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Full Length Research Paper

In vitro propagation of *Alternanthera sessilis* (sessile joyweed), a famine food plant

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A complete protocol for micropropagation of *Alternanthera sessilis* using leaf explants was developed. Leaf explants from *A. sessilis* established on Murashige and Skoog medium were treated with various concentrations and combinations of auxins and cytokinins to determine the best method for callus formation, shoot regeneration and root formation. MS medium supplemented with 1 mg/l BAP and 1 mg/l 2,4-D was determined to be the most suitable for callus induction. After 2 weeks, the callus was then transferred to shooting media which consisted of half strength MS basal medium, 1 mg/l IAA and 1 mg/ l BAP. After 4 weeks, structures were observed with their basal ends embedded on the callus. These structures turned into green colored shoot buds. MS medium supplemented with 1 mg/l IAA and 1 mg/l BAP showed maximum shoot multiplication. For rooting, the optimal medium was half strength MS medium supplemented with 1 mg/l IBA. Rooted plantlets were then transferred to sunbag vessels which provided the high humidity environment. The hardened plants were then successfully established in the soil medium and can function in the natural environment.

Key words: *Alternanthera sessilis*, famine food plant, micropropagation, callus.

INTRODUCTION

Alternanthera sessilis (sessile joyweed), a member of Amaranthaceae family is a weed and occurs in both wetlands and uplands and can grow on a variety of soil types. The plant spreads by seeds, which are wind and water-dispersed and by rooting at stem nodes (Scher, 2004). It is a weed of rice throughout tropical regions and of other cereal crops, sugarcane and bananas. Although it is a weed, it has many utilities. Young shoots and leaves are eaten as a vegetable in southeast Asia (Scher, 2004). The young tips are eaten as a vegetable. The leaf is very rich in iron, vitamin A and dietary fiber. The plant contains protein and soups made with the leaf are given to anaemic patients in rural areas. It contains abundant carotene, therefore it is used for curing night blindness. The plant enhances the secretion of milk in new mothers (Naples, 2005) and it is used as a remedy against intestinal cramps, diarrhoea and dysentery (intestinal disorder), and externally as a cooling agent to treat fever (Naples, 2005). Naples (2005) also reported that *A. sessilis* is used internally against intestinal inflammation, externally to treat wounds, to treat hepatitis, tight chest, bronchitis, asthma, lung troubles, to stop bleeding and as a hair tonic.

Tissue culture techniques are being increasingly exploited for clonal multiplication and *in vitro* conservation of valuable indigenous germplasm threatened with extinction. Greater demand for these plants especially for the purpose of food and medicine is one of the causes of their rapid depletion from primary habitats. Micropropagation offers a great potential for large scale multiplication of such useful species and subsequent exploitation (Boro et al., 1998).

Currently, there are not much published reports on the tissue culture of *A. sessilis*, however there are reports of microprogation performed on other plants from the same family such as *Amaranthus*. In general, the genus *Amaranthus* shows potential with regard to dedifferentiation and morphogenetic processes and the possibility to micropropagate selected genotypes via direct or indirect

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regeneration or via somatic embryogenesis.

In previous research, Bennici et al. (1992) studied 4 different species of Amaranthus (*A. caudatus*, *A. hypochondriacus*, *A. cruentus* and *A. hybridus*) for callus induction, growth and organogenic processes from hypocotyl and epicotyl cultures and found that 5.4 µM of naphthalene acetic Acid (NAA) plus 4.4 µM of benzyl amino purine (BAP) or 2.3 µM of 2,4-dichlorophenoxyacetic acid (2,4-D) plus 2.3 µM of kinetin were the best combinations for callus induction and complete plantlets were obtained from rooted adventitious shoots. Callus production and shoot regeneration from primary callus or hypocotyl explants have been reported by Flores et al. (1982), Flores and Teutonico (1986) in *A. cruentus*, *A. hypochondriacus*, *A. tricolor* and by Bagga et al. (1987) in *A. paniculatus*. Bennici et al. (1997) studied *in vitro* behaviour of *A. cruentus*, *A. hybridus* and *A. hypochondriacus* and found that callus formation occurred from explants from almost all the lines tested. Optimum conditions for shoot induction are high cytokinin/auxin ratio. Strong cytokinin such as BAP or isopentenyl adenine (2-iP) seems to be effective agents for shoot regeneration. The endogenous auxin/cytokinin balance and age dependant competence of explants tissues may play an important role in regeneration (Bennici and Schiff, 1997).

With this background, this study was aimed at successful propagation of *A. sessilis*, a famine food plant under *in vitro* conditions. The objectives of the investigation have been more than fulfilled and many solutions have been found for problems of plant growth, differentiation, morphogenesis and in the application of different growth substances.

MATERIALS AND METHODS

Collection and preparation of explants

The plants were collected from the Kwazulu Natal region of South Africa and were identified using local floral keys. The leaves were used as explants and were washed thoroughly in tap water to remove the impurities and subsequently washed 3 times in sterile distilled water.

Preparation of media for callus induction

The MS medium (Murashige and Skoog, 1962) used in this study was purchased from Sigma-Aldrich, South Africa. The basal MS media supplemented with different concentrations of auxin (2,4-D) and cytokinin (BAP) at 0.5 to 1 mg/l were used for callus initiation. Fresh stock solutions were made twice a month. The pH of the media was always verified to be 5.8. Cefotaxamine 25 mg/l and fungizone 25 mg/l were the antibiotics used to prevent microbial contamination.

Surface sterilization of explants

Three combinations of surface sterilants were tested for their effective sterilization and it was found that the leaves disinfected with 0.1% mercury chloride for 5 min in combination with 40% sodium hypochlorite (active ingredient 50 g/l chlorine) for 20 min were free from any contamination. After chemical sterilization, the explants were thoroughly rinsed 3 times with sterile distilled water under a laminar flow hood.

Callus induction

After sterilization, the leaves were cut into square pieces using sterile scalpel blades. Five leaf squares were placed on each petri plate containing the MS basal medium (catalogue number M9274, Sigma-Aldrich) (42.2 g/l) as well as with different combinations of plant growth regulators in various combinations. All cultures were maintained at 25°C in a 16/8 h light/dark photoperiod in a growth chamber. Visual observations were made every week and the effect of different treatments was quantified on the basis of percentage of explants showing response to callus induction.

Shoot regeneration using callus

Callus obtained from MS medium supplemented with 1 mg/l 2,4-D and 1 mg/l BAP were transferred on shoot regeneration medium having different concentrations of hormones individually or in combination. The cultures were kept in a growth chamber (Polychem Supplies, South Africa) for 16/8 h light/dark photoperiod and observed for shoot formation. Sub-culturing was done every 4 weeks to maintain the culture. Then, the individual shoots were transferred to tissue culture bottles for further multiplication.

Root induction

Regenerated shoots were separated from the cultures individually and used for root induction. The media used for root induction was half strength MS basal medium (21.1 g/l) supplemented with different concentrations of NAA and indole butyric acid (IBA).

Hardening of regenerated plantlets

When the plantlets attained adequate growth by producing 4 - 6 leaves with sufficient root system, they were removed from the culture medium and washed in running tap water carefully to remove the media adhering to roots. They were subsequently transplanted in pots containing sterilized soil. The plants were covered with polyethylene bags and kept in the culture room. After 15 days, the polyethylene bags were removed and well established plants were transferred to natural soil where they were acclimatized to environmental conditions.

RESULTS AND DISCUSSION

Surface sterilization of explants

To identify the ideal surface sterilization conditions of the explants, different combinations of chemical sterilants; sodium hypochlorite and mercury chloride were used. Results are presented in Table 1. Plants were exposed to sterilizing agents for varying durations. Maximum contamination (86 ± 1.41%) was observed when explants were treated with 30% sodium hypochlorite (v/v) for 20 min, followed by combination of 0.1% mercury chloride (m/v) for 5 min and 30% sodium hypochlorite (v/v) for 15

Table 1. Percentage of contamination using different surface sterilizing agents at different exposure times.

Surface sterilizing agent	Duration of Exposure	% Contamination
30 % (v/v) Sodium hypochlorite	20 min	86 ± 1.41
0.1 % (m/v) Mercury chloride + 30 % (v/v) Sodium hypochlorite	5 min + 15 min	52.5 ± 3.53
0.1 % (m/v) Mercury chloride + 40 % (v/v) Sodium hypochlorite	5 min + 20 min	5.5 ± 2.12

Data are mean ± SD (n = 3).

Table 2. Effect of growth regulators on callus induction from leaves of *A. sessilis* (After 2 weeks of culture).

MS medium plus growth hormone	% of leaves from *A. sessilis* forming callus
1 mg/L 2, 4-D + 1 mg/ L BAP	98 ± 2.82
1 mg/L 2, 4-D	0
1 mg/ L BAP	0
0.5 mg/L 2, 4-D + 1 mg/ L BAP	49.5 ± 6.36
0.5 mg/L 2, 4-D + 0.5 mg/ L BAP	77.5 ± 3.53
1 mg/L 2, 4-D + 0.5 mg/ L BAP	53.5 ± 2.12

Data are mean ± SD (n = 3).

min. 0.1% mercury chloride for 5 min and 40% sodium hypochlorite for 20 min showed 5.5 ± 2.12% contamination and was used to sterilize the leaf explants.

Callus induction

Callus initiation was observed on the surface or cut ends of the explants after 14 days of inoculation. The effect of different plant growth regulators and their concentration on callus induction is summarized in Table 2. The best callus induction response for leaf explants of *A. sessilis* was observed on MS medium supplemented with 1 mg/l - 2,4-D and 1 mg/l BAP (Figure 1). No callus induction was noted on 1 mg/l 2, 4-D or 1 mg/l BAP and the explants withered away. When equal concentrations of auxins and cytokinins were supplemented in the media high frequencies of callus induction were noted. When a high concentration of auxin (2,4-D) was used in combination with lower concentration of cytokinin (BAP), a low frequency of callus induction was noted (Table 2). Similar reports of 2, 4-D plus kinetin or NAA plus BAP on callus development was observed in *Amaranthus* by Flores et al. (1982) and Bennici et al. (1992). When a high concentration of cytokinin was used with a low concentration of auxin, a low frequency of callus induction was noted. Hence the callus obtained from MS medium supplemented with 1 mg/l 2, 4-D and 1 mg/l BAP were used for further analyses. The calli were subcultured into fresh medium every 2 weeks.

Table 3. Effect of growth regulators on shoot differentiation from stem explants of *A. sessilis.*

MS medium	Shoot multiplication of *A. sessilis*
1 mg/L IAA + 1 mg/ L BAP	10 shoots per culture
1 mg/L IAA	2 shoots per culture
1 mg/ L BAP	0

Shoot regeneration from callus

Callus obtained from leaf explants of *A. sessilis* were transferred into shooting media which consisted of half strength MS medium, 1 mg/l IAA and 1 mg/l BAP. After 4 weeks, structures were observed with their basal ends embedded on the callus and turned into green colored shoot buds. These structures were transferred into tissue culture bottles consisting of half strength MS basal medium, 1 mg/l IAA and 1 mg/l BAP. After 21 days the individual shoots were cut off and transferred into tissue culture bottles containing MS medium supplemented with 1 mg/l IAA and 1 mg/l BAP. This combination showed maximum shoot multiplication of 10 shoots per culture (Table 3). Bennici et al. (1997) found that high cytokinin: auxin ratio favours shoot regeneration in Amaranthus. In this study, lowest number of shoot regeneration was observed on half strength MS medium supplemented with 1 mg/l IAA only. No shoot multiplication was observed on half strength MS medium supplemented with 1 mg/l BAP. Shoot multiplication was noted 14 days after the cultures were inoculated into the medium.

Rooting of regenerated shoots

For rooting, the shoots developed from the callus of *A. sessilis* were cultured on rooting medium consisting of half strength MS medium containing different concentrations of either NAA or IBA. Optimal rooting was observed on half strength MS medium supplemented with 1 mg/l IBA (Figure 2). Some rooting response was also observed on MS medium supplemented with 1 mg/L NAA. Bagga et al. (1987) found that hypocotyl segments of *A.*

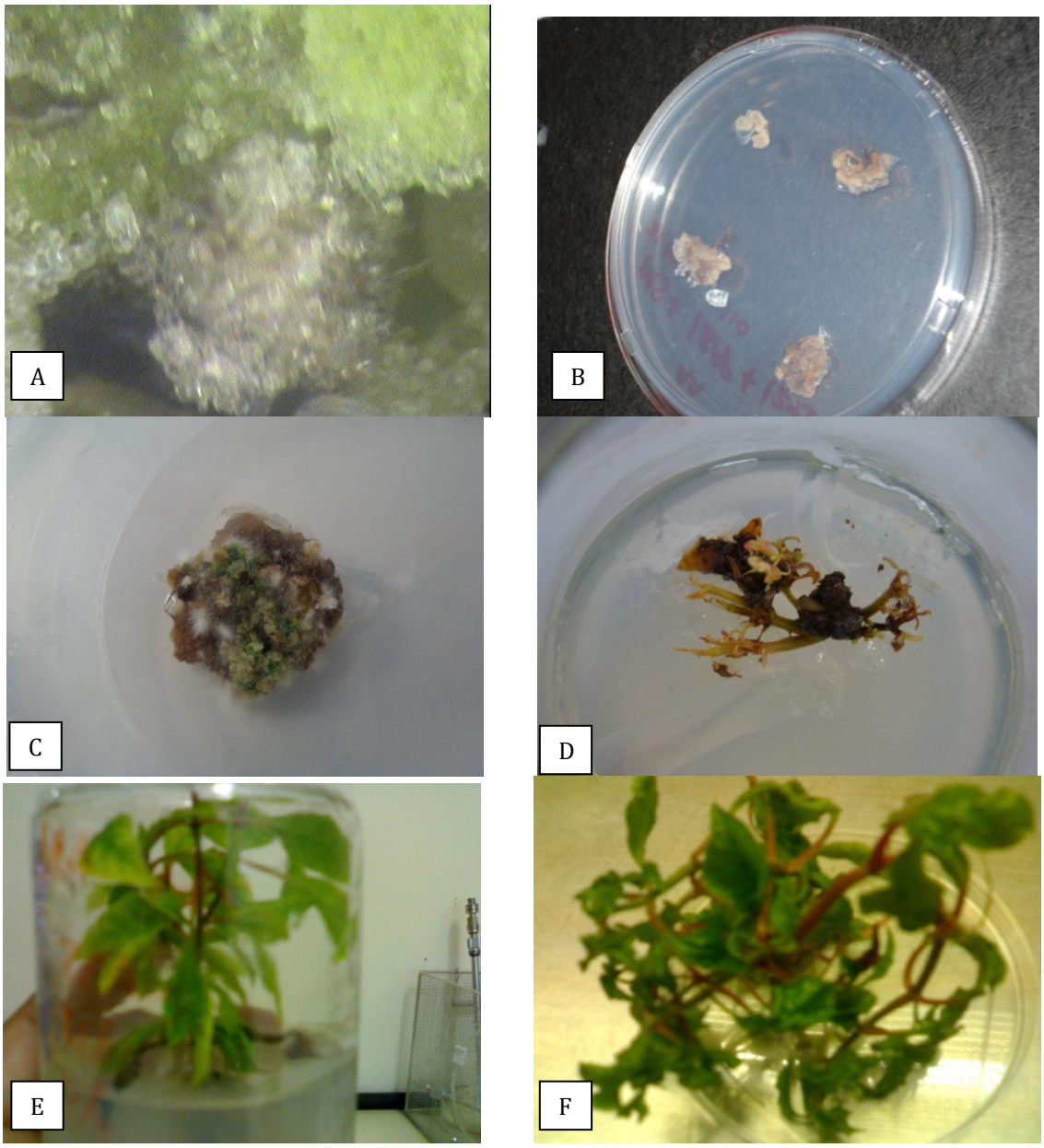


Figure 1. Micropropagation of *A. sessilis*. (A) Callus derived from leaf explants. (B) Callus derived from leaf explants. (C) Shoot formation from callus. (D) Shoot regeneration in tissue culture bottle. (E) Multiple shoot development in a bottle. (F) Multiple shoot development on a petri dish.

paniculatus formed roots on B5 medium supplemented with NAA and Bennici et al. (1992) reported that amaranthus responded well in forming roots with IAA plus kinetin and/or IAA plus BAP.

Hardening of plantlets

The regenerated plantlets from the explant with healthy root and shoot system were transferred after washing with distilled water to remove the media traces from the roots. They were transferred into bottles containing sterilized soil (Figure 2) and covered with sunbag vessels to maintain a high humidity environment for a week. Thereafter the sunbags were removed and the plantlets were transferred into bottles containing normal soil and they were grown under full sunlight.

To summarize, callus formation from the leaf explants of *A. sessilis* was observed in most of the growth regulator combinations but the differences in callus growth were observed depending on the growth hormone combinations used. The best medium for callus induction was

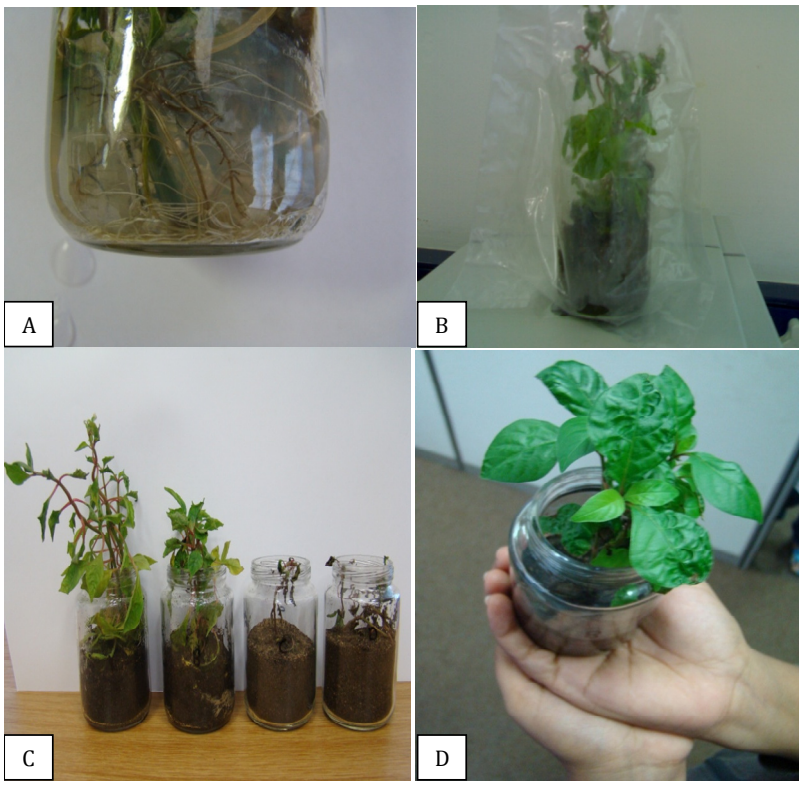


Figure 2. Root development in *A. sessilis*. (A) Rooting in half MS medium supplemented with 1 mg/l IBA. (B) *A. sessilis* plant in a sunbag vessel. (C) Hardened *A. sessilis* plant. (D) Acclimatized plant.

found to be MS medium supplemented with 1 mg/l 2,4-D and 1 mg/l BAP. Better shoot regeneration from the callus of *A. sessilis* was found in MS medium supplemented with 1mg/l IAA and 1 mg/l BAP compared to other combinations of growth regulators. Optimal rooting was observed in MS medium with 1mg/l IBA. The results obtained from this study demonstrate that *A. sessilis* has great potential with regard to dedifferentiation and morphogenetic processes and the possibility to micropropagate these plants.

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