

CHALLENGES AND SOLUTIONS WITHIN *IN VITRO* PROPAGATION OF SWEET POTATO (*IPOMOEA BATATAS* L.)

Mihaela Cioloca^{1*}, Anca Baciuc², Andreea Tican¹, Nina Bărbăscu¹, Monica Popa¹

¹National Institute of Research and Development for Potato and Sugar Beet, Brasov, Romania

²Potato Research and Development Station Târgu Secuiesc, Covasna, Romania

Abstract

The purpose of this study was to find viable solutions for problems encountered during in vitro cultivation of sweet potato (Ipomoea batatas (L.) Lam.). Plant tissue culture are especially suitable for obtaining and maintaining a stock of pathogen-free plants, which can then be multiplied and used as an initial material. Although tissue cultures, carried out under controlled conditions, in a sterile environment, have important applications in plant biotechnologies, however, there are many factors that can influence their success. In addition to the minimum equipment of a plant tissue culture laboratory good function (adequate spaces, equipment, chemicals, laboratory glassware and tools etc.) and trained workers, the success of such an activity also depends on finding the best conditions for the harmonious growth and development of plant organisms, in a controlled environment. These challenges relate in particular to: introducing in vitro conditions of biological material from the in vivo environment; sterilization of the material which will be used as a source of explants; choosing a suitable growth medium, microbial contamination control; establishing optimal parameters in terms of temperature and photoperiod etc. The factors mentioned above vary by species and, more than that, by variety. In this study, the research was carried out over four years, on five Korean sweet potato varieties, at the Research Laboratory for Plant Tissue Cultures of the National Research and Development Institute for Potato and Sugar Beet, Brasov.

Keywords: *sweet potato, in vitro propagation, growth conditions, plantlets, greenhouse transplantation, microbial contamination control*

INTRODUCTION

The sweet potato is considered the seventh most important crop in the world and the fifth most important in developing countries. In Africa and Asia, thousands of people depend on sweet potato for food safety [1].

Although the *in vitro* propagation of *Ipomoea batatas* has not been as successful or common as that of other vegetable crops, several reports concerning tissue culture of sweet potato have appeared in recent years [2]. These contributions have dealt with callus induction in tissue explants followed by plantlet regeneration [3, 4, 5, 6] and with regeneration of plants by meristem culture [3].

Sweet potato is highly recalcitrant in generation and response to tissue culture [7]. It is also commonly propagated using vegetative propagation, a technique that favours propagation of viruses infected materials [8, 9]. Through plant tissue culture, large number of uniform and healthy plants are produced. The use of bud explants was reported to be the best means for regeneration of large planting materials in sweet potato [10]. A number of factors including genotypes, nature and doses of different growth regulators are found to determine the rate of regeneration of sweet potato [11]. Plant growth regulators play a significant role on callus formation, somatic embryogenesis or organogenesis, during the cultivation [12]. Plant growth regulators in combination with other nitrogen sources make up the culture medium that supply all the essential mineral ions required for plant growth and development.

Improved planting materials will motivate growers to cultivate the crops and significantly contribute in poverty alleviation through improved seed quality and through the distribution of improved varieties [13]. Plant cell and tissue culture provides a promising leads toward achieving these [7].

The purpose of this study was to find viable solutions to the problems encountered during the *in vitro* cultivation process of sweet potato (*Ipomoea batatas* (L.) Lam.), which will contribute to the efficiency of this technology.

MATERIALS AND METHODS

The research was conducted over four years at the Research Laboratory for Plant Tissue Cultures of the National Institute for Research and Development for Potato and Sugar Beet, Brasov. This study was possible as a result of the collaboration between NIRDPSB Brasov and RDCAPS Dabuleni, within the ADER project 2.2.2: Development of sweet potato cultivation technologies in the context of climate change and elaboration of measures to promote culture in Romania (2015-2018). The biological material was represented by five korean sweet potato varieties: Yulmi (white flesh), Hayanmi (white flesh), KSP1 (yellow flesh), KSC1 (yellow flesh) and Juhwangmi (orange flesh).

Considering that the micropropagation of sweet potato under controlled conditions was a novelty for our laboratory activity, during this process many challenges were encountered which required finding solutions that allow the specific stages of *in vitro* multiplication technology to be completed in good conditions. The main aspects were related to: finding an efficient method for obtaining sweet potato shoots under laboratory conditions; the choice of the agents used for the biological material sterilization (concentration, exposure time), as well as the application of different methods for microbial contamination control during cultivation; identification of the optimum composition of the medium to ensure the growth and development of sweet potato plantlets.

1. Obtaining the sweet potato shoots in laboratory conditions

To initiate *in vitro* culture, stem shoots from sweet potato plants grown under laboratory conditions can be used. The optimum age of the mother plants is 2-3 months. They must be healthy and have no lateral buds, abundant sprouting being a sign of aging. In this study, two methods have been tested for obtaining sweet potato shoots under laboratory conditions.

In the first method, for tubers sprouting preparation, the following steps were performed: washing of tubers under water jet; drying them on a paper towel; placing tubers on filter paper moistened with water; spraying tubers once a week with gibberellin solution (0.1 mg/l). The tubers were kept under laboratory conditions, providing a temperature of about 20-23 °C and alternating periods of artificial lighting with periods of darkness. To maintain humidity, the filter paper on which the tubers were placed was moistened daily, and for breaking dormancy tubers were sprinkled with gibberellin (Fig. 1).



Fig. 1. Obtaining the sweet potato shoots in laboratory conditions (method 1-up; method 2-down)

The steps of the second method, which consisted in planting sweet potato tubers in pots containing a substrate (peat, pearl and sand), were carried out as follows: preparing and moistening the substrate; horizontal positioning of tubers; spraying tubers with gibberellin solution; covering the tubers with a thin layer of soil; maintenance of the wet substrate by periodic watering and weekly application of a treatment with gibberellin solution. Until the emergence of the shoots were provided optimal conditions of temperature (23-25 °C), the culture vessels being kept in dark conditions and watered periodically, so that the substrate is always moist (Fig. 1). Once a week, gibberellin solution (0.1 mg/l) may be added.

2. Control of microbial contamination during *in vitro* cultivation of sweet potato

In plant tissue culture technology, microbial infections are frequently encountered. Sterilization is the most effective in control of the pathogenic effect of microorganisms. A standard protocol for plant material sterilizing includes the following steps: washing with running water; immersion in a sterilizing solution; rinses repeated with sterile distilled water. Because the nutrient media provide them very good conditions for development, bacteria and fungi invade cultures *in vitro* in a short time unless the asepsis of transfer spaces, culture vessels, environment, working tools and biological material is ensured.

In order to control the level of microbial contamination that occurred during *in vitro* cultivation of sweet potato (Fig. 2), several methods were tested: reducing the amount of sucrose in the culture medium combined with applying an appropriate sterilization treatment; use of an antibiotic culture medium and a broad spectrum product that limits the development of pathogens in plant tissue cultures (PPM™ - Plant Preservation Mixture), at different concentrations; transplanting of sweet potato plantlets from the culture vessels in which microbial infections have occurred in pots located in protected space (greenhouse).

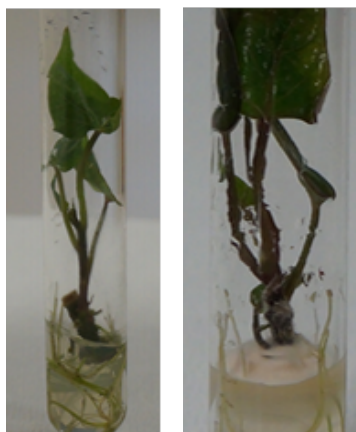


Fig. 2. Healthy culture (left) and contaminated culture (right)

2.1. In the case of the first method, different concentrations of sucrose added to growth medium (20 g/l, 30 g/l and 40 g/l respectively) were tested as well as different time periods in which the biological material was in contact with the sterilizing agent: 70% alcohol (3, 4 and 5 minutes) and 1% sodium hypochlorite (10, 13 and 16 minutes respectively).

2.2. In order to reduce the level of microbial contamination, another method based on adding antibiotics to the growing medium was tested. Before inoculation in antibiotic addition medium, the sweet potato plantlets were taken from the culture vessels that had infections, the large leaves and roots were removed and then fragmented into uninodal cuttings. The following three steps were performed in sterile space, in the laminar air flow hood: immersion of the nodal cuttings in alcohol (70%), for 1 minute; drying on sterilized paper; inoculation on nutrient medium containing antibiotic, without prior rinsing (Fig. 3).

After inoculation of the explants on medium with the addition of antibiotic the culture vessels were incubated in the growth chamber, following the evolution of the explants and the appearance of possible infections. After 24 days from the culture initiation the explants were passed on multiplication medium, without antibiotic. Prior to inoculation, uninodal cuttings were immersed in 4% PPMTM solution for 5 minutes. Then, without rinsing, they were left a few seconds on sterile filter paper and inoculated on the nutrient medium.



Fig. 3. Inoculation on medium with antibiotic of cuttings taken from contaminated cultures

2.3. The growth medium used for plant tissue cultures contains numerous nutrients that can create favorable conditions for bacterial development. Plant cells stimulated to grow in *in vitro* conditions are subject to a higher level of stress compared to the natural environment, which favors the emergence of infections with bacteria that would not normally affect them [14, 15, 16].

As antibiotic use is not encouraged, a new strategy to reduce the losses caused by the presence of microbial infections in the *in vitro* cultivation of sweet potatoes has been tried.



Fig. 4. Transplantation in pots of plantlets from contaminated cultures

For this purpose, plantlets from contaminated *in vitro* cultures were transplanted into protected space (greenhouse), in pots (Fig. 4). The substrate used was of two types: only pearl or a mixture of peat and pearl (1:1). One plantlet was planted in each pot.

After the plants began to develop, NPK 16:16:16 + sulfur complex (12 g/l) was added to the water. This treatment was applied once a month. Approximately two months after transplantation, the vine cuttings obtained could be used as a source of explants to initiate new *in vitro* cultures.

3. Optimization of growth medium composition to ensure both plants development and cost efficiency

Successful establishment of tissue culture is governed by several factors. In the literature, there are numerous studies on *in vitro* multiplication of sweet potato, many of them referring to research on the composition of the nutrient medium. The chemical composition of the culture medium is perhaps the most studied aspect of sweet potato tissue culture. It plays an important role in success of micropropagation.

In this study different growth medium variants has tested (Table 1) in order to optimize the *in vitro* conditions for sweet potato cultivation and the main goal was to find an optimal growth medium that would allow both the good development of *in vitro* cultivated sweet potato plants and cost efficiency. All three culture medium variants were based on MS salts and vitamins [17].

Table 1. Growth medium variants used for sweet potato micropropagation

Medium variants	Components	Amount/1L of medium (g)
MS – Murashige-Skoog, 1962	MS salts including vitamins	4.4
MS – Murashige-Skoog, 1962 NAA GA ₃	MS salts including vitamins	4.4
	Naphthalene acetic acid	0.0003
	Gibberellic acid	0.01
SPPM – Sweet Potato Propagation Medium	MS salts including vitamins	4.4
	Ascorbic acid	0.2
	Calcium nitrate	0.1

	Calcium panthotenate	0.002
	L-Arginine	0.1
	Putrescine-HCl	0.02
	Gibberellic acid	0.01

Sucrose	30
Phyto Agar	9
PPM (ml)	3

The pH of the medium was adjusted to 5.7. Tubes containing 5 ml of culture medium were sterilized in autoclave for 20 minutes at 120 °C.

After 4 weeks of *in vitro* cultivation sweet potato plants were evaluated for following parameters: plant height, leaf number, root number, and root length.

RESULTS AND DISCUSSIONS

Results on obtaining the sweet potato shoots in laboratory conditions

To obtain the shoots, the tubers were kept in laboratory conditions, providing a temperature of about 20-23 °C, adequate humidity and alternating periods of artificial lighting with periods of darkness. In these conditions, after 17 days of initiation, the tubers began to sprout.

Table 2. Dynamics of bud emergence depending on the variety and the method used

Initiation 28.01	FIRST METHOD				
Sprouting	Number of buds				
	Hayanmi	Yulmi	Juhwangmi	KSC1	KSP1
15.02	2	-	-	-	-
26.02	5	-	-	3	-
TOTAL	7	-	-	3	-
Initiation 28.01	SECOND METHOD				
Sprouting	Number of buds				
	Hayanmi	Yulmi	Juhwangmi	KSC1	KSP1
15.02	-	6	-	-	-
26.02	4	7	-	8	11
31.03	5	8	1	5	11
18.04	9	5	7	5	3
TOTAL	18	26	8	18	25

The results regarding the dynamics of bud emergence were different according to variety and method. For example, after one month from the initiation of the experiment, at the KSP1 and Yulmi varieties in the case of the first method the tubers did not sprout at all, while applying the second method we obtained numerous shoots, which had a fast growth rate. By applying the second method, a greater number of shoots were obtained in most of the studied varieties, and the shoots were more vigorous compared to the first method (Table 2).

After approximately 80 days from initiation, all the varieties studied showed viable shoots (second method). The highest number of shoots was recorded in the Yulmi variety (26), followed by KSP1 (25), Hayanmi and KSC1 with the same number of shoots (18), and in the last place was the Juhwangmi variety, which later sprouted and has the lowest number of shoots (8).

Results on the control of microbial contamination that occurred during in vitro cultivation of sweet potato

By using growth medium variants with a lower sucrose content and applying appropriate treatments for sterilizing the biological material, efficient control of microbial infections occurring during *in vitro* cultivation of sweet potato can be achieved. Observations were made every two days for culture contamination and general growth of plantlets for 4 weeks. Regarding the influence of sterilant treatment the lowest percentage of microbial contamination was obtained when 70% ethanol for 4 minutes and 1% sodium hypochlorite for 13 minutes was used (T2). The amount of sucrose in the growth medium also influenced the level of microbial contamination. Thus, the lowest percentage of contamination was obtained on the medium variant with an addition of 20 g/l sucrose, and the highest percentage of contamination was recorded on the medium variant with an addition of 40 g/l sucrose. The combined influence of sterilant treatment and concentration of sucrose in the medium (Table 3) determined the lowest percentage of infection (0%) when 70% ethanol for 4 minutes and 1% sodium hypochlorite for 13 minutes and concentration of 20 g/l sucrose was used.

Table 3. The combined influence of sterilant treatment and concentration of sucrose in the culture medium on the level of microbial contamination, 4 weeks after culture initiation

Sucrose content (g/l) / Sterilant treatments	20	30	40
T1 70% ethanol for 3 min. 1% NaClO for 10 min.	7.69	14.29	18.18
Diff.	-	6.6	10.49
Sign.	-	***	***
T2 70% ethanol for 4 min. 1% NaClO for 13 min.	0	4.55	9.52
Diff.	-	4.55	9.52
Sign.	-	***	***
T3 70% ethanol for 5 min. 1% NaClO for 16 min.	5.26	12	28
Diff.	-	6.74	22.74
Sign.	-	***	***

LSD 5% = 0.76

LSD 1% = 1.07

LSD 0.1% = 1.52

Beside the level of microbial contamination, the percentage of sweet potato plantlets regeneration was also determined. From the analysis of the combined influence of sterilant treatments and the sucrose concentration on the plant regeneration percentage it can be noted that by applying T2 treatment and

by using in the nutrient medium of a quantity of 40 g/l sucrose a higher percentage of regeneration is obtained (95.24%), followed by T3 sterilization treatment and using in the nutrient medium of a 20 g/l sucrose (94.74%).

An attempt was made also to stop the infections by use Rifampicin and PPMTM at different concentrations. After about 3 weeks, no new infections were observed on any of the three medium variants. After 22 days of cultivation on antibiotic-free medium, in the surviving explants, no new infections were observed in any of the varieties. Promising results regarding the decrease of the infection level in sweet potatoes cultivated *in vitro* were recorded when using a culture medium to which Rifampicin (1.2 g/l) and PPMTM (2.8 ml/l) were added.

In order to reduce the losses caused by microbial contamination, in addition to the methods applied under laboratory conditions, another method, under greenhouse conditions, was tested. This consisted in transplanting sweet potato plants, from contaminated cultures, in pots placed in greenhouse. Two types of substrate was used: only pearl and a mixture of pearl and peat (1:1). The survival rate of the plants was higher on the substrate consisting only of pearl (100%) compared to the substrate composed of pearl and peat (75%). The surviving plants had a fast growth rate, the greenhouse conditions being favorable for this crop. Approximately two months after transplantation, the obtained shoots could be used as a source of explants to initiate new *in vitro* cultures.

Table 4 presents the results obtained in greenhouse conditions regarding the number of shoots, shoots length and the number of buds/shoot, on sweet potato plants grown in pots. The average of the five varieties was considered the control variant. Regarding the number of shoots, there were no significant differences between the analyzed varieties. However, the highest number of shoots was obtained by the Yulmi variety, and the lowest was recorded in the KSC1 and KSP1 varieties. Regarding the length of the shoots, there were significant differences between the analyzed varieties. Thus, the best results were recorded by the KSC1 variety, and the lowest values were recorded by the Hayanmi variety (Table 4). Measurements on the number of buds/shoot were also made. In this case there were significant differences between varieties. The best results were obtained in Juhwangmi variety, and the lowest values were recorded in Yulmi variety.

Table 4. The influence of genotype on the number of shoots, shoots length and the number of buds/shoots on sweet potato plants grown under greenhouse conditions

Variety	Shoots number			Shoots length (cm)			Number of buds/shoots		
	Average number	Diff.	Sign.	Average length	Diff.	Sign.	Average number	Diff.	Sign.
Control (varieties avg.)	1.47	-	-	62.58	-	-	16.89	-	-
Yulmi	2.33	0.86	ns	61.09	-1.49	ns	10.61	-6.28	o
Hayanmi	1.33	-0.14	ns	32.98	-29.60	oo	22.33	5.44	ns
KSC1	1.00	-0.47	ns	98.30	35.72	**	13.67	-3.22	ns
KSP1	1.00	-0.47	ns	57.80	-4.78	ns	12.67	-4.22	ns
Juhwangmi	1.67	0.20	ns	62.75	0.17	ns	24.83	7.94	*
LSD values									
Shoots number			LSD 5%	0.59	Shoots length (cm)			LSD 5%	5.68
			LSD 1%	0.83				LSD 1%	7.98
			LSD 0.1%	1.18				LSD 0.1%	11.26

By applying this method, the process of *in vitro* sweet potato multiplication becomes more economically efficient. Plantlets from contaminated *in vitro* cultures, which would otherwise have been eliminated, are used to obtain new sweet potato plants. After only a few weeks of cultivation under greenhouse conditions, involving minimal costs, many shoots can be obtained which, after being removed from the mother plant, are sterilized and reintroduced in the micropropagation process as uninodal cuttings. Mother plants can remain in pots, generating new shoots, until we get the desired number of explants.

Results on the optimization of growth medium composition to ensure bouth plants development and cost efficiency

In order to optimize the *in vitro* conditions for sweet potato cultivation, different growth medium variants has tested. After 4 weeks of *in vitro* sweet potato cultivation growth parameters, expressed as the plant height, leaves number, roots number and root length, were recorded (Table 5).

Table 5. Effect of the nutrient medium on *in vitro* growth of different sweet potato varieties
(4 weeks after initiation)

Medium variants	Plant height (cm)					Means
	Juhwangmi	KSC1	KSP1	Yulmi	Hayanmi	
MSo (control)	2.52	1.97	1.62	2.15	1.30°	2.21
MS + GA ₃ + NAA	2.67	2.78	2.80	1.52°	2.02	
SPPM	2.80	3.78	2.15	1.45°°	1.67	
LSD 5% = 0.44 cm; LSD 1% = 0.58 cm; LSD 0.1% = 0.75 cm						
	Leaves number					Means
	Juhwangmi	KSC1	KSP1	Yulmi	Hayanmi	
MSo (control)	4.17	6.17	4.00	6.17	6.33	5.66
MS + GA ₃ + NAA	4.83	4.50	6.17	5.50	6.00	
SPPM	5.17	6.33	6.17	6.33	7.00*	
LSD 5% = 0.58; LSD 1% = 0.76; LSD 0.1% = 0.99						
	Roots number					Means
	Juhwangmi	KSC1	KSP1	Yulmi	Hayanmi	
MSo (control)	1.83	2.83	2.33	5.67*	1.83	2.70
MS + GA ₃ + NAA	1.33°	1.67°	3.17	3.50	2.50	
SPPM	1.83	3.33	3.00	2.17	3.50	
LSD 5% = 0.53; LSD 1% = 0.70; LSD 0.1% = 0.91						
	Root length (cm)					Means
	Juhwangmi	KSC1	KSP1	Yulmi	Hayanmi	
MSo (control)	4.87	7.92	4.82	8.72	7.93	7.24
MS + GA ₃ + NAA	8.25	5.48	7.57	5.32	6.28	
SPPM	7.50	9.83*	8.58	9.27	6.22	
LSD 5% = 0.97 cm; LSD 1% = 1.28 cm; LSD 0.1% = 1.66 cm						

Sweet potato has a very high genetic variability in its germplasm. Therefore, the differences in tissue culture response across the cultivars might be due to genotypic effect. The largest expense in preparing the growth medium refers to hormone acquisition. The use of a hormone-free culture medium while allowing good plant growth is desirable in plant tissue culture technology.

CONCLUSIONS

The results obtained in this study highlight the possibility of identifying methods that will allow to solve certain problems encountered during the process of *in vitro* multiplication of sweet potato.

Regarding the provision of a suitable explants source for the initiation of *in vitro* cultures, which will be available throughout the year, the results of this study have shown that obtaining sweet potato shoots under laboratory conditions is an efficient, fast and low-cost method. Comparing the two methods applied, the best results were obtained by planting sweet potato tubers on peat, pearl and sand substrate. In this way, a higher number of vigorous shoots were obtained.

For the control of microbial contamination that occurred during *in vitro* cultivation of sweet potato, three methods were tested, two of them in laboratory conditions and one in greenhouse. By applying a suitable sterilization treatment and using a lower concentration of sucrose in the culture medium, it is possible to reduce the level of microbial contamination in the sweet potato *in vitro* initiated cultures. Based on the results obtained in this study, T2 sterilization treatment (70% ethanol for 4 minutes followed by 1% NaClO for 13 minutes) combined with the addition of 20 g/l and 30 g/l sucrose respectively in the nutrient medium, is recommended to reduce the level of microbial contamination in sweet potato initiated *in vitro* cultures.

Regarding the possibility of using antibiotics in the culture medium, promising results regarding the decrease of the infection level in the sweet potato varieties grown *in vitro*, in this experimental study, were recorded in the case of using a culture medium where Rifampicin (1.2 g/l) and PPMTM (2.8 ml/l) were added.

In order to use sweet potato plantlets from contaminated *in vitro* cultures (which would otherwise have been eliminated), they can be transplanted into the greenhouse, using pots with different substrates. About two months after the transplant, shoots are obtained that can be detached from the mother plant and used as a source of explants. By applying this method, numerous shoots used to initiate new *in vitro* cultures are obtained in a short time and with minimal costs. Only one plantlets was planted in each pot, but three plantlets/pot can be planted. Thus, the number of shoots and respectively the number of buds/shoots that can be obtained is higher, and the process becomes more efficient.

To optimize the *in vitro* conditions for sweet potato cultivation, different growth medium variants has tested. Considering the results presented in this study, there are no significant differences regarding measured parameters, depending on the type of growing medium used. Thus, the five sweet potato varieties can be multiplied *in vitro* using the MS basal medium, the use of complex and implicitly more expensive growth medium, not justifiable. In this way, the costs for the preparation of the nutrient medium can be considerably reduced and the micropropagation process is thus more economically efficient.

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REFERENCES

1. Vettorazzi R.G., Carvalho Virginia Silva, Sudré Cláudia Pombo, Rodrigues Rosana, 2017. Developing an in vitro optimized protocol to sweet potato landraces conservation. *Acta Scientiarum. Agronomy*, Vol. 39, nr. 3, p. 359-367.
2. Torres K.C., 1989. Sweet Potato Tissue Culture. In: *Tissue Culture Techniques for Horticultural Crops*. Springer, Boston, MA
3. Alconero R.A., Santiago G., Morales F., Rodriguez F., 1975. Meristem tip culture and virus indexing of sweet potatoes. *Phytopathology* 65:769–773.
4. Litz R.E., Conover R.A., 1978. In vitro propagation of sweet potato. *HortScience* 13:659–660.
5. Liu J.R., Cantliffe D.J., 1983. Somatic embryogenesis and plant regeneration in tissue culture of sweet potato (*Ipomoea batatas*). *HortScience* 18:618.
6. Liu J.R., Cantliffe D.J., 1984. Improved efficiency of somatic embryogenesis and plant regeneration in tissue cultures of sweet potato (*Ipomoea batatas*). *HortScience* 19:588.
7. Abubakar A.S., Yahaya S.U., Shaibu A.S., Ibrahim H., Ibrahim A.K., Lawan Z.M., Isa A.M., 2018. In vitro propagation of sweet potato (*Ipomoea batatas* (L.) Lam.) cultivars. *Agricultural Science Digest*, 38(1) 2018: 17-21.
8. Aloufa M.A., 2002. Some factors affecting the callus induction and shoot formation in two cultivars of Sweet potato (*Ipomoea batatas* L). *Cienciae Agrotecnologia*, 26: 964–969.
9. El-Afifi S.T., Zaghloul M.M., El Saady W.A., Mosaad F.S., 2012. Using tissue culture technique in micropropagation of sweet potato (*Ipomoea batatas*). *Journal of Plant Production, Mansoura Univ.*, 3(7): 2201 – 2209.
10. Doliński R., Olek O., 2013. Micropropagation of sweet potato (*Ipomoea batatas* (L.) Lam.) from node explants. *Acta Sci Pol., Hortorum Cultus* 12(4): 117-127.
11. Shaibu A.S., Abubakar A.S., Lawan Z.M., Ibrahim A.K., Rabi H.M., Muhammad A.I., et al., 2016. Media Optimization and Effect of Surface Sterilization Timing on In Vitro Propagation of Sweet Potato. *Proceedings of the 2nd International Conference on Drylands*.
12. Thorpe T.A., 1994. Morphogenesis and regeneration. In *Plant Cell and Tissue Culture*. [Thorpe TA, Vasil, IK (eds)], Kluwer Academic Publishers. Netherlands, Dordrecht 7-36.
13. Barker I., Andrade M., Labarta R., Mwanga R., Kapinga R., Fuentes S., Low, J., 2011. Sustainable seed systems. *International Potato Center (CIP)*.
14. Bradbury J.F., 1970. Isolation and preliminary study of bacteria from plants. *Rev. Plant Pathol.* 49: 213-218.
15. Bradbury J.F., 1988. Identification of cultivable bacteria from plants and plant tissue cultures by use of simple classical methods. *Acta Horticulture* 225: 27-37.
16. Ram C.J., Kailash C.S., 2011. Endogenous microbial contamination during in vitro culture of sweet potato [*Ipomoea batatas* (L.) Lam]: identification and prevention. *Journal of Agricultural Technology*, 2011, Vol. 7(6): 1725-1731.
17. Murashige T., Skoog F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant* 15, p. 473-479.