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Micropropagation and Assessment of Genetic Stability of *Musa sp.* cv. Williams Using RAPD and SRAP Markers

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THE PRESENT study was conducted to investigate the effect of different concentrations of plant growth regulators on a commercial scale and true-to-type micropropagation of *Musa sp.,* cv. Williams. In addition, assessment of the genetic stability of micropropagated plants using RAPD and SRAP markers. Murashige and Skoog's (MS) medium supplemented with BAP cytokinin and NAA auxin (3.0+0.2mg/l) was found to be the most suitable combination. It gave the highest shoot number per explant; 7.6, 8.4 and 11.2 after 10, 20 and 30 days, respectively. The longest shoots; 4.2, 5.4 and 6cm were obtained on the same media after 10, 20 and 30 days sculturing, respectively. The highest number of well-developed roots (10.4 roots per shoot) was scored for rooting media supplemented with 3mg/l IAA after 20 days culturing. Rooted plantlets were then transferred to pots and grown in the greenhouse followed by successful transfer to the soil. After the seventh sub-culture, the clonal fidelity among the micropropagated plantlets was assessed by RAPD and SRAP markers. Ten RAPD primers generated 38 bands, while four SRAP primers amplified 16 bands. All generated bands were monomorphic among the micropropagated plants compared to mother plant; except primers me1+em2 combination generated only one polymorphic band. *In vitro* micropropagation protocol reported herein could be served as a commercial method for large scale production of disease-free and genetically stable banana.

Keywords: Banana, Growth regulators, Micropropagation, RAPD and SRAP.

Introduction

The banana (*Musa* spp.) belonging to the family Musaceae are one of the world's most important crops. It ranks as the fourth major crop in tropical and subtropical countries and widely grown fruit in the world (Rahman et al., 2013). Most of bananas varieties are polyploids, thus have a complex genetic structure (Ortiz & Swennen, 2014). Bananas are considered as a source of energy and have lots of health benefits. Banana plants are usually propagated by suckers (Robinson & De Villiers**,** 2007**)**. The main negative impact of this method is slow rate of multiplication and transmission of diseases (Hussein, 2012). The traditional clonal or vegetative propagation method appears to be unable to supply the increasing demand for disease free and healthy planting materials of banana. To reduce the aforementioned problems, *in vitro* micropropagation or tissue culture method could be alternative method for banana production. It had been reported by several studies using different explants (Strosse et al., 2006; Resmi & Nair, 2007; Shirani et al. 2009; Ferdous et al. 2015 and Kishor et. al., 2017). Furthermore, meristem culture could be an efficient protocol for virus free banana production and rapid propagation. Tissue culture derived plantlets of banana performed better than the conventional method (Faisal et al., 1998).

Plant growth regulators (PGRs) are inevitable for *in vitro* regeneration of crop plants in any artificial medium. Generally, cytokinin helps in shoot proliferation and auxins helps in rooting of proliferated shoots. However, the requirement of cytokinin and auxins depends on the variety of banana and culture conditions. Cytokinins such as benzyl aminopurine (BAP) is generally known to induce shoot formation from meristematic explants in banana (Abeyaratne & Lathiff, 2002; Madhulatha et al., 2004; Farahani et al., 2008; Buah et al., 2010 and Ferdous et al., 2015). The multiplication rate of adventitious buds under the influence of BAP is one of the responsible factors deciding on

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the efficiency of the micropropagation system. Moreover, combination of BAP and auxins such as IAA, NAA and ABA were used in Banana *in vitro* micropropagation (Keshari & Pradhan, 2016). The most commonly used cytokinin is BAP at a range of 2-5 mgl^{-1} in combination with an auxin at concentration of 0.1-0.2 mgl^{-1} (Reddy et al., 2014).

Molecular markers had become a powerful tool in fingerprinting, genetic analysis and germplasm characterization (Saad-Allah & Youssef, 2018). Additionally, they could be used for quality control in plant tissue culture at early stage of development. Random amplified polymorphic DNA (RAPD) analysis using short arbitrary primers has been established to detect variations among individuals. RAPD technique has been usually used to assess the genetic stability of banana (Maria & Garcia, 2000 and Chaudhary et al., 2015). Li & Quiros (2001) termed a new simple and efficient marker targets open reading frames (ORFs), called, sequence related amplified polymorphism (SRAP)**,** it is adapted for a variety of purposes in different crops, and allows easy isolation of bands for sequencing. So fare, SRAP markers had been used to determine genetic diversity in some crops but this is first report to be used for banana. Based on DNA level some studies had reported somaclonal variations in *Musa* using microsatellites (Buhariwalla et al., 2005). RAPD analysis used to evaluate genetic stability of several micropropagated crops (Farahani et al., 2011 and Goyal et al., 2015). Two important factors were found to affect genetic stability in micropropagation prosses; the subculture number and subculture interval (Etienne & Bertrand, 2003 and Bairu et al., 2006).

The present study was undertaken to establish an efficient micropropagation protocol through direct organogenesis from shoot tips of *Musa* sp.cv. Williams. In addition, to confirm genetic uniformity of micropropagated plants using RAPD and SRAP.

Materials and Methods

Culture media and explant preparation

The present study was conducted at the Genetic Engineering and Tissue Culture Lab. (GETCL), Department of Genetics, Faculty of Agriculture, Kafrelsheikh University, Egypt during 2016-2017. The experiment was carried out using different PGRs combinations supplemented to MS medium (Murashige & Skoog, 1962) on micropropagation and maintenance of *Musa* sp., cv. Williams from El-Mohandseen Laboratory for plant tissue culture, Cairo, Egypt.

Different BAP concentrations (1, 2, 3 and 4mg/l) combined with 0.2mg/l NAA were supplemented to MS medium. The effects of the above combinations on shoot organogenesis were evaluated. The culture media was solidified by adding 8g/l plant agar. Sucrose (30g) was added as a carbon source. The pH of the media was adjusted to 5.8 before autoclaving at 1.5 kg/cm^2 pressure and 121°C for 20 min. The medium was then cooled and kept for three days at room temperature before use. The shoot apex explants were prepared and surface sterilized exactly as described by Ray et. al. (2006).

Shoot induction and multiplication

The apical meristems were then inoculated to each of the culture jar containing 25ml of MS medium supplemented with different concentrations of PGRs as mentioned above. Jars were transferred to incubation chamber and allowed to grow under controlled conditions. The temperature of the growth room was maintained within 25±1°C by an air conditioner. A 16-hour light period was maintained with light intensity of 3000 lux for the growth and development of culture. Initial sub-culturing was done when adventitious shoot buds developed from the explant. Adventitious shoot buds were isolated individually for sub-culturing. Leaf and blackish or brownish basal tissues were removed to expose shoot meristems to induction medium. The sub-culturing was done monthly for seven times.

Rooting and acclimatization

MS medium supplemented with different concentrations (1, 2, 3 and 4mg/l) of indole acetic acid (IAA) were used for root induction. Well-developed shoots (3-5cm in length and 2-3 leaves) were rescued aseptically and cultured on freshly prepared rooting media. Root number per individual shoot was recorded after 10, 20 and 30 days of culture.

Well-rooted plantlets were taken out of the culture jars carefully without damage of the root system. The roots were washed gently under running tap water to remove the medium. Finally, plantlets were transferred to pots containing peat moss and clay soil with a ratio of 1:1 (v/v). All plantlets were hardened in a greenhouse for 30

days with spray irrigation twice a day.

Data collection and analysis

The experiments were set up in completely randomized design with five replicates. Number of shoots per explant, shoots length (cm), number of leaves per explants and number of roots per shoot were recorded after 10, 20 and 30 days of culturing. The significance of differences among means was carried out by Duncan's multiple range tests at $p \leq 0.05$. The results expressed as mean ± SE of five replicate per each treatment and subjected to one-way analysis of variance (ANOVA) using SPSS v.17 (SPSS, Chicago, USA).

Genetic stability assessment

Total genomic DNA was isolated from fresh leaves of seven micropropagated plantlets and the mother plant according to the procedure of Doyle & Doyle (1990). Purified total DNA was quantified and its quality verified by Nanodrop ND-100 P330 Spectrophotometer (IMPLN) Germany. Ten RAPD and four SRAP primers (Table 1) were used for assessment of genetic stability of the micropropagated plantlets after the seventh sub-culture. Polymerase chain reaction (PCR) reaction mixture consisted of 50ng template DNA, 1 x PCR buffer (10mM Tris-HCL pH 8.8, 250mM KCl), 200µM dNTPs, 0.80µM 10-base random primers and 1 unit of *Taq* polymerase, in a total volume of 25µl. For RAPD analysis, PCR was performed as follow; DNA was initially denatured at 94˚C for 4 min, followed by 35 cycles of denaturation for 1 min at 94˚C, primer annealing for 1 min at 32˚C and primer extension for 2 min at 72˚C and finally terminated with an extension of 7 min at 72˚C.

The protocol for SRAP analysis started with initial denaturation at 94°C for 4 min, follow by five cycles of denaturation at 94°C for 1 min, annealing at 35°C for 1 min, and elongation at 72°C for 30 sec, and finally 30 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1 min, and elongation at 72°C for 30 sec, ending with an elongation step for 10 min, at 72°C. The PCR amplified products were mixed with DNA gel loading dye and separated by electrophoresis on a 1.2% (w/v) agarose gels using 1 X TAE buffer. DNA ladder (1kb) was used to confirm the appropriate separation. PCR reactions were repeated at least twice to establish reproducibility of the amplified products. The gels were stained with Red safe and visualized under UV light.

Results and Discussion

The effect of different concentrations of BAP cytokinin combined with 0.2mg/l of NAA auxin on shootmultiplication was summarized in Table 2 and illustrated in Fig. 1. The data showed that, the most efficient PGRs combination for shoot regeneration was 3mg/l BAP + 0.2mg/l NAA. It prompted the highest mean of shoots per explant; 7.6, 8.4 and 11.2 shoots per explant at 10, 20 and 30 days of culturing, respectively. Also, it induced the highest shoot lengths; 4. 2, 5.4 and 6cm after 10, 20 and 30 days, respectively. Consistently, the maximum No., of leaves 4, 10 and 14 leaves/explant after 10, 20 and 30 days, respectively were recorded for the same treatment. So, this medium was selected for further sub-culturing in the present study. For rooting, MS media supplemented with four conc. of IAA (1,2,3 and 4mg/l) were tested. Strong root system with the highest root number per shoot across the studied periods was scored for 3mg/l IAA as presented in Fig. 2. The micropropagated plants were transferred to pots with no morphological abnormalities during hardening period (Fig. 3 c-e).

Several studies suggested cytokinins and auxins combination for successful shoot induction and proliferation in banana (Nauyen & Kozai, 2001; Kagera et al., 2004 and Gebeyehu, 2015). In our study, we providea very simple efficient protocol for commercial banana micropropagation using MS medium supplemented with 3mg/l BAP and 0.2mg/l NAA, followed by 3mg/l IAA for rooting, which induced a vigorous root system. Similarly, previous reports have declared the necessity of using auxins for better rooting (Madhulatha et al., 2006 and Safarpour et al., 2017). According to Gebeyehu (2015) banana plantlets regenerated through tissue culture have higher survival rate and reduce the cost of disease management.

To confirm the genetic stability of micropropagated plantlets, comparison of RAPD and SRAP fingerprinting of seven randomly selected *in vitro* raised plants with their corresponding mother plant were carried out. Ten decamer RAPD primers produced clear and reproducible amplification products. A total of 38 monomorphic bands were scored (Table 1 and Fig 4). SRAP analysis revealeda total of 16 fragments with 6. 25% polymorphism (Table 1). All scored bands were monomorphic excepta band detectedonly in the mother plant using me1+em2 primers combination. A successful micropropagation

protocol should give true-to-type plantlets with no genetic or morphological alteration (Sudipta et al., 2014; Prakash et al., 2016 and Safarpour et al., 2017). Therefore, testing of genetic stability of *in vitro* raised plants is necessary to the banana commercial production. Molecular analysis is an efficient and reliable techniques for screening true to types nature of tissue culture-derived plants (Damasco et al., 1996). In the present study, about 6.25% polymorphism was detected by SRAP analysis among the micropropagated plants. However, RAPD generate monomorphic bands with no polymorphism. The low polymorphism % detected by SRAP may possibly due to repeated subculturing up to seven as reported in earlier studies (Nwauzoma & Jaja, 2013 and Safarpour et al., 2017). The rate of genetic variation induced in the current study is seemingly less than previously reported for banana shoot meristem cultures of 0–70% polymorphism (Smith, 1988 and Vuylsteke et al., 1991). The low genetic variation reported herein might be due duration of subculture cycles and long-term storage.

RAPD technique has been successfully employed by many authors to confirm genetic uniformity and to identify somaclonal variants among tissue culture raised banana plants (Bairu et al., 2011). Somaclonal variation is often induced by the composition of the culture media and subculture cycles. About 5.3% polymorphism was detected by RAPD analysis of micropropagated banana plantlets regenerated using high levels of BAP and Thidiazuron (TDZ) in comparison to plantlets produced under lower concentrations or control conditions (Bidabadi et al., 2010). However, in the present study no genetic variability was revealed by RAPD analysis indicating the suitability of the current protocol to produce true-to-type banana plants. This finding was agreed with those previously stated by Howell et al. (1994), shown no variation between mother and micropropagated plants of *Musa* sp. The present study provides an efficient rapid micropropagation protocol which could be used for preserving *Musa* sp. cv. Williams without risk of genetic instability.

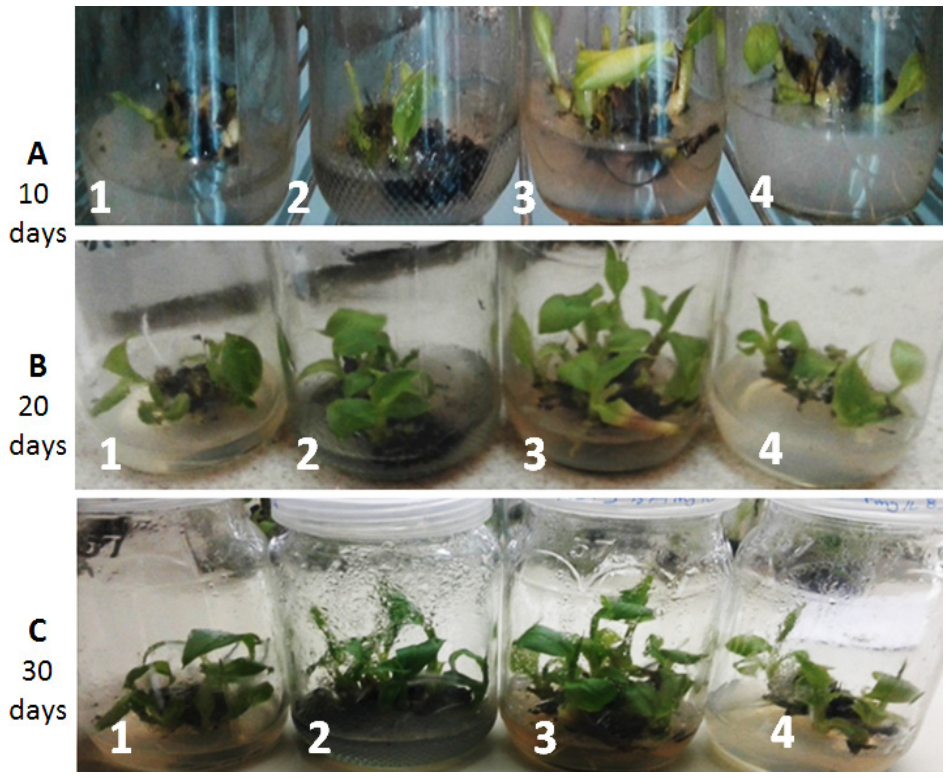

Fig. 1. Initiation of multiple shoot formation after 10, 20 and 30 days of culture on MS medium supplemented with 6-Benzyl amino purine (BAP) and Naphthalene acetic acid (NAA).
(1) 1mg\l BAP +0.2mg/l NAA; (2) 2mg\l BAP +0.2mg/l NAA; (3) 3mg\l BAP +0.2mg/l NAA and (4) 4mg\l BAP +0.2mg/l NAA.

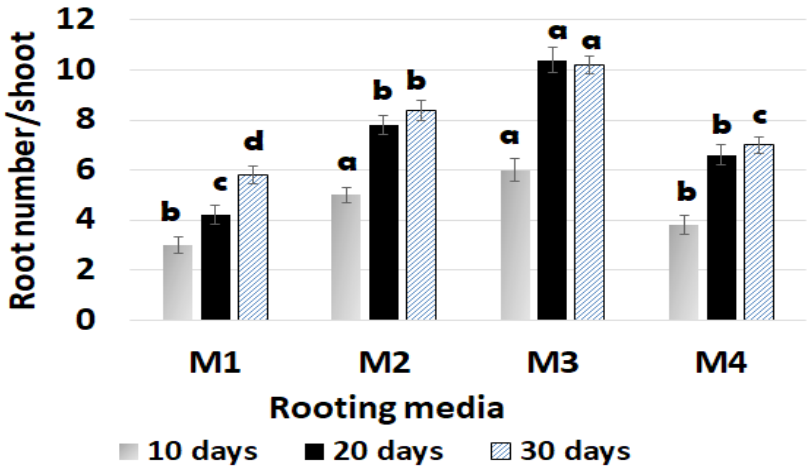

Fig. 2. Effect of different concentrations of Indole acetic acid (IAA) on root induction in banana 'Williams'. M1:1mg/l IAA, M2: 2 mg/l IAA, M3: 3 mg/l IAA and M4: 4mg/l IAA.
Means followed by the same letter in the same column are not significantly different based on Duncan's multiple range tests at $p \leq 0.05$. Bars represent the standard error.

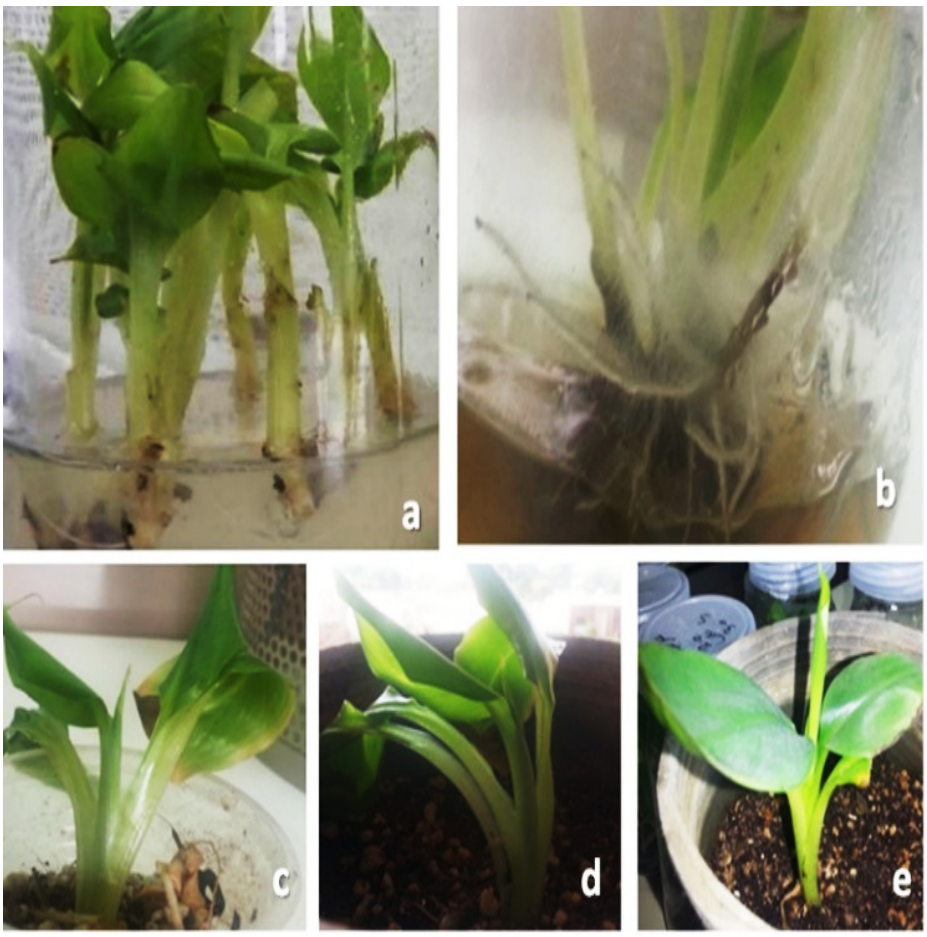

Fig. 3. Different stages of regeneration and hardening of micropropagated *Musa* sp.'Williams', a, shoot multiplication; b, rooting and c- e, hardening.

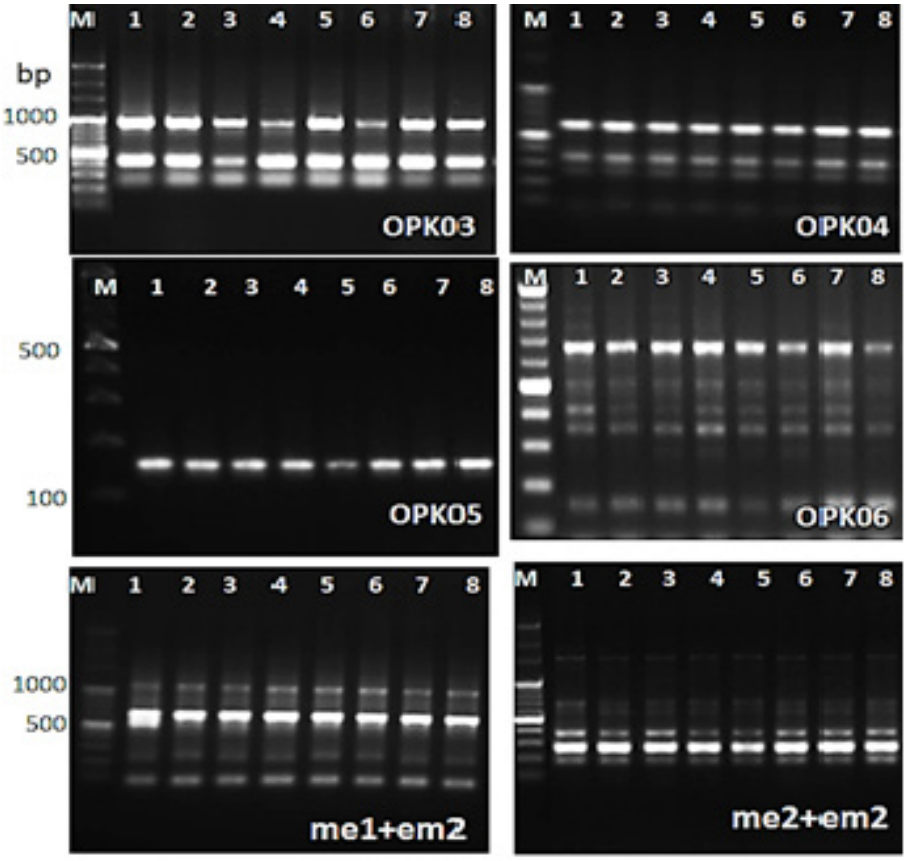

Fig. 4. Representative RAPD and SRAP profiles of micropropagated plantlets obtained from shoot apex culture of banana 'Williams'. 1, mother plant; 2-8 *In vitro* raised plants; M, 1kb DNA ladder.

TABLE 1. RAPD and SRAP primers sequences and number of amplified fragments.

RAPD 5′--3′					
Primer code	**Primer sequences**	**No. of amplified fragments**	**Primer code**	**Primer sequences**	**No. of amplified fragments**
OPK-01	CATTCGAGCC	4	OPK-06	CACCTTTCCC	5
OPK-02	GTCTCCGCAA	5	OPK-07	AGCGAGCAAG	4
OPK-03	CCAGCTTAGG	3	OPK-08	GAACACTGGG	3
OPK-04	CCGCCCAAAC	4	OPK-09	CCCTACCGAC	5
OPK-05	TCTGTCGAGG	1	OPK-10	GTGCAACGTG	4

SRAP 5′--3′			
	Forward	**Reverse**	**No. of amplified fragments**
me 1+em 2	TGAGTCCAAACCGGATA	GACTGCGTACGAATTTGC	4
me 1+em 3	TGAGTCCAAACCGGATA	GACTGCGTACGAATTGAC	4
me 2+em 2	TGAGTCCAAACCGGAGC	GACTGCGTACGAATTTGC	5
me 2+em 3	TGAGTCCAAACCGGAGC	GACTGCGTACGAATTGAC	3

TABLE 2. Effect of different BAP and NAA combinations on shoot length, number of shoots, and leaves after 10, 20 and 30 days culture.

Media	Cultivation period								
	10 days			20 days			30 days		
	Shoot length (cm)	No of shoots/ explant	No. of leaves/ explant	Shoot length (cm)	No of shoots/ explant	No. of leaves/ explant	Shoot length (cm)	No of shoots/ explant	No. of leaves/ explant
M1	3.6 ± 0.4b	3.4± 0.2c	2.2± 0.2b	4.4± 0.2b	4.6± 0.2c	6.8± 0.4c	4.8± 0.4c	7.4± 0.2b	8.4± 0.4c
M2	4.2 ± 0.4	5.0± 0.3b	3. 0± 0.3b	5.4± 0.2a	6.4± 0.4b	8.2± 0.4b	6.0± 0.3ab	8.2± 0.4b	12.0± 0.4b
M3	5.8 ± 0.a4	7.6± 0.2a	4.0± 0.3a	6.0± 0.3a	8.4± 0.4a	10.0± 0.3a	6.8± 0.4a	11.2± 0.4a	14.2± 0.4a
M4	3.4± 0.2b	4.0± 0.3c	2.4± 0.2b	4.2± 0.4b	5.4± 0.4bc	7.6± 0.4bc	5.2± 0.2bc	7.8± 0.4b	9.4± 0.5c

Data represents mean ± SE of five replicates/treatment. ANOVA tested by the Duncans range test at significance level ($p \leq 0.05$). M1,1mg\l BAP +0.2 mg/l NAA; M2, 2mg\l BAP +0.2 mg/l NAA; M3, 3mg\l BAP +0.2 mg/l NAA and M4, 4mg\l BAP +0.2 mg/l NAA.

Conclusion

This study provided an easy and fast method for *in vitro* micropropagation of *Musa* sp. cv. Williams. Low genetic variability was detected by SRAP analysis among mother and the *in vitro*-raised plants. On the other hand, no genetic variation was noticed by the ten tested RAPD primers. *In vitro* micropropagation protocol developed in this study can serve as a commercial method for large scale production of disease-free and genetically stable banana.

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References

Abeyaratne, W.M. and Lathiff, M.A. (2002) *In-vitro* propagation of 'Rathambala' (*Musa* AAA) and the occurrence of phenotypic variations in the pseudostem. *Annals of the Sri Lanka Department of Agriculture,* **4,** 191-197.

Bairu, M.W, Aremu, A.O. and Van Staden, J. (2011) Somaclonal variation in plants: Causes and detection methods. *Plant Growth Regulation,* **63**,147–173.

Bairu, M.W., Fennell, C.W. and Van Staden, J. (2006) The effect of plant growth regulators on somaclonal variation in Cavendish banana (*Musa* AAA cv. Zelig'). *Scientia Horticulturae,* **108,** 347–351.

Bidabadi, S.S., Meon, S., Wahab, Z. and Mahmood, M. (2010) Study of genetic and phenotypic variability among somaclones induced by BAP and TDZ in micropropagated shoot tips of banana (*Musa* spp.) using RAPD markers. *Jornal of Agricutural Scinces,* **2**, 49–60.

Buah, J.N., Danso, E., Taah, K.J., Abole, E.A., Bediako, E.A., Asiedu, J. and Baidoo, R. (2010) The effects of different concentration cytokinins on the in vitro multiplication of plantain (Musa sp.). *Biotechnology* **9**(3), 343–347.

Buhariwalla, H.K., Jarret, R.L., Jayashree, B., Crouch, J.H. and Ortiz, R. (2005) Isolation and characterization of microsatellite markers from *Musa balbisiana*. *Molecular Ecology Resources,* **5**, 327–330.

Chaudhary, D.S., Kajla, A., Poonia, B., Brar, S., and Duhan, J.S. (2015) Molecular assessment of genetic stability using ISSR and RAPD markers in commercial banana cultivar cv. Robusta. *Indian Journal of Biotechnology,* **14**(3), 420–424.

Damasco, O.P, Graham, G.C, Henry, R.J, Adkins, S.W., Smiths, M.K. and Godwin, I.D. (1996) Random amplified polymorphic DNA (RAPD) detection of dwarf off-types in micropropagated Cavendish (*Musa* spp. AAA) bananas. *Plant Cell Reports,* **16** (1-2), 118–123.

Doyle, J.J. and Doyle, J.L. (1990) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Focus,* **12**, 13–15.

Etienne, H. and Bertrand, B. (2003) Somaclonal variation in *Coffea arabica*: Effects of genotype and embryogenic cell suspension age on frequency and phenotype of variants. *Tree Physiology,* **23**, 419–426.

Faisal, S.M., Hoque, M.A. and Quasem, A. (1998) Field performance of *in vitro* plantlets against normal suckers of banana (*Musa sapientum*) cv. Champa. *Plant Tissue Cult*ure, **8**(2), 125–129.

Farahani, F., Aminpoor, H., Sheidai, M., Noormohammadi, Z. and Mazinan,i M.H. (2008) An improved system for *in vitro* propagation of banana (*Musa acuminate* L.) cultivars. *Asian Journal of Plant Scince,* **7**(1),116–118.

Farahani, F., Yari, R. and Masoud, S. (2011) Somaclonal variation in Dezful cultivar of olive (*Oleaeuropaea subsp*. europaea). *Gene Conserve,* **10**, 216–221.

Ferdous, M.H., Billah, A.A.M., Mehraj, H., Taufique, T. and Uddin, A.F.M. (2015) BAP and IBA pulsing for *in vitro* multiplication of banana cultivars through shoot-tip culture. *Journal of Bioscience and Agriculture Research,* **3**(2), 87–95.

Gebeyehu, A. (2015) Effects of different concentrations of BAP (6-Benzyl amino purine) and NAA (Naphthalene acetic acid) on Banana (*Musa* spp.) cv. giant Cavendish shoot proliferation. *International Journal of Plant Science and Ecology,* **1**(2), 36–43.

Goyal, A.K., Pradhan, S., Basistha, C.B. and Sen, A. (2015) Micropropagation and assessment of genetic fidelity of *Dendrocalamus strictus* (Roxb.) nees using RAPD and ISSR markers. *3 Biotech,* **5**, 473–482.

Howell, E.C., Newbury, H.J., Swennen, R.L., Withers, L.A. and Ford-Lloyd, B.V. (1994) The use of RAPD for identifying and classifying *Musa* germplasm. *Genome,* **37**, 328–22.

Hussein, N. (2012) Effects of nutrient media constituents on growth and development of banana (*Musa* spp.) shoot tips cultured *in vitro*. *African Journal of Biotechnology,* **11**, 9001–9006.

Kagera, A.G., Kagera, G.R., Kegera, C.B.M., Van den Houwe, I. and Swennen, R. (2004) Rapid mass propagation and diffusion of new banana verities among small scale farmers in north western Tazania. *African Crop Science Journal,* **12**(1), 7–17.

Keshari, B. and Pradhan, B. (2016) Effects of cytokinins and auxins on micropropagation of *Musa* spp. cv. Yangambi. *International Journal of Environmental and Agriculture Research,* **2**(5),156–158.

Kishor, H., Abhijith, Y.C. and Manjunatha, N. (2017) Micropropagation of native cultivars of Banana- A Critical Review. *International Journal of Pure and Applied Bioscience* **5**(5), 1559–1564. doi: http://dx.doi.org/10.18782/2320-7051.5209.

Li, G. and Quiros, C.F. (2001) Sequence-related amplified polymorphism (SRAP) a new marker system based on a simple PCR reaction, its application to mapping and gene tagging in *Brassica. Theoretical and Applied Genetics,* **103**, 455–461.

Madhulatha, P., Anbalagan, M., Jayachandran, S. and Sakthivel, N. (2004). Influence of liquid pulse treatment with growth regulators on *in vitro* propagation of banana (*Musa* spp. AAA). *Plant Cell, Tissue and Organ Culture,* **76**,189–192.

Madhulatha, P., Kirubakaran, S.I. and Sakthivel, N. (2006) Effects of carbon sources and auxins on *in vitro* propagation of banana. *Biologia Plantarum,* **50**, 782–784.

Maria, D.C.V. and Garcia, E.D. (2000) Analysis of a *Musa* spp. somaclonal variant resistant to yellow sigatoka. *Plant Molecular Biology Reporter,* **18**, 23–31.

Murashige, T. and Skoog, F. (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures *Physiologia Plantarum,* **15**, 473–497.

Nauyen, Q.T. and Kozai, T. (2001) Growth of *in vitro* banana (*Musa* sp.). shoots under photomixotrophic and photoautotrophic conditions. *In vitro Cellular and Developmental Biology,* **37**, 824–829.

Nwauzoma, A.B. and Jaja, E.T. (2013) A review of somaclonal variation in plantain (*Musa* spp): Mechanisms and applications. *Journal of Applied Biosciences,* **67**, 5252–5260.

Ortiz, R. and Swennen, R. (2014) From cross breeding to biotechnology-facilitated improvement of

banana and plantain. *Biotechnology Advances,* **32**(1), 158–169.

Prakash, L., Middha, S.K., Mohanty, S.K. and Swamy, M.K. (2016) Micropropagation and validation of genetic and biochemical fidelity among regenerants of *Nothapodytes nimmoniana* (Graham) Mabb. employing ISSR markers and HPLC. *3 Biotech.* **6**, 1-9.

Rahman, S., Biswas, N., Hassan, M.M., Ahmed, M.G., Mamun, A.N.K., Islam, M.R., Moniruzzaman, M. and Haque, M.E. (2013) Micro propagation of banana (*Musa* sp.) cv. Agnishwar by *in vitro* shoot tip culture. *International J. Biotechnology,* **4**(4), 83–88.

Ray, T., Dutta, I., Saha, P., Das, S. and Roy, S. (2006) Genetic stability of three economically important micropropagated banana (*Musa* spp.) cultivars of lower Indo-Gangetic plains, as assessed by RAPD and ISSR markers. *Plant Cell, Tissue and Organ Culture,* **85**, 11–21.

Reddy, D.R., Suvarna1, D. and Muralidhra, D. (2014) Effects of 6-benzyl amino purine (6-bap) on *in vitro* shoot multiplication of grand naine (*Musa* sp.). *International Journal of Advanced Biotechnology and Research,* **5**, 36–42.

Resmi, L. and Nair, A.S. (2007) Plantlet production from the male inflorescence tips of *Musa acuminata* cultivars from South India .*Plant Cell, Tissue and Organ Culture,* **88**, 333–338.

Robinson, J.C. and De Villiers, E.A. (2007) The cultivation of banana. ARC-Institute for Tropical and Subtropical Crops, Nelspruit, South Africa/Du Roi Laboratory, Letsitele, South Africa.

Saad-Allah, K.M. and Youssef, M.S. (2018) Phytochemical and genetic characterization of five quinoa (*Chenopodium quinoa* Willd.) genotypes introduced to Egypt. *Physiology and Molecular Biology of Plants*. https://doi.org/10.1007/s12298-018-0541-4

Safarpour, M., Sinniah, U.R., Subramaniam, S. and Swamy, M.K. (2017) A novel technique for *Musa acuminata* Colla 'Grand Naine'(AAA) micropropagation through transverse sectioning of the shoot apex. *In vitro Cellular & Developmental Biology-Plant,* **53**(3), 226–238.

Shirani, S., Mahdavi, F. and Maziah, M. (2009) Morphological abnormality among regenerated shoots of banana and plantain (*Musa* spp.) after *in vitro* multiplication with TDZ and BAP from excised shoot tips. *African Journal of Biotechnology,* **8**(21), 5755–5761.

Smith, M.K. (1988) A review of factors influencing the genetic stability of micropropagated bananas. *Fruits,* **43**, 219–223.

Strosse, H., Schoofs, H., Panis, B., Andre, E., Reyniers, K. and Swennen, R. (2006) Development of embryogenic cell suspensions from shoot meristematic tissue in bananas and plantains (*Musa* spp.). *Plant Science,* **170**, 104–112.

Sudipta, K.M., Swamy, M.K., Balasubramanya, S. and Anuradha, M. (2014) Assessment of genetic fidelity, antioxidant enzyme activity and proline content of micropropagated and field grown plants of *Leptadenia reticulata* (Wight & Arn.) an endangered medicinal plant. *Plant Cell Biotechnology and Molecular Biol*ogy, **15**,127–135.

Vuylsteke, D., Swennen, R. and De Langhe, E. (1991) Somaclonal variation in aplantain (*Musa* spp. AAB group) derived from shoot-tip culture. *Fruits,* **46**, 429–439.

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الإكثار الدقيق وتقييم الثبات الوراثي في الموز صنف ويليمز باستخدام دلائل قطع الدنا المتضاعفة عشوائيا RAPD والتباين ذو الصلة بالتتابعات المتضاعفة SRAP

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اجريت الدراسة الحالية لدراسة تأثير التركيزات والاتحادات المختلفة لمنظمات النمو على التكاثر الدقيق للموز صنف ويليمز على النطاق التجاري. بالإضافة إلى تقييم الثبات الوراثي للنباتات الناتجة بواسطة دلائل الـ RAPD وSRAP. أظهرت النتائج ان بيئة موراشيج وسكوج (MS) المضاف اليها 3.0 ملجم/لتر من السيتوكينين بنزيل امينو بيورين BAP و 0.2 ملجم/لتر من الأوكسين حمض خليك النفثالين NAA كانت افضل الاتحادات المستخدمة. وأعطت أعلى متوسطات لتكوين للأفرع الخضرية لكل منفصل نباتي منزرع 7.6 و8.4 و11.2 بعد 10 و20 و30 يوم من الزراعة على التوالي. كما تم الحصول على اعلى قيم لمتوسطات أطوال الأفرع الخضرية 4.2 و5.4 و6سم على نفس البيئة بعد 10 و20 و30 يوم على التوالي. تم تسجيل أعلى عدد من الجذور جيدة النمو (10.4 جذر لكل مجموع خضري) على بيئة التجذير المحتوية على 3 ملجم/لتر إندول حمض الخليك IAA بعد 20 يوم. وبعد ذلك تم نقل النباتات إلى اصص ونمت في الصوبة وتم نقلها بنجاح إلى التربة. وللتأكد من ان النباتات الناتجة من الإكثار الدقيق مطابقة للنبات الأم ولا يوجد بها تغيرات وراثية بعد دورة الاكثار الدقيق السابعة استخدمت تقنيات ال RAPD و SRAP. حيث استخدم 10 بوادئ من ال RAPD انتجت 38 حزمة، بينما استخدم اربعة بوادئ من ال SRAP انتجت 16 حزمة و كانت الحزم الناتجة متماثلة بين النباتات الناتجة من الإكثار الدقيق والنبات الام، ماعدا البادئ me1+em2 نتج عنه حزمة واحدة مختلفة. ويتضح من نتائج الدراسة الحالية انه يمكن استخدام بروتوكول الإكثار الدقيق في المعمل كطريقة تجارية للإنتاج على نطاق واسع للموز الخالي من الأمراض والثابت وراثيا والمشابهة للنبات الأصلي.