THE USE OF RIVER SAND/SAWDUST FOR ACCLIMATIZATION OF *IN VITRO* RAISED SWEET POTATO PLANTLETS

***Ubalua, A. O. and G. C. Nsofor**
Plant Tissue Culture Unit, Biotechnology Research and Development Center, National Root Crops Research Institute (NRCRI) Umudike, PMB 7006 Umuahia, Abia State.
*Contact: alfreduba@yahoo.com

Abstract
Eight weeks old *in vitro* plantlets of two sweet potato varieties (NRSP/05/5A and CIP 50) were hardened on local substrate (river sand/sawdust) and on conventional substrate (jiffy peat) for two weeks in a humidity chamber. The local substrates (river sand/sawdust) compared favourably to the conventional substrate (jiffy peat). Jiffy peat substrate produced a mean plant height of 20.3±5.3 cm (NRSP/05/5A) and 13.5±4.6 cm (CIP 50) while river sand/saw dust produced a competitive growth of 19.8±3.6 and 12.6±3.2 cm for cultivar NRSP/05/5A and CIP 50, respectively. The two varieties responded differently to the composition of the substrates in all the growth parameters considered. Although jiffy peat substrate is superior to river sand/sawdust substrate, the effectiveness of the treatment on the plantlets was not significantly different from the response obtained from river sand/saw dust substrate. The implication is that the variations in response to the treatment by the two sweet potato varieties could be ascribed to their inherent genotypic differences since such a response is genotypically dependent in many species. In all respects, when economics and affordability advantage is considered, the substrate river sand/saw dust is preferred over jiffy peat in the sub-Saharan Africa.

Keywords: River sand, saw dust, jiffy peat, sweet potato plantlets, humidity chamber, hardening.

Introduction

Sweet potato (*Ipomoea batatas* L. Lam) is among the world's most important versatile and underexploited root crops. It is an important food crop after cassava, rice and yam in the tropics. It is cultivated mainly for its storage roots and is probably the food crop that produces the most energy per unit area. Its high-energy value exceeds even that of potato, cassava and other known tubers (Ubalua and Okoroafor, 2013). Based on its calorific yields, nutritional value, adaptability, versatility and vegetative reproduction, sweet potato could in future contribute significantly to world agriculture. It is a major food, feed and industrial raw material in China whose total output in the world is estimated to be more than 80% (Islam 2006; Farmer *et al*., 2007; Liu *et al*., 2011). The crop is characterized by its fast growth, active root formation and development which confers greater survival rate of the seedlings and the expected output (Alam *et al*., 2010). Consequently its formation of a large root system in the early stage promotes robust growth of the seedlings in early development, flourishing stems and leaves that are conducive to overall yield (Deng *et al*., 2012). The orange flesh varieties have sufficient carotene that serves as a good source of vitamin A. Recent studies by Islam (2006) have revealed that sweet potato leaf extracts contains radical scavenging, antimutagenic, anticancer and antibacterial activities. Hence, sweet potato leaves is a veritable source of natural antioxidant. Presently, the total production land mass and yield is approximately 6.6 million ha and 106 million tons respectively (Zhang *et al*., 2009). Recent growth of fast food restaurants and snacks in the sub-Saharan Africa has given sweet potato a new status, new prominence and new potential.

Profitable production of sweet potato through vine cuttings has declined due to its susceptibility to biotic stress. The crop thrives well in marginal soils producing good yield with little demand for either fertilizer or water. Although sweet potato is relatively drought tolerant and can provide good ground cover and often cultivated without pesticides (Ewell, 1990), its productivity is however, limited by a number of both biotic and abiotic constraints (Alam *et al*., 2010). Amongst the 20 viruses that have been identified to infect sweet potato in the world, sweet potato feathery mottle virus (SPFMV, genus *Potyvirus*, family *Potyviridae*) is more prevalent in all sweet potato growing areas while the others are localized to one or more geographic areas (Moyer and Salazar, 1989; Kreuze *et al*., 2000). The most severe sweet potato virus disease (SPVD), usually caused by the dual infection of sweet potato feathery mottle virus (SPFMV) and sweet potato chlorotic stunt virus (SPCSV), can result to 56-98% yield loss (Gibson *et al*., 1998). Thus, SPVD epidemics have been associated with low on-farm productivity and with the disappearance of the once elite cultivars (Gibson *et al*., 1997).

A regular supply of clean materials for planting is therefore necessary for sustainable production (Wang and Valkonen, 2008). These intractable problems associated with virus infestation of the planting materials demands for alternative approach. Interestingly, tissue culture has been identified as a powerful tool to overcome these limitations. One of the tissue culture techniques termed micropropagation aims at rapidly multiplying clonal superior genotypes of disease-free and pest free plants (Smith and Drew, 1990). Strategies for postflask management of *in vitro* cultured sweet potato plantlets have been reported. Cassana *et al*. (2010) conducted an extensive research on the photochemical efficiency of leaves of sweet potato grown in an *in vitro* and *ex vitro* conditions. Semoradova *et al*. (2002) reported on responses of tobacco plantlets during transfer from an *in vitro* to an *ex vitro* conditions. Seelye *et al*. (2003) examined some of the major physiological

events that occurs during *in vitro* transition of plantlets to *ex vitro* environment while Hazarika (2003) reviewed some current and developing methods for acclimatization of *in vitro*-produced plantlets. Despite the successes and the benefits of using jiffy peat for acclimatization, the prohibitive cost of postflask management of tissue-cultured plantlets has been a source of concern in the sub-Saharan Africa. The present study therefore aims at addressing this problem through the use of locally available materials as an alternative hardening substrate to jiffy peat a conventional substrate.

Materials and Methods

Establishment of cultures

Apical shoot-bud (4 mm stem single-node) explants were aseptically excised in a laminar airflow cabinet from proliferating shoot cultures of the two sweet potato cultivars (NRSP/05/5A and CIP 50) after eight weeks of growth in Murashige and Skoog (MS) medium. The pH of the medium was previously adjusted to 5.8 before sterilizing by autoclaving at 121^0C for 20 min. The tender leaves were aseptically removed with sterile forceps and knives in sterile petri dishes in a laminar air-flow cabinet. The nodal cuttings were seeded singly into sterile test tubes containing 10 ml sterile MS medium solidified with 8 g/l of agar and supplemented with 20 g/l of sucrose. The test tubes were sealed properly with parafilm and clearly labeled before growing the cultures at a temperature of 28 ± 2^0C for 16 h photoperiod and 8 h darkness at light intensity of 2000-3000 lux. They were sub-cultured every 2 months in order to generate enough plantlets for hardening.

Hardening/Acclimatization

The plantlets were gently washed with tap water to remove the adhering media on the roots before planting with care onto sterile local weaning substrates (river sand (RS), rice mill waste (RMW) and saw dust (SD)) in various combinations in a 5 x 5 cm transparent polybags alongside the conventional substrate (Jiffy peat). The combinations were: RS/SD (2:1), SD/RMW (2:1) and RS/RMW (2:1). The humidity chamber was generously sprayed with water and kept under a shade. The polybags containing the substrates and the plantlets were watered, labeled and kept in the humidity chamber. On the third day morning after transplanting, three holes of about 1 cm in diameter were made at the sides of the humidity chamber while on the fourth day the humidity in the chamber was reduced by cutting an opening (window) at the lower side of the chamber. A wash bottle containing six grains of fertilizer and water were mixed properly before adequate spraying of the substrates and the humidity chamber as well. The substrates and the humidity chamber were again sprayed with water towards evening time before closing the window. On the morning of the fifth day, the previous window was re-opened and a second window on the opposite side of the chamber was opened. The plants and the chamber were again generously sprayed with water. After 2 and 4 weeks, the following parameters were assessed: plant height, number of leaves and nodes before transplanting into top soil in a 5 x 5 cm black polybags. They plants were watered once a day and occasionally with dilute fertilizer and kept under a shade for another 4 weeks before transplanting to the field.

Experimental design and data analysis

Completely randomized design was used for the experiments. Each treatment was repeated three times and data recorded two and four weeks after the beginning of the treatments. Analysis of variance (ANOVA) was used to compare the number of leaves, nodes and plant height in the conventional and the local substrates. Means were separated using Tukey's test at the level of 5%.

Results and Discussion

Acclimatization/Hardening

Acclimatization of *in vitro* produced plantlets requires that *ex vitro* environmental factors be gradually modified for successful survival and establishment of the transferred plantlets. Such is to help improve the internal structure of the plantlets and to give a more successful establishment. The gradual reduction of the relative humidity in the humidity chamber may have enhanced the survival and the establishment of the *in vitro* plantlets in this study. A fact that was supported by the reports of Cassels and Walsh, (1994) and Seelye et al. (2003) that a reduction in relative humidity leads to increases in plants nutrient uptake and transpiration with associated development of functional stomata for controlling plant water loss. The effects of the substrates and substrates combinations on plant height are as presented in Table 1. Substrates RS, jiffy peat and the RS/SD supported growth for the two cultivars (Table 1). Hence after 4 weeks of adaptation on substrates RS/SD, RS and jiffy peat, the mean plant height ranged from 17.6±4.3 to 20.3±5.2 cm for cultivar NRSP/05/5A in contrast to 10.6±3.4 to 13.6±3.0 cm for cultivar CIP 50. A significant growth of 17.6±4.3 and 12.2±3.3 in height was observed on substrate RS/SD for cultivars A5 and 50 respectively. However, the best result in terms of plant height was obtained from the conventional substrate (jiffy peat), although the effectiveness of this treatment did not differ significantly from that of substrates RS and RS/SD. However, substrates SD/RMW and RMW were not significant for both cultivars in contrast to RS, RS/SD and jiffy peat.

Table 1: Mean plant height of the two sweet potato cultivars hardened on local and conventional substrates

	Mean plant height					
	NRSP/05/5A			CIP 50		
Substrates	**1st 2 weeks**	**2nd 2 weeks**	**Mean**	**1st 2 weeks**	**2nd 2 weeks**	**Mean**
RS	17.2±6.1[a]	19.8±3.6[a]	18.5±4.6[a]	10.3±4.6[b]	10.8±3.4[b]	10.6±3.4[b]
SD	13.6±4.3[b]	15.8±4.4[a]	14.7±3.1[b]	8.6±2.2[b]	8.6±1.2[c]	8.6±0.7[c]
RMW	6.2±1.4[c]	7.4±1.7[c]	6.8±2.6[c]	3.1±1.3[d]	3.3±1.0[d]	3.2±1.2[d]
RS/RMW	10.5±0.8[b]	12.6±2.8[b]	11.6±3.1[b]	5.2±1.4[c]	5.9±2.3[c]	5.6±1.4[c]
RS/SD	16.8±4.2[a]	18.4±7.2[a]	17.6±4.3[a]	11.8±1.8[b]	12.6±3.2[b]	12.2±3.3[b]
SD/RMW	2.5±0.4[d]	3.1±0.7[d]	2.8±0.3[d]	3.4±0.8[d]	3.8±0.3[d]	3.6±0.8[d]
Jiffy peat	20.4±5.2[a]	20.3±5.3[a]	20.3±5.2[a]	13.7±4.6[b]	13.5±4.6[b]	13.6±3.0[b]

Key: Values represents mean ± standard errors for three replications for each treatment. Same letters are not significantly different at p≤0.05. RMW-Rice mill waste; SD-Saw dust; RS-River sand

Interestingly, the *ex vitro* survival and development of the *in vitro* plantlets into well-developed plants was 100% irrespective of the substrates or substrate combinations used except for three incidences of caterpillar attacks. This is in contrast to the *in vitro* plantlets grown on untreated control (unsterilized substrates) that produced a comparatively low survival rate of 58%. Certainly *in vitro* grown plantlets are sterile and are also characterized by some anatomical deficiencies such as low deposition of surface wax, stomatal abnormalities and a non-continuous cuticle (Hazarika, 2003). In view of these factors, the substrates were sterilized to reduce possible biotic stress and the humidity inside the chamber (Fig. 1c) was gradually reduced in order to mimic *ex vitro* conditions and improve the internal structure of the *in vitro* plantlets in order to give a more successful establishment. Thus, Short *et al*., (1987), reported 80% relative humidity for optimum growth and *in vitro* hardening of cultured cauliflower and chrysanthemum. Therefore the survival of the *in vitro* produced plantlets in the first 2 weeks of acclimatization, may suggest that the *in vitro*-formed roots may have enhanced the continued growth and establishment of the plants during the initial acclimatization period. Thus, Leclerc *et al*., (1994) reported a 2 week period of sustained growth by the *in vitro*-formed roots of potato. They further stated that *in vitro*-formed roots not only survived transfer to the substrate but elongated and formed secondary and tertiary root systems with root hairs, implying that *in vitro* formed roots are functional during acclimatization and contributes significantly to the early growth of transplants *ex vitro*. This is in complete contrast to the *in vitro*-produced leaves that were observed not to have significant influence on the growth and establishment of the transplanted plantlets.

The effects of the substrates were evaluated on leaf production by the two sweet potato cultivars (Table 2). After 4 weeks of growth in the substrates, leaf development was poor on substrates RMW and SD/RMW for the two cultivars compared to the other treatments (Table 2). The number of leaves produced after 4 weeks using jiffy peat substrate was significantly higher than that obtained from substrates RMW and SD/RMW but comparable to substrates RS and RS/SD. Mean number of leaves produced on jiffy peat substrate were 9.2±3.1 and 8.4±2.2 while 6.8±1.3 and 6.6±2.1 leaves were produced on substrate RS/SD (Table 2). Number of leaves produced on substrates RS, SD, RMW, RS/RMW and SD/RMW differed significantly from that produced on substrates RS/SD and the conventional substrate. Although, the number of leaves produced on the conventional substrate was slightly higher, they were not significantly different from that produced on RS/SD substrate. *In vitro*-raised plantlets are usually grown in an environmental condition that is different from the natural field conditions and *in vitro*-produced leaves are associated with a number of anatomical and physiological changes compared to greenhouse and field-grown plants. Thus plantlets produced under conventional photomixotrophic conditions, such as high relative humidity has reduced epicuticular wax deposition, poor cuticle development and malfunctional stomata (Zobayed *et al*., 2000). Although *in vitro*–formed leaves may be photosynthetically competent, they are often replaced shortly after transfer to the green house by leaves with higher photosynthetic activity (Huylenbroeck *et al*., 1996).

Table 2: Mean number of leaves produced by the two sweet potato cultivars hardened on local and conventional substrates

	Mean number of leaves					
	NRSP/05/5A			CIP 50		
Substrates	**1st 2 weeks**	**2nd 2 weeks**	**Mean**	**1st 2 weeks**	**2nd 2 weeks**	**Mean**
RS	5.8±0.6[b]	6.2±1.8[b]	6.0±0.7[b]	3.8±1.4[c]	5.4±0.9[b]	4.6±0.5[b]
SD	5.5±1.2[b]	6.8±0.7[a]	6.2±1.3[b]	3.5±0.3[c]	3.9±0.6[c]	3.7±0.6[c]
RMW	4.2±0.3[b]	4.4±1.4[c]	4.3±0.6[c]	2.2±0.4[d]	2.8±0.1[d]	2.5±0.3[d]
RS/RMW	5.8±1.4[b]	6.5±1.3[b]	6.2±1.4[b]	3.6±0.5[c]	3.9±1.2[c]	3.8±0.4[c]
RS/SD	6.6±2.1[a]	6.9±2.4[a]	6.8±1.3[a]	6.0±1.8[b]	7.2±0.8[a]	6.6±2.1[a]
SD/RMW	3.3±1.3[c]	3.2±0.1[c]	3.3±0.5[c]	2.9±0.4[d]	3.2±1.2[c]	3.1±0.2[c]
Jiffy peat	9.3±2.4[a]	9.1±1.6[a]	9.2±3.1[a]	8.2±1.7[a]	8.6±0.5[a]	8.4±2.2[a]

Key: Values represents mean ± standard errors for three replications for each treatment. Same letters are not significantly different at p≤0.05. RMW-Rice mill waste; SD-Saw dust; RS-River sand.

Nodal increase of plants is associated with increases in growth. Table 3 shows the mean number of nodes produced by the two sweet potato cultivars hardened on local and conventional substrates after 4 weeks growth period. The highest numbers of nodes (8.6±1.8 and 6.6±2.1) were obtained on the conventional substrate followed by 7.2±1.4 and 5.2±1.4 on RS/SD

substrate for cultivars NRSP/05/5A and CIP 50 respectively (Table 3). Substrates (RS, RMW, RS/RMW and SD/RMW) were not significant when the number of nodes produced for both cultivars per substrate were considered (Table 3). Differences in *in vitro* grown plant have been reported. Dessai *et al*., (1995) reported differences in nodal production of 27 cultivars of sweet potato during *in vitro* propagation. Ogero *et al*., (2012) evaluated the responses of two sweet potato cultivars when cultured *in vitro* with low cost and conventional media. Possible reasons for variability could be due to the balanced nutrient composition of the conventional substrate and differences in the genetic make-up of the crop species. The overall response of the two sweet potato cultivars on the seven substrates considered in this study is as captured in Table 4.

Table 3: Mean number of nodes produced by the two sweet potato cultivars hardened on local and conventional substrates.

	Mean number of nodes					
	NRSP/05/5A			CIP 50		
Substrates	**1st 2 weeks**	**2nd 2 weeks**	**Mean**	**1st 2 weeks**	**2nd 2 weeks**	**Mean**
RS	5.7±0.8[b]	6.8±1.2[a]	6.3±0.5[b]	3.8±0.2[b]	5.6±1.3[b]	4.7±0.5[a]
SD	6.4±1.3[b]	6.7±2.3[a]	6.6±2.0[a]	4.0±0.2[b]	4.7±1.4[b]	4.4±0.6[b]
RMW	4.6±1.5[c]	5.5±0.7[b]	5.1±0.4[b]	2.9±0.3[c]	3.4±0.6[c]	3.2±1.2[c]
RS/RMW	5.0±0.4[b]	5.6±1.4[b]	5.3±0.5[b]	3.5±1.2[b]	4.6±0.7[b]	4.1±0.8[b]
RS/SD	6.6±2.3[a]	7.8±1.6[a]	7.2±1.4[a]	4.5±1.4[a]	5.8±2.4[b]	5.2±1.3[a]
SD/RMW	3.8±1.2[c]	4.5±0.6[c]	4.2±0.1[c]	3.3±0.7[c]	4.1±1.5[b]	3.7±0.6[b]
Jiffy peat	8.8±2.2[a]	8.4±3.1[a]	8.6±1.8[a]	6.5±0.4[a]	6.6±1.5[a]	6.6±2.1[a]

Key: Values represents mean ± standard errors for three replications for each treatment. Same letters are not significantly different at p≤0.05. RMW-Rice mill waste; SD-Saw dust; RS-River sand.

Generally, the conventional substrate (jiffy peat) out-competed the rest of the substrates (RS, SD, RMW, RS/RMW, RS/SD and SD/RMW), though comparable to RS/SD substrate. Considering plant height, NRSP/05/5A responded well to the conventional substrate and substrate RS/SD in contrast to the performance of cultivar CIP 50 on these substrates. Thus, the obtained plant height on RS/SD substrate and on the conventional substrate for cultivar NRSP/05/5A are significantly different from the values obtained for cultivar CIP 50 on the same substrates. Similarly, number of leaves and nodes produced on the same substrates followed the same pattern for both cultivars, suggesting that the conventional and the RS/SD substrates are better substrates compared to the other substrates. Conclusively, although jiffy peat substrate is superior to RS/SD which is a locally available substrate, RS/SD substrate is thus a considerable alternative when economics and availability is considered.

Acknowledgements

The authors are grateful to the management of National Root Crops Research Institute (NRCRI) Umudike, Umuahia, Abia State for supporting this research.

References

Alam I, Sharmin SA, Naher K, Alam J, Anisuzzaman M, Alam MF. (2010). Effect of Growth Regulators on Meristem Culture and Plantlet Establishment in Sweet Potato (*Ipomoea Batatas* (L.) Lam.). *Plant Omics*. 3:35-39.

Cassana FF, Falqueto AR, Braga EJB, Peters JA, and Bacarin MA (2010). Chlorophyll *a* fluorescence of sweet potato plants cultivated *in vitro* and during ex vitro acclimatization. *Braz. J Plant Physiol*. 22(3): 167-170.

Cassells AC, Walsh C (1994). The influence of gas permeability of the culture lid on calcium uptake and stomatal function in Dianthus microplants. *Plant Cell Tiss Org Cult* 37: 171-178.

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. *Aus J Crop Sci. AJCS* 6(11): 1572-1578.

Dessai AP, Gosukonda RM, Blay E, Dumenyo CK, Medina-Boliva RF, Prakash CS (1995). Plant regeneration of sweet potato (Ipomoea batatas L.) from leaf explants in vitro using a two-stage protocol. *Scientia Horticulturae*. 62: 217-224.

Ewell PT (1990). Sweet potatoes in Eastern and Southern Africa. Paper presented at the workshop on sweet potatoes in the food systems of Eastern and Southern Africa, held in 1990 in Nairobi, Kenya.

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.

Gibson RW, Mwanga ROM, Kasule S, Mpembe I, Carey EE (1997) Apparent absence of viruses in most symptomless field grown potato in Uganda. *Ann Appl Biol* 130:481-490.

Gibson RW, Mpembe I, Alicai T, Carey EE, Mwanga ROM, Seal SE, Vetten HJ (1998). Symptoms, aetiology and serological analysis of sweet potato virus disease in Uganda. *Plant Pathol* 47:95-102.

Hazarika BN (2003). Acclimatization of tissue-cultured plants. *Curr sci*. 85: 12.

Hue S-M, Boyce AN, Somasundram C (2012). Antioxidant activity, phenolic and flavonoid contents in the leaves of different varieties of sweet potato (*Ipomoea batatas*). *Aus J Crop Sci. AJCS* 6(3):375-380.

Huylenbroeck JM van, Piqueras A, Debergh PC (1996). Photosynthesis and carbon metabolism in leaves formed prior and during ex vitro acclimatization of micropropagated plants. *Plant Sci.* 134: 21-30.

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

Kreuze JF, Karyeija RF, Gibson RW, Valkonen JPT (2000). Comparisons of coat protein gene sequences show that East African isolates of Sweet potato feathery mottle virus form a genetically distinct group. *Arch Virol* 145:567-74.

Leclerc Y, Donelly DJ, Seabrook JEA (1994). Microtuberization of layered shoots and nodal cuttings of potato: the influence of growth regulators and incubation periods. *Plant Cell Tiss Org. Cult*., 37: 113-120.

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

Moyer JW, Salazar LF (1989) Virus and virus like diseases of sweet potato. *Plant Dis* 73:451- 455.

Ogero KO, Mburugu GN, Mwangi M, Ngugi MM, Ombor O (2012). Low Cost Tissue Culture Technology in the Regeneration of Sweet Potato (*Ipomoea batatas* (L) Lam). *Res J Bio*. 2 (2): 51-58.

Seelye JF, Burge GK, Morgan ER (2003). Acclimatizing Tissue Culture Plants: Reducing the Shock. Combined Proceedings International Plant Propagators' Society. 90: 53.

Semorádová S, Synková H, Pospíšilová J (2002). Responses of tobacco plantlets to change of irradiance during transfer from *in vitro* to *ex vitro* conditions. *Photosynthetica* 40: 605-614.

Short KC, Warburton J, Roberts AV (1987). *In vitro* hardening of cultured cauliflower and chrysanthemum plantlets to humidity. *Acta Hortic*. 212: 329-334.

Smith MK, Drew RA (1990) Current applications of tissue culture in plant propagation and improvement. *Aus J. Plant Physiol*. 17: 267-89.

Wang QC, Valkonen JPT (2008). Elimination of two viruses which interact synergistically from sweetpotato by shoot tip culture and cryotherapy. *J Virol Meth*. 154: 135–45.

Zhang LM, Wang QM, Liu QC, Wang QC. (2009). Sweetpotato in China. In: Loebenstein G,Thottappilly G, editors. Biology and biotechnology of sweetpotato. *Springer*; p. 325–58.

Zobayed SMA, Afreen F, Kubota C, Kozai T (2000). Water Control and Survival of *Ipomoea batatas* grown photoautotrophically under forced ventilation and photomixotrophically under natural ventilation. *Annals of Botany*. 86: 603-610.

1a (Wild type)

1b (In vitro plantlets in MS medium)

1c (Acclimatization of the in vitro plantlets in the substrates contained in transparent polybags in a humidity chamber).

1d (Acclimatized in vitro plantlets in top soil ready to be transplanted to the field).