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Micropropagation of *Plectranthus edulis* (Vatke) Agnew from meristem cultur

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***Plectranthus edulis* (Vatke) Agnew is a tuber-bearing food crop in Ethiopia. However, its productivity is hampered by shortage of pathogen-free planting materials. Therefore, the objective of this study was to develop micropropagation protocol for this plant using meristem to produce clean planting materials. Mersitems were collected from apical and axillary shoots from Holeta and Welayta areas and cultured on MS medium containing gibberellic acid (GA_3) (1.0 mg l^{-1}), α-naphthalene acetic acid (NAA) (0.1 mg l^{-1}) in combination with 6-benzylaminopurine (BAP) (0.1, 0.5, 1.0, 2.0 and 5.0 mg l^{-1}). Shoots were multiplied on MS medium containing 0.1, 0.5, or 1.0 mg l^{-1} of BAP or 0.5, 1.0, 2.0 or 3.0 mg l^{-1} of Kinetin alone or their combination with 0.05 or 0.1 mg l^{-1} NAA. *In vitro* and *ex vitro* rooting was performed using different types of auxins followed by acclimatization. The highest percentage of shoots initiated from collected mersitem at Holeta (73%) was obtained on MS medium containing 1.0 mg l^{-1} BAP, 1.0 mg l^{-1} GA_3 and 0.1 mg l^{-1} NAA. The highest shoot number explants^{-1} (7.2) was obtained on MS medium containing 1.0 mg l^{-1} Kinetin and 0.1 mg l^{-1} NAA, whereas the highest root number shoot^{-1} (6.2) was obtained from *ex vitro*. All plants derived from Holeta and 96.7% of those from the Welayta survived after acclimatization. These results provided rapid and reproducible conditions for propagation of relatively pathogen-free planting material of this plant.**

Key words: *In vitro* propagation, meristem culture, micropropagation, *Plectranthus edulis* (Vatke) Agnew, shoots multiplication.

INTRODUCTION

Plectranthus edulis (Vatke) Agnew is also known as *Coleus edulis* and an indigenous root crop in Ethiopia. It serves an important part of the diet of the population (PGRC, 1996; IBC, 2005) and is widely used as a major source of tasty carbohydrates in many parts of Ethiopia (Taye et al., 2007). It is one of the major crops cultivated in Oromia

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Abbreviations: GA_3, Gibberellic acid; **NAA,** α-naphthalene acetic acid; **BAP,** 6-benzylaminopurine; **PGR,** plant growth regulator; **IAA,** indole-3-acetic acid; **IBA,** indole-3-butyric acid.

region (Sirika, 2011).

The genus *Plectranthus* has been distributed all over the tropical and subtropical regions of India, Pakistan, Sri Lanka, Tropical East Africa, Brazil and Egypt (Sunilkumar, 2005). *P. edulis* is one of the native tuber crops in Ethiopia (IBC, 2005) and is cultivated in the south and south western parts of Ethiopia whereas the wild species are found throughout the country (PGRC, 1996). It also serves as a medicinal plant and eating the cooked root avoids loss of appetite (Megersa, 2010).

The major constraints of production identified by Taye (2008) are the poor storability of the tubers and the shortage of seed tubers. Seed production of *P. edulis* is an important direction to emphasize for this crop, although the effect of seed quality on performance of the resultant crop is yet unknown. Alternatively, growers of *P. edulis* can continually acquire large numbers of disease-free planting stock from plants micropropagated from mersitem explants.

Reports recently documented the in growing areas of *P. edulis* showed the occurrence of diseases (Taye, 2008). It is propagated vegetatively, and hence it is susceptible to diseases that are carried from one generation to the next through infected planting material. The systemic diseases of viruses, viroids, and mycoplasma and several bacteria are the most devastating in terms of yield loss for root and tuber crops (Bryan, 1983).

The application of plant tissue culture offers valuable ways to overcome many of the problems that are encountered in natural propagation. Meristem culture can be used alone or in combination with thermotherapy to improve the elimination rate of a number of viruses and bacteria (Kartha and Gamborg, 1975). Increased use of rapid multiplication techniques is enabling scientists to select and produce large amounts of pathogen-free material.

Meristem culture can even improve the technique by eliminating systemic pathogens present in the donor plant (Stepan-Sarkissian, 1990). Therefore, the objective of this study was to develop a micropropagation protocol for *P. edulis* using meristem as explant.

MATERIALS AND METHODS

Sterilization of explants

Seed tubers of *P. edulis* that were collected from Holeta (30 km west of Addis Ababa) and Welayta (330 km south west of Addis Ababa) were planted in greenhouse. Approximately 1.5 to 2.0 cm long apical and axillary shoots were collected from two-month-old greenhouse grown mother plants. The shoot explants were washed twice with tap water and detergent, and rinsed with double distilled water twice. Those explants were then surface sterilized with 70% alcohol for 30 s and rinsed three times with sterile double distilled water followed by sterilization by 0.5% sodium hypochlorite containing three drops of Tween 20 for 10 min. After sterilization, the explants were rinsed three times with sterile double distilled water.

Meristem isolation and shoot induction

Leaves were removed and meristems of 0.2 to 0.5 mm diameter with one or two leaf primordia were isolated under a dissecting microscope using sterile forceps, scalpels and hypodermic needles.

The meristems were cultured on 90 mm diameter Petri dishes each containing 20 ml shoot induction medium. The shoot induction medium consisted of MS medium (Murashige and Skoog, 1962) containing different concentrations of BAP (0.1, 0.5, 1.0, 2.0 or 5.0 mg l^{-1}) in combination with 0.1 mg l^{-1} NAA, 1.0 mg l^{-1} GA_3 and supplemented with 30 g l^{-1} sucrose. Experimental design used in shoot induction was a three factor treatment structure in completely randomized design in which total of 5 combinations of three factors (five levels of the BAP, 0.1, 0.5, 1.0, 2.0, 5.0 mg l^{-1} one level of NAA 0.1 mg l^{-1} and one level of GA_3 1.0 mg l^{-1}) was observed. After the pH was adjusted to 5.8, the medium was autoclaved at 121°C for 15 min. Growth regulator-free medium was used as a control in these experiments.

Five explants were cultured per Petri dish with six replications. Cultures were transferred to the same fresh medium every two weeks until shoots were initiated. The cultures were maintained at temperature of 25 ± 2°C under a light intensity of 20 µmol m^{-2} s^{-1} and a 16 h photoperiod provided by cool-white fluorescent lamps. The numbers of meristems induced to shoots were recorded. The experiment was then repeated once. Unless otherwise indicated, all cultures were maintained under these culture conditions.

Shoot multiplication

Initiated shoots were cultured on shoot multiplication medium. Shoot multiplication medium consisted of MS medium with variable concentrations of BAP (0.1, 0.5, or 1.0 mg l^{-1}) or Kinetin (0.5, 1.0, 2.0 or 3.0 mg l^{-1}) alone or their combination with NAA (0.05 or 0.1 mg l^{-1}). Growth regulator-free medium was used as a control. Shoots were cultured in Magenta GA-7 vessels containing 50 ml medium. For each treatment, a total of 30 explants were used. The treatment structure was considered as a completed two-factorial design with three levels of BAP (0.1, 0.5, and 1.0 mg l^{-1}) alone or with either four levels of Kinetin (0.5, 1.0, 2.0 or 3.0 mg l^{-1}) or two levels of NAA (0.05 and 0.1 mg l^{-1}) augmented with a control (0 mg l^{-1} BAP, 0 mg l^{-1} Kinetin and 0 mg l^{-1} NAA). The effects of different treatments were quantified on the basis of number of shoots $explants^{-1}$ for each treatment. Number and length of shoots $explants^{-1}$ were recorded after four weeks of culture. The whole experiment was repeated once.

Rooting

Rooting was performed both *in vitro* and *ex vitro*. *In vitro* rooting was carried out in two ways: firstly, shoots were cultured on both full and half strength MS media containing 1.0 mg l^{-1} NAA, IAA or IBA. Secondly, shoots were immersed into 5.0 mg l^{-1} IBA for 5 min before transferring into growth regulator-free MS medium. Shoots were kept for a week in darkness and were then transferred to a 16-h photoperiod for three weeks. In *ex vitro* rooting, shoots were transferred to plastic pots filled with soil, compost, and sand in a 2:1:1 ratio respectively following immersion into 5.0 mg l^{-1} IBA for 5 min and were then covered with polyethylene bags. The polyethylene bags were removed from the pots after a week under greenhouse condition. Experimental design used in rooting was a two factor treatment structure in completely randomized design in which five out of six combination of two factors (two levels of the MS salt half and full strength alone or with three levels of hormone 1.0 mgl^{-1}NAA, 1.0 mgl^{-1}IAA and 5.0 mgl^{-1} IBA) together with one *ex vitro* rooting was observed. Rooting was evaluated in terms of rooting percentage, root number, and the mean root length after

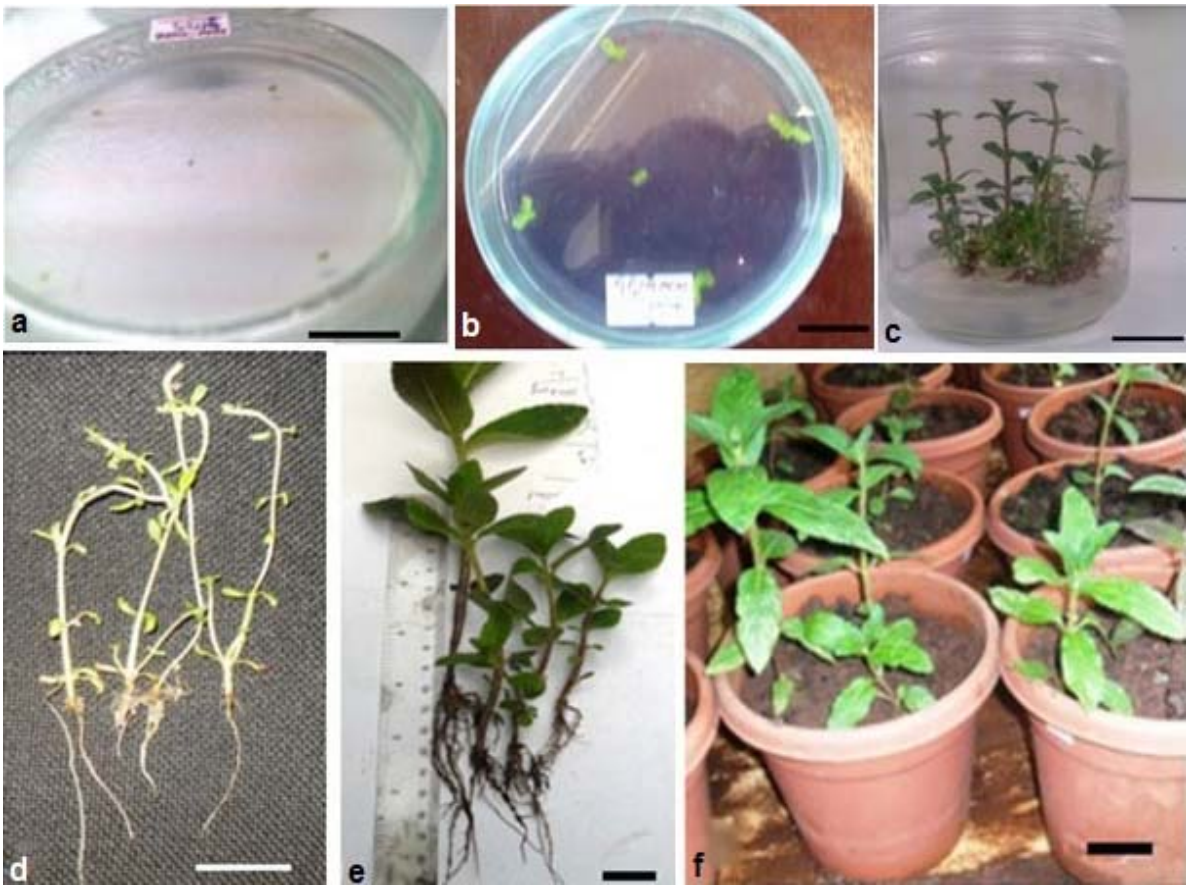

Figure 1. Mersitem culture on MS medium containing 1.0 mg/l BAP in combination with 1.0 mg/l GA_3 and 0.1 mg/l NAA. **(a)** After one week. **(b).** After 20 days. **(c).** Shoots cultured on MS medium containing 1.0 mg/l BAP. **(d).** Shoots rooted on half strength MS medium containing 1.0 mg/l IBA. **(e).** Shoots rooted *ex vitro* by immersing in 5.0 mg/l IBA for 5 min: Bars = 2 cm. **(f).** Plants after acclimatization: Bar = 5 cm.

one month of culturing.

Acclimatization

After four weeks in rooting medium, roots were washed thoroughly to remove residual medium and transferred to plastic pots containing a mixture of soil, compost and sand in a ratio of 2:1:1, respectively. Each pot was covered with a polyethylene bag and kept in the greenhouse. The polyethylene bags were removed after a week and the number of plants that survived in the greenhouse was recorded after a month. Furthermore, the pots were transferred to external environment and watered as necessary. The experimental design used in acclimatization phase was a one-factor treatment structure in a completely randomized design. The total number of plantlets transferred to pots was 240; each treatment represented by 60 plantlets (from *ex vitro* and *in vitro* rooted Holeta and Welayta origin).

Statistical analysis

Statistical analysis of quantitative data was carried out by the SPSS computer software version 16. Data were subjected to analysis of variance and variables that showed significant difference were compared by the LSD at 5% probability.

RESULTS

Shoot induction from meristem

The use of solid MS medium containing BAP, GA_3 and NAA resulted in the development of single or multiple shoots from a meristem. An earlier sign of growth from treated meristem was noticeable within seven days after culture of the explants (Figure 1a to c).

ANOVA results showed that cytokinin concentrations significantly affected percentages of shoot induction ($p \leq 0.05$), although no statistically significant difference was seen between plants of the two collection areas in percentage of shoot induction. The highest percent shoot induction (73%) was achieved from MS medium containing 0.1 mg/l and 1.0 mg l^{-1} BAP in combination with 1.0 mg l^{-1} GA_3 and 0.1 mg l^{-1} NAA (Table 1).Percentages of shoot induction in these treatments ranged from 52 to 73%. At higher concentrations of BAP (2.0 or 5.0 mg l^{-1}) combined with 0.1 mg l^{-1} NAA and 1.0 mg l^{-1} GA_3 stunted adventitious shoots were observed.

Table 1. Effect of different concentrations of BAP combined with GA_3 and NAA on shoot induction from meristem explants of *P. edulis* after six weeks of culture.

Plant growth regulators			Percentage of shoot induction	
BAP (mg l^{-1})	GA_3 (mg l^{-1})	NAA (mg l^{-1})	Holeta	Welayta
0.0	0.0	0.0	10	13
0.1	1.0	0.1	73	60
0.5	1.0	0.1	53	57
1.0	1.0	0.1	72	65
2.0	1.0	0.1	53	58
5.0	1.0	0.1	58	52

Table 2. Percentage of shoots producing multiple shoots, number and length of shoots per explant that were cultured on MS medium containing different concentration of BAP, Kinetin and NAA.

PGR (mg/l)			Explants with multiple shoots (%)		Number of shoots explant^{-1} ± SD		Shoot length (cm) ± SD	
BAP	Kinetn	NAA	Holeta	Welayta	Holeta	Welayta	Holeta	Welayta
0	0	0	16.6	0.0	1.2±0.4^{a}	1.0±0.0^{a}	7.2±1.5^{a}	7.3±1.4^{a}
0.1	0	0	73.3	76.7	2.1±0.8^{bd}	2.1±0.8^{b}	4.0±1.5^{b-f}	4.4±1.7^{b-j}
0.5	0	0	100	96.7	4.51.2±^{cg}	4.1±1.3^{c-g}	2.8±1.5^{c}	3.0±1.6^{c}
1.0	0	0	100	100	4.9±1.6^{cj}	4.8±1.5^{c-i}	2.6±1.3^{c}	2.5±1.2^{d}
0.1	0	0.05	78.3	85	2.7±1.1^{bdf}	3.2±1.6^{eg}	3.3±2.1^{c-g}	4.1±2.5^{bef}
0.5	0	0.05	68.3	95	2.7±1.7^{bdf}	4.5±2.4^{cdf}	3.2±1.6^{ceg}	3.2±1.5^{ci}
1.0	0	0.05	100	100	5.8±2.0^{ej}	4.9±1.9^{dfi}	3.0±1.5^{c}	3.0±1.7^{ci}
0.1	0	0.1	75	86.7	3.1±1.4^{dfh}	3.2±1.7^{eg}	4.7±2.0^{bd}	4.3±2.1^{be}
0.5	0	0.1	100	100	4.6±1.6^{cgj}	4.0±1.5^{cfg}	3.3±1.1^{ceg}	3.2±1.5^{ci}
1.0	0	0.1	100	100	4.5±1.9^{cg}	5.2±2.4^{d-j}	3.3±1.6^{ceg}	3.2±1.7^{ci}
0	0.5	0	86.7	85	3.3±1.5^{d-h}	3.2±1.4^{eg}	4.2±1.9^{b-g}	4.2±1.9^{bef}
0	1.0	0	90	100	4.1±1.7^{cgh}	4.7±1.4^{c-i}	4.3±1.8^{bdf}	4.2±1.9^{bef}
0	2.0	0	100	100	4.7±1.8^{cgj}	4.7±1.9^{c-i}	3.9±1.9^{b-f}	3.9±1.9^{b-g}
0	3.0	0	100	100	4.7±2.1^{cgj}	4.7±2.1^{c-i}	3.8±1.5^{b-g}	3.8±1.5^{fg}
0	0.5	0.05	86.7	93.3	3.5±1.8^{fgh}	3.7±1.7^{ceg}	4.6±2.1^{bd}	4.7±2.2^{bhj}
0	1.0	0.05	95	90	4.3±2.3^{cg}	4.0±2.3^{cfg}	4.6±1.9^{bd}	4.2±2.0^{be}
0	2.0	0.05	100	100	4.1±2.4^{cgh}	3.9±2.3^{c-g}	4.1±2.1^{b-g}	4.3±2.4^{beh}
0	3.0	0.05	100	100	6.6±4.1^{i}	6.2±4.2^{hj}	4.0±1.7^{bfg}	4.3±1.8^{be}
0	0.5	0.1	100	98.3	5.7±1.3^{ej}	5.2±1.6^{d-j}	4.5±1.4^{bd}	4.6±1.7^{bhj}
0.0	1.0	0.1	100	93.3	7.2±2.1^{i}	5.9±2.8^{dhij}	2.9±0.9^{c}	3.3±1.4^{ci}
0.0	2.0	0.1	100	96.7	5.3±2.8^{cej}	4.6±2.7^{cdfi}	3.2±1.3^{ce}	3.3±1.4^{ci}
0.0	3.0	0.1	100	100	5.5±2.3^{cej}	5.1±1.6^{dfi}	3.5±1.1^{cefg}	4.7±1.6^{bhj}

Means followed by the same letter within a column were not significantly different at 5% probability.

Shoot multiplication

Shoot number was highly influenced by concentration and type of the growth regulators. The highest number of shoots explant^{-1} (7.2) was obtained on MS medium supplemented with 1.0 mg l^{-1} Kinetin and 0.1 mg l^{-1} NAA from shoots of Holeta origin. The highest number of shoots explant^{-1} (6.2) of Welayta origin was obtained on MS medium containing 3.0 mg l^{-1} Kinetin and 0.05 mg l^{-1} NAA (Table 2). Generally, the number of shoots explant^{-1} was higher on media containing Kinetin than on those containing BAP. After 35 days of culture, most of shoots cultured on plant regulator (PGR) free medium (83.4% of Holeta and all explants of Welatya) produced only a single shoot with highest mean shoot length of 7.2 and 7.3 cm from Holeta and Welayta, respectively. The length of shoots was affected by sources of the plant, as well as types and concentrations of the growth regulators

Table 3. Effect of MS salt strength and types of auxin on rooting, number of roots shoot^{-1} and root length of *P. edulis*.

Treatment		Rooted shoots (%)		Number of roots explant^{-1} ± SD		Root length (cm) ± SD	
MS salt strength	**Auxin (1.0 mg l^{-1})**	**Holeta**	**Welayta**	**Holeta**	**Welayta**	**Holeta**	**Welayta**
Full strength	0	60	31.7	1.9±1.8^{a}	0.8±1.2^{a}	1.7±1.0^{ac}	1.0±0.7^{ad}
Full strength	NAA	30	35.0	0.6±1.1^{bce}	0.9±1.5^{a}	1.0±0.6^{bc}	0.9±0.3^{ad}
Full strength	IAA	10	33.3	0.2±0.5^{bc}	0.8±1.4^{a}	1.5±0.4^{abc}	1.5±0.5^{bd}
Full strength	IBA	95	61.7	3.2±1.5^{d}	1.61±1.5^{b}	1.6±1.0^{ac}	2.0±1.4^{cd}
Half strength	IAA	25	10	1.0±1.8^{be}	0.1±0.3^{ce}	1.6±0.5^{ac}	1.5±0.4^{abc}
Half strength	IBA	100	76.7	3.1±0.9^{d}	2.2±1.4^{d}	1.5±0.9^{c}	1.1±0.2^{ad}
*Half strength	0	38.3	16.7	0.7±0.9^{bce}	0.3±0.8^{e}	0.9±0.4^{bc}	0.9±0.2^{ad}
**Ex vitro*	0	100	100	6.2±1.9^{f}	5.4±0.9^{f}	3.3±1.1^{d}	3.3±0.9^{e}

Plants of treatments indicated by asterisk, *Half strength and **Ex vitro* were grown on growth regulator free MS medium and potting mix respectively, but the shoots were dipped into 5.0 mg l^{-1} IBA for 5 min before culturing on growth regulators free medium and potting mix. Means followed by the same letter within a column were not significantly different at 5% probability.

(Table 2). Shoots cultured on higher concentration of BAP (1.0 mg l^{-1}) have significantly lower shoot length compared to explants cultured on medium containing a lower concentration of BAP (0.1 mg l^{-1}).

Shoots cultured on medium containing 1.0 mg l^{-1} BAP combined with 0.05 mg l^{-1} NAA produced more shoots than those cultured on the same concentration of BAP alone. Mention-worthily among shoots cultured on PGR free MS medium, 75% of Holeta and 91.7% of Welayta showed spontaneous rooting. On the other hand, spontaneous rooting of 30 and 20% were exhibited by shoots of Holeta and Welayta origin, respectively, on MS medium containing 1.0 mg l^{-1} Kinetin and 0.1 mg l^{-1} NAA.

Rooting and acclimatization

Rooting was observed three weeks after culturing the shoots on root induction medium, and most shoots developed roots by the fourth week (Figure 1d). The Holeta area plants had a better rooting response as compared to Welayta area plants in most cases (Table 3).

Effect of MS salt strength and auxins on root induction

Shoots rooted better on half strength MS medium than full strength medium. The highest percentage of rooting (100%) was exhibited by shoots of Holeta origin when, only 76.7% of the shoots of Welayta origin produced roots on half strength MS medium containing 1.0 mg l^{-1} IBA. In the same medium, shoots of Holeta origin produced a mean root number of 3.1 whereas those of Welayta origin produced 2.2 (Table 3).

Ex vitro exhibited 100% rooting and 6.2 and 5.4 mean roots shoot^{-1} from Holeta and Welayta origin, respectively, and produced the longest mean root length of 3.3 cm in both cases. In *ex vitro* rooting, shoots of Holeta origin showed a higher percentage of survival (90%) compared to shoots of Welayta origin which exhibited 76.7% survival. *In vitro* rooted shoots showed a better survival percentage during acclimatization than e*x vitro* rooted shoots. Rooting percentage, number of roots shoot^{-1}, and root length were significantly different resulting from these treatments in both Holeta and Welayta origins. *Ex vitro* grown shoots showed well developed leaf and root structures (Figure 1e). After acclimatization of one month in greenhouse, 100% of the plants of Holeta origin and 96.7% of plants of Welayta origin survived (Figure 1f). All plants that were transferred to external environment while still in pots were survived after one month.

DISCUSSION

Shoot induction from meristem

MS medium containing 1.0 mg l^{-1} GA_3 and 0.1 mg l^{-1} NAA in combination with different concentrations of BAP exhibited direct shoot initiation from meristem explants. Similar results was observed in yams by Acedo (2006) using MS medium containing the growth regulators used in the present study, although the medium the author used was liquid. Other species like sweet potato and cassava also showed good response towards plant regeneration in MS medium in the presence of BAP combined with auxins as reported by various authors (Acedo, 2006; Wondimu et al., 2012). Dagnino et al. (1991) also reported that different cultivars of *Ipomoea batatas* responded differently to different concentrations of GA_3 yet it had no effect on growth of *Coracao alado* and promoted multiple shoot in 'Mae de Famillia' cultivars.

Shoot development without tissue callusing was effected only when the three growth regulators were combined (Acedo, 2006). Armin et al. (2011) found that the best shoot induction response of purple colored sweet potato was obtained on MS medium supplemented with 1.0 mg l^{-1}

BAP. Beyene et al. (2010) also reported the use of 0.5 or 1.0 mg l^{-1} BAP in combination with 1.0 mg l^{-1} GA_3 and 0.01mg l^{-1} NAA gave morphologically good looking shoots in cassava varieties. These findings were consistent with the results of the present study in which MS medium supplemented with 1.0 mg l^{-1} BAP, 0.1 mg l^{-1} NAA and 1.0 mg l^{-1} GA_3 proved superior in terms of shoot initiation from meristem in both mother plants collected from Holeta and Welayta.

Meristems cultured *in vitro* on shoot culture medium containing only low amounts of growth regulators produced one shoot, but they can be induced to form multiple shoots on cytokinin containing medium (Puonti-Kaerlas, 1998). In our study, most of the meristems induced multiple shoots.

The shoots induced from higher cytokinin (2.0 to 5.0 mg l^{-1} BAP) treatments were numerous, but grew slowly, formed callus like structure at their base, and were not effectively multiplied in successive subcultures. Such shoot phenotypes were reported also by Yasmin et al. (2011) in potato; most of combinations of higher concentration of BAP and NAA explants formed shoots and calli at the base except on 0.5 mg l^{-1} BAP and 0.5 mg l^{-1} NAA which regenerated shoots without callus formation.

Usually, no exogenous growth regulators are required if meristems contain two or more leaf primordia (Altaf, 2009). But the report on potato proliferation by Yasmin et al. (2011) showed that growth of explants is slow in media devoid of growth regulator. This result agree with the present study where the least shoot induction percentage was observed in growth regulators free medium (about 10% from Holeta and 13% from Welayta); growth was slow and shoots were stunted.

All combinations of growth regulators tested led to shoot induction from meristem during the early stage. Each treatment showed different patterns and qualities of growth during later stages. All these results showed that 1.0 mg l^{-1} BAP combined with 1.0 mg l^{-1} GA_3 and 0.1 mg l^{-1} NAA in MS solid medium is the optimum concentration to induce shoots from meristem of *P. edulis*.

Shoot multiplication

Application of BAP and NAA in shoot multiplication MS medium decreased shoot formation and rooting of single nodes of 'Agria' and 'Marfona' cultivars of potato. The best medium for shoot formation and rooting was modified solid MS medium without NAA and BAP (Armin et al., 2011). Contrary to this report, in our study, both BAP and Kinetin performed well in multiple shoot induction. However, Kinetin exhibited higher shoot number explant^{-1} than BAP, alone or in combination with NAA. This was in agreement with the result of Salehi (2006) who obtained the best shoot proliferation on MS medium containing 3.0 mg l^{-1} kinetin and 0.5 mg l^{-1} NAA or 1.0 mg l^{-1} BA and 1.0 mg l^{-1} NAA for cultivars of *Dianthus caryophyllus* species. On the other hand, using BAP (0.5 mg l^{-1}) alone was found to be sufficient for high numbers of shoot formation (Nagib et al., 2003).

On the other hand, Badoni and Chauhan (2009) reported that combinations of higher concentration of Kinetin (1.0 mg l^{-1}) and low concentration of NAA resulted in the least mean shoot height and number of nodes in potato cultivar 'Kufri Himalini'. In the present study, the highest mean number of multiple shoots was observed in 1.0 mg l^{-1} Kinetin in combination with 0.1 mg l^{-1} NAA for Holeta and 3.0 mg l^{-1} Kinetin in combination with 0.05 mg l^{-1} NAA for Welayta. These shoots originated from different sites and showed variation in the number of shoots at different concentrations of NAA and Kinetin. This is possibly due to the presence of genotype difference between plants collected from these two sites as different genotypes of the same plant species respond differently in tissue culture (Dagnino et al., 1991; Feyissa et al., 2005; Jamshieed et al., 2010). *P. edulis* treated with PGR free medium exhibited strong apical dominance with little tendency to branch. Due to this, most of the controls had only a single shoot and the mean length of the shoots was maximum, a result of apical dominance.This agree with the findings of Ezeibekwe et al. (2009) who reported in white yam that shoots cultured on PGRs free medium resulted in the longest shoot. The result of this study also showed that the number of shoots increased and the mean length of shoots decreased as the concentration of BAP increased. This is due to the fact that BAP decreases apical dominance.

A comparatively lower response was recorded when BAP or Kinetin was added alone in the medium of the water yam (Behera et al., 2009). In our study, when both types of cytokinins were used alone, they produced multiple shoots and Kinetin was slightly more effective. When BAP or Kinetin was combined with NAA in multiplication medium, the frequency of multiple shoot development was significantly improved as indicated by significant difference in the number of shoots between explants treated with 1.0 mg l^{-1} BAP alone and its combination with 0.05 mg l^{-1} NAA. This was in agreement with the report of Adeniyi et al. (2008) as there was significant BAP x NAA interaction indicating that the effectiveness of each of the phytohormones in inducing shoots was influenced by the presence or absence of the other. The work of Hoque (2010) also supports this finding as kinetin induces multiple shoots but the rate of shoot multiplication was slightly higher in combination with IAA.

Rooting and acclimatization

In this study, among the *in vitro* rooting cultures, the maximum number of roots shoot^{-1} was observed in half strength MS medium containing 1.0 mg l^{-1} IBA. Statistically significant differences were seen in the percentage of rooted shoots between full and half strength MS medium of the

same IBA concentration ($p \geq 0.05$). Ahmad et al. (2003) reported the highest root number per explants of peach rootstock on half strength MS medium supplemented with 3.0 mg l^{-1} IBA. The auxins, NAA and IBA were used by Behera et al. (2009) to induce rooting from *in vitro* raised shoots of white yam. In their study half strength MS basal medium containing NAA (2.0 mg l^{-1}) exhibited better rooting response than that of IBA (2.0 mg l^{-1}).

The overall best result in rooting was observed in *ex vitro* rooting condition. Although *ex vitro* rooting showed lower percent of survival (90 and 76.7% from Holeta and Welayta respectively) compared to acclimatization of *in vitro* rooted shoots (100 and 96.7% from Holeta and Welayta respectively), it showed the highest result in percentage of root induction, number of roots explant^{-1} and length of root. This is promising in terms of cost reduction by avoiding the *in vitro* rooting. This *ex vitro* rooting reduced the cost of *in vitro* rooting by 99.2%. Reducing the cost of *in vitro* production is a key issue for increasing the application of the method. Rooting of microcuttings *in vitro* is expensive and can even double the price of the cutting. Acclimatization of *in vitro* rooted plantlets was highly successful in that most plants survived. All explants of Holeta origin and 96% of Welayta origin survived and established as healthy plant. Similar results were observed by Wondimu et al. (2012) in sweet potato who reported 100% survival. The present study is the first of its kind in tissue culture of *P. edulis*. In addition to the direct application in disease-free production of this crop, the present protocol contributes to further biotechnological research of this crop.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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