academicJournals

Vol. 13(28), pp. 2830-2839, 9 July, 2014
DOI: 10.5897/AJB2013.13287
Article Number: 6765C5E45881
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Effects of plant growth regulators on *in vitro* cultured nodal explants of cassava (*Manihot esculenta* Crantz) clones

Yirssaw Demeke[1]*, Wondyifraw Tefera[2], Nigussie Dechassa[3] and Bekele Abebie[4]

[1]School of Plant and Horticultural Sciences, College of Agriculture, Hawassa University; P. O. Box: 05, Hawassa, Ethiopia.
[2]Plant Biotechnology Division, Jimma Agricultural Research Center; P. O. Box: 192, Jimma, Ethiopia.
[3]Department of Plant Sciences, Haramaya University; P. O. Box: 138, Haramaya, Ethiopia.
[4]Department of Plant Sciences, School of Agriculture, Adama University P. O. Box: 193, Assela, Ethiopia.

Received 16 September, 2013; Accepted 26 May, 2014

Cassava (*Manihot esculenta* Crantz) is one of the major food security crops in Ethiopia. Recently, clean planting materials of improved cassava cultivars are in high demand. A limitation, however, is the low multiplication ratio (1:10) of the crop *via* conventional methods. Thus, a study was undertaken to develop an efficient *in vitro* mass propagation protocol for two elite cassava clones, 44/72-NR and 44/72-NW. Combination of different plant growth regulators (PGRs); four concentrations of 6-benzylaminopurine (BAP) and kinetin (Kin) on shoot multiplication and that of α-naphthaleneacetic acid (NAA) and BAP each at four concentration combination on root induction were assessed. The experiments were factorial laid out in a completely randomized design (CRD) with PGRs as one-factor and clones as another, replicated five times. Significant ($p< 0.05$) interaction effects were observed in response to shoot multiplication and root induction treatments within six weeks of culture. Murashige and Skoog (MS) medium containing BAP and Kin each at 0.75 mg/L gave an average of 7.30 shoots per explant than other media combinations. Consecutively, the regenerated cassava shoots produced an average of 6.14 roots within four weeks in a 0.5 mg/L NAA medium and were successfully acclimatized and transferred to field.

Key words: Cassava, *Manihot esculenta*, clone, *in vitro,* nodal bud, plant growth regulators.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a major food crop for about 800 million people in sub-Saharan Africa and other parts of the world (Taye, 2009). Although reliable statistical information on the area of production and productivity of cassava in Ethiopia is lacking, the crop had long been in cultivation particularly in the Southern, South Western and Western parts of the country (IAR, 1981). In all those growing regions, the farmers are paying more attention to the crop due to its being reliable and cheap source of food available year round. In addition, it is considered as the only flexible and alternative crop to the poor farmers due to its acceptable yield

*Corresponding author. E-mail: yirssawdemeke@gmail.com. Tel: +251-913172991. Fax: +251-462206711/462205421.

under marginal soil and drought prone conditions with minimum inputs (FDRE, 2002). Despite its great potential to attain food security, cassava has gained little attention in the national agricultural research system of Ethiopia. However, a remarkable growth has been registered in its production within the last decade compared with other crops (Amsalu, 2003).

Most recently, the Ethiopian government has considered cassava as one of the major food security crops. It has also been given top research priority with special emphasis on its promotion and expansion to all drought prone and degraded regions of the country (FDRE, 2002). Consequently, there is an urgent need for disease free and high quality cassava planting materials in the national production system. Towards this aim, Hawassa Agricultural Research Center (HaARC) has released two cassava varieties, *Qulle* and *Kello* for the Southern region (HaARC, 2008) and Jimma Agricultural Research Center (JARC) is about to release a number of varieties for the South Western parts of the country. The limitations, however, are the low multiplication ratio (1:10) of the crop using traditional production methods and systemic infections, which hamper the progress in replacing susceptible or non-adapted varieties, or in expanding cassava into new areas. Thus, tissue culture techniques could be a feasible option to overcome these problems.

Plant tissue culture techniques were developed five decades ago (Santana et al., 2009) and since then, they have been recognized as powerful tools for studying and solving basic and applied problems of cassava production and productivity like many other crops (Robert and Dennis, 2000; Adane, 2009). This technique is faster and requires less space than that required for conventional methods of preparing cuttings (Loyola-Vargas and Vazques-Flota, 2006). In this regard, available research findings (Were et al., 2004; Le et al., 2007) have long proved the tissue culture techniques to be the only realistic and efficient means for supplying large volumes of true-to-type clean planting materials of any new high-value crop variety like cassava within short period. However, multiplication of tissues requires the optimization of the plant growth regulators (PGRs) concentrations in the MS media and these PGR requirements vary with species since PGRs often determine the course of morphogenesis (Staden et al., 2008; IITA, 2009). Earlier, Konan et al. (2006) and Acedo (2009) revealed that shoot multiplication of many crops including cassava could be enhanced with a relatively higher concentration of cytokinins (disrupt apical dominance of the shoot tips); while rooting is promoted by the use of auxins. Kane (2005) also reported cytokinins, 6-benzylaminopurine (BAP) and kinetin (Kin) and auxin, α-naphthaleneacetic acid (NAA) as the most widely used and effective (0.01-10 mg/L) PGRs for shoot multiplication and root induction, respectively.

Smith et al. (1986) and Konan et al. (1997) were the first to report success in the production of an average of 5 to 6 shoots per bud from *in vitro* culture of cassava, although the field survival rates recorded were low (62 - 74%). Several workers have since these earlier studies also succeeded in *in vitro* nodal culture of cassava (Konan et al*.,* 2006; Medina et al., 2006; Escobar et al., 2009). Despite these reported successes, no work has so far been done to develop an *in vitro* mass propagation protocol for those elite cassava clones in Ethiopia, and development of such a protocol is urgently needed to ensure rapid mass propagation and dissemination of the improved cassava clones to respond to the prevailing high interest to increase cassava production and introduce it into new potential areas of the country. In view of all these, the objective of this study was to develop an efficient *in vitro* mass propagation protocol for two elite cassava clones, 44/72-NR and 44/72-NW.

MATERIALS AND METHODS

Study location

The research work was conducted in the Tissue Culture Laboratory of the Plant Biotechnology Division, Jimma Agricultural Research Centre (JARC), Jimma, Ethiopia. The center is located 363 km south west of the capital, Addis Ababa at 7°46' N latitude and 36°0'E longitude.

Genetic materials

Two preferred cassava clones, 44/72-NR and 44/72-NW, obtained from JARC were selected due to their best performance across the South Western region of Ethiopia with respect to early maturity, high productivity, and resistance to diseases and pests compared to other cultivars tested.

Stock plant establishment

Twenty-five to thirty cm long mature stem cuttings with 5-8 nodes of both clones were taken from the horticulture research field of JARC and soaked in a 0.3% (w/v) Kocide-101 solution for five minutes followed by rapid rinsing under running tap water, twice. The cuttings were planted in plastic pots of 2 L volume filled with a pre-sterilized potting mix of forest soil, well-decomposed coffee husk, and red sand at the respective ratio of 1:1:2 by volume (JARC experience) and the stock plants were established in the maintenance greenhouse of the biotechnology division at an average temperature of 25 ± 2°C.

Explant sterilization and initiation

Three-to-five-week old sprouted nodal buds of 1.5 - 2.0 cm length were collected from both clones, washed thoroughly once under running tap water using a liquid detergent and kept soaked in a 0.3% (w/v) Kocide-101 solution for 30 min followed by rapid rinsing three times with double distilled water in the laminar flow hood cabinet. The nodal buds were then soaked in 70% (v/v) ethyl alcohol for 1 min and immediately rinsed three times with double distilled water before the actual sterilization with sodium hypochlorite (NaOCl). After disinfection in 0.1% (v/v) NaOCl with 1-3 drops of Tween-20 (wetting agent) for 10 min and washing with double distilled water thrice, all the dead and chlorine affected tissues of the nodal bud explants were removed under aseptic conditions.

The nodal buds were then placed in test tubes containing solid Murashige and Skoog (MS-1962) medium supplemented with 100 mg/L myo-inositol, 1 mg/L thiamine, 1.6 mg/L GA_3, 0.01 mg/L NAA with 3% (w/v) sucrose (carbon source) and 0.8% (w/v) agar (gelling agent) at 5.8 pH (IITA, 2009) for initiation. The test tubes were incubated at 23-24°C under 16/8 h light/dark cycles and a light intensity of 1000 Lux. The nodal buds of the two clones normally took 1-2 months to develop into a plantlet ready for sub culture.

Treatments and experimental design

The initiated 4-8 weeks old plantlets from the two clones were multiplied through stem cuttings containing single node. The nodal buds were placed onto full strength solid MS basal medium in Magenta culture vessels containing different concentrations and combinations of PGRs namely 6-Benzylaminopurine (BAP) and Kinetin (Kin) each at four concentrations (0, 0.75, 1.5, and 2.25 mg/L) in all combinations for shoot multiplication and α-naphthalene acetic acid (NAA) at four concentrations (0, 0.5, 1, and 1.5 mg/L) in all combination with BAP at four concentrations (0, 2.5, 5, and 7.5 mg/L) for root induction (Table 1). Before conducting the rooting experiment, the initiated mini *in vitro* derived shoots were transplanted onto a fresh growth regulator free MS basal medium for five weeks in order to avoid any carry over effect of NAA used at the initiation stage.

The two clones were equally and randomly treated while all exogenous factors were held constant except the factors being considered. In all cases, the brim of each test tube and/or Magenta culture vessel was flamed together with their caps prior to closing and sealed with a strip of Para film. The vessels were clearly labelled with the media code, date of inoculation as well as name of variety and incubated at 25 ± 2°C, 16 h photoperiod with a light intensity of 2000-3000 Lux from cool white 40 watt florescent bulbs. Completely Randomized Design (CRD) in factorial arrangement with PGRs as one-factor and clone as another factor with five replications and five nodal buds per replication was used. In order to ensure reliability of the results, the experiments in the study were repeated twice.

Acclimatization and transfer of plantlets

After the plantlets had produced 3-5 leaves and initiated roots within four weeks, they were removed from the glass jar and transferred to a container of warm (10°C) double distilled water and gently rinsed to remove the agar-media off the roots followed by immersion into a 0.3% (w/v) Kocide-101 solution (prophylaxis measure). Finally, 30 plantlets (15 plantlets from each clone) were planted in six plastic pots of 2 L volume filled with a pre-sterilized potting mix of forest soil, well-decomposed coffee husk, and red sand at the respective ratio of 1:1:2 by volume (JARC experience) and acclimatized in a 70% shade netted greenhouse for two successive weeks followed by 30% shaded greenhouse for a week and then transferred to the environment with ambient conditions.

Data collected

After 2-3 weeks, the plantlets were studied for various growth analysis parameters and transferred to green house. During the research work, data on shoot length (cm), number of nodes/plantlet, number of leaves/plantlet, number of shoot/plantlet, number of roots/plantlet, root length (cm)/plantlet, shoot fresh and dry weights (g) as well as root fresh and dry weights (g) and root to shoot weight ratio were recorded on 1600 samples taken from shooting and rooting experiments each consisting of 800 samples.

Data analysis

The average data estimated from the raw data collected for each trait in the two experiments were subjected to analysis of variance (ANOVA) using statistical analysis software (SAS), version 9.2. Log and arcsine transformation techniques were applied for all counted and percentage data of each trait respectively, in order to fulfil the assumptions of ANOVA (Montgomery, 2005) and mean comparesons were undertaken according to Student-Newman-Keuls multiple-range test (SNK) at the alpha level of 5%.

RESULTS AND DISCUSSION

Effects of cytokinins on *in vitro* shoot multiplication of cassava

Different concentrations and combinations of cytokinins, BAP and Kin, were tested to compare their effectiveness on shoot regeneration of cassava using axillary nodal bud culture. Shoot regeneration of the two cassava clones were influenced differentially by the concentrations and combinations of the cytokinins used (Table 2). Both BAP and Kin had a significant ($P \leq 0.01$) effect on the number of shoots, leaves, and nodes, as well as shoot length, shoot fresh and dry weights whilst only leaf number was significantly ($P \leq 0.05$) affected by clone type (Table 2). Similarly, all shoot growth and development parameters considered were significantly ($P \leq 0.01$) affected by BAP x Kin interaction. The effect of interaction between BAP and clone type on shoot fresh and dry weight parameters was also significant ($P \leq 0.01$). However, Kin × clone and BAP × Kin × clone interactions had no significant effects on all the shoot multiplication parameters evaluated.

The full strength solid MS basal medium supplemented with BAP and Kin combination (both at 0.75 mg/l) resulted in regeneration of the highest mean maximum values on almost all shoot growth parameters considered namely, number of shoots (7.30), leaves (5.67), and nodes (5.65), shoot length (5.05 cm), shoot fresh (2.55 g), as well as dry (2.20 g) weights per explant (Table 3). This could be associated with the synergistic effect of the two cytokinins when combined, and their effectiveness even at low concentrations as earlier reported (Onuoch and Onwubiku, 2007; Staden et al., 2008) in cassava. Both authors observed similar effects of the two cytokinins when combined at concentrations as high as 0.75-1 mg/L. Growth and development inhibitory effect of the two cytokinins were reported when they were combined at very high concentrations (Berrie, 1984; FFTC, 2009) in cassava, which corroborates with our present observation. In general, combining BAP and Kin (both above 0.75 mg/L) has no additional advantage as almost all of the evaluated shoot growth parameters failed to improve at higher concentrations and combinations of the two cytokinins (Table 3).

In accordance with the results of this study, previous reports in cassava (Kartha et al., 1984; Ogburia, 2003; Konan et al., 2006) and Egyptian sweet potato (El Far et

Table 1. Shoot multiplication and rooting treatment combinations arrangement.

Experiment type	Hormone Source (mg/L)	Treatment combination															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Shoot Multiplication	BAP	0	0	0	0	0.75	0.75	0.75	0.75	1.5	1.5	1.5	1.5	2.25	2.25	2.25	2.25
	Kin	0	0.75	1.5	2.25	0	0.75	1.5	2.25	0	0.75	1.5	2.25	0	0.75	1.5	2.25
Root regeneration	BAP	0	0	0	0	2.5	2.5	2.5	2.5	5	5	5	5	7.5	7.5	7.5	7.5
	NAA	0	0.5	1.0	1.5	0	0.5	1.0	1.5	0	0.5	1.0	1.5	0	0.5	1.0	1.5

Table 2. Mean square estimates of *in vitro* induced shoot parameters of cassava explants as affected by different concentrations and combinations of BAP, Kin and clone (ANOVA).

Treatment	DF	Shoot multiplication parameters/explant					
		Shoot				Number of	
		Number (No)	Length (cm)	Fresh Wt (g)	Dry Wt (g)	Leaves (No)	Nodes (No)
BAP	3	2.665**	3.602**	1.600**	1.379**	2.670**	1.302**
Kin	3	1.28**	0.680**	0.218**	0.252**	0.425**	0.225**
BAP x Kin	9	0.52**	0.479**	0.171**	0.150**	0.386**	0.098**
Clone	1	0.01	0.021	0.001	0.007	0.028*	0.530
BAP x Clone	3	0.04	0.009	0.042**	0.029**	0.049	0.016
Kin x Clone	3	0.04	0.024	0.001	0.001	0.007	0.002
BAP x Kin x Clone	9	0.09	0.015	0.004	0.004	0.010	0.014
R^2 (%)		77.0	89.8	85.9	88.9	88.5	73.1

*, ** indicates that Mean square values of shoot parameters considered are significant at 0.05 or 0.01 probability level, respectively.

al., 2009) showed a reduction of shoot growth characters in response to in vitro culture when BAP and Kin were combined at higher concentrations.

Significant ($P < 0.01$) differences were observed between the two clones for the number of leaves regenerated (Supplementary Data). The mean number of leaves regenerated per explant from clone 44/72-NR was 7% higher than the number of leaves regenerated from clone 44/72-NW. This variation in number of leaves between the clones could be associated with the variation in the amount of the endogenous cytokinins available in the explant buds rather than the effect of the externally applied ones. In agreement with the results of the present study, Escobar et al. (2009) reported that variations between clones in their response to similar treatments were primarily due to the presence of variable levels of cytokinin in explant buds.

Moreover, the leaves produced from clone 44/72-NR were morphologically good looking and had deep green colour than of 44/72-NW (Figure 1). This variation in the colour of the regenerated leaves might be due to the two cytokinins which enhanced chlorophyll development in the leaves of clone 44/72-NR as compared with the leaves of clone 44/72-NW, which also suggests that chlorophyll development at least in these two cassava clones is controlled by different genes. Consistent with this result, Konan et al. (2006) and Wondwosen (2009) also reported differential responses of different cassava clones *in vitro* for number of leaves regenerated.

As shown in Figure 2, the highest mean shoot fresh (1.94 g) and dry (1.56 g) weights per explant were observed in both clones on MS medium supplemented with 0.75 mg/L BAP. This shows the effectiveness of BAP at lower concentration in inducing axilary shoot proliferation and development of chlorophyll and confirms the report of Kulaeva et al. (2002) which demonstrated that the enhanced development of etioplasts especially grana in response to low BAP could accelerate the photosynthetic rate and thereby influence the shoot fresh and dry weights of the two clones. The result also corroborates the earlier submission by Najma and Uzman (2001) who reported the potential of BAP to elicit a maximum response at appreciably lower concentrations.

Effects of auxin and cytokinin on *in vitro* root induction of cassava

In this experiment, different concentrations and combinations of NAA and BAP were tested to compare their effectiveness on root regeneration of cassava. All the characters evaluated were found to be influenced by the different concentrations and combinations of the NAA and BAP applied. The number of main roots, root length, root fresh and dry weights, and root to shoot weight ratio

Table 3. Interaction effect of BAP and Kin on shoot growth and development of cassava explants after six weeks of *in vitro* culture.

Cytokinin source		Growth parameters/explants					
		Shoot				Number of	
BAP (mg/L)	Kin (mg/L)	Number (No)	Length (cm)	Fresh weight (g)	Dry weight (g)	Leaves (number)	Nodes (number)
0.00	0.00	1.60^{k}	1.53^{f}	0.55^{g}	0.36^{gh}	2.33^{f}	2.34^{fg}
	0.75	4.60^{c}	3.82^{b}	1.70^{b}	1.25^{c}	3.10^{d}	3.18^{c}
	1.50	3.90^{de}	3.82^{b}	1.51^{c}	1.04^{d}	2.74^{e}	2.70^{de}
	2.25	3.10^{fg}	3.09^{d}	1.30^{d}	0.84^{e}	2.40^{f}	2.38^{ef}
0.75	0.00	5.40^{b}	3.69^{b}	1.81^{b}	1.42^{b}	4.02^{b}	3.61^{b}
	0.75	7.30^{a}	5.05^{a}	2.55^{a}	2.20^{a}	5.67^{a}	5.65^{a}
	1.50	4.10^{d}	3.41^{c}	1.55^{c}	1.08^{d}	3.29^{c}	3.38^{bc}
	2.25	3.20^{f}	3.12^{d}	1.24^{d}	0.80^{e}	2.84^{e}	2.86^{d}
1.50	0.00	3.90^{de}	3.53^{c}	1.42^{c}	0.98^{d}	2.72^{e}	2.76^{de}
	0.75	3.70^{e}	3.04^{d}	1.05^{e}	0.65^{f}	2.83^{e}	2.84^{d}
	1.50	2.90^{fgh}	2.11^{e}	0.78^{f}	0.43^{g}	2.21^{fg}	2.24^{fgh}
	2.25	2.40^{i}	1.55^{f}	0.68^{f}	0.34^{gh}	2.08^{gh}	2.10^{fgh}
2.25	0.00	2.80^{gh}	1.97^{e}	0.50^{gh}	0.25^{hi}	2.13^{gh}	2.12^{fgh}
	0.75	2.60^{hi}	1.37^{f}	0.42^{ghi}	0.20^{i}	1.98^{hi}	1.97^{fgh}
	1.50	2.00^{j}	0.93^{g}	0.37^{hi}	0.16^{i}	1.88^{i}	1.88^{gh}
	2.25	1.80^{jk}	0.65^{h}	0.31^{i}	0.12^{i}	1.80^{i}	1.82^{h}
CV (%)		9.84	8.05	8.14	6.97	6.68	6.00

Means followed by the same letter within a column are not significantly different at 5% probability level, according to Student-Newman-Keuls multiple-range test (SNK).

characters were significantly ($P \leq 0.01$) affected by both NAA and BAP (Table 4).

Similarly, there was significant ($P \leq 0.01$) difference between the two clones for all the root growth parameters evaluated except root fresh weight and root to shoot weight ratio. Also, NAA × BAP interaction had significant ($P \leq 0.01$) effect on all root growth parameters examined. The interaction effect of NAA and clone was observed to be significant ($P \leq 0.05$) only on main root length, while the effect of BAP × clone interaction had non-significant effect on all parameters considered. Moreover, only main root length was significantly ($P \leq 0.01$) influenced by NAA × BAP x clone interactions.

The lower auxin-cytokinin combination (0.5 mg/l NAA and 2.5 mg/L BAP) led to a considerable increase in the mean main root number (3.86), root fresh (3.57 g), and dry (2.08 g) weights per explant (Table 5). Likewise, the maximum mean root to shoot weight ratio (3.04) per explant was recorded in 1.0 mg/L NAA and 2.5 mg/L BAP combination enriched medium. This could be associated with the synergistic effect of auxin and cytokinin, when both were applied together at lower concentrations. This is in line with the earlier submission by Staden et al. (2008) who reported synergistic effect of NAA and BAP in cassava when combined at lower concentrations. Also, treatment combinations of 1.5 mg/L NAA with 5.0 and 7.5 mg/L BAP resulted in significant ($P > 0.05$) reduction in mean root fresh and dry weights (gm) per explant, respectively. Previously, Konan et al. (2006) and Zimmermann et al. (2009) found an antagonistic effect of higher BAP on NAA activity on root induction of selected cassava clones, which corroborates the results of the present study.

On the other hand, the shoots of both clones cultured on MS medium with 0.5 mg/L NAA alone had numerous long main roots and very few secondary roots (Figure 3). This is a well documented unique effect of auxin (NAA) that regulates numerous developmental processes such as tissue swelling, cell division, cell elongation, and formation of adventitious roots when it is fortified at lower concentrations (Wilmoth et al., 2005). From the results of this study, it was observed that combining auxin and cytokinin in root induction medium has no additional benefit since NAA *per se* is enough to achieve the required results. Similar findings with this result were reported previously in cassava (Ogburia, 2003; Le et al., 2007; Escobar et al., 2009; Wondwosen, 2009) and Egyptian sweet potato (El Far et al., 2009).

Significant ($P \leq 0.01$) variations among the two clones were observed for the number of main root and root dry weight characters. Clone 44/72-NR produced the maximum mean number of main root (2.86) and root dry

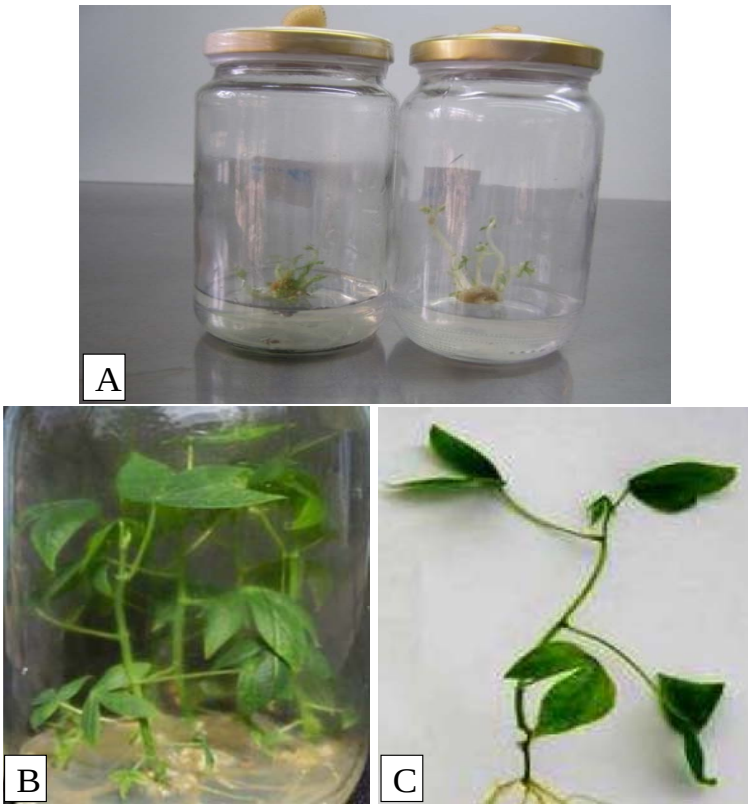

Figure 1. Morphological variations between the two cassava clones: *in vitro* proliferated shoots of clone 44/72-NR (right) and 44/72-NW (left) in 0.75 mg/L BAP and Kin media after two weeks of culture. (A), *In vitro* derived shoots of clone 44/72-NR (B) and 44/72-NW (C) in 0.75 mg/L BAP and Kin media after six weeks of culture.

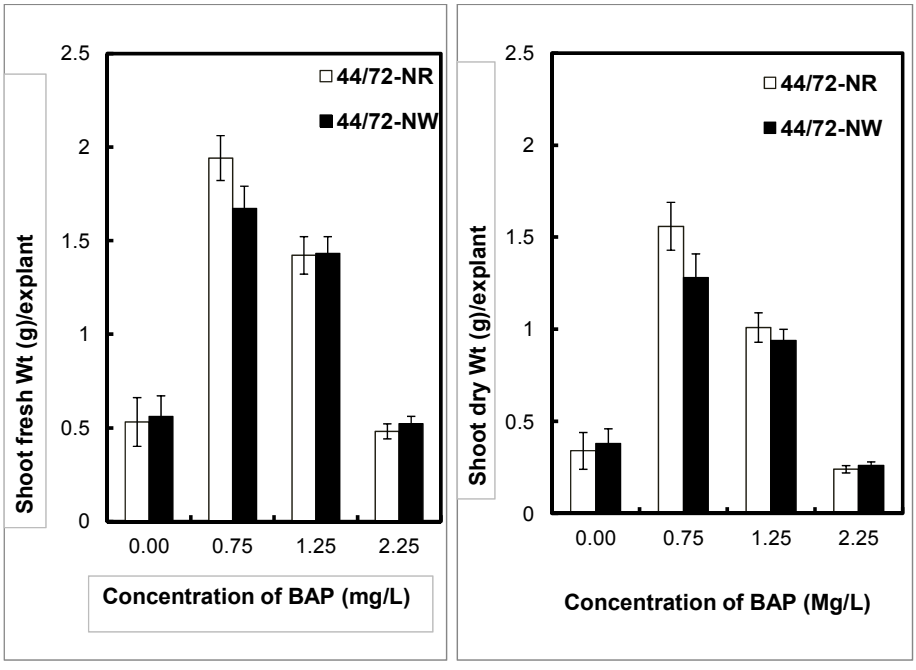

Figure 2. Interaction effect of BAP and clone on fresh and dry weights of cassava shoots after six weeks of *in vitro* culture.

Table 4. Mean square estimates of *in vitro* induced root parameters of cassava explants as affected by different concentrations and combinations of NAA, BAP and clone (ANOVA).

Treatment	DF	Root parameters/explant				Root : shoot weight ratio
		Number (No)	Length (cm)	Fresh weight (g)	Dry weight (g)	
NAA	3	1.210**	4.561**	2.997**	1.713**	0.259**
BAP	3	2.718**	3.794**	3.156**	2.507**	5.041**
NAA x BAP	9	0.141**	0.473**	0.555**	0.508**	0.102**
Clone	1	0.226**	0.299**	0.040	0.105**	0.042
NAA x Clone	3	0.001	0.029*	0.024	0.003	0.004
BAP x Clone	3	0.012	0.015	0.015	0.012	0.016
NAA x BAP x Clone	9	0.012	0.020**	0.019	0.013	0.013
R^2 (%)		91.2	96.7	92.1	92.1	78.0

*, ** indicate that Mean square values of root parameters considered are significant at 0.05 or 0.01 probability level, respectively.

Table 5. Interaction effect of NAA and Kin on in vitro root induction of cassava shoots after six weeks of culture.

Auxin source	Cytokinin source	Root growth parameters/explant			
NAA (mg/L)	Kin (mg/L)	Number (No)	Fresh weight (g)	Dry weight (g)	Root : shoot weight Ratio
0.0	0.0	3.50^{d}	3.21^{c}	2.05^{b}	2.50^{c}
	2.5	2.48^{g}	3.19^{c}	1.91^{c}	2.17^{d}
	5.0	2.10^{h}	1.83^{f}	1.09^{f}	0.86^{g}
	7.5	1.58^{k}	1.51^{g}	0.86^{g}	0.19^{i}
0.5	0.0	6.14^{a}	7.83^{a}	5.95^{a}	3.16^{a}
	2.5	3.86^{c}	3.57^{b}	2.08^{b}	2.56^{c}
	5.0	2.74^{f}	2.31^{e}	1.31^{e}	1.06^{fg}
	7.5	1.97^{i}	1.46^{gh}	0.81^{g}	0.54^{h}
1.0	0.0	4.13^{b}	2.55^{d}	1.82^{c}	2.83^{b}
	2.5	3.15^{e}	2.22^{e}	1.46^{d}	3.04^{ab}
	5.0	2.18^{h}	1.43^{gh}	0.90^{g}	1.19^{f}
	7.5	1.67^{jk}	1.17^{i}	0.71^{gh}	0.17^{i}
1.5	0.0	2.50^{g}	1.59^{g}	1.07^{f}	1.59^{e}
	2.5	2.25^{h}	1.29^{hi}	0.81^{g}	2.32^{cd}
	5.0	1.78^{j}	0.98^{j}	0.58^{hi}	1.20^{f}
	7.5	1.58^{k}	0.85^{j}	0.47^{i}	0.25^{i}
CV (%)		5.27	6.60	7.03	12.32

Means followed by the same letter within a column are not significantly different at 5% probability level, according to Student-Newman-Keuls multiple-range test (SNK).

weight (1.57 g) per explant as compared with clone 44/72-NW (Table 6) even when the *in vitro* rooting environment was the same for both. This might be due to the genetic variation that exists between the two clones. It could also be associated with long and high number of main and secondary roots (Supplementary Data) of clone 44/72-NR when compared with that of clone 44/72-NW, which directly increase number of main root and root dry weight. Furthermore, this clone was produced higher number of leaves in the shooting phase (Figure 1), which might have helped in supplying higher amount of photosynthates to the root which is a strong sink. Corroborate with this finding, Smith et al. (1986) and Acedo (2009) in cassava, Anura (2009) and Geleta (2009) in sweet potato previously reported the responses of root number and dry weight as it was different for different cassava clones *in vitro*.

Acclimatization of *in vitro* derived plantlets

The ultimate success of *in vitro* propagation lies in the

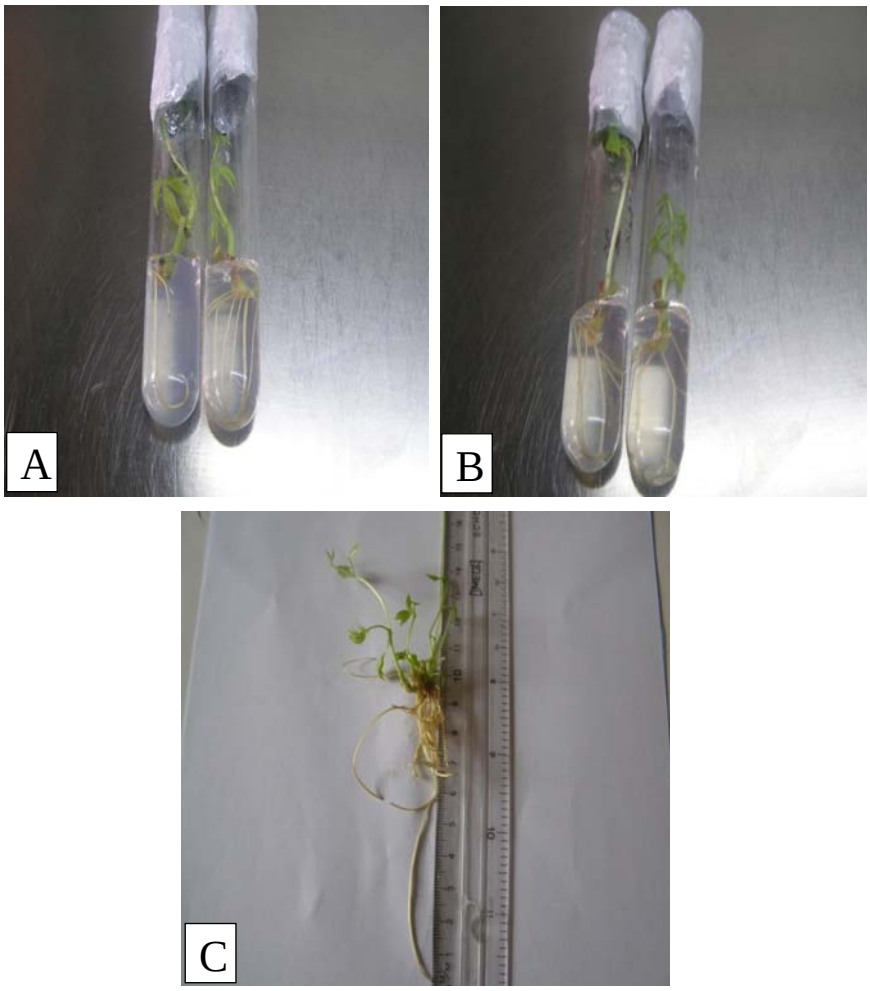

Figure 3. *In vitro* root development of clone 44/72- NR (A) and 44/72- NW (B) in 0.5 mg/L NAA media after four weeks of culture. Root length = 5.5 cm (C).

Table 6. Variation in number and dry weight of roots between two cassava clones after six weeks of *in vitro* culture

Clone	Parameters per explant	
	Number of roots (No)	Dry weight of root (g)
44/72-NR	2.86[a]	1.57[a]
44/72-NW	2.59[b]	1.42[b]

Means followed by the same letter within a column are not significantly different at 5% probability level, according to Student-Newman-Keuls multiple-range test (SNK)

successful establishment of plantlets in the soil. In this study, the *in vitro* rooted plantlets of both clones were planted onto a commonly recommended pre-sterilized potting mix of forest soil: well decomposed coffee husk: red sand (1:1:2 ratio by volume) to evaluate their survival rate under open field condition. Out of a total of 50 plantlets (25 plantlets from each clone) taken for final acclimatization, 93.3% of 44/72-NR and 86.7% of 44/72-NW survived and all plants were successfully transferred to field (Figure 4).

Conclusion

From the study, it was observed that *in vitro* shoot growth and development using nodal bud explants of the two cassava clones were best attained when the *in vitro* initiated plantlets were inoculated onto full strength solid MS medium supplemented with 0.75 mg/L BAP in combination with the same level of Kin. Likewise, the best *in vitro* root induction in the two cassava clones was observed when the *in vitro* derived shoots of both clone

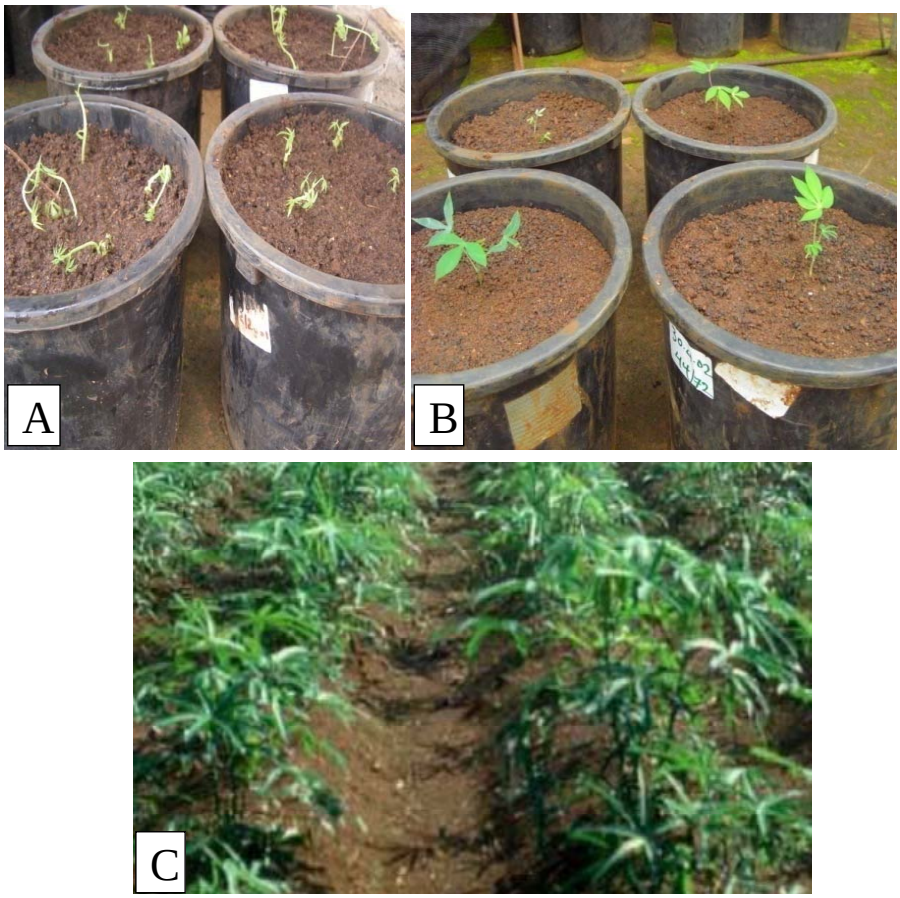

Figure 4. *Ex vitro* acclimatization of *in vitro* raised cassava plantlets: initiated plantlets during first day of acclimatization (A), seedlings after two weeks (B), survived seedlings under open field condition after 60 days (C).

were cultured in MS medium containing only 0.5 mg/L NAA. Thus, from our study, it can be concluded that for effective micro-propagation of the two cassava clones, MS medium need to be standardized in such a way that the shoot multiplication medium could be supplemented with a combination of BAP and Kin (each at 0.75 mg/L) and 0.5 mg/L NAA for root induction.

ACKNOWLEDGEMENTS

We gratefully acknowledged all institutions and individuals involved during the research work.

REFERENCES

Acedo VZ (2009). Meristem culture and micropropagation of cassava. J. Root Crops. 45: 65-78.

Adane A (2009). A Review on agricultural biotechnology research and development in Ethiopia. Afr. J. Biotechnol. 8: 7196-7204.

Amsalu N (2003). Genetic variations in cassava at Jimma, Southwest Ethiopia. J.Trop. Sci. 46: 171-175.

Anura H (2006). Tissue culture and meristem culture in sweet potato. Unpublished M.Sc. Thesis, School of Graduate Studies, University of Hannover, Germany. 71p.

Berrie AM (1984). Germination and Growth Regulators. In: Advanced Plant Physiology, W.B. Walcom, (eds.). English Language Book Society/Longman, England. 987 p.

El Far MMM, Mangoury KEl, Elazab HEM (2009). Novel plant regeneration for Egyptian sweet potato (*Ipomoea batatas* L.) Abees cultivar via indirect organogenesis stimulated by initiation medium and cytokinin effects. Aust. J. Basic Appl. Sci. 3: 543-55.

Escobar R, Munoz L, Roca WM (2009). Cassava Micropropagation for Rapid "Seed Production" Using Temporary Immersion Bioreactors. International Center for Tropical Agriculture (CIAT). Cali, Colombia. [Online] Available from: http:// www. redbio. Org /portal/ encounters /enc2001/ posters /07/ pdf/Sin%20registro5.pdf. [Accessed on 21January, 2010].

FDRE (2002). Industrial Development Strategy. [Amharic Version].

FFTC (2009). Micropropagation of cassava suspension culture derived from nodal explants. Kor. J. Plant Tiss. Cult. 27:185-189.

Geleta D (2009). In vitro Production of Virus Free Sweet Potato (*Ipomoae batatas* L.) by Meristemculture and Thermotherapy. Unpublished M.Sc. Thesis, School of Graduate Studies, Addis Ababa University, Ethiopia. 65 p.

HaARC (2008). Ministry of Agriculture and Rural Development, Crop Development Department, Crop Variety Register Book. June 2006, Addis Ababa, Ethiopia. p.119.

IAR (1981). Research on Horticultural Crops in Ethiopia. IAR, Addis Ababa, Ethiopia.

IITA (2009). Cassava Processing and Gene Banking Manual. [Online] Available from: www.iita.org. [Accessed on 19 February, 2010].

Kane ME (2005). Shoot Culture Procedure in Plant Development and Biotechnology. In: R. N. Trigiano, and D. J. Gray, (eds.). CRC Press,

Boca Raton, Washington D.C.
Kartha KK, Gamborg OL, Constable F, Shylock JP (1984). Regeneration of cassava plants from apical meristem. J. Plant Sci. 2: 107-113.
Konan NK, Schopke C, Cárcamo R, Beachy RN, Fauquet C (1997). An efficient mass propagation system for cassava (*Manihot esculenta* Crantz) based on nodal explants and axillary bud-derived meristems. Plant Cell Reports. 16: 444-449.
Konan NK, Sangwan RS, Sangwan NBS (2006). Efficient *in vitro* shoot regeneration system in cassava (*Manihot esculenta* Crantz). J. Plant. Breed. 113: 227-236.
Kulaeva O, Burkhanova E, Kavaiko N, Selvankina S, Porfirova S, Maslova G, Zemlychenko Y (2002). Chloroplast effect on the leaf response to cytokinin. J. Plant Physiol. 159:1309-1316.
Le BV, Anh BL, Soytong K, Danh ND, Anh Hong LT (2007). Regeneration of cassava (*Manihot esculenta* Crantz) plants. J. Agric. Technol. 3: 123-127.
Loyola-Vargas VM, Vazquez-Flota F (2006). Plant Cell Culture Protocols (2nd ed.). Methods in Molecular Biology, 318: 3-8. In: Loyola-Vargas VM, Vazquez-Flota F (eds.). Human Press Inc. New Jersy. 672 p.
Medina RD, Faloci MM, Gonzalez AM, Mroginski LA (2006). *In vitro* cultured primary roots derived from stem segments of cassava (*Manihot esculenta*) can behave like storage organs. Ann. Bot. 209: 444-449.
Montgomery D (2005). Design and Analysis of Experiments (6th ed.). John Wiley and Sons. Inc, USA. 792 p.
Murashige T, Skoog F (1962). A Revised medium for rapid growth and bioassays with tobacco tissue culture. J. Plant Physiol. 9: 473-497.
Najma Y, Uzman K (2001). Effect of Growth Hormones: GA_3, IAA and Kinetin on the External Morphology and Flowering of *Phaseolus aureus* L. Department of Botany, University of the Punjab, Quaid-e-Azam campus, Lahore. 454 p.
Ogburia MN (2003). Somatic embryogenesis, plantlet regeneration and micropropagation of cultivars and F_1 hybrids of *Manihot esculenta* Crantz. Biol. Plan. 137: 429-432.
Onuoch CI, Onwubiku NJC (2007). Micropropagation of cassava (*Manihot esculenta* Crantz) using different concentration of N-6-benzyl amino purin (BAP). J. Engin. and Appl. Sci. 2: 1229-1231.
Robert N, Dennis J (2000). Plant Tissue culture Concepts and Laboratory Exercises (2nd ed.). CRC press. 454 p.
Santana MA, Romay G, Matehus J, Vicente-Villardón J L, Demey JR (2009). A simple and low-cost strategy for micropropagation of cassava (*Manihot esculenta* Crantz). Afr. J. Biotechnol. 8 (16): 3789-3897.
Smith MK, Biggs BJ, Scott KJ (1986). *In vitro* propagation of cassava (Manihot esculenta Crantz). J. Plant Cell, T. and Org. Cult. 6: 221-228.
Staden JV, Zazimalova E, George EF (2008). Plant Growth Regulators II: Cytokinin, their Analogues and Antagonists. In: George EE, Hall MA, and De Klerk G, (eds.). 3: 205-226.
Taye B (2009). Cassava, Africa's Food Security Crop. [Online] Available from: http://www.worldbank.org/htm/cigar/newsletter/Mar96/4cas2.htm. [Accessed on 16March, 2009].
Were HK, Winter S, Maiss E (2004). Virus infecting cassava in Kenya. J. Plant Dise. 88: 17-22.
Wilmoth JC, Wang S, Tiwari SB, Joshi AD, Hagen G, Guilfoyle TJ, Alonso JM, Ecker JR, Reed JW (2005). NPH4/ARF7 and ARF19 promote leaf expansion and auxin-induced lateral root formation. Plant J. 43: 118-130.
Wondwosen A (2009). Micropropagation of selected cassava varieties. Unpublished M.Sc. Thesis, School of Graduate Studies, Addis Ababa University, Ethiopia. 45 p.
Zimmermann TW, Williams K, Joseph L, Wiltshire J, Kowalski JA (2009). Rooting and acclimatization of cassava (*Manihot esculenta*) *ex vitro*. A Proceeding organized by ISHS at International Symposium on Biotechnology of Temperate Fruit Crops and Tropical Species.[Online]Availablefrom:http://www.actahort.org/members/showpdf?Booknrarnr=738_97.[Accessed on 7May, 2009].