DOI : 10.5958/0974-0112.2015.00069.9

Indian J. Hort. 72(3), September 2015: 353-358

Influence of hermetic and non-hermetic culture rooms on biochemical and *in vitro* morphological characters of potato cultivars

E.P. Venkatasalam*, Sumita Sharma, K.K. Pandey, Shilpa Sharma, Vandana Thakur, Richa Sood and B.P. Singh

ICAR-Central Potato Research Institute, Shimla 171 001, Himachal Pradesh

ABSTRACT

In this study, effect of hermetic and non-hermetic culture rooms on potato (*Solanum tuberosum* L.) microplantlet morphological as well as biochemical characters were evaluated in 17 cultivars belonging to different maturity groups. Significant interaction between culture room and cultivar was observed for both morphological and biochemical characters. Non-hermetic culture room was found better for vigorous plant growth without any morphological abnormalities as compared to hermetic culture room. Besides, non-hermetic culture room increased the chlorophyll *a* (151.7%), chlorophyll *b* (94.3%), total chlorophyll (133.0%), carotenoids (158.33%) and phenols (41.5%) that favoured acclimatization to *ex vitro* conditions. Therefore, culture room ventilation is a must for obtaining vigorous microplantlet growth without any morphological abnormalities.

Key words: Potato, micropropagation, culture room, tissue culture, ventilation.

INTRODUCTION

Micropropagation is a efficient method for producing large number of genetically uniform pathogen-free plants in short time. In general, growth of a particular crop during micropropagation depend on many factors, such as genotype, explant, composition of basic medium, growth regulators, gelling agent, light intensity and its quality, photoperiod, temperature, culturing vessels, vessel closure types as well as chemical environment of culture room (Walker, 14).

In plant tissue culture, vessel closure and culture room environment have been found to affect the growth of plantlets (Lentini *et al.*, 6), however, traditional micropropagation protocols have ignored the same. Hermetically sealed culture vessels have higher humidity, besides it also inhibits gaseous exchange thereby altering the concentration of carbon dioxide and ethylene in headspace of culture vessel. *In vitro* cultures of potato are sensitive to ethylene induced growth abnormalities (flaccidity, vitrification, abnormal swelling of the stem, excessive leaf senescence, aerial rooting, overall growth reduction *etc.*) when culture are grown in tightly sealed culture vessel (Sarkar *et al*., 11).

Micro-environmental conditions in culture room can be controlled by technique similar to greenhouse control, such as enhancing natural/ forced ventilation. Controlled micropropagation systems, especially under forced ventilation have been shown to produce morphologically superior as well as physiologically normal plants (Zobayed *et al*., 16). During mass multiplication of potato cultivars we have observed differences in growth of microplantlets grown in non-hermetic (naturally ventilated) and hermetic (insulated) culture rooms. Therefore, in the present investigation, the effect of non-hermetic and hermetic culture rooms on biochemical and *in vitro* morphological characters of potato cultivars was studied.

*Corresponding author's E-mail: venkat_ep@yahoo.co.in

MATERIALS AND METHODS

The study was carried out during 2010-11 at 31° 06' N, 77° 10' E and 2,160 m (latitude, longitude, msl) at Central Potato Research Institute, Shimla with 17 tetraploid (2n = 4x = 48) potato (*Solanum tuberosum* L. ssp. *tuberosum*) cultivars, *viz.*, Kufri Arun (KAR), Kufri Badshah (KBD), Kufri Bahar (KBR), Kufri Chandramukhi (KCM), Kufri Chipsona-3 (KC-3), Kufri Chipsona-4 (KC-4), Kufri Frysona (KFS), Kufri Girdhari (KGD), Kufri Himalini (KHM), Kufri Jyoti (KJT), Kufri Kanchan (KKN), Kufri Khyati (KKY), Kufri Lauvkar (KLK), Kufri Pukhraj (KPR), Kufri Pushkar (KPK), Kufri Sindhuri (KSD) and Kufri Surya (KSY) belonging to different maturity groups. Three double-node cuttings (DNCs) dissected from middle portion of the micro-plants were cultured per test tube (25 × 150 mm) containing 13 cm^3 semi-solid (7.0 g l^{-1}) Murashige and Skoog (9) medium supplemented with 30 g l^{-1} commercial sugar, 4.19 µM D-calcium pantothenate, GA_3 0.29 µM and NAA 0.05 µM. Culture tubes closed with cotton plugs were incubated in two different culture rooms, *viz.*, hermetic and non-hermetic. The experiment was carried out in a factorial (2 × 17) completely randomized design (CRD) with

two different culture rooms and 17 cultivars over a period of 28 days. Each treatment comprised of four replicates and each replicate consisted of three test tubes. The culture tubes were incubated under 16 h photoperiod (irradiance of 60 µmol m^{-2} s^{-1}) at temperature of 22 ± 1°C. After 28 days of culture, observations were recorded on microplant morphological growth parameters such as shoot height (cm); number of leaves, nodes and roots; inter-nodal and root length (cm); fresh as well as dry mass (mg) as described by Venkatasalam *et al*. (13). Biochemical parameters like chlorophyll (chl *a*, chl *b* and total chl), carotenoids and phenol contents were also estimated. Chlorophyll contents and carotenoids were estimated as per the method described by Arnon (1), whereas, phenols by Goldstein and Swain (3). The carbon dioxide (CO_2) content of growth room was measured during the growth period using hand held CO_2 analyzer. The data were analyzed statistically using the software AGRES for obtaining analysis of variance and means were separated according to the least significant differences (LSD) at 0.05 level of probability.

RESULTS AND DISCUSSION

After 28 days of culturing significant ($p \leq 0.05$) effect due to culture room-types and cultivars was observed on all the microplant morphological characters, *viz.*, shoot height, number of leaves, number of nodes, inter-nodal length, root length and fresh as well as dry weight and biochemical characters, *viz.*, chlorophyll *a*, chlorophyll *b*, total chlorophyll, carotenoids and phenols. Significant interaction effect of cultivar with culture room was observed on microplant morphological characters like shoot length, number of leaves, number of nodes and inter-nodal length as well as all the biochemical characters. The CO_2 content of non-hermetic growth room was equal to atmospheric CO_2 content (~300 ppm), whereas, CO_2 content of hermetic culture room was > 400 ppm.

In general after 28 days, microplants grown in non-hermetic culture room recorded significantly maximum chlorophyll *a* [1.51 mg g^{-1} (f.m)], chlorophyll *b* [0.68 mg g^{-1} (f.m)] as well as total chlorophyll [2.19 mg g^{-1} (f.m)] contents. In most of the cultivars, chlorophyll contents were invariably more in cultures grown in non-hermetic culture room. However, culture room types did not show any significant effect on chlorophyll contents of Kufri Bahar and Kufri Kanchan. Among the cultivars, Kufri Frysona recorded significantly maximum chlorophyll *a* [2.03 mg g^{-1} (f.m)], chlorophyll *b* [0.97 mg g^{-1} (f.m)] as well as total chlorophyll [2.99 mg g^{-1} (f.m)], however, chlorophyll *b* was at par with Kufri Arun. The cultivars Kufri Bahar, Kufri Chandramukhi, Kufri Chipsona-4 and Kufri Kanchan recorded significantly minimum chlorophyll *a*, chlorophyll *b* as well as total chlorophyll levels (Table 1).

Ethylene is well known to induce chlorophyll breakdown leading to senescence and leaf abscission (Park *et al*., 10). In our study, we also observed a sharp decrease in chlorophyll contents in cultures grown in hermetic culture room. Presumably, the ethylene level in hermetic culture room might be physiologically active enough to break chlorophyll, thereby reducing chlorophyll contents. Similar conclusion was also demonstrated by Chanemougasoundharam *et al*. (2) in potato during culture vessel closure types as well as Park *et al*. (10) during sealed and ventilated gaseous microenvironment on hyperhydricity of potato shoots *in vitro*.

In case of carotenoids, Kufri Kanchan [0.037 mg g^{-1}(fm)] recorded significantly maximum, which was at par with Kufri Frysona, Kufri Bahar and Kufri Khyati whereas, Kufri Arun and Kufri Sindhuri [0.011 mg g^{-1}(fm)] the minimum and was at par with Kufri Chipsona-4, Kufri Chandramukhi, Kufri Lauvkar, Kufri Badshah and Kufri Chipsona-3. In the interaction, most of the cultivars grown in non-hermetic culture room recorded the maximum carotenoids, however, culture room types did not have any effect on cultivars having least carotenoids content. Among the cultivars, Kufri Frysona recorded significantly higher phenols, which was at par with Kufri Chandramukhi, Kufri Chipsona-3, Kufri Pukhraj and Kufri Pushkar, whereas, Kufri Bahar the least (Table 1). In the interaction, phenol showed same trend as that of carotenoids. Hence, non-hermetic culture room would lead to photoautotrophy of plantlets, which further favours the acclimatization to *ex vitro* conditions (Serret *et al*., 12).

Microplant shoot height was significantly influenced by cultivar, culture room types and their interaction. Hermetic culture room resulted in the production of maximum shoot height (7.8 cm) as compared to non-hermetic (6.1 cm). The former resulted in the production of weak and lanky microplants with hooked stem apices as compared to latter. After twenty-eight days, culture room types did not show any significant effect on shoot height in Kufri Arun, Kufri Kanchan, Kufri Khyati, Kufri Lauvkar, Kufri Pukhraj and Kufri Pushkar, whereas, hermetic culture room showed significantly increase in the shoot height in Kufri Badshah, Kufri Bahar, Kufri Chandramukhi, Kufri Chipsona-3, Kufri Chipsona-4, Kufri Frysona, Kufri Girdhari, Kufri Himalini, Kufri Jyoti, Kufri Sindhuri and Kufri Surya (Fig. 1). This may be due to genetic nature of cultivars, genetically controlled *in vitro* response has already been reported previously (Miller *et al.*, 8).

Table 1. Effect of non-hermetic and hermetic culture rooms on biochemical characters of potato *in vitro* plantlets.

Cultivar	Chl *a* [mg g^{-1} (fm)]			Chl *b* [mg g^{-1} (fm)]			Total Chl [mg g^{-1} (fm)]			Carotenoids [mg g^{-1} (fm)]			Phenols [mg 100 g^{-1} (fm)]		
	G1	G2	Mean	G1	G2	Mean	G1	G2	Mean	G1	G2	Mean	G1	G2	Mean
Kufri Arun	2.03	1.12	1.57	1.08	0.49	0.78	3.11	1.60	2.36	0.013	0.008	0.011	554.4	607.8	581.1
Kufri Badshah	1.67	0.29	0.98	0.76	0.21	0.48	2.43	0.50	1.46	0.019	0.008	0.014	639.1	346.5	492.8
Kufri Bahar	0.64	0.17	0.41	0.32	0.13	0.22	0.96	0.30	0.63	0.053	0.008	0.031	404.7	381.9	393.3
Kufri Chandramukhi	0.69	0.34	0.51	0.31	0.26	0.28	0.99	0.59	0.79	0.015	0.010	0.013	796.3	372.2	584.3
Kufri Chipsona-3	2.17	0.37	1.27	1.00	0.25	0.63	3.17	0.62	1.90	0.021	0.013	0.017	732.1	434.6	583.4
Kufri Chipsona-4	1.15	0.28	0.72	0.44	0.18	0.31	1.60	0.46	1.03	0.014	0.010	0.012	448.4	433.4	440.9
Kufri Frysona	2.41	1.65	2.03	1.07	0.86	0.97	3.48	2.51	2.99	0.047	0.016	0.032	718.3	819.8	769.1
Kufri Girdhari	1.29	0.43	0.86	0.75	0.24	0.50	2.04	0.67	1.36	0.043	0.013	0.028	627.6	305.8	466.7
Kufri Himalini	0.60	1.07	0.84	0.30	0.59	0.44	0.90	1.66	1.28	0.033	0.007	0.020	486.1	505.1	495.6
Kufri Jyoti	1.45	0.62	1.03	0.39	0.45	0.42	1.83	1.07	1.45	0.035	0.019	0.027	777.3	459.6	618.5
Kufri Kanchan	0.46	0.23	0.34	0.25	0.18	0.22	0.71	0.41	0.56	0.046	0.028	0.037	463.7	398.7	431.2
Kufri Khyati	1.99	0.29	1.14	0.72	0.24	0.48	2.71	0.53	1.62	0.035	0.022	0.029	487.9	429.3	458.6
Kufri Lauvkar	1.68	0.55	1.12	0.74	0.29	0.52	2.42	0.85	1.63	0.016	0.009	0.013	646.2	246.4	446.3
Kufri Pukhraj	1.68	0.87	1.28	0.77	0.42	0.59	2.45	1.29	1.87	0.041	0.009	0.025	748.2	485.0	616.6
Kufri Pushkar	2.27	0.98	1.63	0.89	0.59	0.74	3.16	1.57	2.36	0.040	0.011	0.026	757.1	496.2	626.7
Kufri Sindhuri	1.40	0.35	0.87	0.70	0.21	0.45	2.10	0.55	1.32	0.014	0.007	0.011	567.1	312.5	439.8
Kufri Surya	2.18	0.57	1.37	1.0	0.30	0.65	3.17	0.87	2.02	0.043	0.011	0.027	593.6	350.6	472.1
Mean	1.51	0.60	1.06	0.68	0.35	0.51	2.19	0.94	1.57	0.031	0.012	0.022	614.6	434.4	524.5
	V	G	VG	V	G	VG	V	G	VG	V	G	VG	V	G	VG
$CD_{0.05}$	0.37	0.13	0.52	0.19	0.06	0.26	0.51	0.17	0.71	0.008	0.003	0.011	121.41	41.64	171.70

G1 = Non-hermetic culture room; G2 = Hermetic culture room

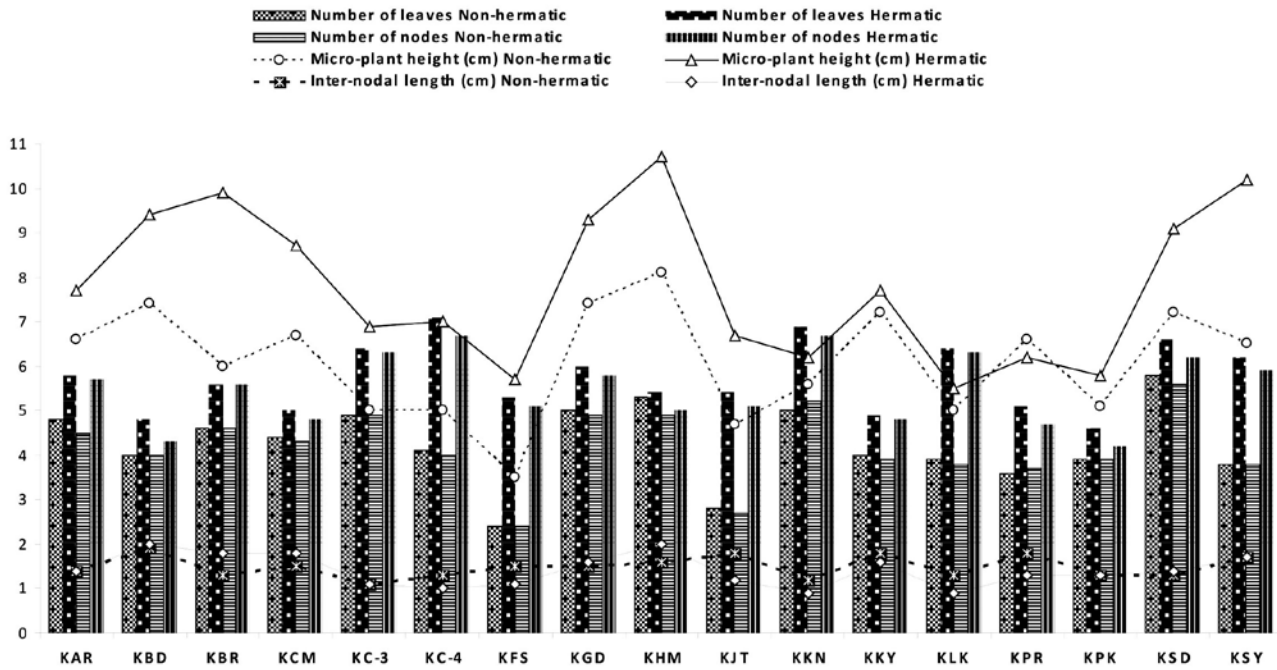

Fig. 1. Effect of non-hermetic and hermetic culture rooms on morphological characters of potato *in vitro* plantlets.

The growth abnormalities may be due to accumulation of ethylene in hermetic culture room (Jackson *et al*., 4). The poor growth of microplants in hermetic culture room can be attributed to the inhibitory effect of ethylene as well as to the non-utilization of CO_2 due to low density and high number of non-functioning stomata (Lai *et al*., 5). Further, it could be understood that the improved growth under non-hermetic culture room are a function of balanced CO_2 supply enabling the plants to benefit from net photosynthetic assimilate production (Zobayed *et al*., 15). Similar results were also reported in potato by Chanemougasoundharam *et al*. (2) in least permeable vessel closure types.

Number of leaves as well as nodes was also significantly influenced by cultivar, culture room types and their interaction. Culture room types had significant effect on number of leaves (5.5) and nodes (5.7) per plant, most of the cultivars grown in hermetic culture room recorded significantly more number of leaves as compared to non-hermetic culture room, whereas, later resulted in production of vigorous plants with more leaf area as compared to hermetic culture room. However, culture room types did not show any significant effect on number of leaves and nodes in Kufri Badshah, Kufri Chandramukhi, Kufri Himalini, Kufri Pushkar and Kufri Sindhuri. Cultivar Kufri Kanchan recorded significantly maximum number of leaves and nodes, which was at par with Kufri Sindhuri (Fig. 1). This may be due to low level of accumulation of competitive inhibitors in the non-hermetic culture room (Zobayed *et al*., 16; Chanemougasoundharam *et al*., 2). Controlled micropropagation systems, especially under forced ventilation have been shown to produce morphologically superior as well physiologically normal plants (Zobayed *et al*., 15, 16).

Internodal length was significantly influenced by cultivar, culture room type and their interaction. Among the culture room types non-hermetic recorded significantly more inter-nodal length (1.5 cm) as compared to hermetic culture room (1.4 cm). In cultivars Kufri Arun, Kufri Badshah, Kufri Chipsona-3, Kufri Girdhari, Kufri Pushkar, Kufri Sindhuri and Kufri Surya, culture room types did not have any significant effect on inter-nodal length. Significant increase in inter-nodal length was recorded in cultivars Kufri Chipsona-4, Kufri Frysona, Kufri Jyoti, Kufri Kanchan, Kufri Khyati, Kufri Lauvkar and Kufri Pukhraj when grown in non-hermetic culture room however, maximum inter-nodal length was developed in cultivars Kufri Bahar, Kufri Chandramukhi and Kufri Himalini when the plants were grown in hermetic culture room. Cultivar Kufri Badshah recorded significantly maximum inter-nodal length, which was at par with Kufri Himalini (Fig. 1).

Microplant root number was significantly influenced only by cultivar and Kufri Arun recorded statistically maximum number of roots followed by Kufri Badshah, whereas, Kufri Chandramukhi had minimum. Root length of microplant was significantly influenced by cultivar as well as types of culture room. Microplants grown in non-hermetic culture room produced significantly longer roots (6.6 cm) as compared to hermetic culture room (6.0 cm) but later resulted in production of aerial roots. Among the cultivars Kufri Frysona registered significantly maximum root length (9.3 cm), which was at par with Kufri Bahar (8.9 cm), whereas, Kufri Chandramukhi the minimum (2.4 cm) (Table 2).

Fresh as well as dry mass of microplant was significantly influenced by cultivar and culture room type. Cultivars Kufri Frysona, Kufri Jyoti and Kufri Khyati recorded significantly maximum fresh as well as dry mass, whereas, Kufri Bahar, Kufri Chipsona-3 and Kufri Sindhuri the minimum. In general, cultivars grown in non-hermetic culture room (323.1 and 27.9 mg) resulted production of more fresh as well as dry mass as compared to hermetic culture room (290.2 and 24.0 mg) (Table 2). The increase in dry mass of the plantlets grown in non-hermetic culture room may be due to low relative humidity. Similar results were also obtained in non-hermetically closed culture vessels by Majada *et al*. (7). However, in contrast Zobayed *et al*. (16) and Chanemougasoundharam *et al*. (2) reported higher fresh mass in hermetically sealed culture vessels.

The present study illustrated that the plantlets grown in non-hermetic culture room fostered vigorous microplant growth without any morphological abnormalities and induced higher chlorophyll, carotenoids and phenols content, which leads to more resistant to biotic and a biotic stresses. Hence, non-hermetic culture rooms are better suited than hermetic culture ones. Therefore, this study suggests that *in vitro* development is an early function of environmental conditions in spite of fact that the basic form is genetically controlled.

REFERENCES

1. Arnon, D. 1949. Copper enzymes in isolated chloroplasts polyphenoloxidase in *Beta vulgaris. Plant Physiol*. **24**: 1-15.

2. Chanemougasoundharam, A., Sarkar, D., Pandey, S.K. and Al-Biski, F. 2004. Culture tube closure-type affects potato plantlet growth and chlorophyll contents. *Biol*. *Plant*. **48**: 7-11.

3. Goldstein, J.L. and Swain, T. 1963. Changes in tannin in ripening frits. *Phytochem*. **2**: 371-83.

Table 2. Effect of non-hermetic and hermetic culture rooms on morphological characters of potato *in vitro* plantlets.

Cultivar	No. of roots			Root length (cm)			Fresh weight (mg)			Dry weight (mg)		
	G1	G2	Mean	G1	G2	Mean	G1	G2	Mean	G1	G2	Mean
Kufri Arun	8.0	7.9	7.9	6.1	6.6	6.4	339.8	220.5	280.1	28.1	21.9	25.0
Kufri Badshah	7.8	7.6	7.7	5.0	3.9	4.5	294.8	234.0	264.4	27.6	24.1	25.9
Kufri Bahar	4.7	5.0	4.8	9.5	8.4	8.9	210.2	236.1	223.2	19.7	20.8	20.3
Kufri Chandramukhi	3.4	2.7	3.1	2.5	2.2	2.4	322.8	330.8	326.8	28.2	28.6	28.3
Kufri Chipsona-3	4.8	4.6	4.7	8.2	8.2	8.2	185.0	177.3	181.1	18.2	18.0	18.1
Kufri Chipsona-4	5.7	4.8	5.3	7.1	7.9	7.5	295.8	276.0	285.9	27.0	22.8	24.9
Kufri Frysona	7.0	6.0	6.5	10.4	8.3	9.3	405.7	345.7	375.7	37.7	29.2	33.5
Kufri Girdhari	4.7	5.5	5.1	5.4	5.3	5.3	254.6	250.1	252.3	21.0	17.9	19.5
Kufri Himalini	4.5	4.3	4.4	6.4	6.2	6.3	298.8	314.0	306.4	23.1	23.0	23.0
Kufri Jyoti	5.6	7.2	6.4	6.5	5.5	6.0	449.6	344.0	396.8	38.1	27.5	32.8
Kufri Kanchan	4.7	4.5	4.6	6.8	5.5	6.1	357.9	316.1	337.0	34.4	28.3	31.4
Kufri Khyati	6.3	4.8	5.6	7.4	6.2	6.8	491.3	397.0	444.1	40.9	25.6	33.3
Kufri Lauvkar	5.2	6.1	5.6	5.2	4.1	4.7	295.8	353.3	324.6	20.9	24.3	22.6
Kufri Pukhraj	4.1	5.4	4.7	6.3	6.2	6.2	360.8	328.7	344.7	29.8	28.3	29.0
Kufri Pushkar	8.7	6.6	7.7	4.0	3.9	4.0	264.8	277.5	271.1	18.2	23.5	20.9
Kufri Sindhuri	6.3	4.8	5.5	8.2	7.9	8.1	270.5	201.5	236.0	20.6	20.5	20.6
Kufri Surya	5.8	5.0	5.4	7.1	6.8	6.9	394.4	331.7	363.0	40.9	24.2	32.6
Mean	5.7	5.4		6.6	6.0		323.1	290.2		27.9	24.0	
Factor	V	G	VG	V	G	VG	V	G	VG	V	G	VG
$CD_{0.05}$	1.1	NS	NS	1.0	0.4	NS	70.3	24.1*	NS	5.9	2.0	NS

G1 = Non-hermetic culture room; G2 = Hermetic culture room
* Significant only at (p≥0.05)

4. Jackson, M.B., Abbott, A.J., Belcher, A.R., Hall, K.C., Butler, R. and Cameron, L. 1991. Ventilation in plant tissue culture and effects of poor aeration on ethylene and carbon dioxide accumulation, oxygen depletion and explant development. *Ann*. *Bot*. **67**: 229-37.

5. Lai, C.C., Lin, H.M., Nalawade, S.M., Fang, W. and Tsay, H.S. 2005. Hyperhydricity in shoot cultures of *Scrofularia yoshimurae* can be effectively reduced by ventilation of vessels. *J*. *Plant Physiol*. **162**: 355-61.

6. Lentini, Z., Mussell, H., Mutschler, M.A. and Earlem, E.D. 1988. Ethylene generation and reversal of ethylene effects during development *in vitro* of rapid cycling *Brassica campestris* L. *Plant Sci*. **54**: 75-81.

7. Majada, J.P., Fal, M.A. and Sanchez-Tames, R. 1997. The effect of ventilation rate on proliferation and hyperhydricity of *Dianthus caryophyllus* L. *In vitro Cell Dev*. *Biol*. **33**: 62-69.

8. Miller, P.R., Amirouche, L., Stauchbury, T. and Matthews, S. 1985. The use of plant growth regulators in the micropropagtion of slow growing potato cultivars. *Potato Res*. **28**: 479-86.

9. Murashige, T. and Shoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol*. *Plant*. **15**: 473-97.

10. Park, S.W., Jeon, J.H., Kim, H.S., Park, Y.M., Aswath, C. and Joung, H. 2004. Effect on sealed and vented gaseous micro-environments on the hyperhydricity of potato shoots *in vitro*. *Scientia Hort*. **99**: 199-205.

11. Sarkar, D., Sood, K.C., Chakrabarti, S.K. and Naik, P.S. 1999. Growing of potato microplants in the presence of alginate silver thiosulphate capsules reduces ethylene-induced culture abnormalities during minimal growth conservation *in vitro. Plant Cell Tiss*. *Org*. *Cult*. **68**: 79-89.

12. Serret, M.D., Trillas, M.I., Matas, J. and Aruas, J.L. 1997. The effect of different closure types, light and sucrose concentrations on carbon isotope composition and growth of *Gardenia jasminoides* plantlets during micropropagation and subsequent acclimation *ex vitro. Plant Cell Tiss*. *Org*. *Cult*. **47**: 217-30.

13. Venkatasalam, E.P., Latawa, Jyoti, Sharma, Shilpa, Sharma, Sumita, Sharma, Ashwani Kumar, Sharma, Sneh, Patial, Rishu and Singh, Sarjeet. 2011. *In vitro* and *in vivo* performance of potato cultivars for different seed production systems. *Potato J*. **38**: 149-54.

14. Walker, P.N., Heuser, C.W. and Heinemann, P.H. 1988. Micropropagation: studies of gaseous environments. *Acta Hort*. **230**: 145-51.

15. Zobayed, S.M.A., Amstrong, J. and Amstrong, W. 1999. Evaluation of a closed system, diffusive and humidity induced convective through flow ventilation on the growth and physiology of cauliflower *in vitro. Plant Cell Tiss*. *Org*. *Cult*. **59**: 113-23.

16. Zobayed, S.M.A., Amstrong, J. and Amstrong, W. 2001. Micropropagtion of potato: Evaluation of closed, diffusive and forced ventilation on growth and tuberization. *Ann*. *Bot*. **87**: 53-59.

Received : December, 2012; Revised : October, 2014;
Accepted : February, 2015