British Biotechnology Journal
12(2): 1-7, 2016, Article no.BBJ.23903
ISSN: 2231–2927, NLM ID: 101616695

SCIENCEDOMAIN *international*
www.sciencedomain.org

Factors Affecting on Induction of Microrhizomes in Ginger (*Zingiber officinale* Rosc.), Cultivar Local from Sri Lanka

D. B. R. Swarnathilaka[1*], N. S. Kottearachchi[2] and W. J. S. K. Weerakkody[2]

[1]Department of Export Agriculture, Plant Tissue Culture Research Station, Walpita, Sri Lanka.
[2]Department of Biotechnology, Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, Sri Lanka.

Authors' contributions

This work was carried out in collaboration with all authors. Author DBRS conducted the experiment, involved in data collection, analysis, interpretation and wrote all the drafts of the manuscript. Author NSK guided and reviewed the experimental design and drafts of the manuscript. Author WJSKW guided and reviewed the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BBJ/2016/23903
Editor(s):
(1) Marli Camassola, Institute of Biotechnology, University of Caxias do Sul, Brazil.
Reviewers:
(1) M. K. Yadav, Sardar Vallabhbhai Patel University of Agriculture and Technology, India.
(2) T. Pullaiah, Sri Krishnadevaraya University, India.
Complete Peer review History: http://sciencedomain.org/review-history/13345

Original Research Article

Received 28th December 2015
Accepted 28th January 2016
Published 18th February 2016

ABSTRACT

Aims: *In-vitro* produced microrhizomes in ginger are favored as planting material over other conventional planting materials as they are free from soil born pathogens. This study was conducted to develop an efficient protocol for production of healthy microrhizomes including the investigation of effect of different growth regulators, sucrose concentration and photoperiod exposure levels.
Place and Duration of Study: The entire study was conducted in plant tissue culture research unit of the Department of Export Agriculture, Walpita, Sri Lanka between November 2013 and August 2015.
Methodology: *In-vitro* produced shoots established in hormone free medium were cultured in Murashige and Skoog (1962) (MS) medium fortified with eight treatments of 6-benzylaminopurine (BAP) (0.0, 2.0, 0.4 and 6.0 mg L^{-1} of BAP) and Naphthalene acetic acid (NAA) (0.1 and 0.2 mg L^{-1} of NAA) structuring in factorial design to study the effect of growth regulators for formation of

**Corresponding author: E-mail: thilakaswarna@yahoo.com;*

microrhizomes. *In-vitro* produced shoots were cultured on MS medium fortified with different concentration of sucrose (30, 60, 90 and 120gL^{-1}) separately. Effect of the solid/liquid nature of the medium and the different photoperiod level on induction of microrhizome was also studied.

Results: Results revealed that medium containing 4.0 mgL^{-1} BAP with 0.1 mgL^{-1} NAA showed the best response followed by 6.0 mgL^{-1} BAP with 0.1 mgL^{-1} NAA for induction of microrhizomes within 60 days. Increased level of NAA did not enhance mocrorhizome induction. Results of different concentration of sucrose revealed that MS medium fortified with 90 g L^{-1}sucrose recorded the highest fresh and dry weight of microrhizomes followed by the treatment with 60 g L^{-1} sucrose. However, plantlets supplemented with more than 90 g L^{-1} of sucrose exhibited lower weight of microrhizomes, but higher root induction and root fresh weight probably due to accumulation of water. Different photoperiod exposure levels revealed that 16 hrs of light and 4 hrs of dark condition with solid medium produced highest fresh weight (3.72 g) and highest number of microrhizomes (9.6).

Conclusion: Murashige and Skoog (1962) medium supplemented with 4 mgL^{-1} BAP, 0.1 mgL^{-1} NAA and 90 gL^{-1} sucrose in solid form with 16-h photoperiod for 10 weeks of culture duration were the best conditions for induction microrhizomes in ginger, cultivar Local.

Keywords: Growth regulators; media; microrhizome; photoperiod.

1. INTRODUCTION

Ginger (*Zingier officinale* Rosc.) of the family Zingiberaceae is an important herbaceous plant primarily used as a spice and in medicine at the global level. Commercially it is grown for its aromatic rhizomes, which are utilized in herbal medicine, food, beverage and form of important raw materials in pharmaceutical industries [1]. Rhizomes of local ginger are small and the fibrous flesh is somewhat ash-white in color. The taste and the aroma of local ginger is comparatively higher than other imported varieties. The use of rhizomes is a routine vegetative propagation method for *Zingiber officinale*, and many rhizomes are required because the efficiency of vegetative propagation is low. According to Bhagyalakshmi and Singh [1], conventional multiplication produces only 10-15 lateral buds from the rhizome of a single plant after 8 months. Rhizome is the economically used part of the ginger plant. Therefore it restricts the availability of ginger rhizome seeds, needs for the cultivation. The availability of good quality seed is a major constraint in ginger seed production due to progressive accommodation of diseases and pest in seed stocks. It is reported that the three-fold increase in the production of rhizomes could be possible by the effective control of the diseases [2]. Moreover since pathogenic fungi, bacteria or viruses are readily transmitted through traditional practices, it is important to develop a micro propagation technique and to make available the pathogen free ginger germplasm for commercial use. Availability of good quality healthy rhizome is a pre-requisite for a successful ginger industry.

The *in-vitro* technique is applied to overcome the seed needs in the formation of micro rhizome. Ginger microrhizomes were induced by few authors i.e. Sharma and Singh [3] who found that, MS medium supplemented with 8 mg/L BAP produced the microrhizomes. Bhat et al. [4] and Zheng et al. [5] indicated that, ginger rhizome formation *in-vitro* is affected by many factors, including photoperiod, mineral nutrition, culture methods (liquid or solid culture), and carbon source. In addition, Rout et al. [6] concluded that, the indication under 24-h photoperiod helped in production of ginger rhizomes and Roh et al. [7] reported that young plantlets of ginger formed rhizomes when cultured on MS medium containing sucrose 90 gL^{-1}. And also Archana et al. [8] developed ginger microrhizome protocol for large scale production using MS medium supplemented with externally added Ammonium nitrate.

There are many advantages in *in-vitro* produced microrhizomes over conventional planting materials. Microrhizomes can be easily stored and transported as they do not require any culture medium or any other special measures to prevent contamination. Microrhizome can be used for germplasm conservation because it is more efficient and can be stored longer duration) [9]. Tyaagi et al. [10] and Annisuzzaman et al. [11] have stated that micro rhizome can be planted directly without acclimatization process. Recently *in-vitro* formation of storage organs such as bulbs, corms, tubers and rhizomes came into focus because they can be used as a pathogen free seeds and especially microrhizomes are recommended to use in

regions with high disease pressure. Hence, the present investigation was carried out to highlight an effective, reliable and reproducible protocol for *in-vitro* production of ginger microrhizomes. Therefore, in this study, experiments were designed to find out the best concentration of cytokinin and sucrose and photoperiod exposure level for successful production of microrhizome in Ginger.

2. MATERIALS AND METHODS

2.1 Culture Initiation for Microrhizome Induction

Aseptic shoots approximately 3-4 cm long, which were derived from the *in-vitro* established culture of Local variety of ginger were used as explants for induction of microrhizomes. After separating the fully grown shoots originated from the axillary rhizome buds, the small shoots with the size of 3-4 cm long were again cultured on fresh medium without any growth hormone for another four weeks to avoid carryover effects of growth regulators.

2.2 Detection of the Effect of Growth Regulators on Microrhizome Induction

In-vitro produced shoots with 3-4 cm shoot height, established in hormone free medium, were cultured in Murashige and Skoog (1962) [12] (MS) medium fortified with eight treatments of 6-benzylaminopurine (BAP) (0.0, 2.0, 0.4 and 6.0 mg L^{-1} of BAP) and Naphthalene acetic acid (NAA) (0.1 and 0.2 mg L^{-1} of NAA) structuring in factorial design to study the effect of growth regulators for formation of microrhizome. Each treatment consisted of 10 individual culture bottles to provide 10 replicates. Data in each culture bottle were recorded separately on number of rhizomes, fresh weight of rhizome, number of buds, shoot height, and number leaves after 60 days of culturing.

2.3 Detection of the Effect of Sucrose Concentration on Microrhizome Induction

In-vitro produced shoots with 3-4 cm shoot height established in hormone free medium were cultured on MS medium supplemented with 4 mgL^{-1}BA, 0.25 mgL^{-1} NAA and different consecrations of sucrose (30, 60, 90, and 120 gL^{-1}) separately. All the cultures were incubated at 26±2℃ with 16/8 hr light and dark period. Data were collected on number of buds, shoot height, number of leaves, wet and dry weight of shoots, roots and rhizome.

2.4 Detection of the Effect of Photoperiod and Solid/Liquid form of the Medium on Formation of Microrhizomes

In-vitro produced shoots with (about 3-4 cm height established in hormone free medium were collected and cultured on solid and liquid forms of MS medium supplemented with 4 mgl^{-1}BA, 0.1 mgL^{-1} NAA, and 90 gL^{-1}sucrose. Varying level of photoperiod 0 (dark) hr, 4 hr, 8 hr and 16 hr were provided to examine their effect on formation of microrhizome and all the cultures were incubated at room temperature (26±2℃). Data were collected on, number of microrhizomes, fresh weight of microrhizome, number of shoots, shoot height, shoot and root fresh weight and percentage of cultures that induced microrhizomes.

2.5 Data Analysis

The data were analyzed by ANOVA using SAS 9.2 software. Means were compared by the least Significant Difference ($LSD_{0.05}$).

3. RESULTS AND DISCUSSION

3.1 Effect of BAP and NAA on Induction of Microrhizomes

The results obtained in the present investigation using different concentration of growth regulators showed a significant influence on microrhizome production. Swelling of basal portion was observed after 25-30 days. Among the 8 different combinations of growth regulators, 4 mgL^{-1} BAP together with 0.1 mgL^{-1} NAA exhibited a better response than the other treatments in terms of mean number of microrhizomes (8.16) (Table 1). However, it did not show any promising result on weight gaining of microrhizomes. In this medium maximum weight obtained was not exceeded 0.625 g and it may be due to low strength (3%) of sucrose. There was no significant difference between the treatment with 4 or 6 mgL^{-1} BAP with either 0.1 mgL^{-1} or 0.2 mgL^{-1} NAA in the formation of number of microrhizomes.

Sharma et al. [13] have reported that optimum production of microrhizome has been achieved in turmeric cultured in MS basal medium supplemented with 5.0 mgL^{-1} BAP and 0.5 mgL^{-1} NAA. However in the present study, increase of

NAA did not directly affect on increasing of the number and the weight of the microrhizomes but the shoot growth was increased considerably. Shirgurkar et al. [14] reported that 5 mgL^{-1}BAP completely inhibited microrhizome production and producing only multiple shoots in turmeric. According to their results, weight of the microrhizome was gradually increased up to 4.0 mgL^{-1} BAP. Nayak [15] and Sharma and Singh [3] have also reported that 5 mgL^{-1} BA in *C. aromatica* and 8 mgL^{-1} BA in ginger respectively enhanced the microrhizome production. Abbas et al. [16] stated that MS medium supplemented with 9 mg/l BAP indicated the best results of *in-vitro* rhizome formation under light conditions while supplementation of MS medium with 6 mg/l BAP was more favorable for microrhizomes induction under darkness in ginger. Observations of our study are in accordance with the results reported by Anisuzzaman, et al. [11] who reported that highest frequency of rhizome induction was recorded in 4.0 mg L-1 BAP containing medium. in *Curcuma zedoaria*.

3.2 Effect of Sucrose on Microrhizome Induction in Ginger

Ginger plantlets in the MS medium containing 90 gL^{-1} sucrose showed the highest weight of microrhizome with mean value of 8.553 g (wet) and 0.771 g (dry) followed by the medium with 60 gL^{-1} sucrose (Table 2). Basal part of the shoots started swelling after 20 days of culturing, which could be a signal for processing of rhizome formation. During this period, cultures produced a fresh aromatic odor.

Rhizome formation was induced *in-vitro* at the basal region of each shoot when the concentration of sucrose was increased from 30 gL^{-1} to 90 gL^{-1}. However, by increasing the concentration of sucrose from 90 gL^{-1} to120 gL^{-1}, a high number of small shoot buds were induced (Table 2) while rhizome fresh and dry weight were slightly decreased. Similar results, high number of *in-vitro* shoot buds, were obtained by Al-Khateeb [17] on date palm (*Phoenix dactylifera* L.) and the paper reported that this kind of increment in shoot bud formation was due to the osmotic stress created by high amount of sucrose.

Present results was comparable to the report of Abbas et al. [16] who also reported that MS medium supplemented 60-90 g/L sucrose were the best level of carbon source for induction of ginger microrhizomes. Islam et al. [18] also found that, nine percent sucrose was to be the most suitable for microrhizome production and gave the largest microrhizome of *Curcuma longa in-vitro*. Singh et al. [19] have shown that 8% sucrose was the optimum level for induction of microrhizome in ginger varieties. The enhanced rate of *in-vitro* microrhizome formation with increasing concentration of sucrose may be attributed to the presence of high carbon energy in sucrose since rhizomes mostly contain carbohydrates and sucrose [15]. Arora et al. [20] reported that high concentration of sucrose remarkably promotes the *in-vitro* formation of storage organs such as bulbs, corms and tubers. Abbott and Belcher [21] stated that the sucrose is the most critical stimulus for tuber induction of potato. Chirangini and Sharma [22] who studied the induction of microrhizome in *Zingiber cassumunar* reported that, 0.81 g of average fresh weight was observed on MS medium supplemented with 5% sucrose. Furthermore, Martin [23] found that, the bulbs were formed at the base of each shoot on medium supplemented with 6% or 8% sucrose and exhibited an increased tendency to develop into microrhizomes in *Ipsea malabarica.* El-Sawy et al. [24] reported that sucrose was an important factor for microtuber formation and the highest tuber formation was achieved when 12% sucrose was added to the culture medium of potato. Ondo et al. [25] also reported about mass production of microtubers with large sizes in yam cultured in high sucrose containing media. In the present study, among the different concentration of sucrose, MS medium supplemented with 120 gL^{-1} sucrose exhibited the low weight in wet and dry form of microrhizome (5.330 g-wet and 0.583 g-dry) and the highest root formation with average of 12.191 g of fresh root weight. However the amount of dry matter content of the roots was reduced to 0.374 g. Accordingly it can be assumed that the amount of accumulation of water may increase with the increased concentration of sucrose and it probably be due to the induction of water stress related signaling pathways. We have experienced similar results in ginger cultivations at the field level under the water stress condition.

3.3 Effect of Photoperiod and Solid/Liquid Form of the Medium on Formation of Microrhizome

Microrhizomes produced under 4, 8 and 16 photo period in MS solid treatments significantly increased the fresh weight than the cultures produced in the liquid medium (Table 3).

Table 1. Influence of growth regulators on microrhizome production in ginger cultivar, Local

Concentration of NAA mgL^{-1}	Concentration of BAP mgL^{-1}	Number of microrhizomes	Wet weight of micorhizome g	Shoot height cm	Number of leaves
0.1	0.0	2.00 c	0.019 e	4.48 c	6.0 d
	2.0	5.33 b	0.328 c	5.48 b	12.33 c
	4.0	8.16 a	0.652 a	6.50 a	14.67 ab
	6.0	7.83 a	0.542 b	6.38 a	13.83 bc
0.2	0.0	2.33 c	0.105 d	4.13 c	6.50 d
	2.0	6.16 b	0.362 c	7.08 a	15.50 a
	4.0	5.66 b	0.526 b	7.25 a	15.66 a
	6.0	7.50 a	0.503 b	7.08 a	14.16 ab

Within a column, means followed by the same letters are not significantly different at p=0.05

Table 2. Influence of sucrose for induction of micro rhizome

Sugar gL^{-1}	Number of buds	Shoot Height cm	Number of leaves	Wet weight g			Dry weight g		
				Shoot	Root	Rhizome	Shoot	Root	Rhizome
30	8.429 bc	10.428 a	42.286 a	12.257 a	5.240 c	2.621 c	0.596 a	0.433 a	0.346 c
60	7.714 c	10.300 a	41.714 a	13.341 a	7.026 b	6.337 b	0.659 b	0.644 b	0.533 b
90	9.571 b	9.429 a	31.429 b	4.016 b	6.673b	8.553 a	0.187 c	0.231 c	0.771a
120	23.714 a	7.286 b	33.714 b	3.053 b	12.191a	5.330 c	0.286 b	0.374 b	0.583 b

Within a column, means followed by the same letter are not significantly different at p=0.05

Table 3. Influence of photoperiod and physical form of the medium for induction of micro rhizome

Status of the media	Photo period hrs	Number of rhizome	Rhizome weight (wet) gm.	Number of Shoots	Shoot height cm	Shoot weight (wet) gm.	Root weight gm.
Liquid	0(Dark)	2.4e	1.35 e	10.6 a	5.70 a	1.202 c	0.58 bc
Liquid	4	6.4 c	1.39 de	9.8 a	3.92 de	0.604e	1.28 ab
Liquid	8	4.4 d	1.92 c	9.0 a	3.34 e	0.558 e	0.39 c
Liquid	16	4.6 d	2.25 b	10.8 a	4.10 cde	0.704 de	0.44 c
Solid	0(Dark)	6.4 c	1.81 cd	11.0 a	5.26 ab	1.538 ab	1.31 ab
Solid	4	8.2 ab	2.71 b	11.2 a	5.06 ab	1.004 cd	0.62 bc
Solid	8	7.2 bc	2.43 b	9.2 a	4.5 bcd	1.240 bc	1.87 a
Solid	16	9.6 a	3.72 a	10.0 a	4.9 abc	1.730 a	1.58 a

Within a column, means followed by the same letter are not significantly different at p=0.05

The highest performance was obtained in solid cultures incubated under light condition with 16-h/day producing 3.72 g of average fresh weight of rhizomes and 9.6 number of microrhizomes (Fig. 1). Plantlets incubated under dark condition have produced microrhizome with the lowest average of fresh weight in both liquid (1.35 g) and in solid (1.81 g) MS media. But incubation under complete darkness reduced the number and size of the microrhizomes significantly. When comparing with dark condition, the increase in the productivity of the plants incubated under light condition can be attributed to increase in the photosynthetic rate that caused increasing of storable energy in storage part (rhizome). Abbas et al. [16] reported that MS medium supplemented with 9 mg/L BAP and 60-90 g/L sucrose under 16-h photoperiod within 10 weeks of cultivation were the best conditions for ginger microrhizome induction. In this study, we fixed the level of BAP to 4 mg/l when detecting best sucrose concentration and photoperiod exposure levels. Therefore further experiments could be proposed by fixing 90 g/L sucrose and 16-h photoperiod with varying level of BAP above 4 mgL^{-1} BAP. Also, it is necessary to examine the field performance of the microrhizomes comparatively with conventional rhizomes with regards to yield and other agronomic performance before recommending this protocol for commercial growers.

Fig. 1. Microrhizomes induced under solid MS +4mgL^{-1}BAP +0.1 4mgL^{-1}NAA + 90gL^{-1} sucrose with 16 hr photoperiod

4. CONCLUSION

Murashige and Skoog (1962) medium, supplemented with 4 mgL^{-1} BAP, 0.1 mgL^{-1} NAA and 90 gL^{-1} sucrose, in solid condition under the 16-h photoperiod with 10 weeks of culture duration were the best conditions for induction of microrhizomes in ginger.

ACKNOWLEDGEMENTS

Department of Export Agriculture, Sri Lanka is greatly acknowledged for providing financial assistance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Bhagyalakshmi, Singh. Meristem culture and propagation of a variety of ginger (*Zingiber officinale* Rosc.) with a high yield of oleoresin. J. Hort. Sci. 1988;63:321-327.
2. Balachandran SM, Bhat SR, Chandel KPS. *In-vitro* clonal multiplication of bananas. Acta Horticulturae.1990;275:307-313.
3. Sharma TR, Singh BM. *In-vitro* microrhizome production in *Zingiber officinale* Rosc. Plant Cell Rep. 1995;15: 274–277. Available:http://dx.doi.org/10.1007/bf00193735
4. Bhat SR, Chandel KPS, Kacker A. *In- vitro* induction of rhizomes in ginger (*Zingiber officinale* Rosc). Ind. J. Exp. Bot. 1994;32: 340-344.
5. Zheng Y, Liu Y, Ma M, Xu K. Increasing *in-vitro* microrhizome production of ginger (*Zingiber officinale* Roscoe). Acta Physiologiae Plantarum. 2008;30:513–519. Available:http://dx.doi.org/10.1007/s11738-008-0149-3
6. Rout GR, Palai SK, Samantaray S, Das P. Effect of growth regulator and culture conditions on shoot multiplication (*Zingiber officinale* Rosc.) *in-vitro*. *In-vitro* Cell. Dev. Biol. - Plant. 2001;37(6):814-819.
7. Roh KH, Ium TS, Lee JH, Choi IL, Choi YH, Jang YS. *In-vitro* propagation and tuberization of plantlet regenerated from shoot-tip culture in ginger. Korean J. Plant Tissue Culture. 1996;239(3):129-134.
8. Archana CP, Pillai GS, Balachadran I. *In-vitro* microrhizome induction in three high yielding cultivars of *Zingiber officinale* Rosc. and their phytopathological analysis. International Journal of Advanced Biotechnology and Research. 2013;4(3): 296-300.
9. Nayak S, Naik PK. Factors effecting *in-vitro* microrhizome formation and growth in *Curcuma longa* L. and improved field performance of micropropagated plants. Science Asia. 2006;32:31-37.
10. Tyagi RK, Agrawal A, Yusup A. Conservation of Zingiber germplasm through *in-vitro* rhizome formation. Sci. Hort. 2006;108:210-219.
11. Anisuzzaman M, Sharmin SA, Mondal SC, Sulana R, Khalekuzzaman M, Alam MF. *In-vitro* microrhizome induction in *Curcuma zedoaria* (Christm.) Rsoscoe- A conservation prioritized medicinal plant. Journal of Biological Sciences. 2008;1-5.
12. Murashige T, Skoog F. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Plant Physiol. 1962;15: 473-497.
13. Sharma I, Deka AC, Sharma S, Sharma TC. High frequency clonal propagation and microrhizome induction of *Curcuma longa* (cv. lakadong_- A rich source of curcumin of north east India. The Bioscan. 2011; 6(1):11-18.
14. Shirgurkar MV, John CK, Nadgauda RS. Factors affecting *in-vitro* microrhizome production in turmeric. Plant Cell Tiss. Org. Cult. 2001;64:5-11.
15. Nayak S. *In-vitro* multiplication and microrhizome induction in *Curcuma aromatic* Salisb. Plant Growth Regulation. 2000;32(1):41–47.

16. Abbas M, Aly U, Taha H, Gabar ES. *In-vitro* production of microrhizomes in ginger *Zingiber officinale Rosco*). J Microbiol Biotech Food Sci. 2014;4(2):142-148.
17. Al-khateeb A. Regulation of *in- vitro* bud formation of date palm (*Phoenix dactylifera* L.) cv. Khanezi by different carbon sources. Bio Resource Technology. 2008b; 99:6550-6555.
18. Islam MA, Klopstech K, Jacobsan HJ. Efficient procedure for *In-vitro* microrhizome induction in *Curcuma longa* L. (Zingiberaceae) – a medicinal plant of tropical Asia. Plant Tissue Cult. 2004; 14(2):123-134.
19. Singh TD, Chakpram L, Devi HS. Induction of Microrhizome using silver nitrate in *Zingiber officinale* Rosc. var Baishey and Nadia. Indian Journal of Biotechnology. 2014;13:256-262.
20. Arora JS, Singh K, Grewal HS, Chanana YR. *In-vitro* corm production from nodal bud and cormel tips in Gladiolus. In: Plant tissue culture. Islam AS, (Ed.), Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi. 1996;50-53.
21. Abbott AJ, Belcher AR. Potato tuber formation *in- vitro* and its agriculture application. Eds. Withers LA, Alderson PG, Butterworths, London, Plant Tissue Cult. 1986;113-122.
22. Chirangini P, Sharma GJ. *In-vitro* propagation and microrhizome induction in *Zingiber officinale* (Roxb). An antioxidant rich medicinal plant. J of Food Agriculture and Environment. 2005;3(1):139-142.
23. Martin KP. Clonal propagation. Encapsulation and reintroduction of *Ipsea malabarica* (Reichb. F.) J. D. Hook., an endangered orchid. *In -vitro* Cellular and Development Biology – Plant. 2003;39(3): 322–326.
Available:http://dx.doi.org/10.1079/ivp2002399
24. El-sawy A, Bekheet TS, Aly UI. Morphological and molecular characterization of potato microtubers production on coumarin inducing medium. International Journal of Agriculture & Biology. 2007;9(5):675–680.
25. Ondo OP, Kevers C, Dommes J. Effects of reducing sugar concentration on *in-vitro* tuber formation and sprouting in yam (*Dioscorea cayenensis – D. rotundata* complex). Plant Cell, Tissue and Organ Culture. 2009;99(1):55–59.
Available:http://dx.doi.org/10.1007/s11240-009-9575-1

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/13345