academicJournals

Vol. 5(1) pp. 1-6, April 2014
DOI: 10.5897/IJBMBR2013.0178
Article Number: 9C9B96443879
ISSN 2141-2154

http:// www.academicjournals.org/IJBMBR

International Journal for Biotechnology and Molecular Biology Research

Full Length Research Paper

In vitro effects of gibberellic acid and sucrose concentration on micropropagation of two elite sweet potato cultivars in Rwanda

Valery Ndagijimana[1], Jane Kahia[3]*, Theodore Asiimwe[2], Peter Yao Sallah[1], Bancy Waweru[2], Isidore Mushimiyimana[2], Jean Ndirigwe[2], Sindi Kirimi[4], Damien Shumbusha[2], Peter Njenga[5], Modeste Kouassi[6] and Edmond Koffi[6]

[1]Faculty of Agriculture, National University of Rwanda (NUR), P. O. Box117, Huye, Rwanda.
[2]Rwanda Agriculture Board (RAB), P. O. Box 5016. Kigali, Rwanda.
[3]World Agroforestry Centre (ICRAF) Cote d' Ivoire Country Program Cocody Mermoz, Abidjan, Côte d'Ivoire | 08 BP 2823 ABIDJAN 08.
[4]International Potato Center (CIP), Sub-Saharan Africa Region, P. O. Box 25171, Nairobi, Kenya.
[5]Jomo Kenyatta University of Agriculture and Technology, Box 62000, Nairobi, Kenya.
[6]Centre National de Recherche Agronomique (CNRA), Laboratoire Central de Biotechnologies (LCB), 01 BP 1740 Abidjan 01, Côte d'Ivoire.

Received 22 November, 2013; Accepted 29 March, 2014

The current study aimed at evaluating the effect of gibberellic acid (GA_3) and sucrose on *in vitro* propagation of two elite sweet potato cultivars (Ukerewe and Gihingamukungu). Nodal explants from *in vitro* growing plantlets were harvested and cultured on Murashige and Skoog media supplemented with 2.5, 5, 10, 20 and 40 µM, GA_3. In a separate experiment, sucrose was evaluated at 30, 60, 90, 120, 150, 180 and 210 mM. For Ukerewe, the explants cultured on medium supplemented with 10 µM GA_3 recorded the longest (2.78 ± 0.36 cm) microshoots. On the other hand, cultivar Gihingamukungu explants cultured on media supplemented with 2.5 GA_3 µM produced the longest ((3.23 ± 0.40 cm) microshoots. Nodal explants from the two cultivars cultured on media supplemented with sucrose 150 mM yielded the longest microshoots (2.51 ± 0.26 and 2.34 ± 0.24 cm, respectively). From the results of the current study, it can be concluded that for micropropagation of the cultivar Ukerewe 10 µM GA_3 should be used while 2.5 GA_3 µM should be used for micropropagtion of cultivar Gihingamukungu. The regenerated plantlets were successfully weaned in the greenhouse. The protocol developed in this research will open new prospects for massive propagation of the elite sweet potato cultivars in Rwanda.

Key words: Ukerewe, Gihingamukungu, nodal explants, microshoot.

INTRODUCTION

Sweet potato (*Ipomoea batatas*) is a high yielding crop which is ranked second in the world after potatoes (Deng et al., 2012). It belongs to the family *Convolvulacea* (Xiansong, 2010) and it originated in America (Burden,

*Corresponding author. E-mail: janekahia@yahoo.co.uk.

2005). Sweet potato is a major food; feed and industrial raw material in China whose total output in the world is estimated to be more than 80% (Farmer et al., 2007; Islam, 2006; Liu, 2011). Studies by Islam (2006) have revealed that sweet potato leaf extracts contains, antimutagenic, anticancer and antibacterial properties. Sweet potato is propagated by stem cuttings in Rwanda. This method of propagation is associated with increase in viral load over time on the planting materials and bacterial diseases that affect sweet potato in the major production zones of the country (ISAR, 2008). To eradicate these constraints, there is a need to develop and transfer new techniques for producing pathogen-free clonal planting materials which can help to significantly increase the potential yield of sweet potato (Zhang, 1995).

Multiplication by tissue culture techniques provides a viable alternative to the traditional methods of sweet potato propagation (Bachou, 2002) and could permit the production of relatively uniform plants on a massive scale in a shorter period of time (Mutandwa, 2008). *In-vitro* propagation of clonally propagated crops offers promise for rapid multiplication of quality planting materials and sustained optimal agricultural productivity. The production of plants *in vitro* is independent of season and can continue throughout the year. Sweet potato plants propagated by tissue culture mature earlier are more robust; leading to accelerated growth than the plants propagated through conventional methods (ASARECA, 2008).

Nodal explants are occasionally cultured on media supplemented with GA_3 to increase the length of shoots during multiplication or prior to rooting (Moshkov et al., 2008).

The most characteristic effects of GA_3 on shoot growth are increased inter-node extension, increased leaf growth and enhance apical dominance. The elongated shoots are then subdivided to serve as starting mother stock culture for another multiplication cycle.

The concentration of sucrose is one of the factors controlling the induction and growth of *in vitro* shoots (Gibson, 2000; Gurel and Gulsen, 1998). The optimum sucrose level for shoot development may vary among species and genotypes (Nowak et al., 2004). The concentration at which sugar is used has a great impact on the photosynthetic abilities of plantlets (Desjardins et al., 1995) and the relative success of subsequent acclimatization process. The optimum sucrose concentration as an efficient carbon source has been examined in tissue cultures systems of some plant species, such as *Coffea Arabica* cultivar Ruiru 11 (Kahia, 1999), *Paederiafoetida* (Amin et al., 2003) and *Elaeocarpus robustus* (Rahman et al., 2004). The current study aimed at evaluating the effect of different concentrations of gibberellic acid (GA_3) and sucrose on microshoots proliferation in two elite sweet potato cultivars.

MATERIALS AND METHODS

Plant materials

The study was carried out at the plant tissue culture laboratory of Rwanda Agriculture Board (RAB) located in Rubona, Southern Province of Rwanda (Altitude: 1630 m 2°29'07''S, 29°47'49''E). The two sweet potato cultivars were initially propagated *in vitro* by nodal culture at the Kenya Agricultural Research Institute, Muguga Plant Quarantine Services in Kenya and distributed to Rwanda through the Rwanda Agriculture Board.

Preparation of media

In the first experiment, nodal explants were cultured in Murashige and Skoog (MS) (1962) media supplemented with GA3 evaluated at 2.5, 5, 10, 20 and 40 µM, ascorbic acid (100 µM), calcium nitrate (50 µM), L-arginine (50 µM), 100 mg/l myo-inositol and 90 mM sucrose. In the second experiment, MS media was supplemented with 30, 60, 90, 120, 150, 180 and 210 mM sucrose. The medium pH was adjusted to 5.8 before gelrite was added and media heated to dissolve it. Ten (10) ml MS medium with supplements was dispensed into 25 x125 mm test tubes and steam sterilized in an autoclave at 1.06 kg cm^2 and 121ºC for 15 min.

Inoculation and incubation

Inoculation was carried out under aseptic conditions in a laminar airflow hood in the laboratory in Rubona. Explants with two nodes were dissected from *in vitro* growing cultures using sterile blade and forceps and the leaves were cut off. They were cultured into test tubes containing 10 ml medium under evaluation. Twenty (20) test tubes were used per treatment and these were sealed with parafilm before incubating them in a growth room maintained at 25 ± 2°C under the cool white fluorescent lights and 16 h photoperiod with a photon flux density of about 60 µ mol m^{-2} s^{-1} and 70-80% relative humidity.

Transplanting

The *in vitro* regenerated sweet potato plantlets were carefully removed from the test tubes and the roots gently cleaned with running tap water to remove the gelrite. The plantlets were then taken to the green house where they were soaked with 2% fungicide (Redomil) for 20 min. A weaning pot was filled with sterile potting mixture consisting of top soil, sand and manure mixed in the ratio of 3:2:1(w/w). The vessel was placed in a basin containing water to allow the potting mixture to take up water until the top became moist. The pot was then removed from the basin and the plantlets carefully planted using sharp wooden sticks. The plantlets were irrigated once a week with tap water for the first two weeks and twice a week thereafter.

Data collection

Collection of data on the number of microshoots, roots and their lengths was carried out one week after inoculation and on a weekly basis for four weeks during incubation. While in the glasshouse the number of surviving plantlets was observed.

Experimental design and data analysis

The trials were laid out in a Completely Randomized Design. The number of shoots and roots was counted while the length was

Table 1. Effect of different GA_3 concentrations (µM) on *in vitro* shoots proliferation and roots regeneration of Ukerewe sweet potato.

Concentration	Number of microshoots	Length of shoots (cm)	Number of roots	Length of roots (cm)
2.5	5.03 ± 0.62[a]	1.65 ± 0.22[b]	4.60 ± 0.58[b]	5.50 ± 0.62[a]
5	4.53 ± 0.51[a]	1.82 ± 0.21[b]	3.83 ± 0.33[b]	3.96 ± 0.43[b]
10	5.10 ± 0.68[a]	2.78 ± 0.36[a]	4.73 ± 0.37[b]	4.24 ± 0.47[b]
20	4.77 ± 0.64[a]	1.52 ± 0.19[b]	7.43 ± 0.69[a]	3.57 ± 0.32[b]
40	5.40 ± 0.73[a]	1.58 ± 0.18[b]	7.03 ± 0.61[a]	3.81 ± 0.30[b]
P value	0.8988	0.0013	<.0001	0.0256
LSD	1.7899	0.6714	1.51	1.2549

Values represent means ± SE. Means within a column followed by different letters are significantly different at $P = 0.05$. LSD: least significant difference test (LSD).

Table 2. Effect of different GA_3 concentrations (µM) on *in vitro* shoots proliferation and roots regeneration of Gihingamukungu sweet potato.

Concentration	Number of microshoots	length of shoots (cm)	Number of roots	length of roots (cm)
2.5	5.33 ± 0.59[a]	3.23 ± 0.40[a]	3.83 ± 0.30[a]	4.15 ± 0.31[a]
5	5.23 ± 0.54[a]	2.08 ± 0.26[b]	2.70 ± 0.25[b]	4.10 ± 0.21[a]
10	5.07 ± 0.53[a]	2.18 ± 0.26[b]	3.83 ± 0.39[a]	4.51 ± 0.26[a]
20	5.57 ± 0.69[a]	1.74 ± 0.19[b]	4.26 ± 0.27[a]	3.23 ± 0.24[b]
40	4.93 ± 0.57[a]	1.49 ± 0.16[b]	4.36 ± 0.39[a]	4.05 ± 0.30[a]
P value	0.9521	0.0001	0.0040	0.0198
LSD	1.6431	0.7432	0.9222	0.7586

Values represent means ± SE. Means within a column followed by different letters are significantly different at $P = 0.05$. LSD: least significant difference test (LSD).

measured in centimeter. The collected parameters were analyzed using SAS software (SAS Institute, 2001). Analyses of variance (ANOVA) were computed for each parameter and the data were summarized as mean ± SE of each parameter and presented in tables. Means were separated using least significant difference test (LSD) with 5% of level of significance

RESULTS

The effects of GA_3 on shoot proliferation and root growth from Ukerewe nodal explants is presented in Table 1. GA_3 at all the concentrations evaluated did not have a significant difference in the number of microshoots per explant. However, GA_3 evaluated at a concentration of 10 µM yielded the highest mean shoot length (2.78 ± 0.36) which was significantly different (P<.0001) from all other concentrations evaluated. The highest mean number (7.43 ± 0.69) of roots was observed on medium supplemented with 20 µM GA_3.

The effects of GA_3 on shoot proliferation and root growth from Gihingamukungu nodal explants is presented in Table 2. The media supplemented with 20 µM GA_3 produced the highest (5.57 ± 0.69) mean microshoot which was not significantly different from all other GA_3 concentrations evaluated. Explants cultured on medium supplemented with 2.5 µM GA_3 produced microshoots with the highest (3.23 ± 0.40) mean length. On the other hand, the highest (4.36 ± 0.39) mean number of roots was achieved on medium supplemented with 40 µM GA_3 which was not significantly different from the numbers obtained with 2.5, 10 and 20 µM. Plate 1A shows the regenerated plantlets from explants cultured on medium supplemented with different GA_3 concentrations.

The effects of different concentrations of sucrose on shoot proliferation and root growth from Ukerewe sweet potato nodal explant is presented in Table 3. Increasing the concentration of sucrose from 30 to 150 mM increased the number of microshoots fivefold. The media supplemented with 180 mM sucrose produced the highest (5.93 ± 0.62) mean number of microshoots per explant which was not significantly different from the numbers produced on medium supplemented with sucrose 120, 150 and 210 mM. Nodal explants cultured on media supplemented with 150 mM sucrose produced the highest (2.51 ± 0.26) mean shoot length which was significantly higher at P <.0001 than the length obtained using the lower sucrose concentrations (30, 50 and 90 mM). The highest (7.47 ± 0.74) mean number of roots was produced on the medium supplemented with 210 mM sucrose.

The effects of sucrose concentration on shoot

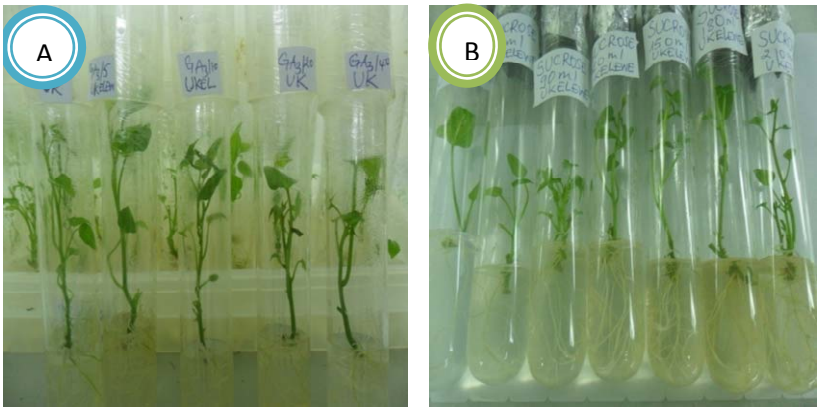

Plate 1. A: Regenerated sweet potato plantlets from nodal explants cultured on 2.5, 5, 10, 20 and 40 µM gibberellic acid **B:** *In vitro* regenerated plantlets showing profuse rooting on media supplemented with sucrose 120, 150 and 210 mM (from left to right).

Table 3. Effect of different sucrose concentrations (mM) on *in vitro* shoots proliferation and roots regeneration of Ukerewe sweet potato.

Concentration	Number of microshoots	Length of shoots (cm)	Number of roots	Length of roots (cm)
30	1.07 ± 0.08^{d}	0.600 ± 0.03^{c}	0.30 ± 0.09^{f}	0.22 ± 0.12^{e}
60	2.60 ± 0.31^{c}	1.35 ± 0.11^{b}	2.87 ± 0.27^{e}	3.05 ± 0.26^{d}
90	4.43 ± 0.47^{b}	1.67 ± 0.17^{b}	4.83 ± 0.44^{dc}	4.09 ± 0.40^{c}
120	5.23 ± 0.48^{ba}	2.45 ± 0.28^{a}	4.47 ± 0.39^{d}	4.61 ± 0.34^{bc}
150	5.90 ± 0.55^{a}	2.51 ± 0.26^{a}	7.03 ± 0.60^{ba}	5.74 ± 0.33^{a}
180	5.93 ± 0.62^{a}	2.41 ± 0.24^{a}	6.10 ± 0.42^{bc}	5.00 ± 0.38^{ba}
210	5.67 ± 0.47^{ba}	2.33 ± 0.23^{a}	7.47 ± 0.74^{a}	5.15 ± 0.34^{ba}
P value	<.0001	<.0001	<.0001	<.0001
LSD	1.2759	0.5715	1.3014	0.903

Values represent means ± SE. Means within a column followed by different letters are significantly different at $P = 0.05$. LSD: least significant difference test (LSD).

proliferation and root growth from Gihingamukungu nodal explants is presented in Table 4. The media supplemented with 210 mM sucrose produced the highest (4.93 ± 0.36) mean number of microshoot per explant. On the other hand, nodal explant cultured on media supplemented with 150 mM sucrose produced the highest (2.34 ± 0.24) mean shoot length and highest (5.30 ± 0.42) mean number of roots. Rooting was observed in all the cultures (Plate 1B).

The effect of GA_3 and sucrose on regeneration efficiency in the two cultivars is presented in Table 5. There were no significant difference in the responses of two cultivars to the gibberellic acid concentrations evaluated in terms of the number and length of microshoots. The effect of sucrose was significantly higher in cultivar Ukerewe compared to Gihingamukungu for all the parameters evaluated. The plantlets were successful weaned in the greenhouse with an average 60% survival rate (data not shown)

DISCUSSION

The production of plants from nodal explants has proven to be the most generally applicable and reliable method of regenerating true-to-type *in vitro* plantlets (George et al., 2008). The elongation of microshoot is one of the crucial aspects in developing protocols for regeneration of sweet potato. In order to promote elongation and accelerate microshoot development, and gibberellic acid (GA3) is sometimes added to the culture medium. In previous studies on micropropagation of sweet potato, the effect of GA_3 on elongation of microshoot has been evaluated and it was used at various concentrations e.g. 40 mg/L (Dagnino et al., 1991) and 0.02 g/L (Luo et al., 2006). During the current study, GA_3 promoted induction and elongation of microshoots in both the cultivars evaluated. These results were interesting as Dagnino et al. (1991) reported that GA_3 had no influence on culture of sweet potato meristem of cultivar curacao Alado

Table 4. Effect of different sucrose concentrations (mM) on *in vitro* shoots proliferation and roots regeneration of Gihingamukungu sweet potato

Concentration	Number of microshoots	Length of shoots (cm)	Number of roots	Length of roots (cm)
30	0.33 ± 0.09^{c}	0.11 ± 0.03^{b}	1.46 ± 0.32 ^{c}	1.41 ± 0.21 ^{d}
60	0.63 ± 0.10^{c}	0.51 ± 0.11^{b}	1.80 ± 0.24 ^{c}	1.62 ± 0.18 ^{d}
90	3.60 ± 0.45^{b}	1.83 ± 0.25^{a}	2.36 ± 0.20^{c}	2.25 ± 0.18 ^{c}
120	4.87 ± 0.54^{a}	1.98 ± 0.19^{a}	3.60 ± 0.30^{b}	2.81 ± 0.09^{b}
150	4.60 ± 0.39^{ba}	2.34 ± 0.24^{a}	5.30 ± 0.42 ^{a}	4.26 ± 0.25^{a}
180	4.90 ± 0.45^{a}	2.27 ± 0.23^{a}	4.46 ± 0.42^{ba}	4.80 ± 0.21^{a}
210	4.93 ± 0.36^{a}	1.87 ± 0.25^{a}	5.13 ± 0.38^{a}	3.03 ± 0.16^{b}
P value	<.0001	<.0001	<.0001	<.0001
LSD	1.0319	0.5619	0.9505	0.5396

Values represent means ± SE. Means within a column followed by different letters are significantly different at P = 0.05. LSD: least significant difference test (LSD).

Table 5. Comparing the effect of GA_3 and sucrose on shoot proliferation and root Regeneration of Ukerewe and Gihingamukungu sweet potato cultivars

Variety	GA_3 and sucrose	Number of shoots	Length of shoots (cm)	Number of roots	Length of roots (cm)
Ukerewe	GA_3	4.97 ± 0.28^{a}	1.87 ± 0.11^{a}	5.53 ± 0.26^{a}	4.22 ± 0.21^{a}
Gihingamukungu	GA_3	5.23 ± 0.26^{a}	2.14 ± 0.13^{a}	3.80 ± 0.15^{b}	4.01 ± 0.12 ^{a}
P value		0.4996	0.1080	<.0001	0.3874
LSD		0.757	0.3343	0.6037	0.4734
Ukerewe	Sucrose	4.40 ± 0.21^{a}	1.90 ± 0.09^{a}	4.72 ± 0.23^{a}	3.98 ± 0.17^{a}
Gihingamukungu	Sucrose	3.40 ± 0.19^{b}	1.56 ± 0.09^{b}	3.44 ± 0.16^{b}	2.89 ± 0.11^{b}
P value		0.0007	0.0101	<.0001	<.0001
LSD		0.5555	0.2548	0.5648	0.3965

GA_3: gibberellic acid, LSD: least significant difference test (LSD).

whereas meristem of cultivar Mae de Familai cultured on medium supplemented with GA_3 formed multiple shoots. In cassava, Villaluz (2005) reported that when used singly, GA_3 did not elicit any growth response from nodal explants however, Mushiyimana et al. (2011) reported that nodal explants cultured on MS medium supplemented with 40 µM GA_3 produced the highest mean number of microshoots and length. A possible explanation **f**or such disparity seems to be genotypic difference. During the current study, prolific rooting was achieved in all the cultures. The reason for this could be the fact that GA_3 and sucrose are known to induce roots (Kochba et al., 1973; Calamar and De klerk, 2002). The presence of roots is of paramount importance because a good root system is essential for successful acclimatization of the plantlets and subsequent growth in the field since roots facilitate the absorption of nutrients and water from the soil (Xiansong, 2010).

In the current study, increasing the concentration of sucrose from the 90 mM (the concentration that is routinely used) resulted in increased mean number of microshoots per explant for both sweet potato cultivars. The results of the current work agree with earlier reports that indicate that there are cases where higher concentrations > 3% of sucrose may be beneficial (Boggetti, 1997; Kahia, 1999; Van Huylenbroeck and Debergh, 1996). However, it contradicts other reports which have shown that high sucrose concentration was detrimental to shoot formation. These authors have suggested that the osmotic level in the medium with high sucrose concentration may be inhibitory to shoot growth and development (Karimet al., 2007; Nowak et al., 2004).

A genotype dependent one step protocol for the micropropagation of elite sweet potato cultivar Ukerewe, and Gihingamukungu, was developed. This protocol will provide the basis for the mass production of studied cultivars through *in vitro* techniques. This is the first report on evaluating the effect of different concentrations of sucrose to regenerate sweet potato plantlets.

Conflict of interests

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

ACKNOWLEDGEMENTS

The authors would like to thank the Rwanda Agriculture Board (RAB) Management for allowing them to use their facilities to carry out this work. The assistance rendered by the Tissue culture laboratory staff at RAB Rubona is highly appreciated.

REFERENCES

Amin MN, Rahman MM, Manik MS (2003). *In vitro* clonal propagation of *Paederiafoetida* L. A medicinal plant of Bangladesh. Plant Tissue Cult.13:117-123.

Association for Strengthening Agriculture Research in Eastern and Central Africa (ASARECA) (2008). Enhancement of cassava and sweet potato tissue culture and conservation technologies in Eastern and Central African region. Proceedings of Tissue culture workshop, Bujumbura.

Bachou H (2002). The Nutrition Situation in Uganda. Nutrition. 18(4):356-358.

Boggetti B (1997). Development of micropropagation and possible genetic transformation system for cashew (*Anacardium occidentale*). PhD Thesis University of London.

Burden D (2005). Sweet potato profile, agriculture marketing and resource center (AgMRC). A National information resource for value added agriculture. Iowa State University.

Calamar A, Geert-Jan-de K (2002). Effect of sucrose on root regeneration in apple. Plant Cell Tissue Org. Cult. 70:207-212.

Dagnino DS, Carelli MD, Arrabal RF, Esquibel MA (1991). Effect of Gibberelic acid on *Ipomoea batatas* regeneration from meristem culture. Presquisa Agropecuaria Brasilia 26:259-262.

Deng XP, Cheng YJ, Wu XB, Kwak SS, Chen W, Egrinya A (2012). Exogenous hydrogen peroxide positively influences root growth and exogenous hydrogen peroxide positively influences root growth and metabolism in leaves of sweet potato seedlings. Australian J. Crop Sci. 6(11):1572-1578.

Desjardins Y, Hdider C, de Riek J (1995). Carbon nutrition *in vitro*: regulation and manipulation of carbon assimilation in micropropagated system. In: *Automation and Enviromental Control in Plant Tissue Culture* (Aitken-Christie, J., Kozai, T. and Smith, M. A.,eds), Kluwer Academic Press. pp. 441-471.

Farmer M, Li X, Feng G, Zhao B, Chatagnier O, Gianinazzi S, Gianinazzi-Pearson V, Van Tuinen D (2007). Molecular monitoring of field-inoculated AMF to evaluate persistence in sweet potato crops in China. Appl. Soil Ecol. 35:599-609.

George EF, Hall MA, De Klerk GJ (2008). The background, Plant propagation by tissue culture Springer Verlag, Dordrecht, The Netherlands

Gibson SI (2000). Plant sugar response pathway part of a complex regulatory web. Plant Physiol. 124:1532-1539.

Gurel S, Gulsen Y (1998). The effects of different sucrose, agar and pH levels on *in vitro* shoot production of Almond (*Amygdaluscommunis* L.). Turk. J. Bot. 22:363-373.

Institute of Agronomic Sciences of Rwanda (ISAR) Sweet Potato Program (2008).

Islam S (2006). Sweet potato (*Ipomoea batatas* L.) Leaf its potential effect on human health and nutrition. J. Food Sci. 71(2):R13-R121.

Kahia WJ (1999). *In vitro* propagation of the Disease resistant cultivar – Ruiru 11. PhD thesis, University of London.

Karim MZ, Yokota S, Rahman MM, Eizawa J, Saito Y, Azad MAK, Ishiguri F, Iizuka K, Yoshizawa N (2007). Effects of the Sucrose Concentration and pH Level on Shoot Regeneration from Callus in *Arariaelata* Seem. Asian J. Plant Sci. 6:715-717.

Kochba JB, Spiegel- Roy P, Kochba M (1973). Stimulation of roots of citrus embryoids by Gibberelllic acid and adenine sulphate. Ann. Bot. 38:795-802.

Liu Q (2011). Sweet potato omics and biotechnology in China. Plant Omics J. 4(6):295-301.

Luo HR, Santa Maria M, Benavides J, Zhang DP, Zhang YZ (2006). Rapid genetic transformation of sweet potato *(Ipomoea batatas* (L.) Lam) via organogenesis. Afr. J. Biotechnol. 5(20):1851-1857.

Moshkov GI, Novikova EV, Hall MA (2008). Plant Growth Regulators III: Gibberellins, ethylene, abscisic acid, their analogues and inhibitors; miscellaneous compounds. In: *Plant propagation by tissue culture* (Edwin, M. A.J. and George, F., 3rd Edition), The Netherlands: Springer. p. 22.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Plant Physiol. 15:473-497.

Mushiyimana I, Emannuel H, Gervais G, Peter Yao KS, Safia K, Felix G, Theodore A, Jane K, Daphrose G (2011). Micro-Propagation of Disease Resistant Cassava Variety in Rwanda. Rwanda J. 24:49-57.

Mutandwa E (2008). Performance of Tissue cultured sweet potatoes among smallholders in Zimbabwe. J. Agrobiotechnol. Manag. Econ. 11(1).

Nowak B, Miczynski K, Hudy L (2004). Sugar uptake and utilization during adventitious bud differentiation on *in vitro* leaf explant of WegierkaZwykla plum (*Prunusdomestica*). Plant Cell Tissue Org. Cult. 76:255-260.

Rahman MM, Amin MN, Ahmed R (2004). *In vitro* rapid regeneration from cotyledon explant of native olive (*Elaeocarpusrobustus*Roxb). Asian J. Plant Sci. 3:31-35.

SAS Institute (2001). SAS / STAT user's guide. Version 8.2. SAS Institute, Cary, USA

Van Huylenbroeck JM, Debergy PC (1996). Impact of sugar concentration *in vitro* photosynthesis and carbon metabolism during *ex vitro* rooting acclimatization of *Spathiphyllum* plantlets. Plant Physiol. 96:298-304.

Villaluz ZA (2005). **I**mprovement of *in vitro* techniques for rapid meristem development and mass propagation of Philippine cassava (*Manihot esculenta* Crantz). Plant Cell Reports 9:356-359.

Xiansong Y (2010). Rapid production of virus-free plantlets by shoot tip culture *in vitro* of purple-coloured sweet potato (*Ipomoea batatas (L.) Lam*.). Pak. J. Biol. 42(3):2069-2075.

Zhang L (1995). Progress of Research and application of virus free sweet potato seeds in Shandong. Proceedings of the first Chinese-Japanese symposium of sweet potato and potato. Beijing Agriculture University.Press, Beijing.