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Protocol optimization for *in vitro* propagation of two Irish potato (*Solanum tuberosum* L.) varieties through lateral bud culture

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Potato is a versatile vegetable that is eaten all around the year. The objective of this study was to establish a protocol for *in vitro* micropropagation of Gudene and Belete popular varieties in Ethiopia from lateral bud explants. For shoot induction and multiplication, lateral bud explants were cultured on MS basal medium supplemented with different concentration levels of PGRS. Rooting was done on the same media type with different concentration levels of IBA and NAA in combination. The rooted plantlets were then acclimatized under poly-house conditions by transplanting the plantlets regenerated on moist soil mixture of loam soil, sand and compost in 2:1:1 ratio respectively. Results showed that shoot initiation, shoot multiplication and root formation responses were significant (P<0.05) at different hormone levels and combinations. 91.67 and 87.5% of explants survived and initiated for Gudenie and Belete varieties, respectively on shoot initiation MS basal medium supplemented with combination of 2.0 mg/l BAP and 1.0 mg/l IAA. In both varieties, number of nodes/explant, number of shoots/explant and shoot length/explant were significantly (P<0.05) higher at 0.5 mg/l BAP and 2 mg/l Kn. Number of days to shoot emergence was also found to be shorter at this level of hormonal combination than other treatments. Number of roots/shoot, root length/shoot, root fresh and dry weight were significantly affected due to growth regulators combination. The acclimatization experiment showed that plantlets of both varieties survived better on the sterilized soil mixture (loam red soil, san and compost) in a ratio of 2:1:1 with better Gudene performance as compared to the second variety.

Key words: Explants, auxin, cytokinin, *in vitro* propagation, MS media and lateral buds.

INTRODUCTION

Irish potato *(Solanum tuberosum* L.*)* is one of the world's most economically important tuber crops belonging to the family Solanaceae. It is a versatile vegetable that is eaten all around the year. It is considered to be the fourth major food crop of the world following rice, wheat and maize (Mustafa and Sarker, 2002). It contains about 79% water, 18% starch, 2% protein, 1% vitamins, minerals and many trace elements (Ahmad et al., 2011), though it is best

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known for its carbohydrate contents. The annual diet of an average global citizen in the first decade of the 21st century included about 33 kg of potato (Food and Agriculture Organization - FAO, 2008). Moreover, it is used as industrial raw material apart from its daily consumption by humans (Hoque, 2010).

In Ethiopia, potato production is expanding from highland areas to mid and lowland parts of the country due to its potential for production in a short period of time, high yield per unit area, source of food and cash crops to large number of food-insecure smallholder farmers and pastoralists in the country. It is the first root crop in production area coverage 70,131.32 ha with average productivity of 13.45 ton/ha (Central Statistical Agency - CSA, 2016) which is very low below world productivity (FAO, 2012). Nowadays, the main production season for potato represents only 22% (34,000 ha), while the off-season production is around 128,000 ha in northern and central Ethiopia (Haverkort et al., 2012).

Production of the crop could fill the gap in food supply during the hungry months of July to August before the grain crops are being harvested. Currently, in Ethiopia, potato grown on around 164,146 ha producing an estimated total tuber yield of 940,087 tons. This implies that average yield in the country reaches only 18.6 t/ha when the potential for small holder is around 25 t/ha (CSA, 2016). Among many, the major causes for the low productivity identified were as a result of many factors being identified as the causes for this low yield in Ethiopia and most of the East African countries, but the lack of high quality seed tuber is the major one which seems to explain most of the yield reduction. Moreover, unavailability of seed tubers, lack of well adapted cultivars, prevalence of diseases and insect pests, drought and frost at high altitude and extreme temperature at very low altitudes are some of the problems that contribute to the reduced yield of potato (Tsoka et al., 2012).

In vitro regeneration and micro propagation methods ar e now widely used for disease free plantlets regeneration and mass multiplication of plant material, germplasm conservation and for the improvement of crops through biotechnological methods. *In vitro* Irish potato production technology is a more advanced technology over traditional method of Irish potato production with respect to optimal yield uniformity, disease free planting material and true to type plants. Mass multiplication of tissue culture plants could also be done in a short time. The development of an optimal micro propagation protocol would have a major significance in the propagation of well adapted and better yielding varieties of good agronomic traits of Irish potato clones for its distribution to farmers (Rabbani et al., 2001).

So far, very limited efforts have been made for *in vitro* micro propagation of Irish potato in the country. In order to meet the objectives of mass propagation within short period of time and produce disease free seed tubers to benefit potato producers from tissue culture technique and contribute towards improvement of productivity, it is important to optimize efficient micro-propagation protocol as per facilities available which needs to be optimized. Few researchers have reported their research on Irish potato tissue culture. For example, Hussain et al. (2005) investigated micro propagation of potato from lateral bud explants on MS basal medium with plant growth regulators (auxins and cytokinin). Molla et al. (2011) also studied the effect of growth regulators (IAA, IBA, NAA, BAP and Kin) on direct regeneration of potato. However, only partial success and a low rate of multiplication was obtained with very limited work done on *in vitro* propagation of the two potato varieties (Belete and Gudene) from lateral meristem in Ethiopia. Therefore, this paper was prepared to share the information generated from the study conducted with the objective to optimize efficient micro propagation protocol for Irish potato varieties in Areka plant tissue culture laboratory conditions.

MATERIALS AND METHODS

Description of the study area

The study was conducted at Plant Tissue Culture Laboratory of Areka Agricultural Research Center (AARC), in Southern Nations Nationalities and People's Regional State (SNNPRS), Wolaita Zone. It was located 300 km to the south of Addis Ababa and 3 km away from Areka town at an elevation of 1800 m.a.s.l. The annual rainfall of the area was 1520 mm and the average maximum and minimum temperatures are 26 and 14°C, respectively (AARC).

Explants source and surface sterilization

Two Irish potato varieties (Gudenie and Belete) sprouted tubers were collected from Areka Agricultural Research Center and were planted in pots in green house to be used as mother plants for explants source of the study. Young and healthy shoot explants (1.0 to 2.0 cm long) containing lateral buds were cut using sterilized surgical bloods and washed with double distilled water (DDW). Thereafter, explants were dipped in 70% alcohol for one minute and immediately washed with distilled water. Thereafter, they were sterilized in the laminar air flow cabinet with chloride in “Berekina” local bleach with 5% active chlorine diluted to 1.25% as replacement of Sodium hypochlorite for 8 min accompanied by continuous shaking. Surface sterilized segments were washed 4 to 5 times with sterilized distilled water.

Stock solution preparation

Murashige and Skoog (1962) basal medium supplemented with 30 g/l sucrose as a source of carbon and agar (6 g/l) as gelling agent was used throughout this research activity. Initially, full strength stock solutions of macronutrients, micronutrients and vitamins and other organic supplements were separately prepared. To do so, appropriate amount of each nutrient was weighted in grams per liter and dissolved in DDW consecutively in such a way that the next nutrient was added after the first one was completely dissolved. After all the components were completely dissolved using magnetic

stirrer, the solution was poured into plastic bottles and stored at +4°C until used, for maximum of four weeks.

Plant growth regulators stock solution preparation

Plant growth regulators (PGRs) were prepared in 1 mg/ml concentration. The PGRs used for the study were the cytokinins: 6-benzyl amimopurine (BAP) and Kinetin (KN), and the auxins: indol-3- butyric acid (IBA), indol acetic acid (IAA) and Naphthalene acetic acid (NAA). The powdered crystal of the PGRs was first weighed and dissolved in 3-4 drops of 1 N NaOH, and 1 N HCl based on the type of PGR (NaOH for auxins and HCl for cytokinins). Upon complete dissolution, the solution of each PGR was poured into labeled 50-ml plastic bottles and filled with double distilled water (DDW) to the required volume. Then gently stirred and stored at a temperature of +4°C for short term use (a week) and -5°C for long term use (up to a month) until used.

Culture media preparation

In this study, the culture medium used for shoot initiation, multiplication and root induction contained full strength of MS basal medium (Murashige and Skoog, 1962) composed of 100 ml macronutrient, 10 ml micronutrient, and 10 ml vitamin per liter, supplemented with 30 g/l sucrose as a source of carbon and agar (6 g/l) as gelling agent with or without (for control) PGRs, throughout the experiment. Finally, the pH of all media was adjusted to 5.8 using 1 N NaOH and/or 1 N HCL after addition of agar. Because the agar used has shown slight increase in pH after addition to the media, it was then autoclaved at 121°C for 20 min.

Experimental design

For all experiments from shoot initiation to rooting stage, completely randomized (CRD) with factorial arrangement replicated thrice was used.

Acclimatization

The *in vitro* well rooted Irish potato plantlets were taken out gently from each PGR treatments of the culture media jar and washed under running tap water to remove traces of agar that prevent the absorption of nutrients from the acclimatization culture substrates by roots. The plantlets were transplanted into acclimatization plastic cell tray filled with culture substrates of moist red soil, sand soil and compost soil or their mixture in 2:1:1 ratio, respectively. The plantlets were covered with white transparent polythene plastic bag to maintain high humidity, and the plantlets were acclimatized in an open greenhouse environmental condition within the same room by removing the polyethylene sheet and red cheese cloth and irrigated with tap water every day.

Plastic cover was partially removed after a week and completely removed after two weeks; thereafter regenerated plantlets were grown to maturity. Finally, after three weeks, percent of plantlets successfully hardened during growing and died were counted measured by the number of survived shoot lets and died shoot lets from the total transferred plantlets.

Data collection and analysis

Data for number of clean explants, average number of days for shoot emergence, mean number of bud per explants, mean shoot number, shoot length, days to root induction, root number, root length, and acclimatization percentage was carefully collected.

Data Analysis

Data collected at each stage was analyzed using SAS 9.2 version at probability level 0.05 in which means were separated by LSD test method.

RESULTS AND DISCUSSION

Lateral bud survival and shoot initiation

The highest percent number of explants shoot that initiated new shoots was recorded for Gudenie (91.67%) and Beletse (87.50%) varieties at PGR combination of 2 mg/l BAP with 1 mg/l IAA for both varieties (Table 1). Though the values were not significantly different, Gudene variety showed more response. Previously, some other researchers (Hussain et al., 2005; Molla et al., 2011) reported better shoot induction in other potato varieties cultured on MS medium supplemented with BAP and IAA in 2 mg/l to 0.5 mg/l combination and their results are somehow in agreement with the result of this study. With the rise of BAP to IAA concentrations up to 2 to 1 mg/l, shoot induction appeared to increase, but later decreased.

Effect of cytokinins on *in vitro* shoot multiplication

The result analysis showed significant difference at $P<0.05$ level test for number of nodes/explants, number of days to shoot emergence, number of shoot /explant, shoot fresh and dry weight between treatments in both varieties (Table 2). For Gudenie variety, number of nodes/explant, number of shoots/explant and shoot length/explant were significantly higher at 0.5 mg/l BAP and 2 mg/l Kn combination than at other treatments. Number of days to shoot emergence was also found to be shorter at this level of hormonal combination even if it is not significant statistically. Similar pattern had also been observed in Belete variety too, but the response of Gudenie (Figure 1) in terms of the mentioned parameters was better than that of Belete variety, suggesting that varietal difference evokes varying response to the same PGRs. In the case of Belete variety, 0 mg/l BAP to 2 mg/l Kn combination treatment had similar effects as that of 0.5 mg/l BAP to 2 mg/l Kn.

In many plants, multiple shoots can be obtained from the shoot tips or axillary buds by administering BAP or KIN (Bhat et al., 2010; Azar et al., 2011; Thiruvengadam et al., 2011). In line with this study, Mustafa and Sarker(2002) reported that some potato varieties treated with BAP showed better response in terms of shoot per explant, shoot length and number of nodes. The synergistic effect of BAP and Kn for increased shoot

Table 1. Percentage of initiated after 30 days of culturing.

Variety	Hormone combination		No. of explants/tree	No. of initiated explants	No. of explants that died	Shoot Initiation percentage
	BAP (mg/l)	IAA (mg/l)				
Gudenie	0	0	9	5.37	3.63	59.67^{cde}
	1	0.5	9	6.37	2.63	70.83^{c}
	2	1	9	8.25	0.75	91.67^{a}
	3	1.5	9	6.00	3.00	66.67^{cd}
	4	2	9	6.00	3.00	66.67^{cd}
Belete	0	0	9	5.00	4.00	55.56^{cde}
	1	0.5	9	6.75	2.25	75.00^{bc}
	2	1	9	7.87	1.13	87.50^{ab}
	3	1.5	9	6.37	2.63	70.83^{c}
	4	2	9	6.37	2.63	70.83^{c}
LSD						13.24
CV (%)						10.41

Values are means and means represented by the same lowercase letter within a column are not significantly different, whereas those with different letters are significantly different.

Figure 1. Well regenerated potato plantlets ready for acclimatization.

multiplication rate and proliferation was also reported on *B. tulda* and *M. baccifera* (Waikhom and Louis, 2014).

Effect of auxins on *in vitro* root induction

The development of healthy root system was required for the successful establishment of *in vitro* regenerated shoots to adapt to the external environments. For this, those shoots with 1 cm and more in height were taken and transferred onto rooting medium that contained full strength MS supplemented with different IBA and NAA in different concentrations solely or in combination. Compared to the control, both IBA and NAA alone or in

Table 2. Effect of different concentrations of cytokinins (BAP and Kin) combination on the morphogenetic *in vitro* responses of experimental varieties.

Variety	Hormone combination		No. of nodes per explant (n)	No. of days to shoot emergence (n)	No. of shoots per explant (n)	Shoot Length per explant (cm)	Shoot fresh weight per explant (g)	Shoot dry weight per explant (g)
	BAP (mg/l)	KN (mg/l)						
Gudenie	0	0	3.67^{m}	0.00^{e}	0.00^{n}	0.00^{k}	0.*05*^{j}	0.01^{f}
	0.5	0.5	6.67^{cdef}	3.33^{cd}	5.67^{ghij}	4.83^{efghi}	0.11^{efghi}	0.05^{cdef}
	1	1	8.33^{b}	3.17^{d}	7.67^{bcd}	7.50^{a}	0.21^{abcde}	0.07^{abcde}
	1.5	1.5	5.67^{fghi}	3.67^{bcd}	4.67^{jklm}	5.25^{def}	0.13^{fghi}	0.05^{cdef}
	2	2	4.33^{jkl}	3.33^{cd}	3.67^{m}	4.17^{hij}	0.15^{bcdefghi}	0.06^{cdef}
	0.5	0	7.33^{bcd}	3.67^{bcd}	7.33^{bcde}	4.83^{efghi}	0.17^{bcdefg}	0.08^{abcd}
	1	0	8.33^{b}	3.33^{cd}	8.33^{b}	5.50^{cde}	0.13^{efghi}	0.09^{abc}
	1.5	0	4.67^{ijkl}	3.83^{abcd}	6.00^{fghi}	4.50^{fghij}	0.14^{cdefghi}	0.06^{bcdef}
	2	0	3.67^{l}	4.00^{abcd}	5.33^{hijk}	4.08^{ij}	0.25^{ab}	0.08^{abcd}
	0	0.5	4.00^{kl}	4.67^{a}	4.33^{klm}	3.67^{j}	0.23^{abcd}	0.11^{ab}
	0	1	5.67^{fghi}	4.00^{abcd}	5.00^{ijkl}	4.67^{efghi}	0.15^{bcdefghi}	0.07^{bcde}
	0	1.5	6.33^{defg}	3.83^{abcd}	6.00^{fghi}	4.67^{efghi}	0.13^{fghi}	0.06^{bcdef}
	0	2	7.00^{cde}	3.33^{cd}	6.67^{defg}	5.83^{cd}	0.12^{efghi}	0.05^{cdef}
	0.5	2	9.67^{a}	3.17^{d}	10.67^{a}	7.167^{ab}	0.08^{ghi}	0.02^{ef}
	2	0.5	6.33^{defg}	3.33^{cd}	7.00^{cdef}	4.67^{efghi}	0.28^{a}	0.12^{a}
Belete	0	0	0.00^{m}	0.00^{e}	0.00^{n}	0.00^{k}	0.07^{hi}	0.02^{ef}
	0.5	0.5	6.33^{defg}	3.83^{abcd}	5.67^{ghij}	5.33def	0.11^{efghi}	0.06^{cdef}
	1	1	6.67^{cdef}	3.67^{bcd}	7.00^{cdef}	6.83^{ab}	0.17^{bcdefg}	0.08^{abcd}
	1.5	1.5	4.67^{ijkl}	4.00^{abcd}	4.67^{jklm}	5.17^{defg}	0.12^{efghi}	0.05^{cdef}
	2	2	3.67^{l}	4.67^{a}	4.00l^{m}	4.50^{fghij}	0.13^{efghi}	0.05^{cdef}
	0.5	0	6.67^{cdef}	4.33^{ab}	5.67^{ghij}	4.67^{efghi}	0.13^{defghi}	0.04^{def}
	1	0	7.67^{bc}	3.83^{abcd}	6.67^{defg}	5.00^{defgh}	0.18^{abcdefg}	0.05^{cdef}
	1.5	0	5.00^{hijk}	4.67^{a}	5.33^{hijk}	4.33^{ghij}	0.13^{defghi}	0.05^{cdef}
	2	0	5.33^{ghij}	4.17^{abc}	5.00^{ijkl}	4.17^{hij}	0.20^{abcdef}	0.08^{abcd}
	0	0.5	6.00^{efgh}	3.67^{bcd}	5.33^{hijk}	4.00^{ij}	0.18^{abcdefg}	0.08^{abcd}
	0	1	6.33^{defg}	4.67^{a}	5.67^{ghij}	4.67^{efghi}	0.15^{bcdefghi}	0.06^{bcdef}
	0	1.5	6.67^{cdef}	3.17^{d}	7.00^{cdef}	5.33^{def}	0.13^{efghi}	0.05^{cdef}
	0	2	7.33^{bcd}	3.50^{bcd}	8.33^{b}	7.33^{a}	0.14^{bcdefghi}	0.05^{cdef}
	0.5	2	7.33^{bcd}	3.33^{cd}	8.00^{bc}	6.33^{bc}	0.17^{bcdefgh}	0.07^{abcde}
	2	0.5	5.67^{fghi}	4.67^{a}	6.33^{efgh}	4.33^{ghij}	0.24^{abc}	0.10^{abc}
LSD			1.04	0.85	1.04	0.85	0.11	0.05
CV (%)			10.98	14.62	11.01	10.92	7.02	11.09

Values are means and means represented by the same lowercase letter within a column are not significantly different, whereas those with different letters are significantly different at P<0.05. No. = number, expt. = explants and wt = weight.

combination showed significant positive effect on root formation of shoots of both varieties of Irish potato at all concentration levels. The lowest number of day to rooting response per/shoot was 6.33 at 1 mg/l IBA with 0.25 mg/l NAA in Gudenie and 7.33 at 0.5 mg/l IBA with 0.5 mg/l NAA in Belete varieties; the highest root number per/shoot was 23.34 in Gudene and 17.33 in Belete at 1 mg/l IBA with 0.25 mg/l NAA concentration in both varieties; the highest length of root per/shoots was 8.33 cm in Gudenie and 5.33 cm in Belete at 1 mg/l IBA with 0.25 mg/l NAA concentration in both varieties; whereas the highest root fresh weight per/shoots was 0.19 at 0.5 mg/l IBA with 0.5 mg/l NAA in Gudenie and 0.24 at 1.0 mg/l IBA with 0.25 mg/l Kin in Belete varieties. Similar trend was also observed with regard to root dry weight. In both varieties, number of roots/shoot, root length/shoot and root fresh and dry weight had significantly higher values at 1 mg/l IBA to 0.25 mg/l NAA combination treatment than other treatments (Table 3). Though both varieties performed well at 1 mg/l IBA to 0.25 mg/l NAA

Table 2. Effect of different concentrations of cytokinins (BAP and Kin) combination on the morphogenetic *in vitro* responses of experimental varieties.

Variety	Hormone combination		No. of nodes per explant (n)	No. of days to shoot emergence (n)	No. of shoots per explant (n)	Shoot Length per explant (cm)	Shoot fresh weight per explant (g)	Shoot dry weight per explant (g)
	BAP (mg/l)	KN (mg/l)						
Gudenie	0	0	3.67^{m}	0.00^{e}	0.00^{n}	0.00^{k}	0.*05*^{j}	0.01^{f}
	0.5	0.5	6.67^{cdef}	3.33^{cd}	5.67^{ghij}	4.83^{efghi}	0.11^{efghi}	0.05^{cdef}
	1	1	8.33^{b}	3.17^{d}	7.67^{bcd}	7.50^{a}	0.21^{abcde}	0.07^{abcde}
	1.5	1.5	5.67^{fghi}	3.67^{bcd}	4.67^{jklm}	5.25^{def}	0.13^{fghi}	0.05^{cdef}
	2	2	4.33^{jkl}	3.33^{cd}	3.67^{m}	4.17^{hij}	0.15^{bcdefghi}	0.06^{cdef}
	0.5	0	7.33^{bcd}	3.67^{bcd}	7.33^{bcde}	4.83^{efghi}	0.17^{bcdefg}	0.08^{abcd}
	1	0	8.33^{b}	3.33^{cd}	8.33^{b}	5.50^{cde}	0.13^{efghi}	0.09^{abc}
	1.5	0	4.67^{ijkl}	3.83^{abcd}	6.00^{fghi}	4.50^{fghij}	0.14^{cdefghi}	0.06^{bcdef}
	2	0	3.67^{l}	4.00^{abcd}	5.33^{hijk}	4.08^{ij}	0.25^{ab}	0.08^{abcd}
	0	0.5	4.00^{kl}	4.67^{a}	4.33^{klm}	3.67^{j}	0.23^{abcd}	0.11^{ab}
	0	1	5.67^{fghi}	4.00^{abcd}	5.00^{ijkl}	4.67^{efghi}	0.15^{bcdefghi}	0.07^{bcde}
	0	1.5	6.33^{defg}	3.83^{abcd}	6.00^{fghi}	4.67^{efghi}	0.13^{fghi}	0.06^{bcdef}
	0	2	7.00^{cde}	3.33^{cd}	6.67^{defg}	5.83^{cd}	0.12^{efghi}	0.05^{cdef}
	0.5	2	9.67^{a}	3.17^{d}	10.67^{a}	7.167^{ab}	0.08^{ghi}	0.02^{ef}
	2	0.5	6.33^{defg}	3.33^{cd}	7.00^{cdef}	4.67^{efghi}	0.28^{a}	0.12^{a}
Belete	0	0	0.00^{m}	0.00^{e}	0.00^{n}	0.00^{k}	0.07^{hi}	0.02^{ef}
	0.5	0.5	6.33^{defg}	3.83^{abcd}	5.67^{ghij}	5.33def	0.11^{efghi}	0.06^{cdef}
	1	1	6.67^{cdef}	3.67^{bcd}	7.00^{cdef}	6.83^{ab}	0.17^{bcdefg}	0.08^{abcd}
	1.5	1.5	4.67^{ijkl}	4.00^{abcd}	4.67^{jklm}	5.17^{defg}	0.12^{efghi}	0.05^{cdef}
	2	2	3.67^{l}	4.67^{a}	4.00l^{m}	4.50^{fghij}	0.13^{efghi}	0.05^{cdef}
	0.5	0	6.67^{cdef}	4.33^{ab}	5.67^{ghij}	4.67^{efghi}	0.13^{defghi}	0.04^{def}
	1	0	7.67^{bc}	3.83^{abcd}	6.67^{defg}	5.00^{defgh}	0.18^{abcdefg}	0.05^{cdef}
	1.5	0	5.00^{hijk}	4.67^{a}	5.33^{hijk}	4.33^{ghij}	0.13^{defghi}	0.05^{cdef}
	2	0	5.33^{ghij}	4.17^{abc}	5.00^{ijkl}	4.17^{hij}	0.20^{abcdef}	0.08^{abcd}
	0	0.5	6.00^{efgh}	3.67^{bcd}	5.33^{hijk}	4.00^{ij}	0.18^{abcdefg}	0.08^{abcd}
	0	1	6.33^{defg}	4.67^{a}	5.67^{ghij}	4.67^{efghi}	0.15^{bcdefghi}	0.06^{bcdef}
	0	1.5	6.67^{cdef}	3.17^{d}	7.00^{cdef}	5.33^{def}	0.13^{efghi}	0.05^{cdef}
	0	2	7.33^{bcd}	3.50^{bcd}	8.33^{b}	7.33^{a}	0.14^{bcdefghi}	0.05^{cdef}
	0.5	2	7.33^{bcd}	3.33^{cd}	8.00^{bc}	6.33^{bc}	0.17^{bcdefgh}	0.07^{abcde}
	2	0.5	5.67^{fghi}	4.67^{a}	6.33^{efgh}	4.33^{ghij}	0.24^{abc}	0.10^{abc}
LSD			1.04	0.85	1.04	0.85	0.11	0.05
CV (%)			10.98	14.62	11.01	10.92	7.02	11.09

Values are means and means represented by the same lowercase letter within a column are not significantly different, whereas those with different letters are significantly different at P<0.05. No. = number, expt. = explants and wt = weight.

combination treatment, performance of Gudenie explants is better than Belete variety. This shows that as in the case of shooting response, varietal difference evokes varying rooting response to the same PGRs. Asma Rabbani (2001) reported that the best rooting response in plants such as *S. tuberosum* L., was observed when IBA concentrations is at higher proportion than NAA in a combination of the two. The work of Khadiga (2009) also showed that use of 2.5 mg/l of IBA is good to improve root initiation of potato plantlets which agrees with the present finding that rooting increases with high IBA concentration when treated with relatively higher amount of IBA in a combination of the two auxins.

Acclimatization

Results showed that plantlets grown on a mix of moist red soil: sand soil: and compost in 2:1:1 ratio hardened very well compared to other substrates used. Survival

Table 3. Effect of different concentrations of auxins (IBA and NAA) combination on the morphogenetic responses of Irish potato varieties on in *vitro* root induction.

Variety	Hormone combination		Days to rooting response (n)	Root no. per shoot (n)	Length of root per shoot (cm)	Root fresh weight per shoot (g)	Root dry weight per shoot (g)
	IBA (mg/l)	NAA (mg/l)					
Gudenie	0	0	12.33^{abc}	1.33^{lm}	1.67^{l}	0.09^{ef}	0.04^{cde}
	0.25	0.25	8.00^{hijkl}	16.67^{bc}	5.83^{c}	0.13^{bcdef}	0.04^{bcd}
	0.5	0.5	6.67^{kl}	19.00^{b}	7.17^{b}	0.19^{abc}	0.05^{abcd}
	0.75	0.75	9.33^{efghi}	10.00^{def}	5.17^{cde}	0.11^{ef}	0.06^{abcd}
	1	1	10.33^{def}	8.67^{efgh}	3.83^{fghij}	0.17^{bcde}	0.05^{abcd}
	0.25	0	11.33^{bcd}	6.33^{ghijk}	3.17^{hijk}	0.15^{bcdef}	0.06^{abcd}
	0.5	0	10.33^{def}	9.00^{efg}	3.83^{fghij}	0.12^{def}	0.05^{bcd}
	0.75	0	8.67^{fghij}	12.67^{d}	4.33^{defg}	0.14^{bcdef}	0.05^{abcd}
	1	0	6.67^{kl}	15.67^{c}	5.33^{cd}	0.12^{def}	0.05^{abcd}
	0	0.25	8.00^{hijkl}	12.67^{d}	3.50^{ghijk}	0.15^{bcdef}	0.06^{abcd}
	0	0.5	9.67^{defgh}	8.33^{efghi}	3.08^{ijk}	0.13^{bcdef}	0.05^{abcd}
	0	0.75	10.67^{cde}	7.33^{fghij}	2.83^{jk}	0.12^{cdef}	0.04^{bcd}
	0	1	13.33^{a}	4.67^{jk}	2.50^{kl}	0.13^{bcdef}	0.05^{abcd}
	0.25	1	8.33^{ghijk}	15.67^{c}	4.67^{def}	0.12^{cdef}	0.04^{cde}
	1	0.25	6.33^{l}	23.33^{a}	8.33^{a}	0.17^{abcde}	0.06^{abcd}
Belete	0	0	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{g}	0.00^{e}
	0.25	0.25	9.33^{efghi}	12.33^{d}	4.17^{efgh}	0.08^{f}	0.04^{cde}
	0.5	0.5	7.33^{jkl}	15.67^{c}	4.83^{cdef}	0.12^{cdef}	0.05^{abcd}
	0.75	0.75	10.00^{defg}	9.00^{efg}	3.50^{ghijk}	0.13^{bcdef}	0.06^{abcd}
	1	1	9.33^{efghi}	6.00^{hijk}	2.83^{jk}	0.13^{cdef}	0.05^{abcd}
	0.25	0	10.67^{cde}	4.67^{jk}	2.83^{jk}	0.12^{def}	0.04^{bcd}
	0.5	0	10.33^{def}	8.00$_{efghi}$	3.17^{hijk}	0.10^{ef}	0.04^{cde}
	0.75	0	9.67^{defgh}	9.00^{efg}	3.50^{ghijk}	0.09^{f}	0.03^{de}
	1	0	7.67^{ijkl}	12.67^{d}	4.50^{defg}	0.16^{bcdef}	0.06^{abcd}
	0	0.25	9.33^{efghi}	10.00^{def}	3.08^{ijk}	0.19^{abc}	0.07^{abcd}
	0	0.5	10.33^{def}	7.00^{ghij}	2.67^{kl}	0.14^{bcdef}	0.06^{abcd}
	0	0.75	11.33^{bcd}	5.67^{ijk}	2.50^{kl}	0.12^{cdef}	0.05^{abcd}
	0	1	13.00^{ab}	3.67^{kl}	1.67^{l}	0.19^{abcd}	0.08^{abc}
	0.25	1	9.67^{defgh}	10.66^{de}	4.00^{fghi}	0.21^{ab}	0.09a
	1	0.25	7.67^{ijkl}	17.33^{bc}	5.33^{cd}	0.24^{a}	0.08^{ab}
LCD			1.68	2.99	1.01	0.08	0.04
CV (%)			11.18	8.15	6.22	9.20	12.36

Values are means and means represented by the same lowercase letter within a column are not significantly different, whereas those with different letters are significantly different at P<0.05. No. = number, and wt = weight.

rates were 93.33% and 86.66% for Gudene and Belete varieties, respectively (Table: 4). However, for plantlets which acclimatized in unsterile soil mix the survival rates were 53.33 and 46.66% for Gudene and Belete varieties, respectively. This was severely affected by cutworms which cut down the stem from the bottom. Also, some leaves were dried up and consequently detached from the shoots. This may be due to unrestricted loss of water from their leaves or low hydraulic conductivity of roots and root-stem connections (Pospišilova et al., 1999).This result also revealed the highest survival rate on the Gudenie variety plantlets as compared to Belete Irish potato variety after 15 days in the open greenhouse environments. Further observation of survived individual plantlets in the greenhouse revealed no aberrant phenotypes (Table 4).

Conclusions

From the results of experiments conducted for different stages it is possible to conclude that the study provided optimal protocol for micro-propagation of two popular varieties of Irish potato through lateral bud culturing on

Table 4. Effect of different sterilized culture substrates on the survival rate of *in vitro* regenerated plantlets of Gudenie and Belete Irish potato varieties during acclimatization in greenhouse.

Variety	Type of culture substrates	Total no. of explants transferred	No. of survived explants	No. of explants that died	% of survived explants	% of explants that died
Gudenie	Loam soil alone	15	11.83^{bc}	4.25^{bc}	73.33^{bc}	26.67^{bc}
	Sand soil alone	15	9.00^{c}	6.70^{b}	60.00^{cd}	40.00^{ab}
	Compost soil alone	15	12.33^{bc}	3.66^{c}	80.00^{ab}	20.00^{cd}
	Mixture in 2:1:1 ratio	15	14.74^{a}	1.83^{d}	93.33^{a}	6.67^{d}
Belete	Loam soil alone	15	9.33^{c}	6.15^{b}	60.00^{cd}	40.00^{ab}
	Sand soil alone	15	7.83^{d}	8.00^{a}	46.66^{d}	53.34^{a}
	Compost soil alone	15	11.67^{bc}	4.33^{bc}	73.33^{bc}	26.67^{bc}
	Mixture in 2:1:1 ratio	15	13.01^{b}	2.67^{cd}	86.66^{ab}	13.34^{cd}
Significance			**	**	*	*
LSD			0.48	0.96	1.27	7.48
CV (%)			5.38	8.16	3.14	3.47

Values are means and means represented by the same lowercase letter within a column are not significantly different, whereas those with different letters are significantly different at P<0.05.

MS basal medium supplemented with appropriate concentrations of different PGRs in sole or combination. This protocol can thus be utilized to micro-propagate disease free and high quality planting materials of the two varieties to boost its production.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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