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Full Length Research Paper

A possible cultivation system towards genetic improvement of *Plectranthus edulis* (Vatke) Agnew from shoot tip and nodal explants

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Plectranthus edulis **Vatke belongs to the family of Lamiaceae, which occurs both as a wild and cultivated species. The major constraint in the cultivation of *P. edulis* is its low productivity due to shortage of planting materials and incidence of pests and diseases. In this study, an efficient protocol was established for the micropropagation of *P. edulis* germplasm using shoot tip and nodal explants. Explants were sterilized using different concentrations of Sodium hypochlorite (NaOCl) for different times of exposure. MS medium supplemented with different types and concentrations of auxin and cytokinin were used for culture initiation, shoot multiplication and root induction. NaOCl at a concentration of 2% and exposure time of 5 min gave 74.50±0.5% of clean culture for nodal and 69.83±0.76 from shoot tip. Six-Benzylaminopurine at 1.5 µM was found to be an optimum concentration for shoot induction, yielding 91.67±0.58% for nodal and 85.57±0.51% for shoot tip explants 3 weeks after culture. The combination of 2.0 µM BAP with 1.0 µM IAA was found to be the optimum concentration yielding 10.28±0.06 and 6.12±0.01 shoots per explants for nodal and shoot tip, respectively for shoot multiplication. Half strength MS medium with 2.0 µM IBA and 1.0 µM NAA gave the highest rooting percentage (97.00±0.28) with optimum root number (33) and length (3 cm). Up on acclimatization and transplanting, 83% survival efficiency was observed on soil mix ratio of 2:1:1 decomposed coffee husk, forest soil and sand, respectively. There were no observable variations with respect to morphology and growth characteristics to the greenhouse raised parent plants. The results obtained in this study permit the development of mass propagation protocol that could enable large scale commercial production of this highly demanded cultivar true-to type and provide a possible system towards genetic improvement of the crop.**

Key words: Explants, micropropagation, nodal culture, microshoots, plant growth regulators, plantlet.

INTRODUCTION

Plectranthus edulis Vatke is a tuber crop plant belongs to the family of Lamiaceae, in which the genus *Coleus* consists of over 350 tuber bearing and non-tuber bearing species. Although the origin of *P. edulis* was from Ethiopia, currently, it is widely distributed in Asia, Australia and other African countries (Codd, 1985), growing in mid and high altitude areas ranging from 1880 to 2200 m above sea level (Demissie, 1991; Greenway, 1944; Ryding, 2000).

P. edulis was a major traditional food crop for the rural

communities of southwest Ethiopia. Nowadays, the potential uses of this indigenous crop have become deteriorated and the natural populations are rapidly disappearing as a result of the shortage of planting materials and attack by pests and microbial diseases (Ryding, 2000; Pratibha et al., 2011) to use for conventional propagation methods by tubers and stem cuttings.

Although several protocols have been developed for the micrpropagation of the species of genus Coleus such as *Plectranthus forskohlii* (Reddy et al., 2001; Praveena et al., 2012), *Plectranthus Blumei* (Smith and Murashige, 1982; Rani et al., 2006; Gaurav et al., 2010; Pratibha et al., 2011). *In vitro* propagation of *P. edulis* has recently been reported by Tsegaw and Feyisaa (2014) using meristem culture but there was no studies reported on the use of nodal and shoot tip explants and their sterilization experiments to avoid contamination yet. Therefore, there is need to develop plant tissue culture techniques for this species. One of the most important application of tissue culture as a tool of biotechnology is its application in further genetic improvement of the species and in the production of disease free plant materials. Besides, it enables production of large number of plantlets in a short period of time as well as conservation of germplasm under controlled conditions in small spaces with reduced labor requirement (Abraham, 2009). Moreover, each variety requires its own regeneration protocol (Gonzalez et al., 1999). The aim of this study was to develop a micropropagation protocol for *P. edulis* using shoot tip and nodal explants.

MATERIALS AND METHODS

Plant material

Healthy tubers of *P. edulis* were obtained from the Institute of Biodiversity Conservation (IBC), located in Addis Ababa (the capital city of Ethiopia), planted in a tin pot contained a sterilized soil that had a mixture of loam soil, coffee husk and sand (2:1:1, respectively), kept and grown under greenhouse condition of the College of Natural Science, Jimma university until used for experiment. The growing plant materials were daily watered with tap water and sprayed with 0.3% Mancozeb at 15 days interval to control fungal infection. Two months old, healthy and vigorous plants were used as a source of explants.

Culture medium and growth regulators stock preparation

MS media was prepared by dissolving 4405.19 gl^{-1} of the readily available medium with vitamins (company name) with sucrose (30 g) in double distilled water. The pH of the solution was adjusted to 5.7 to 5.8 using 0.1 N HCl or 1N KOH before making up to final volume. For solidification, agar powder (Company name) 0.8% (w/v) was added to the moderately warm solution and then, melted by constant stirring. The medium (25 ml) was dispensed into borosilicate test tubes, plugged with cotton, covered with aluminum foil and autoclaved at 121°C for 20 min in a vertical autoclave. Different growth regulators viz. 6-benzylamino purine (BAP), Kinetin, α-naphthalene acetic acid (NAA), and indol-3-butyric acid as stock solutions of 1 mg/L and required amount for specific concentrations were added to the medium before autoclaving.

Explant preparation and sterilization

Healthy and juvenile plants were taken from mother stock *P. edulis* maintained in greenhouse. Both shoot tip (1 to 1.5 cm long) and nodal explants (1.5 to 2 cm long) were excised and sterilized with different concentrations of NaOCl for different times of exposure, washed three times with tap water and detergent using sponge. Thereafter, the explants were kept under running water for 10 min and finally washed with double distilled water.

Culture initiation

Sterilized explants were cultured on agar solidified (0.8% agar-agar) full strength MS basal medium supplemented with different concentrations of BAP (0, 0.5, 1.0, 1.5, and 2.0 mg/L) and Kinetin (0, 1.0, 2.0, and 3.0 mg/L) to be tested for shoot induction rate of shoot tip and nodal explants in CRD design in 4x3x2 factorial combinations.

Shoot multiplication

Shoot buds initiated from those explants that had responded well to the prevailing culture conditions were transferred singly onto a shoot multiplication MS medium containing different concentration and combination of BAP and NAA. The experiment was thus being arranged in a 4x3x2 factorial in CRD. After two-three weeks, cultures proliferating shoot clumps were divided and sub cultured on to a fresh medium of similar composition.

Root induction

Well-developed microshoots obtained from shoot multiplication media were transferred for rooting on agar solidified (0.8% agar-agar) half strength MS basal medium was supplemented with 3% sucrose and different concentrations of IBA and NAA. The experiment was laid with treatment of five concentrations for IBA (0, 0.5, 1.0, 1.5, 2.0 and 2.5 mg/L) and three concentrations for NAA (0, 0.5, 1, and 1.5 mg/L) auxins in CRD in 5 x 3 factorial combination.

Acclimatization

Plantlets with well developed from rooting media were isolated, washed and then treated in light polyethylene pot covered by 70% shade net above it. The system was designed to give high humidity (80 to 90%) to prevent desiccation for ten days, prior to their transfer to a shade house. Starting from the 15th day, the RH within the system was reduced to gradually to 60% at the end of the month. After the month, the plantlets were transferred to a 70% shade net, where they were retained for a month. Later they were transferred to a 30% shade net and maintained there for three weeks. The numbers of survived plantlets were recorded in each step.

Experimental design and treatments

All experiments were laid in a Completely Randomized Design (CRD) with factorial treatment combinations, having three replications per treatment and five explants per jar under each replicate. All the experiments were repeated two times to ensure reproducibility of the results and the average of these two were considered for analysis. Prior to laying the multiplication and rooting experiments, sufficient explants were made to multiply till the desired numbers of explants are generated. At all times, explants were cultured on a PGR-free medium prior to their use for an experiment; so as to avoid any sort of carryover effects from the previous culture medium they were retained. Controls were set for each experiment with zero concentration of the analyses considered.

Data recording

After sterilization experiment, the number and percentage of explants affected by contamination and tissue death was recorded during the first two weeks of culture for shoot tip and nodal explants independently. For the second experiment, the number and/or percentage of explants forming shoot buds was recorded after four weeks for shoot tip and nodal explants independently. Number of shoots proliferated from each shoot bud on multiplication media was counted at three weeks interval during sub culturing. The number of roots (including the main roots and their branches), shoot length and the length of the roots was recorded after three weeks of culture for experiment four.

Data analysis

Average of the data collected from the two repetitions for each experiment were independently subjected to statistical analysis using the SAS statistical software (version 9.2) and ANOVA was constructed, followed by mean separation using appropriate procedures (REGWQ). When ANOVA indicated significant treatment effects (5, 1 or 0.1%) based on the F-test, probability level of 0.05 ($p \leq 0.05$) was considered to determine which treatments were statistically different from the other treatments.

RESULTS AND DISCUSSION

Effects of NaOCl concentrations and exposure time on sterilization of *P. edulis* explants

The analysis of variance showed that the concentration of active chlorine in sodium hypochlorite solution, time duration of explants exposure to the sterilants and interaction of concentrations to time duration had very highly significant effect ($p < 0.0001$) on both of contamination and clean culture of shoot tip and nodal explants .Very highly significant difference had also been revealed between the two types of explants (treatment * explants = $p < 0.0001$) indicating that the level of contamination and clean culture was influenced by the concentration of NaOCl and duration of exposure time and the mean average value for contamination and clean culture of node exceeded shoot tip. The highest rate of clean culture (69.83±0.76%) was obtained from treatment concentration of 1% active chlorine (in NaOCl solution) with five minute exposure duration for shoot tip explants. For nodal explants, the same percentage concentration and time exposure duration was found to be the most effective treatment combination with mean average result of 74.50±0.50 clean culture (Table 1). The highest percentage of contamination were observed from nodal and shoot tip explants due to the low concentration NaOCl (0.5%) at short exposure of time (three and five minute) for both types of explants. In the higher concentration of sodium hypochlorite (2% NaOCl) and long exposure of time (seven and nine minutes), percentage of contamination was very low but tissue death is highest.

In the present study, one percent sodium hypochlorite treatment of five minutes exposure of time was optimum for sterilization of *P. edulis* explants (Table 1). Different scholars use mercuric chloride for explant surface sterilization (Bhattacharya and Bhattacharya, 2001; Rani et al., 2006) but using mercuric chloride for surface sterilization is not environmentally friendly so that it is not recommendable.

Effect of different concentration and combination of BAP and Kinetin shoot initiation on nodal and shoot tip explants of *P. edulis*

Aseptic shoot tips and nodal cultures were transferred on MS media fortified with different concentrations of BAP in combination with kinetin for four weeks to determine optimum medium for shoot induction of *P. edulis*. The an-

Table 1. Interaction effect of Sodium hypochlorite concentrations and its time of exposure on sterilization offshoot tip and nodal explants of *P. edulis.*

Conc. NaOCl	Duration (min)	% contamination		% Clean culture		% Tissue death	
		Nodal (Mean ±StdDev)	Shoot tip (Mean ±StdDev)	Nodal (Mean ±StdDev)	Shoot tip Mean ±StdDev	Nodal (Mean ±StdDev)	Shot tip Mean ±StdDev
0.5	3	89.76±0.68^{a}	87.73+0.64^{a}	10.24±0.65^{k}	12.27± 0.63^{l}	0.00±0.00^{n}	0.00±0.00^{l}
0.5	5	79.83±0.76^{b}	75.00±0.50^{b}	17.17±1.26^{j}	22.16±0.76^{g}	2.50±0.50^{m}	3.40±0.52^{k}
0.5	7	68.40±0.52^{c}	65.83±0.76^{c}	25.60±0.36^{n}	29.00±1.00^{f}	5.76±0.68^{l}	5.76±0.68^{j}
0.5	9	59.76+ 0.68^{d}	57.73+0.64^{d}	32.24+0.74^{g}	34.27+0.86^{e}	8.40+0.52^{kl}	8.50+0.50^{l}
1	3	49.93±0.90^{e}	48.76±0.68^{e}	40.07±1.10^{f}	42.00±1.00^{d}	10.60±0.52^{k}	9.40±0.52^{l}
1	5	15.83±0.76^{f}	20.83±0.76^{f}	74.00±0.40^{a}	69.16±1.89^{a}	11.83±0.76^{k}	10.43±0.51^{l}
1	7	13.90±0.85^{g}	18.60±0.52^{g}	60.10±1.15^{b}	62.00±1.00^{b}	21.43±0.51^{j}	19.73±0.64^{h}
1	9	12.40+ 0.52^{g}	16.43+0.51^{n}	54.00+0.80^{c}	53.57+0.51^{c}	29.73+0.64^{i}	29.76+0.68^{g}
1.5	3	10.66 ±0.76^{h}	13..83±0.76^{i}	51.34±1.32^{d}	40.00±1.00^{d}	37.83±0.76^{n}	47.73+0.64^{f}
1.5	5	10.60±0.52^{h}	10.60±0.52^{j}	43.40±1.96^{e}	29.40±1.21^{f}	55.90±0.85^{g}	59.83±0.76^{e}
1.5	7	8.40±0.52i	8.40±0.52^{k}	20.00±1.00^{i}	20.00±0.30^{gh}	69.76±0.68^{f}	72.50±0.50^{d}
1.5	9	6.43+0.51^{j}	6.43+ 0.51^{l}	18.33+1.04^{ij}	17.57+1.24^{h}	76.50+0.50^{e}	75.76±0.68^{c}
2	3	5.60±0.52^{j}	5.83±0.76^{l}	9.40±0.76^{k}	10.00±0.50^{j}	85.83±0.76^{d}	75.43+0.51^{c}
2	5	3..83±0.76^{k}	2.60±0.52^{m}	7.17±0.64^{l}	7.40±0.52j^{k}	89.76±0.68^{c}	89.73±0.64^{b}
2	7	2.60+ 0.52^{k}	2.50+ 0.50	5.40+ 0.50^{l}	5.63+0.40k^{l}	92.60+0.52^{b}	92.40±0.52^{b}
2	9	00.00±0.00^{l}	0.00±0.00^{n}	2.00±0.20^{m}	3.00±0.70^{l}	98.73±0.64^{a}	96.40±0.52^{a}
CV		*2.37*	*2.18*	*3.15*	*3.69*	*1.96*	*2.0*

Means with the same letters in a column are not significantly different from each other by Ryan - Einot - Gabriel - Welsch Multiple Range Test (REGWQ) at α= 5%.

alysis of variance (Table 2) showed that the interaction with BAP and type of explants had very highly significant effect (p< 0.0001) on the shoot induction rate. Interaction effect of explants type with BAP on rate of shoot induction was found to be highly significant (BAP*Explant).The response of shoot tip and nodal explants to a given concentration of BAP was not the same that the nodal explants gave greater response than shoot tip explants (Table 2).

The highest rate of shoot induction (91.67±0.58%) was achieved on MS medium supplemented with 1.5 mg/L BAP from nodal and 85.57±0.5% on 1.5 mg/L BAP from shoot tip on MS media (Table 2). For both shoot tip and nodal explants, MS basal media added with 1.5 mg/L BAP used alone were found to be optimum media for *in vitro* shoot initiation of *P. edulis*.

In combination of BAP and Kinetin, the highest percentage of induction was observed at 1.5 mg/L BAP and 3.0 mg/L Kinetin (73.20±1.05% and 70.56±1.39%) for nodal and shoots tip explants, respectively (Table 2). From the given concentrations, high concentration of BAP and kinetin and a medium with free growth regulator resulted in low percentage of shoot induction. Therefore, BAP proved to be a more effective than Kinetin for multiple shoot induction of *P. edulis.* The present result was in accordance with the result of Vasile et al. (2006) achieved high regeneration shoot induction from *P. blumei*, using 1.5 mg/L of BAP. Similar result was also reported by Pratibha et al. (2011) that 1.5 mg/L BAP was found to be optimum for shoot initiation. High BAP concentration decreased the shoot production either by inhibition of shoot initiation or by encouraging callusing (Figure 1).

Effect of different concentration and combination of BAP and NAA on shoot multiplication of *P. edulis*

Those shoot buds induced well on the prevailing shoot induction medium were transferred to MS media supplemented with BAP (1.0 to 3.0 mg/L) alone and in combination with (1 to 3 mg/L) NAA. Cultures were sub cultured twice and the effect of hormones on *in vitro* shoot multiplication of *P. edulis* cultivar was evaluated.

In this study, the significance of BAP and its interaction with NAA were considered. The ANOVA revealed that the concentration of BAP both alone and together with NAA had very highly significant effect (p < 0.0001) on shoot multiplication rate. Shoot buds raised from nodal explants responded exceeds shoot tip shoot multiplication and this indicating the significant effect of explants at this stage.

In this study, maximum number of shoot proliferation 10.28±0.06 and 6.12±0.01 was obtained on MS medium containing 2.0 mg/L BAP and 1.0 mg/L NAA from nodal and shoot tip, respectively (Table 3). Similar results were reported by Rani et al. (2006). Some of the previous studies, where BA and NAA were found to be useful in shoot multiplication from nodal segments and shoot tip explants of various other plants, e.g. *Jasminum officinale*

Table 2. The effects of different concentrations of BAP and Kinetin alone and in combination on MS medium for percentage of shoot induction of *P. edulis* shoot tip and nodal explants.

Conc. of PGRS		Explant	
		Nodal	Shoot tip
BAP(mg/l)	Kin(mg/L)	Mean±STD	Mean±STD
0	0	10.56±0.51^{l}	9.60±0.52^{o}
0	1	40.92±0.88^{k}	35.62±1.07^{n}
0	2	41.87±0.26j^{k}	40.86±1.02^{m}
0	3	43.62±0.67^{j}	42.65±0.56^{m}
0.5	0	81.25±0.90^{c}	74.32±0.58^{c}
0.5	1	53.08±0.88^{l}	50.18±1.04^{l}
0.5	2	57.57±0.38^{n}	53.72±0.62^{k}
0.5	3	58.33±0.32^{n}	57.66±0.57^{j}
1	0	85.50±0.50^{b}	77.76±0.67^{b}
1	1	61.58±0.51^{g}	60.58±1.41^{hi}
1	2	64.78±1.07^{f}	62. 35±1.52^{hg}
1	3	66.67±1.15^{f}	63.56±1.25^{fg}
1.5	0	91.67±0.58^{a}	85.57±0.51^{a}
1.5	1	70.68±1.00^{e}	66.00±0.99^{ef}
1.5	2	71.47±1.04d^{e}	67.38±1.19^{e}
1.5	3	73.20±1.05^{d}	70.56±1.39^{d}
2	0	66.63±0.54^{f}	64.58±1.00^{fg}
2	1	61.25±1.56^{g}	59.68±0.58^{ij}
2	2	56.65±0.56^{n}	54.65±0.56^{k}
2	3	53.37±0.54^{i}	50.40±1.21^{l}
CV		1.34	1.70

Means within a column followed by the same letters are not statically significant at p< 0.01 by Ryan - Einot - Gabriel - Welsch Multiple Range Test (REGWQ).

(a) (b) (c) (d) (e)

Figure 1. Micropropagation of *P. edulis*; (a) Shoot induction on 1.5 mg/l BAP, (b) Shoot multiplication on 2.0 mg/l of BAP and 1.0 mg/l IAA, c) Rooting on half - strength medium with 2 mg/l IBA and 1.0 mg/l NAA, d) Plantlets ready to transfer on sterile soil mix. e) Acclimatization on 2:1:1 top soil, coffee husk and sand soil mix (f) plantlets under greenhouse condition 6 weeks after transfer.

(Bhattacharya, 1997), *Vanilla planifolia* (George and Ravishankar, 1997), *Aristolochia indica* (Manjula et al., 1997), *Vitex negundo* (Kannan and Jasrai, 1998), *Syzygium travancoricum* (Anand et al., 1999) and *Ancistrocladus abbreviatus* (Bringmann et al., 1999). However, in certain other *Plectranthus* species, *P. forskohlii* (Sen and Sharma, 1991) and *P. parviflorus* (Ponsamuel et al., 1994), BA (2 mg/L) alone was sufficient for formation of multiple shoots from nodal segments and shoot tips. Similarly, Hiregoudar et al. (2005) also reported that addition of BA (2 µM) alone to MS medium is responsible for shoot induction. In the present study, among all the combinations and concentrations, the longest shoots 4.51±0.04 cm and 3.45±0.09 cm were observed on the medium containing 2.0 mg/L BAP with 1.0 mg/L NAA for both nodal and

Table 3. Effect of different concentrations and combinations of BAP and NAA treatments on shoot multiplication of *P. edulis.*

Levels of PGR		Nodal		Shoot tip	
BAP (mg/L)	NAA (mg/L)	Shoot number Mean ± StdDev	Shoot length Mean ± StdDev	Shoot number Mean ± StdDev	Shoot length Mean ± StdDev
0	0	2.41±0.01^{kl}	2.12±0.06^{l}	2.49±0.07^{gh}	1.99±0.01^{e}
0	1	2.70±0.10	2.96±0.07^{hi}	2.78±0.05^{e}	2.02±0.02^{e}
0	2	2.68±0.02^{fgh}	2.15±0.03^{hi}	2.51±0.01^{gh}	2.07±0.01^{de}
0	3	2.88±0.07^{d}	2.25±0.06g^{hi}	2.85±0.02^{cde}	2.13±0.02^{de}
1	0	2.54±0.06^{ijk}	2.97±0.03^{c}	2.43±0.03^{hij}	2.07±0.06^{de}
1	1	2.38±0.04^{l}	2.56±0.06^{e}	2.65±0.02^{f}	2.62±0.10^{c}
1	2	2.62±0.01	2.19±0.04^{ghi}	2.19±0.04^{m}	2.80±0.10^{bc}
1	3	2.52±0.06^{ijk}	2.75±0.07^{d}	2.31±0.01^{kl}	2.19±0.08^{de}
1.5	0	2.85±0.01^{de}	2.48±0.06^{ef}	2.80±0.04^{de}	2.91±0.06^{b}
1.5	1	2.55±0.01^{hijk}	2.51±0.04e	2.35±0.02^{jkl}	2.75±0.12^{bc}
1.5	2	2.46±0.06^{jkl}	3.18±0.05^{b}	2.53±0.03^{gh}	2.70±0.03^{c}
1.5	3	2.57±0.01^{ghij}	2.14±0.04^{hi}	2.91±0.01^{c}	2.15±0.04^{de}
2	0	3.35±0.05^{b}	2.30±0.10^{gh}	3.58±0.03^{b}	2.27±0.06^{d}
2	1	10.28±0.06^{a}	4.51±0.04^{a}	6.12±0.01^{a}	3.45±0.09^{a}
2	2	3.23±0.05^{c}	1.70±0.05^{j}	2.60±0.02^{gf}	2.92±0.02^{b}
2	3	3.18±0.06^{c}	2.21±0.04^{ghi}	2.90±0.05^{cd}	2.10±0.07^{de}
3	0	2.71±0.04^{f}	2.35±0.02^{fg}	2.25±0.06^{mi}	2.03±0.02^{e}
3	1	2.52±0.02^{ijk}	2.28±0.11^{ghi}	2.45±0.05^{hij}	2.17±0.09^{de}
3	2	2.75±0.02^{ef}	2.52±0.04^{e}	2.57±0.04^{gf}	2.15±0.08^{de}
3	3	2.45±0.04^{jkl}	2.89±0.05^{cd}	2.39±0.02^{ijk}	2.20±0.09^{de}
CV		1.58	2.36	1.38	2.98

Means within a column followed by the same letters are not statically significant at p< 0.01 by Ryan - Einot - Gabriel - Welsch Multiple Range Test (REGWQ).

Table 4. Effect of various concentrations of IBA and NAA on rooting of proliferated shoots of *P. edulis* cultured on half - strength MS medium.

Conc. of PGRs (µM)		Rooting (%)	Shoot height (cm)	Root number	Root length (cm)
IBA(mg/L)	NAA(mg/L)	(Mean ± SD)	(Mean ± SD)	(Mean ± SD)	(Mean ± SD)
0	0	49.71±0.47^{n}	2.70±0.26^{n}	4.60±0.10^{m}	1.07±0.04^{jk}
0	0.5	70.95+0.39^{f}	3.17±0.22^{fgh}	8.30±0.43^{gh}	1.45±0.09^{ghi}
0	1	85.25+0.97^{c}	3.32±0. 24^{fg}	19.32±0.71^{e}	1.59±0.20^{fgh}
0	1.5	51.17±0.67^{mn}	2.71±0.14^{n}	4.83±0.20l^{m}	1.52±0.17^{ghi}
0.5	0	59.32±0.88^{l}	3.48±0.19^{ef}	7.80±0.26^{ghi}	2.11±0.14^{cde}
1	0	84.39+ 0.61^{c}	4.65±0.30^{bcd}	26.07±0.55^{c}	2.15±0.08^{bcd}
1.5	0	90.76+1.11^{b}	4.90±0.18^{b}	30.57±0.24^{b}	2.47±0.04^{b}
2	0	97.00+ 0.28^{a}	5.90±0.09^{a}	32.73±0.14^{a}	2.95±0.08^{a}
2.5	0	68.55±0.71^{g}	2.60±0.07^{i}	17.37±0.01^{f}	1.75±0.01^{efg}
CV		0.99	5.53	2.19	6.03

(±). Means within a column followed by the same letters are not statically significant at α= 5 % by Ryan - Einot - Gabriel - Welsch Multiple Range Test (REGWQ).

shoot tip explants, respectively. A medium free growth regulators and a medium with high concentration of BAP alone and in combination with NAA resulted in low multiplication rate (Table 3). Length of shoots that were obtained from nodal explant was longer than observed from shoot tip explants.

Effect of different concentrations of IBA, and NAA for *in vitro* root initiation of *P. edulis*

The highest rooting percentage (97.00+ 0.28) was obtained on half-strength MS medium at 2.0 mg/L of IBA followed by 85.25+0.97% at 1 mg/L of NAA (Table 4).

Among the given concentrations auxins with higher concentration resulted in less rooting percentages. Naphthalene acetic acid (NAA) at a concentration of 1.5 mg/L resulted in less percentage of rooting (51.17±0.67) that was less than the root induced from all the rest at high and low concentration. The longest shoot (5.90±0.09 cm) was obtained from a medium that contained 2.0 mg/L 1BA followed by 3.32±0.24 cm from 1.0 mg/L of NAA. Smallest shoot height 2.00±0.23 cm were obtained from 2.5 mg/L of IBA.

The highest mean number 32.73±0.14 of roots were obtained from 2.0 mg/L of IBA followed by 19.32±0.71 on1.0 mg/L NAA. Highest concentrations of auxins resulted in less number of root. Relatively, less number of roots 2.60±0.07 was obtained from IBA at 2.5 mg/L (Table 4). Similar results were reported by Rani et al. (2006) observed that 1/2-MS with 2 mg/L IBA was found to be the best treatment for induction of roots. Root induction decreased with increase in concentration of IBA. NAA resulted in comparatively lesser number of roots. In this study, half - strength MS medium supplemented with IBA (0.5 1.0, 1.5, 2.0 and 2.5 mg/L) and NAA (0.5 1.0 and, 1.5 mg/L) were evaluated and relatively 2.0 and 1.0 mg/L IBA and NAA respectively gave good rooting percentage. The concentrations beyond these led to a decrease in the number of roots and root length per rooted explant and rooting rate. Similar findings on some other plants, e.g.

Elaeagnus angustifolia (Iriondo et al., 1995), *Asparagus robustus* (Nayak and Sen 1998), *Eucalyptus tereticornis*

(Sharma and Ramamurthy, 2000) and *Hemides musindicus* (Sreekumar et al., 2000).The root elongation phase is very sensitive to auxin concentration, and it is inhibited by high concentration of auxin in the rooting medium. Daffalla et al. (2011) reported that roots may require a less concentration of auxin to grow, but root growth is strongly inhibited by its higher level because at this level, auxin induces the production of ethylene, a root growth inhibitor.

Acclimatization *in vitro* derived *P. edulis* plantlet

The establishment of *in vitro* plantlets under different environmental conditions was greatly affected in terms of survival percentage of plantlets. In the present study, the plantlets showed 83.4% survival efficiency. The plantlets transferred under net house conditions resulted in the best establishment, whereas no plantlets could be established under direct field conditions (Figure 1).

Conclusion

One percent concentration of NaOCl solution for five minute exposure time were found to be optimum treatment for sterilization of shoot tip and nodal explants of *P. edulis*. The maximum percentage of shoot induction (91.67±0.58) and (85.57±0.51) was observed on an MS medium supplemented with 1.5 mg/L BAP from nodal and shoot tip explants respectively. MS basal medium supplemented with 2.0 mg/L BAP and 1.0 mg/L NAA resulted in 10.28±0.06 shoot number with best and vigor morphological appearance. Best rooting percentage was achieved on half strength MS basal media containing 2.0 mg/L IBA which resulted mean values of 97.00+ 0.28 with 32.73±0.14 root number, followed by half strength MS basal media containing 1 mg/L NAA which resulted 85.25+0.97 with 19.32±0.71 root number, 1.59±0.20 cm root length and 3.32±0.24 shoot length. Those plantlets well performed *in vitro* showed 85% survival efficiency after hardening and acclimatization on soil mix ratio of 2:1:1 decomposed coffee husk, top forest soil and sand respectively.

Conflict of Interest

The authors have not declared any conflict of interest.

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