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Micropropagation of *Sauropus androgynus* (L.) Merr.—An important green leafy vegetable

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A micropropagation protocol has been developed for *Sauropus androgynus* (L.) Merr. using uninodal explants in Murashige and Skoog (MS) medium supplemented with various concentrations of cytokinins, benzyl adenine (BA) and kinetin (Kn). Shoot induction was observed in 1.0 mg/L BA and 0.1 mg/L Kn after 25 d and more number of shoots was achieved in shoot induction medium. Rooting was induced from shoots in MS medium supplemented with various concentrations of indole 3-butyric acid (IBA) and naphthalene acetic acid (NAA). All the shoots were rooted in 0.5 mg/L IBA and 0.2 mg/L NAA after 25 d. Rooted plants were transferred to soil and successfully acclimatized.

Keywords: Leafy vegetable, micropropagation, nutrition, *Sauropus androgynus*

Sauropus androgynus (L.) Merr., sweet leaf (Katuk, Chekkurmanis), is a shrub belonging to the family Euphorbiaceae, growing in the warm humid tropical regions and used as a leafy vegetable. The plant is reported to have an upright stem reaching a height of 2.5 cm and bears dark green oval leaves 5-6 cm long[1]. *S. androgynus* was introduced to India from Malaysia in the 1950s for its nutritional and medicinal properties. The succulent shoots and tender leaves of the plant are used in culinary preparation and are noted for their high nutritive value. It is, therefore, popularly known as “multivitamin green” and “multi minerals packed leafy vegetable”. The raw leaves have an exceptionally pleasant taste and are an important green vegetable in Borneo[2]. The leaf of *S. androgynus* has superior nutritive value compared to other commonly consumed leafy vegetables in India[3]. The leaf juice is used against eye ailments[4].

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The plant is usually propagated by vegetative means but the rate of multiplication is quite low[5]. Although an effort has been made for *in vitro* propagation of *S. androgynus* on different media and growth regulators using meristem, young leaves, nodal and internodal segments as explants, but the process gave only limited number of shoots per culture[1]. Therefore, present study was undertaken to develop an efficient *in vitro* propagation protocol for large scale cultivation and exploitation of this economically important plant species.

Young shoots were obtained from juvenile plants of *S. androgynus* grown at M S Swaminathan Research Foundation, Chennai. Tender nodes were extracted as explants by cutting off the petioles of the young branches along within the leaves. The explants were washed to remove the exudates under running tap water for 1 h and then rinsed with distilled water for 5 times. The explants were surface sterilized with 0.1% (w/v) mercuric chloride solution for 5 min and subsequently washed 5 times in sterile distilled water to remove traces of surface sterilant.

The nodes were trimmed at both the ends and implanted vertically in MS medium[6] supplemented with different concentrations of BA (0.1-2.5 mg/L) and Kn (0.01-0.25 mg/L). After 25 d, the explants were evaluated in terms of length and number of shoots per explant. For multiplication, shoots were subcultured in MS medium containing BA (1.0 mg/L) and Kn (0.1 mg/L). The explants were monitored for multiple shoot induction after 25 d.

Newly developed shoots were excised under sterile conditions and were transferred to ½ MS basal medium supplemented with different concentrations of NAA (0.05- 5 mg/L) and IBA (0.1-0.8 mg/L) individually for rooting. The data were collected after 30 d measuring root lengths and number of roots per individual shoot.

The MS medium contained 30 g/L (3%) sucrose, and 0.8% bacteriological grade agar (Qualigens Fine Chemicals, Mumbai, India). The *p*H of the medium was adjusted to 5.8 with 1 *N* HCl or 1 *N* NaOH before autoclaving. The medium was autoclaved at 121°C for 15 min. The cultures were incubated at 27±2°C room temperature and at 80% relative humidity under

a photoperiod of 16 h provided by cool white fluorescent tubes with light intensity of 2000 lux. The newly developed shoots were transferred to fresh medium after 25 d for subculturing.

Plantlets with well developed roots were removed from the culture medium and washed under running tap water to remove the solidified agar. Rooted *in vitro* shoots were planted initially in sterile vermiculate pots for 15 d and then they were transferred to polybags containing sand:garden soil:farm yard manure (2:2:1). The well established plants were transferred to the shaded nursery until planted in the field. All the experiments were repeated thrice with 25 cultures per treatment and analyzed for mean±SE.

Initiation of shoots was observed in almost all concentrations of cytokinins studied. However, maximum number of shoots and shoot length was observed on the medium containing 1.0 mg/L BA and 0.1 mg/L Kn after 25 d (Table 1; Fig. 1A). After 25 d, regenerated shoots were selected for subculture. The shoots were subcultured on the MS medium supplemented with 1.0 mg/L BA and 0.1 mg/L Kn as the maximum growth was observed in this combination. Explants growing on higher concentrations of BA and Kn showed callus formation at the proximal edge of the nodal explants. Frequent subculturing in the same medium at every 25 d resulted in increase in number of shoots (17 to 45 shoots per explant) and height (4-7 cm) in 92.5% of the explants (Fig. 1B). Frequent subculturing of *in vitro* shoots did not show any morphological variation.

The shoots cultured on ½ MS medium supplemented with 0.5 mg/L IBA and 0.25 mg/L NAA achieved maximum roots per shoots (Table 2; Fig. 1C). The root development was poor when IBA and NAA were added to the medium separately. The rooted saplings were transferred to vermiculate pots and hardened in a growth chamber for 15 d. Then they were transferred to polybags containing sand:garden soil:farm yard manure (2:2:1) (Fig. 1D). After 45 d, the hardened saplings were planted in the field. More than 82% plants were survived in the field and growth of plants was observed normal.

Our study revealed that node is an useful explant for *in vitro* rapid production of plantlets. The current protocol can be useful for rapid propagation of this economically important species for large scale cultivation and sustainable utilization.

Table 1—Effects of cytokinins on shoot initiation from uninodal explants of *S. androgynous*

Hormone (mg/L)		% explants formed shoots	Mean shoot length (cm±SE)	No. of shoot buds per explant (mean±SE)
BA	Kn			
0.1	0.01	21	1.05±0.11*	3.23±0.24
0.5	0.05	32	1.22±0.14	3.44±0.35
1.0	0.10	78	2.07±0.15	10.13±0.29
1.5	0.15	31	0.92±0.12	3.32±0.14
2.0	0.20	30	0.84±0.14	3.29±0.17
2.5	0.25	22	0.73±0.13	3.41±0.13

Each value represents mean±SE of 25 explants in three repeated experiments

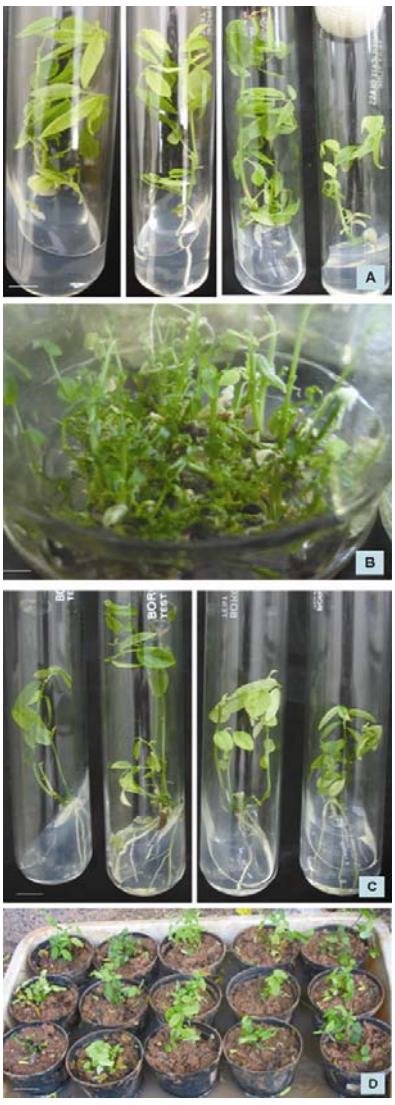

Fig. 1 (A-D)—Micropropagation of *S. androgynous*: (A) Shoot induction; (B) Shoot multiplication in MS medium with 1.0 mg/L BA and 0.1 mg/L Kn after 25 d, bar=8 mm; (C) Roots were regenerated from shoots grown on ½ MS medium with 0.5 mg/L IBA and 0.25 mg/L NAA after 30 d, bar=5 mm; & (D) Hardening of *in vitro* rooted plants, bar=6 mm.

Table 2—Effect of auxins on rooting response of *S. androgynous* in ½ MS medium

Hormone (mg/L)		No. of shoots (mean±SE)	Mean shoot length (cm±SE)	No. of roots (mean±SE)	Mean root length (cm±SE)
IBA	NAA				
0.1	-	0.82±0.12*	0.79±0.12	1.71±0.11	0.43±0.07
0.2	-	0.73±0.14	1.32±0.15	1.53±0.13	0.36±0.05
0.3	-	1.24±0.22	1.46±0.21	1.38±0.14	0.55±0.09
0.4	-	1.62±0.13	2.51±0.24	2.92±0.16	0.27±0.04
0.5	-	3.29±0.26	4.32±0.24	3.58±0.21	1.42±0.06
0.6	-	2.44±0.17	3.25±0.23	2.71±0.16	0.18±0.03
0.7	-	1.38±0.12	2.31±0.22	1.64±0.24	0.34±0.07
0.8	-	1.42±0.23	1.36±0.31	1.32±0.10	0.35±0.08
-	0.1	1.55±0.22	0.45±0.14	0.58±0.08	0.27±0.06
-	0.2	4.27±0.16	2.29±0.23	1.97±0.13	1.46±0.12
-	0.3	2.44±0.13	1.71±0.18	0.26±0.07	0.38±0.08
-	0.4	1.53±0.12	0.68±0.11	0.64±0.11	0.15±0.03
-	0.5	1.31±0.12	0.89±0.12	0.45±0.07	0.14±0.02
0.1	0.05	1.24±0.13	1.72±0.22	1.52±0.21	0.16±0.03
0.2	0.1	1.87±0.12	2.65±0.31	2.31±0.23	0.28±0.02
0.3	0.15	2.32±0.13	3.51±0.21	3.42±0.21	1.31±0.11
0.4	0.2	3.51±0.17	4.73±0.32	4.65±0.24	1.25±0.14
0.5	0.25	6.48±0.25	7.64±0.37	6.78±0.18	2.55±0.13
0.6	0.3	3.32±0.12	4.72±0.28	3.51±0.15	1.23±0.12
0.7	0.35	2.75±0.13	3.54±0.15	2.68±0.17	0.17±0.05
0.8	0.4	1.44±0.18	2.66±0.16	2.34±0.22	0.19±0.04

*Each value represents mean±SE of 25 explants in three repeated experiments

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