0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg l^{-1}), indole-3-acetic acid (IAA) (0.0, 0.1 and 0.2 mg l^{-1}), 3% sucrose, 0.1 g l^{-1} myo-inositol and 0.5% Bacto agar at pH 5.7. The medium, with all the supplementation except IAA, was autoclaved at 121°C for 20 minutes at 15 psi. Then filter sterilised IAA was added to the media while in the laminar air flow cabinet. The medium (10 ml) was dispensed into a glass pyrex test tube (18 × 150 mm), plugged with cotton wrapped in one layer of cheese cloth. Each treatment was replicated 12 times. Explants were subcultured three times onto fresh medium at 25-day intervals. The fourth subculture was done using growth regulator-free medium to eliminate any carry-over effect of hormons (Shibli *et al.* 1996). The number of shoots, average shoot length and the percentage of explants regenerated were recorded at the end of the experiment.

Rooting

For *in vitro* rooting, 16-week-old well-elongated leafy shoots (1–4 cm) from the hormone-free subculture were transferred onto half-strength MS medium supplemented with 3-indole butyric acid (IBA) at six various concentrations (1.0–5.0 mg l^{-1}), 0.1 g l^{-1} myo-inositol, 0.5% Bacto agar and 3% sucrose at pH 5.7. The medium (10 ml) was dispensed into glass pyrex test tubes (25 × 150 mm).

The test tubes were plugged with polypropylene caps and autoclaved at 121 °C for 20 min at 15 psi. Each treatment consisted of 16 test tubes with one leafy shoot each. The percentage of explants that produced roots and the number of roots were calculated after 40 days.

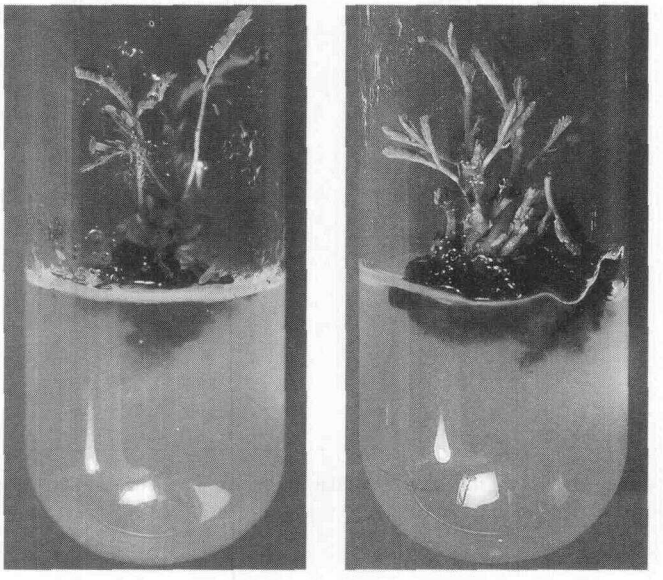
The factorial split-plot arrangement in CRD was used to analyse the data. Analysis of variance (ANOVA) and the least significant difference (LSD) were calculated at probability level 0.05 using the statistical analysis system (SAS 1996, Version 6.03).

Results and discussion

Twenty-one combinations of BA and IAA in MS medium were tested to determine the optimum growth regulator combination for the two species (Table 1). *Acacia nilotica* with 1.0 and 0.1 mg l^{-1} and 2.5 and 0.0 mg l^{-1} of BA and IAA respectively, produced the greatest number of shoots per explant (Figure 1). However, only one single shoot was produced from the axillary bud of *A. tortilis* at the same concentrations, in contrast with *A. nilotica* which showed proliferation of stems (Figure 2).

Both species produced shoots on hormone-free MS medium. However, only *A. nilotica* could produce more than one shoot from each explant. The absence of IAA from the media enhanced shoot proliferation in both species and increased the length of shoots, although it was not significant (Table 2).

The interaction between species and BA was significant (Table 3). *Acacia tortilis* with 0.5 mg l^{-1} BA showed the best results for number and length of shoot. With *A. nilotica*, 1.5 mg l^{-1} BA was the best combination for enhanced shoot production while BA at 0.5 mg l^{-1} produced the tallest shoot. During the present investigation, the explants produced callus.



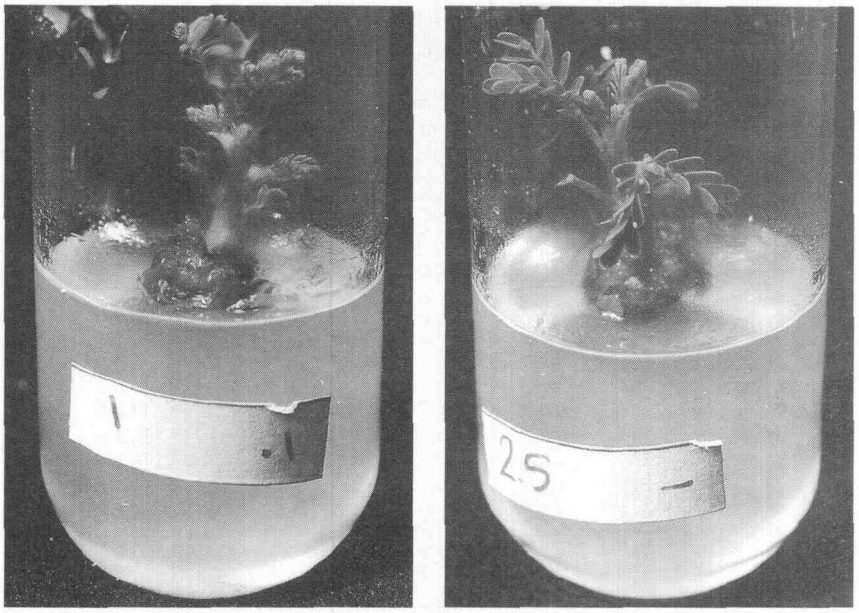
(a) (b)

Figure 1 Shoot proliferation of *Acacia nilotica* as effected by combinations of BA and IAA in MS media: (a) 1.0 mg l^{-1} BA and 0.1 mg l^{-1} IAA and (b) 2.5 mg l^{-1} BA and 0.0 mg l^{-1} IAA

Table 1 Effect of combination of BA and IAA in MS medium on node explants of *Acacia tortilis* subsp. *raddiana* and *A. nilotica*

Species	Growth regulator (mg l^{-1})		Average number of shoots/explant	Shoot length (mm)	Explant proliferation (%)
Acacia tortilis	BA	IAA			
	Control		0.25 m[+]	0.58 a	25
	0.0	0.1	0.17 m	0.41 a	16
	0.0	0.2	0.08 m	0.16 a	8
	0.5	0.0	0.59 J-m	12.1 a	58
	0.5	0.1	0.59 J-m	6.00 a	58
	0.5	0.2	0.50 j-m	1.8 a	50
	1.0	0.0	0.41 k-m	7.1 a	33
	1.0	0.1	0.50 j-m	3.7 a	50
	1.0	0.2	0.50 j-m	8.3 a	50
	1.5	0.0	0.67 i-m	8.2 a	58
	1.5	0.1	0.50 j-m	5.7 a	33
	1.5	0.2	0.25 m	2.4 a	25
	2.0	0.0	0.33 lm	3.1 a	33
	2.0	0.1	0.25 m	2.8 a	25
	2.0	0.2	0.33 lm	2.2 a	33
	2.5	0.0	0.25 m	1.4 a	25
	2.5	0.1	0.41 k-m	6.6 a	33
	2.5	0.2	0.33 lm	2.2 a	33
	3.0	0.0	0.42 k-m	6.8 a	42
	3.0	0.1	0.33 lm	5.4 a	33
	3.0	0.2	0.33 lm	3.9 a	33
Acacia nilotica	BA	IAA			
	Control		0.92 g-m[+]	11.3 a	75
	0.0	0.1	1.25 e-k	11.3 a	92
	0.0	0.2	0.91 g-m	13.3 a	83
	0.5	0.0	2.08 b-e	23.4 a	92
	0.5	0.1	1.17 m	16.9 a	67
	0.5	0.2	0.83 j-m	17.3 a	75
	1.0	0.0	2.08 b-e	10.3 a	92
	1.0	0.1	2.91 ab	13.1 a	83
	1.0	0.2	1.33 d-j	7.4 a	75
	1.5	0.0	2.75 a-c	13.2 a	83
	1.5	0.1	2.17 b-d	7.2 a	75
	1.5	0.2	2.00 c-f	5.6 a	58
	2.0	0.0	1.83 d-f	7.1 a	58
	2.0	0.1	1.5 d-i	6.5 a	58
	2.0	0.2	1.75 d-j	9.0 a	83
	2.5	0.0	3.58 a	10.3 a	92
	2.5	0.1	1.92 c-f	10.0 a	83
	2.5	0.2	0.92 g-m	4.7 a	50
	3.0	0.0	2.08 b-e	7.8 a	83
	3.0	0.1	1.33 d-j	3.5 a	75
	3.0	0.2	1.67 d-m	7.5 a	42

[+] Means within each column followed by similar letters are not significantly different at 0.05 probability level.



(a) (b)

Figure 2 Shoot proliferation of *Acacia nilotica* as effected by combination of BA and IAA in MS media: (a) 1.0 mg l^{-1} BA and 0.1 mg l^{-1} IAA and (b) 2.5 mg l^{-1} BA and 0.0 mg l^{-1} IAA

Table 2 The single effect of IAA in MS medium on node explant of *Acacia tortilis* subsp. *raddiana* and *A. nilotica*

Species	Concentration of IAA (mg l^{-1})	Average number of shoots/explant	Shoot length (mm)
Acacia tortilis	0.0	0.42 d[+]	5.6 a
	0.1	0.39 d	4.4 a
	0.2	0.33 d	3.0 a
Acacia nilotica	0.0	2.19 a	11.9 a
	0.1	1.75 b	9.8 a
	0.2	1.35 c	9.3 a

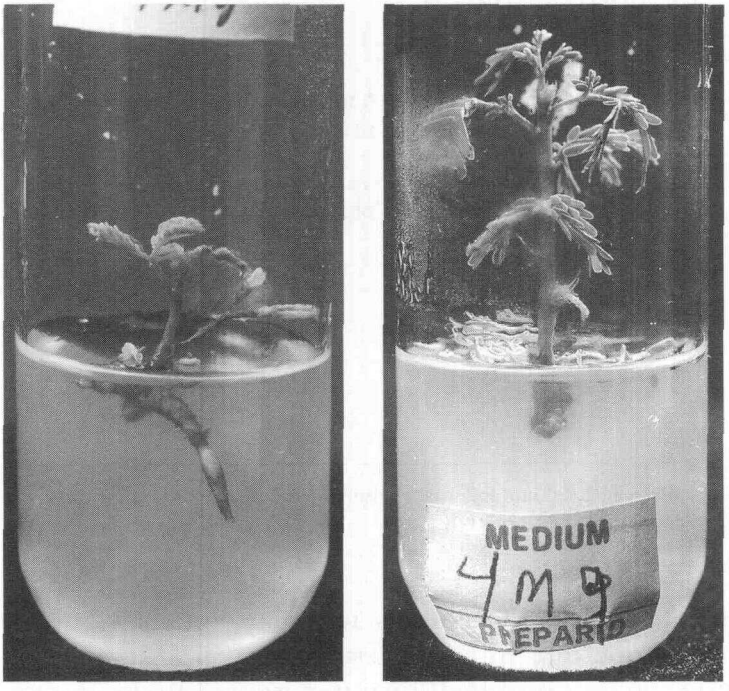
[+]: Means within each column followed by similar letters are not significantly different at 0.05 probability level.

Acacia tortilis failed to produce roots with any level of IBA while *A. nilotica* shoots developed roots directly at the base of the shoots with no intervening callus. However, no root proliferation was observed in the control (Table 4). This may be because *A. nilotica* requires the presence of auxins for root initiation. IBA at 4.0 mg l^{-1} gave the best results with 62.5% rooting. Figure 3 shows the root proliferation of *A. tortilis* and *A. nilotica* in MS media supplemented with 4.0 mg l^{-1} IBA.

Table 3 Single effect of BA in MS medium on node explant of *Acacia tortilis* subsp. *raddiana* and *A. nilotica*

Species	Concentration of BA (mg l^{-1})	Average number of shoots/explant	Shoot length (mm)
Acacia tortilis	0.0	0.16 f [+]	0.4 g
	0.5	0.56 ef	6.6 c-f
	1.0	0.47 f	6.3 c-f
	1.5	0.47 f	5.4 d-f
	2.0	0.31 f	2.7 fg
	2.5	0.33 f	3.4 e-g
	3.0	0.36 f	5.4 d-f
Acacia nilotica	0.0	1.03 de	11.9 b
	0.5	1.37 cd	19.1 a
	1.0	2.11 ab	10.3 bc
	1.5	2.31 a	8.7 b-d
	2.0	1.7 bc	7.5 c-e
	2.5	2.14 ab	8.3 b-d
	3.0	1.7 bc	6.3 c-f

[+]: Means within each column followed by similar letters are not significantly different at 0.05 probability level.



(a) (b)

Figure 3 Root proliferation of (a) *Acacia tortilis* and (b) *A. nilotica* as effected by 4.0 mg l^{-1} IBA on rooting MS media.

Table 4 Effect of IBA in half-strength MS medium on rooting of shoots of *Acacia tortilis* subsp. *raddiana* and *A. nilotica*

Species	Concentration of IBA (mg l^{-1})	Number of roots/explant	Length of root (mm)	% of rooting
Acacia tortilis	0.0	0.61c+*	6.1a	0.0
	1.0	0.61c	6.1a	0.0
	2.0	0.61c	6.1a	0.0
	3.0	0.61c	6.1a	0.0
	4.0	0.61c	6.1a	0.0
	5.0	0.61c	6.1a	0.0
Acacia nilotica	0.0	0.61c	6.1a	0.0
	1.0	0.85bc	8.7a	33.3
	2.0	0.92b	8.5a	50.0
	3.0	1.03b	8.6a	33.3
	4.0	1.3a	8.6a	62.5
	5.0	1.03b	8.3a	33.3

+ Means within each column followed by similar letters are not significantly different at 0.05 probability level.

* Data were transformed by square root transformation before analysis according to Compton (1994)

In the present investigation, *A. nilotica* with 1.5 mg l^{-1} BA produced the greatest number of shoots per explant. The controls developed one to two shoots only. As cited by Dewan *et al.* (1992), Mathur and Chandra (1983) reported development of plantlets in mature nodal explants of *A. nilotica* on auxin containing media, where auxin was essential for shoot differentiation. On the other hand, Coleman and Ernst (1990) found that cytokinin was required for shoot differentiation.

Nandwani (1995) reported that BA containing auxin enhanced shoot induction in *A.tortilis* subsp. *raddiana.* However, our investigation with *A. tortilis* showed that not more than one shoot was obtained in all treatments; this can be attributed to the type and age of explant. This result is in agreement with Badji *et al.* (1993), who obtained not more than one shoot of *A. senegal* on MS medium with 5.0×10^{-7} M zeatin. Cultures with apices are able to proliferate buds at higher rates than those with axillary buds (Debergh & Read 1991). Hypocotyls and cotyledon explants of *A. tortilis* subsp. *raddiana* produce only callus while cotyledonary node segments are found to be best for the multiple shoot induction (Nandwani 1995).

It can be concluded that MS media containing optimum hormone combinations was developed successfully for propagation of *A. nilotica* through nodal segment culture. In addition, *A. tortilis* subsp. *raddiana* was successfully propagated via nodal segment *in vitro.*

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