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In Vitro Propagation of *Satureja Abyssinica* (Benth.) Briq. – A Valuable Medicinal Plant

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Abstract
Satureja abyssinica is an endangered medicinal plant. The aim of this study was to develop micropropagation protocol for S. abyssinica using shoot tips. Cultures were initiated on MS media containing various concentrations of BAP (6-benzyl amino purine) and KIN (Kinetin). The best culture initiation (100%) with 16.30±0.95 mean number of shoot was attained in the presence of 1.5 mg/l BAP. In shoot multiplication media, different concentrations and combinations of BAP, KIN and NAA (α-Naphthalene acetic acid) were used. Maximum number of shoot (20.53±2.59) was obtained on MS medium fortified with 1.25 mg/l KIN. The greatest shoot height (3.41±0.36) was achieved in the presence of 0.5 mg/l BAP. The highest induction (100%) and maximum mean number of root (25.80±2.19) was performed on ½ MS medium augmented with 2.0 mg/l IBA (Indole-3- butyric acid). Best root length (1.98±0.09) was obtained on medium supplemented with 1.0 mg/l IBA. On rooting media, best mean number of shoot (5.46±0.79) and shoot length (5.80±0.69 cm) were received on medium containing 0.2 mg/l NAA and 0.5 mg/l IAA respectively. Highest survival rate (96%) of plantlets was achieved during acclimatization. The present study is point out a great micropropagation protocol for this medicinally important taxon.
Keywords: Medicinal plant, Micropropagation, *Satureja abyssinica*, Shoot tip

Abbreviations

BAP	6-Benzylaminopurine
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
IBC	Institute of Biodiversity Conservation
KIN	Kinetin
MS	Murashige and Skoog 1962
NAA	α-Naphthaleneacetic acid
PGRs	Plant growth regulators

INTRODUCTION

Medicinal plants have been identified and used throughout human history. Most cultures have a tradition of using plants medicinally. In Ethiopia, there is a tradition of using medicinal plants to treat different human and livestock ailments. About 70-90% of the population relies on traditional medicine mainly from medicinal plants for their primary health-care needs (Vasisht et al., 2002; Lehoux et al., 2012).

Cultivation of many medicinal plants is often not viable because of extended production times and very difficult to cultivate as they have low germination rates and very specific ecological requirements (Vines, 2004). According to Vasisht et al. (2002) in Asia and Africa more than 80% of medicinal plant supply comes from the wild sources. About 90% of medicinal plants used by industries are collected from the wild and over 70% of collections involve destructive harvesting (Sharma, et al., 2010). In Ethiopia there is no tradition of cultivating medicinal plants. Around 87% of medicinal plants are harvested from wild population except few herbs that are grown in the backyards (IBC, 2007; Lehoux et al., 2012; Malik et al., 2012). The destructive harvesting leads to endangered genetic stocks and diversity of medicinal plants.

Essential oils from numerous *Satureja* species were showed therapeutic activities such as antifungal and antimicrobial (Mihajilov-Krstev et al., 2010; Adiguzel et al., 2006), antioxidant, antidiabetic, antihyperlipidemic and stimulate reproduction (Abdollahi et al., 2003), antibacterial (Bensouici et al., 2013), antinociceptive and anti-inflammatory (Hajhashemi et al., 2002). *S. abyssinica* is a threatened medicinal plant in Ethiopia and locally known as “Mutansa”. The specie is annual or perennial herb in *Lamiaceae (Labiatae)* family, stem erect, branched with very short hairs and grows up to a height of 5-80 cm. It is growing in stony slopes, grassland at 900-2700 meters above sea level (Ryding, 2006). Essential oils were identified from aerial part of *S. abyssinica* and its *in vitro* antimicrobial and antifungal activities were investigated (Ketema et al., 2007). In Ethiopian traditional medicine, *S. abyssinica* is used to relieve headache, stop menstruation, relieve stomach pains, and treat various infectious diseases and skin inflammations (Teshome, et al., 2002).

The species propagate by seed but due to overexploitation, ecological change, overgrazing, agricultural

expansion and deforestation, it resulting in scarce distribution throughout the country which leads to threat the genetic stock. There is no report on conventional cultivation or *in vitro* micropropagation of this taxon for conservation or medicinal consumption. People collect the whole plant or before seed set and this is seriously damage the species. Therefore, the aim of this study was to overcome all these obstacles and to develop micropropagation protocol from shoot tip explants for production of genetically identical propagules and germplasm preservation of this threatened species of high medicinal value.

Materials and methods

Source of explant and surface disinfection

Matured seeds of *S. abyssinica* were collected from Oromia region, Arsi Zone, Amigna district, Sade-Wale Kebele from a particular place called Golja. Seeds were tightly sealed in cotton cloth and washed with detergent under running tap water. Then, surface disinfected with 70% (v/v) alcohol for 8 min, followed by sodium hypochlorite solution containing two drops of Tween-20 for 15 min. Finally, rinsed three to five times with sterile distilled water and sown in culture vessels (baby food jars of 6 cm diameter) containing 40 ml plant growth regulators-free MS (Murashige et al., 1962) medium. MS medium was enriched with 30 g/l sucrose (w/v), 8 g/l agar (w/v), pH was adjusted to 5.8 and autoclaved at 121ºC with a pressure of 105 Kpa for 15 min. The cultures were maintained in growth room under light intensity of 40 µmol m^{-2} s^{-1} and 16 h photoperiod provided by cool-white fluorescent lamps at a temperature of 25±2 ºC with 60% - 70% relative humidity.

Shoot initiation

Shoot tips obtained from *in vitro* germinated seedlings were used for culture initiation. Roots were excised from seedlings and cultured on MS media containing various concentrations of BAP (0.0, 0.5, 1.0, 1.5, 2.0 mg/l), KIN (0.0, 0.5, 1.0, 1.5, 2.0 mg/l) and BAP in combination with KIN. The medium was supplemented with 30 g/l sucrose (w/v) and pH was adjusted to 5.8 before addition of 8 g/l agar (w/v). A total of 18 explants per treatment with 9 explants per Magenta jars (7 cm diameter) were used. The cultures were maintained in culture room under light intensity of 40 µmol m^{-2} s^{-1} and 16 h photoperiod provided by cool-white fluorescent lamps at a temperature of 25±2 ºC with 60% - 70%.relative humidity. After 4 weeks, data were recorded as percentage of frequency, number of shoots per explant and shoot length.

Shoot multiplication

Shoot multiplication medium was enriched with different concentrations of BAP (0.0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0 mg/l), KIN (0.0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0 mg/l), BAP in combination with KIN and each in combination with 0.25 mg/l NAA. Ten shoots per culture vessel and 3 replications per treatment were used. All subsequent subcultures were done to fresh medium of the same composition at 4 weeks intervals and data were collected on number of shoots per explant and shoot length. Cultures were maintained in growth room with the same culture condition as for shoot initiation.

Root induction

Shoots obtained from best multiplication medium were used for root induction. Half strength MS medium supplemented with various concentrations of auxin (IBA, NAA and IAA), 15 g/l sucrose (w/v) and 8 g/l agar (w/v) were used. Similar concentrations (0.0, 0.02, 0.2, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mg/l) were used for each type of auxin (IBA, NAA and IAA). pH of the medium was adjusted to 5.8 prior to autoclaving at 121ºC with a pressure of 105 Kpa for 15 min. Thirty explants per treatment and 6 shoots per culture vessel with 5 replicates were used. Cultures were maintained in growth room with the same culture condition as for culture initiation and shoot multiplication experiments. The percentage of shoots producing roots, number and length of roots, number and length of shoots were recorded after 30 days.

Acclimatization

Well rooted plantlets were carefully removed from the culture vessels and gently washed under running tap water to avoid all media residues. Then, planted in plastic pots (20 cm diameter upper) containing sand - red soil - compost in ratio of 2:1:2 respectively. The potted plantlets were covered with polyethylene bags for a week and kept in greenhouse. The survived plantlets were recorded after 60 days of acclimatization.

Experimental design and data analysis

A completely randomized design (CRD) was used throughout the experiments. Data were subjected to analysis of variance (ANOVA) using statistical data analysis software SPSS version 20.0 at 5% probability level.

Results

Culture initiation

Best inductions were observed after 10 days from the beginning of culture. Culture on MS medium supplemented with BAP and KIN alone and BAP in combination with KIN, showed a significant difference at P = 0.05 (Table 1). All cultures were displayed high quality appearance except 0.5 mg/l BAP medium induced vitrified shoots with a mean number of 14.75±1.09 (Figure 1). Highest initiation percentage (100%) and maximum mean number (16.30±0.95) of shoot were obtained on medium augmented with 1.5 mg/l BAP. In case of KIN, maximum number (8.65±2.38) and highest percentage (100%) of shoot initiation were observed at a concentration of 2.0 mg/l. Among MS media containing different concentrations of BAP in combination with KIN, highest percentage (100%) and maximum number (11.65±3.31) of shoot were recorded on MS medium enhanced with 1.5 mg/l BAP + 0.5 mg/l KIN. In the present study, MS media supplemented with BAP alone were found to be the superior over KIN alone or BAP in combination with KIN for shoot induction in this taxon. The greatest shoot height (2.65±0.29 and 2.42±0.22) was achieved on control and 2.0 mg/l KIN medium respectively.

Table 1. Efficiency of BAP and KIN on shoot initiation from *in vitro* germinated seedlings

Treatments (mg/l)		Initiation	No. of shoots/explant	shoot length (cm)	Remarks
BAP	KIN	frequency (%)	Mean ± SE	Mean ± SE	
0.0	0.0	95	3.80 ± 0.82^{efg}	2.65 ± 0.29^{a}	-
0.5	-	95	14.75 ± 1.09^{ab}	1.75 ± 0.15^{b}	Vitrified/glass-like appearance
1.0	-	100	2.05 ± 0.23^{fi}	0.90 ± 0.10^{c}	-
1.5	-	100	16.30 ± 0.95^{a}	1.62 ± 0.15^{b}	-
2.0	-	100	5.15 ± 0.94^{df}	1.47 ± 0.15^{bc}	-
0.5	0.5	85	1.30 ± 0.12^{ghi}	0.72 ± 0.10^{c}	-
1.0	0.5	100	2.80 ± 0.33^{efh}	1.07 ± 0.12^{c}	-
1.5	0.5	100	11.65 ± 3.31^{bc}	1.50 ± 0.13^{bc}	-
2.0	0.5	100	6.20 ± 0.94^{de}	1.40 ± 0.15^{bc}	-
-	0.5	90	2.70 ± 0.62^{efi}	1.47 ± 0.26^{bc}	-
-	1.0	90	1.65 ± 0.18^{fi}	0.82 ± 0.15^{c}	-
-	1.5	95	2.65 ± 0.39^{efi}	1.40 ± 0.23^{bc}	-
-	2.0	100	8.65 ± 2.38^{cd}	2.42 ± 0.22^{a}	-

Means within columns having different letter (lower case) in superscript are significantly different at $p < 0.05$.

Figure 1. Shoot initiation from *in vitro* germinated seedling on MS medium enriched with **a)** 1.5 mg/l BAP, **b)** 0.5 mg/l BAP, c) 0.5 mg/l KIN. Bars =.1 cm

Multiplication

Effect of BAP and KIN

An efficient proliferation rate was observed from 10-15 days. A significant difference was detected from culture on various concentrations of KIN and BAP at P = 0.05 after 4 weeks (Table 2). High-quality, attractive culture and maximum mean number (20.53±2.59) of shoot were attained on medium containing 1.25 mg/l KIN. The next highest mean number (16.86±3.10) of shoot was obtained in the presence of 1.75 mg/l BAP. Shoots on all culture media had healthy appearance and grew vigorously (Figure 2). Maximum shoot length (3.41±0.36) was achieved on MS medium supplemented with 0.5 mg/l BAP.

Table 2. Effect of different concentrations of BAP and KIN on shoot proliferation from shoot tips of *S. abyssinica*

Treatment (mg/l) **BAP**	No. of shoots/ explant Mean ± SE	Shoot length (cm) Mean ± SE	Remarks
0.00	3.43±0.44^{e}	2.73±0.27^{b}	-
0.25	11.60±1.90^{c}	1.68±0.18^{cd}	-
0.50	9.76±1.45^{ce}	3.41±0.36^{a}	-
0.75	15.30±1.93^{b}	2.26±0.24^{bc}	-
1.00	9.76±2.10^{ce}	1.33±0.93^{d}	-
1.25	13.96±1.75^{bd}	1.96±0.21^{c}	-
1.50	13.53±2.03^{bd}	1.48±0.09^{cd}	-
1.75	16.86±3.10^{b}	1.16±0.05^{d}	-
2.00	15.16±2.27^{b}	1.46±0.07^{cd}	-
KIN			
0.25	9.06±1.06^{cd}	2.68±0.31^{b}	-
0.50	5.30±0.68^{de}	1.32±0.10^{d}	-
0.75	11.03±1.33^{c}	2.51±0.34^{bc}	-
1.00	11.36±1.55^{c}	1.46±0.15^{cd}	-
1.25	20.53±2.59^{a}	1.86±0.22^{cd}	-
1.50	9.43±1.00^{ce}	1.80±0.10^{cd}	-
1.75	12.93±1.49^{bd}	1.53±0.12^{cd}	-
2.00	12.86±1.48^{bc}	1.56±0.12^{cd}	-

Means within columns having different letter (lower case) in superscript are significantly different at $p < 0.05$.

Synergistic effect of BAP and KIN

Among the various combinations of BAP and KIN tested, the MS medium augmented with (0.5 mg/l BAP + 0.5 mg/l KIN, 0.5 mg/l BAP + 1.0 mg/l KIN, 1.5 mg/l BAP + 0.5 mg/l KIN, 2.0 mg/l BAP + 0.5 mg/l KIN) were produced maximum mean number of shoots (12.20±2.07, 12.60±2.42, 12.56±1.84, 12.43±1.61) per explant respectively. Between all these means there was no significant difference (Table 3). Plant growth regulators-free MS medium resulted in highest average (2.73±0.27) of shoot length. Cultures under this combination were appeared healthy but poor in proliferation rate as compare to BAP and KIN alone.

Table 3. Effect of BAP in combinations with KIN on shoot proliferation of *S. abyssinica*

Treatment (mg/l) BAP	KIN	No. of shoots/ explant Mean ± SE	Shoot length (cm) Mean ± SE	Remarks
0.0	0.0	3.43±0.44^{d}	2.73±0.27^{a}	-
0.25	0.5	4.70±0.68^{ce}	1.55±0.14^{c}	-
0.25	1.0	3.56±0.52^{d}	1.21±0.08^{d}	-
0.25	1.5	4.43±0.56^{cd}	2.03±0.18^{b}	-
0.25	2.0	3.83±0.27^{d}	1.76±0.16^{bc}	-
0.5	0.5	12.20±2.07^{a}	1.21±0.08^{d}	-
0.5	1.0	12.60±2.42^{a}	1.28±0.08^{d}	-
0.5	1.5	3.76±0.32^{d}	1.76±0.17^{b}	-
0.5	2.0	5.26±1.04^{ce}	1.25±0.07^{d}	-
0.75	0.5	6.86±1.15^{bd}	1.60±0.14^{c}	-
0.75	1.0	6.36±1.35^{ce}	1.53±0.10^{ce}	-
0.75	1.5	3.33±0.56^{de}	1.66±0.15^{c}	-
0.75	2.0	4.26±0.34^{ce}	1.76±0.10^{bd}	-
1.0	0.5	7.20±0.98^{bd}	1.50±0.10^{ce}	-
1.0	1.0	8.16±1.20^{b}	1.61±0.13^{c}	-
1.0	1.5	7.06±0.88^{bd}	1.43±0.11^{ce}	-
1.0	2.0	5.96±1.09^{ce}	1.61±0.12^{c}	-
1.25	0.5	9.96±1.31^{ab}	1.46±0.10^{ce}	-
1.25	1.0	6.13±0.84^{ce}	1.53±0.10^{ce}	-
1.25	1.5	7.96±2.00^{b}	1.31±0.08^{d}	-
1.25	2.0	4.80±0.53^{ce}	1.45±0.10^{ce}	-
1.5	0.5	12.56±1.84^{a}	1.48±0.08^{ce}	-
1.5	1.0	8.56±0.93^{b}	1.30±0.09^{d}	-
1.5	1.5	11.36±1.44^{a}	1.23±0.07^{d}	-
1.5	2.0	5.13±0.48^{ce}	1.73±0.09^{bd}	-
1.75	0.5	7.73±1.29^{b}	1.55±0.14^{c}	-
1.75	1.0	6.46±1.03^{ce}	1.48±0.11^{ce}	-
1.75	1.5	5.90±0.86^{ce}	1.71±0.15^{bd}	-
1.75	2.0	4.73±0.50^{ce}	1.43±0.08^{cd}	-
2.0	0.5	12.43±1.61^{a}	1.18±0.06^{de}	-
2.0	1.0	7.40±1.66^{bc}	1.36±0.08^{d}	-
2.0	1.5	5.00±0.66^{ce}	1.78±0.13^{b}	-
2.0	2.0	4.50±0.61^{ce}	2.03±0.15^{b}	-

Means within columns having different letter (lower case) in superscript are significantly different at $p < 0.05$.

Synergistic effect of BAP and NAA

The MS medium containing various concentrations of BAP in combination with low level of NAA was produced shoot that not significantly different from plant growth regulators-free medium (Table 4). Shoot proliferation and elongation rate was declined. MS medium containing 0.25, 0.5, 0.75 mg/l BAP each in combination with 0.25 mg/l NAA were produced spontaneous roots. Highest mean of shoot length (2.73±0.27) was obtained on control medium.

Table 4. Influence of different concentrations of BAP in combination with NAA on shoot multiplication of *S. abyssinica*

Treatment (mg/l)		No. of shoots/ explant Mean ± SE	Shoot length (cm) ± SE	Mean	Remarks
BAP	NAA				
0.0	0.0	3.43 ± 0.44^{ab}	2.73 ± 0.27^{a}		-
0.25	0.25	2.46 ± 0.32^{bcd}	1.23 ± 0.10^{c}		-
0.5	0.25	2.90 ± 0.42^{bcd}	1.30 ± 0.07^{c}		-
0.75	0.25	2.20 ± 0.24^{d}	1.56 ± 0.16^{bc}		-
1.0	0.25	1.76 ± 0.21^{d}	1.76 ± 0.20^{b}		-
1.25	0.25	1.76 ± 0.20^{d}	1.38 ± 0.11^{bc}		-
1.5	0.25	1.96 ± 0.21^{d}	1.60 ± 0.16^{bc}		-
1.75	0.25	4.03 ± 0.59^{a}	1.45 ± 0.09^{bc}		-
2.0	0.25	3.26 ± 0.25^{ac}	1.18 ± 0.06^{c}		-

Means within columns having different letter (lower case) in superscript are significantly different at $p < 0.05$.

Synergistic effect of KIN and NAA

On MS medium containing KIN in combination with NAA, the poorest shoot proliferation rate with poor shoot quality was observed. Mean number of shoot per explant in this treatment was lower than on plant growth regulators-free MS medium (Table 5). Maximum number of shoot (3.43±0.44) and shoot length (2.73±0.27) were achieved on plant growth regulators-free medium.

Table 5. Influence of different concentrations of KIN in combination with NAA on shoot multiplication of *S. abyssinica*

Treatment (mg/l)		No. of shoots/ explant Mean ± SE	Shoot length (cm) ± SE	Mean	Remarks
KIN	NAA				
0.0	0.0	3.43 ± 0.44^{a}	2.73 ± 0.27^{a}		-
0.25	0.25	1.90 ± 0.18^{b}	1.96 ± 0.14^{b}		-
0.5	0.25	2.20 ± 0.32^{b}	1.36 ± 0.10^{c}		-
0.75	0.25	2.33 ± 0.30^{b}	2.01 ± 0.14^{b}		-
1.0	0.25	2.23 ± 0.33^{b}	1.65 ± 0.13^{bc}		-
1.25	0.25	2.00 ± 0.20^{b}	1.98 ± 0.20^{b}		-
1.5	0.25	2.30 ± 0.24^{b}	2.05 ± 0.17^{b}		-
1.75	0.25	1.86 ± 0.23^{b}	2.10 ± 0.18^{b}		-
2.0	0.25	1.70 ± 0.17^{b}	2.08 ± 0.18^{b}		-

Means within columns having different letter (lower case) in superscript are significantly different at $p < 0.05$.

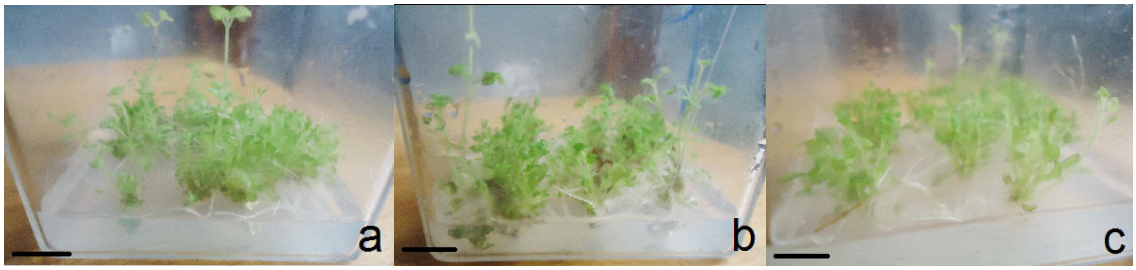

Figure 2. Shoot multiplication on MS medium enriched with **a)** 1.25 mg/l KIN, **b)** 1.75 mg/l BAP, **c)** 1.75 mg/l KIN. Bars = 1 cm.

Root induction

Within two weeks, all explants were responded well excluding those cultured on medium containing various concentrations of IAA. Mean comparison between IBA, IAA and NAA showed a significant difference ($P < 0.05$) in all measured parameters. In all media the nature of root was superficially spread on the surface of the

medium and this is very important to easily detach from medium during acclimatization. In the presence of 2.0 mg/l IBA, maximum number (25.80±2.19) of root with greatest frequency (100%) was recorded. The next highest mean (20.93±1.54) of root was achieved on medium fortified with 2.5 mg/l NAA (Table 6, Figure 3). However, very thin and weakest root formation was achieved on MS medium fortified with IAA. At a concentration of 0.02 mg/l IAA compact calli were produced. The maximum root length (1.98±0.09) was obtained on medium supplemented with 1.0 mg/l IBA. On rooting medium, best mean number (5.46±0.79) and mean length (5.80±0.69) of shoot were received in the presence of 0.2 mg/l NAA and 0.5 mg/l IAA respectively.

Table 6. Efficiency of IBA, NAA and IAA on root number, root length, shoot number and shoot length of *S. abyssinica*

Treatment(mg/l)	Root formation frequency (%)	Mean number of roots	Mean root length (cm)	Mean no. of shoot	Mean shoot length (cm)
IBA					
0.0	40	1.26 ± 0.32^{g}	0.86 ± 0.22^{c}	1.13 ± 0.06^{d}	4.36 ± 0.51^{b}
0.02	86.6	3.40 ± 0.60^{fg}	1.86 ± 0.17^{a}	3.33 ± 0.66^{bc}	2.06 ± 0.13^{cd}
0.2	86.6	2.80 ± 0.46^{fg}	1.38 ± 0.17^{b}	3.30 ± 0.54^{bc}	1.83 ± 0.14^{cd}
0.5	100	17.50 ± 1.60^{cd}	1.76 ± 0.16^{a}	3.37 ± 0.74^{b}	2.60 ± 0.21^{c}
1.0	100	21.16 ± 1.65^{b}	1.98 ± 0.09^{a}	2.83 ± 0.43^{bc}	2.65 ± 0.17^{c}
1.5	100	21.00 ± 1.86^{bd}	1.45 ± 0.09^{b}	2.00 ± 0.27^{cd}	2.66 ± 0.18^{c}
2.0	100	25.80 ± 2.19^{a}	1.36 ± 0.07^{b}	2.00 ± 0.30^{cd}	2.63 ± 0.17^{c}
2.5	100	23.26 ± 1.64^{ab}	1.21 ± 0.05^{bc}	2.10 ± 0.29^{cd}	2.20 ± 0.16^{cd}
3.0	100	22.23 ± 2.06^{ab}	1.05 ± 0.02^{c}	1.56 ± 0.25^{cd}	2.63 ± 0.36^{c}
IAA					
0.02	0	0.00 ± 0.00^{g}	0.00 ± 0.00^{e}	0.76 ± 0.21^{d}	0.66 ± 0.16^{d}
0.2	93.3	6.10 ± 0.77^{ef}	1.41 ± 0.12^{b}	2.10 ± 0.29^{cd}	4.80 ± 0.53^{ab}
0.5	93.3	5.26 ± 0.75^{f}	0.53 ± 0.06^{d}	2.00 ± 0.36^{cd}	5.80 ± 0.69^{a}
1.0	83.3	6.90 ± 0.91^{ef}	0.59 ± 0.08^{d}	2.50 ± 0.33^{c}	5.30 ± 0.60^{ab}
1.5	73.3	4.60 ± 1.04^{fg}	0.82 ± 0.17^{c}	1.83 ± 0.33^{cd}	4.06 ± 0.55^{b}
2.0	96.6	9.36 ± 1.24^{e}	0.90 ± 0.11^{c}	1.66 ± 0.20^{cd}	5.63 ± 0.61^{a}
2.5	90	2.80 ± 0.45^{fg}	0.31 ± 0.03^{d}	1.63 ± 0.21^{cd}	4.13 ± 0.42^{b}
3.0	100	3.36 ± 0.53^{fg}	0.41 ± 0.03^{d}	1.76 ± 0.14^{cd}	4.86 ± 0.45^{ab}
NAA					
0.02	36.6	1.93 ± 0.59^{fg}	0.45 ± 0.11^{cd}	3.23 ± 0.59^{bc}	2.61 ± 0.13^{c}
0.2	100	14.10 ± 1.03^{d}	1.75 ± 0.07^{a}	5.46 ± 0.79^{a}	4.73 ± 0.31^{b}
0.5	100	17.30 ± 1.59^{c}	1.46 ± 0.08^{b}	2.30 ± 0.37^{c}	4.66 ± 0.47^{b}
1.0	100	15.76 ± 1.66^{d}	1.00 ± 0.00^{c}	1.00 ± 0.00^{d}	2.13 ± 0.19^{cd}
1.5	100	20.60 ± 1.34^{bc}	1.28 ± 0.06^{bc}	1.96 ± 0.26^{cd}	2.33 ± 0.16^{cd}
2.0	100	20.76 ± 1.88^{bd}	1.00 ± 0.00^{c}	1.16 ± 0.20^{d}	1.73 ± 0.17^{cd}
2.5	100	20.93 ± 1.54^{bd}	1.18 ± 0.04^{bc}	1.63 ± 0.35^{cd}	1.43 ± 0.18^{d}
3.0	100	15.10 ± 1.25^{d}	1.00 ± 0.00^{c}	1.36 ± 0.16^{d}	1.56 ± 0.14^{d}

Means within columns having different letter (lower case) in superscript are significantly different at $p < 0.05$.

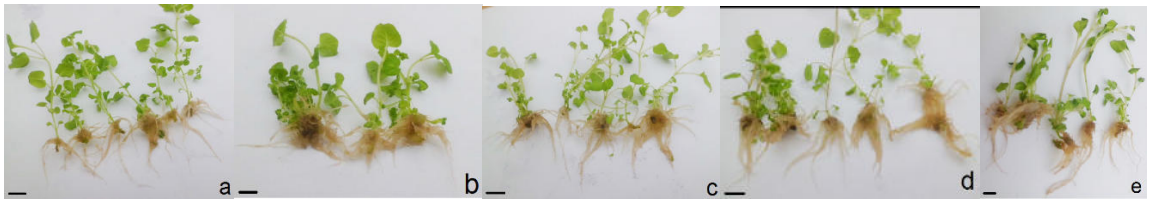

Figure 3. Root induction on ½ MS medium enriched with **a)** 2 mg/l IBA, **b)** 3 mg/l IBA, **c)** 1.5 mg/l NAA, **d)** 2.5 mg/l NAA, **e)** 2 mg/l NAA. Bars = 1 cm.

Acclimatization

Under greenhouse conditions plantlets were successfully established with 96% survival rate and displayed the typical feature of species (Figure 4).

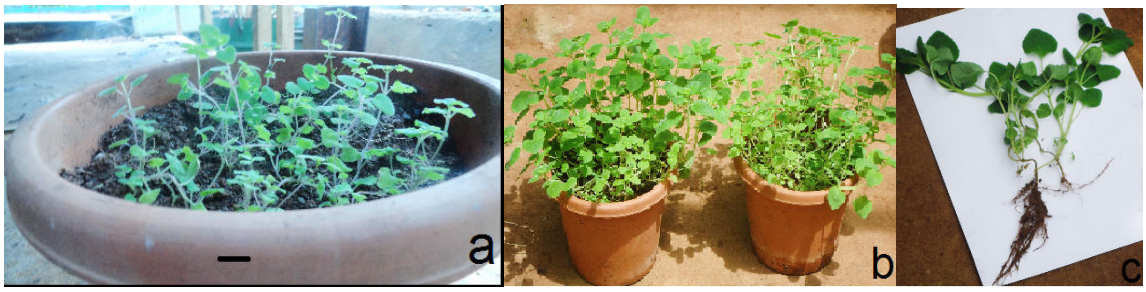

Figure 4. Acclimatization of plantlets of *S. abyssinica* in greenhouse. **a)** After 60-days, **b)** After 90-days, **c)** uprooted 90-days old plant. Bars = 1 cm.

Discussion

Shoot initiation

Shoot initiation was performed with 12 different concentrations of BAP, KIN and combination of them. Results of the present study revealed that, 1.5 mg/l BAP was the optimal concentration for shoot initiation. Effect of KIN was poor as compared to BAP alone or BAP plus KIN. This indicated that BAP was found to be superior over KIN in culture initiation and shoot bud break. Shoot induction efficiency of BAP was also observed in *Solanum nigrum* (Rathore et al., 2013), *Achyranthes bidentata* (Hossain et al., 2013), *Alternanthera sessilis* (Gnanaraj et al., 2011), *Ruta graveolens* (Bohidar et al., 2008). In present study, vitrified shoots were noticed in the presence of 0.5 mg/l BAP and this is in agreement with (Naml et al., 2010) who reported vitrification in *Hypericum retusum* due to BAP. Further increasing BAP concentration alone or in combination with KIN was reduced mean number of initiated shoot. However, at higher concentration KIN encouraged mean number of shoot. Similarly, Baskaran et al. (2005) reported the same phenomena in *Eclipta alba*.

Multiplication

Sixty-five different concentrations of plant growth regulators viz. BAP, KIN alone and each in combination with 0.25 mg/l NAA were performed for shoot multiplication. Results of different concentrations revealed that BAP, KIN alone were better than BAP plus KIN and each in combination with NAA. The maximum shoot per explant was observed in the presence of 1.25 mg/l KIN. The investigation revealed that KIN was the most effective cytokinin than BAP for shoot multiplication in this species. Previous reports showed the efficiency of KIN in mass propagation of some medicinal plant such as *Majorana hortensis* (Tejavathi et al., 2012), *Operculina turpethum* (Sebastinraj et al., 2013). The effectiveness of KIN not only to overcome apical dominance, stimulated high number of shoot buds and released lateral buds from dormancy in shoot proliferation but micro-shoots form this cytokinin also attained high quality with attractive morphology as compared to other treatments. Shoot proliferation degrees were increased with increase in concentrations of BAP and KIN independently or in combination with each other. However, supra-optimal concentrations of BAP and KIN showed significant reduction in multiple shoot formation, which agree with Shiferaw et al. (2015) in *Glinus lotoides,* Chen et al. (2006) in *Bupleurum kaoi*, Baskaran et al. (2005) in *Eclipta alba,* Balaraju et al. (2008) in *Vitex agnus-castus*, Rasool et al. (2009) in *Prunella Vulgaris*.

Different plant species are reacting differently to different hormone type. In present investigation, explants showed very poor response in the presence of NAA. There was positive interaction effect of BAP × NAA relatively in Shoot proliferation than KIN × NAA. This shown the induction capacity of BAP in shoot bud break than KIN when combined with NAA. Mean number of shoot on medium containing KIN plus NAA was lower than on control medium and some shoots were died. Results of this study indicated that the presence of NAA in combination with cytokinin (BAP & KIN) exhibited negative effect on shoot multiplication and this is in agreement with (Fracaro et al., 2001) report on *Cunila galioides.* However, reports in other medicinal plants such as *Holostemma ada- kodien* (Pushparajan et al., 2014), *Withania somnifera* (Duhan et al., 2014), *Polianthes tuberosa* (Naz et al., 2012), *Hypericum retusum* (Naml et al., 2010), *Lippia filifolia* (Peixoto et al., 2006) were disagreement to present finding. An average of shoot per explant and poor culture quality such as drying of shoot, induction of spontaneous root suggested the negative impact of NAA and presence of it in shoot proliferation of this plant species could be inappropriate. This could be due to the presence of high levels of endogenous NAA and addition of exogenous NAA in the medium resulted in increased the total level of auxin which led to increase apical dominancy, reduced shoot proliferation and promote root induction.

Root induction

Largest mean of root, root length, no callus, sturdy and health appearance plantlets were observed in the presence of IBA. IBA was found to be the superior over NAA and IAA for root induction in this taxon. Several scholars were reported the efficiency of IBA in different medicinal plant species including *Glinus lotoides*

(Shiferaw et al., 2015), *Psoralea corylifolia* (Pandey et al., 2013), *Curculigo orchioides* (Nagesh, 2008), *Chlorophytum borivilianum* (Bera et al., 2009), *Stevia rebaudiana* (Jitendra et al., 2012), *Origanum sipyleum* (Oluk et al., 2009), *Solanum nigrum* (Rathore et al., 2013), *Cunila galioides* (Fracaro et al., 2001), *Achyranthes aspera* (Sen et al., 2014), *Vitex agnus-castus* (Balaraju et al., 2008), *Majorana hortensis* (Tejavathi et al., 2012), *Operculina turpethum* (Sebastinraj et al., 2013).

Although, best root induction was achieved on MS medium enriched with different concentrations of NAA, the overall analyzed parameters such as average of root and root length were lower. However, maximum root induction efficiency of NAA was reported in *Lippia filifolia* (Peixoto et al., 2006), *Clitoria ternatea* (Rout, 2004) and *Thymus satureioides* (Aicha at al., 2013). Root induction capability of IAA was poor and large number of rootless shoots were observed. This is parallel with previous report in *Ruta graveolens* (Bohidar et al., 2008). However, Echeverrigaray et al. (2010) reported the efficiency of IAA over IBA and NAA in root induction.

Acclimatized plantlets were grown vigorously and there was no abnormal plants were observed during acclimatization process. The plantlets having more than one branch with abundant leaves were sturdy and survived easily than plantlets with a single shoot and less leaf. This could be enhanced the photosynthesis capability of plantlets.

In Conclusion, *In vitro* propagation of *S. abyssinica* using MS medium supplemented with 1.25 mg/l KIN for shoot proliferation and 2.0 mg/l IBA for root induction were very important for mass propagation and germ preservation of this medicinally important plant.

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