academicJournals

Vol. 13(34), pp. 3522-3530, 20 August, 2014
DOI: 10.5897/AJB2014.13935
Article Number: 835492446844
ISSN 1684-5315
Copyright © 2014
Author(s) retain the copyright of this article
http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Shoot regeneration, biochemical, molecular and phytochemical investigation of *Arum palaestinum* Boiss

Mai M. Farid[1], Sameh R. Hussein[1]*, Lamiaa F. Ibrahim[1], Mohammed A. El Desouky[2], Amr M. Elsayed[2] and Mahmoud M. Saker[3]

[1]Department of Phytochemistry and Plant Systematic, National Research Center, +12311 Cairo, Egypt.
[2]Department of Biochemistry, Cairo University, Cairo, Egypt.
[3]Department of Plant Biotechnology, National Research Center, +12311 Cairo, Egypt.

Received 20 May 2014; Accepted 14 July, 2014

***Arum palaestinum* Boiss. populations are in danger of extinction in the wild. Thus, there is a need to establish a reliable strategy for multiplying this valuable medicinal plant. In the present study, seeds and tissue culture of *A. palaestinum* were subjected to biochemical, molecular and phytochemical analysis. Obtained results indicated that the best medium for shoots proliferation was Murashige and Skoog (MS) medium supplemented with 5 mg/L benzyl adenine (BA) and 0.1 mg/L naphthalene acetic acid (NAA). The regenerated shoots were rooted on half strength MS medium containing 1 mg/L NAA and 2 g/L charcoal. Tissue culture derived plantlets were successfully acclimatized under *ex vitro* conditions. Protein analysis referred that, the difference in protein profiles in the examined samples suggests that a real genetic change might have occurred. Obtained results of the inter simple sequence repeat (ISSR) revealed variation between the regenerated plants and mother plant while the phytochemical investigation revealed that, 10 phenolic compounds (seven flavones, one flavonol and two phenolic acids) were identified using HPLC analysis and five compounds were detected in the plant for the first time. Genetic characterization and chemical investigation of seeds and *in vitro* cultures reported herein, is the first report for *A. palaestinum*.**

Key words: Black calla lily, *in vitro* culture, inter simple sequence repeat (ISSR), sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), isozyme, phenolic compounds.

INTRODUCTION

Arum palaestinum Boiss. (Black Calla Lily) is one of about 26 species of the *Arum* genus belonging to family Araceae (Boyce, 1993; Mayo et al., 1997; Al-Lozi et al., 2008; Makhadmeh et al., 2010) native to Europe,

*Corresponding author. E-mail: sameh_reda@hotmail.com.

Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

Northern Africa, Western Asia, with the highest species diversity in Mediterranean region (Dessouky et al., 2007a). Black lily is a typical “cryptic” species, since its appendix emits mainly ethyl acetate, producing a smell of rotten fruit. In many countries, the aerial parts of *A. palaestinum* are considered ornamental plant, animal fodder and edible after being soaked in salty water or dried. The plant is used in folk medicine to cure several chronic diseases such as stomach acidity, atherosclerosis, cancer, and diabetes (Al-Eisawi, 1982; Al-Lozi et al., 2008; Makhadmeh et al., 2010).

Previous work on the characteristics of secondary metabolites of the Araceae family indicated the presence of polyphenols, alkaloids, proanthocyanidins, flavones, flavone *C*-glycosides and flavonols (Williams et al., 1981; Kite et al., 1997). The phytochemical investigation of *A. palaestinum* resulted in the isolation of two *C*-glucoside flavones: isoorientin and vitexin. The effects of isoorientin on rat isolated aorta, ileum, trachea and uterus and on guinea pig uterus were studied by Afifi et al. (1999). A novel alkylated piperazine were also isolated and showed a significant cytotoxicity against cultured tumor cell lines (El- Desouky et al., 2007a). Polyhydroxy alkaloid compound in addition to; caffeic acid, isoorientin, luteolin, vicenin and 3,6,8-trimethoxy 5,7, 3', 4'-tetrahydroxy flavone were isolated by El- Desouky et al. (2007b). Recently, Aboul-Enein et al. (2012) assured potential antitumor effect of *A. palaestinum* extract. It is worth to mention that all published reports of phytochemical studies used leaves and flowers of *A. palaestinum* and provide evidences for strong antitumor activities of its extract.

The successful use of plant biotechnology techniques in production of secondary metabolites, mass propagation and conservation of rare species dates back to early eighty’s and is well discussed by Engelmann (2004). However, survey of published data indicated that there is only one published manuscript on *in vitro* culture of *A. palaestinum* via somatic embryogenesis (Shibli et al., 2012).

Genetic markers derived from electrophoretic analysis can be used to survey the level of genetic diversity within and among populations and also for taxonomic purpose (Hamrick and Godt, 1989). Isozyme analysis is a highly appropriate method for identifying genomic allele components as well as supplementing DNA analysis. Since the 1930s, electrophoresis in conjunction with the zymogram technique has been used as a tool for the study of heritable variation. Isozymes are widely used because of their relative efficiency and cost effectiveness, particularly in studies of intra and inter specific variation (Johnson et al., 2007; Siva and Krishnamurthy, 2005; Johnson et al., 2010; Smila et al., 2007).

Recently, several DNA markers have been successfully employed to assess the genomic stability/instability in regenerated plants. Among the markers, the inter-simple sequence repeat (ISSR) has been favored because of their sensitivity, simplicity, and cost effectiveness (Yang et al., 1996). The aim of this study was establishment of applicable tissue culture system coupled with monitoring of genetic stability of tissue culture derived clones (*in vitro* plants) for rapid mass propagation, conservation and future biotechnology based production of pharmaceutically bioactive ingredients of black calla lily. The second objective of this study was the genetic characterization and phytochemical investigations for both *in vitro* produced plants and *in vivo* plants, for a better understanding of genetic relationship and the discovery of new potent bioactive substances.

MATERIALS AND METHODS

Plant material

Seeds of *A. palaestinum* were collected from their growing habitats in Bergesh protected area, Irbid, Jordan, Latitude: 32°25'43.17 and Longitude: 35°46'47.01 in February 2012.

Tissue culture of *A. palaestinum*

Seeds of *A. palaestinum* were decoated under sterile conditions of air laminar flow cabinet. The decoated seeds were surface sterilized by immersion in 70% ethanol for 60 s, and then immersed in 20% sodium hypochlorite (NaOCl) solution for 20 min. Seeds were then rinsed three times with sterile distilled water and cultured on basal Murashige and Skoog medium (MS) (1962) containing 3% sucrose and 4.4 g/L of MS, salts without growth regulators and solidified with 2.8 g/L gelrite and kept in incubation room under dark condition for 48 h. The *in vitro* germinated seedlings (2- month-old) about 4-6 cm in height were used as a source of starting plant materials (Figure 1a). During germination, callus was proliferated directly from seeds in some samples as shown in Figure 1b then different explants (leaves, stems, root and corms) were excised from the *in vitro* seedlings (two months old) and cultured on six different regeneration media as illustrated in Table 1. All media contained 4.4 g/L MS basal salts, 30 g/L sucrose and solidified with 2.8 g/L gelrite. The proliferated shoots were multiplied on MS medium supplemented with 0.1 mg/L NAA and 5 mg/L BA (medium 6). Number and length of shoots, and roots were recorded. Shoots developed on regeneration media were rooted on half strength basal MS medium (2.2 g/L MS salts), containing 30 g/L sucrose and supplemented with 1 mg/L NAA and 2 g /L charcoal and solidified with 2.8 g/L gelrite and all culture were incubated in temperature controlled growth room at 27 ± 1°C for 16 h daily light system under light intensity (Ca 50 µmol m^{-2} s^{-1}) and subcultured monthly in fresh medium. Complete plantlets (shoots and roots) were transplanted to mixture of 1:3 vermiculite and soil in plastic pots and placed in greenhouse for acclimatization

Protein analysis

For SDS-PAGE protein patterns and Isozyme analysis, 300 mg of regenerated shoots from corms of *A. palaestinum* cultured on the six tested regeneration media and mother plant were extracted according to the method of Gottlieb (1981)

The separating gel of 10% acrylamide was prepared following the method of Laemmli (1970). The method of Weber and Osborne (1969) was used to determine the apparent (subunit) molecular weight of proteins dissolved or extracted in the presence of SDS

Figure 1. a) *In vitro* germinated seeds of *A. palaestinum*. **b)** Seeds derived callus during germination.

Table 1. Structure of regeneration media used.

Media code	BA (mg /L)	NAA (mg /L)	Thiamine (mg/L)	KH_2PO_4 (mg/L)	Glycine (mg/L)	Adenine sulphate (mg/L)
1	5	-	70	170	100	50
2	1	0.1	-	-	-	-
3	5	0.5	-	-	-	-
4	5	-	-	-	-	-
5	5	0.1	-	-	-	-
6	5	0.1	70	170	100	50

while isozyme gel was stained for α-Esterase enzyme according to the protocols described by Soltis et al. (1983).

Molecular analysis using ISSR

Fourteen ISSR primers were used for the control mother plant and tissue culture raised plantlets. PCR amplification was performed in 25 µl reaction mixture each containing 0.25 µl 0.5 U Taq DNA polymerase, 2.5 µl 0.2 mM dNTPs (dATPs, dCTPs, dGTPs and dTTPs), 5 µl (5X) colourless reaction buffer, 20.4 ng (3 µl) genomic DNA and 3 µl of 10 pmole primers, and 11.25 µl sterile distilled water. The thermocycler program for ISSR was 95°C for 3 min; 92°C for 2 min; 44 cycles of 43°C for 1 min; 72°C for 2 min; 72°C for 10 min and at 4°C for soaking; 100 bp DNA ladder (Biogene) was used. The banding profile of ISSR was scored using Labimage program. The polymorphism was estimated as follow:

Percent of polymorphism = (Number of polymorphic bands / Total Number of Bands) × 100.

Phytochemical investigation

The seeds (8 g) and the air dried *in vitro* shoots (47 mg), roots (140 mg) and callus (71 mg) of *A. palaestinum* were extracted with 70% methanol at room temperature for three times. The crude filtered extracts were concentrated under reduced pressure in a rotary evaporator to give a residue which dissolved in methanol. The isolation and identification of the compounds were carried out by using two dimension paper chromatography method with stander samples and confirmed by analyzing the extract on an Agilent HPLC 1200 series equipped with diode array detector (Agilent Technologies, Waldbronn, Germany). Chromatographic separations were performed using a waters column C18. The binary mobile phase consisted of (A) acetonitrile and (B) 0.1% acidified water with formic acid. The elution profile was: 0-1 min 100% B (isocratic), 1-30 min 100-70% B (linear gradient), 30-35 min 70-20% B (linear gradient). The flow rate was 0.3 ml/min and the injection volume was 5 µl. Chromatograms were recorded at 278 nm. This analysis enabled the characterization of phenolics on the basis of their retention time and UV spectra.

The retention time of the isolated compounds were compared with those of standard samples which were selected according to the compounds previously isolated from *A. palaestinum* and the Araceae family by the Phytochemistry and Plant Systematic Department, National Research Center.

Statistical analysis

All data were subjected to analysis of variance ANOVA to test the significance in the all experiments. The least significant difference (LSD) at $P< 0.05$ level was calculated according to the statistical

Table 2. Regeneration of shoots from corms of *A. palaestinum* cultured on six tested regeneration media.

Media type	Number of shoots	Length of shoots (cm)	Number of Roots	Length of root (cm)
1	$1.3^{a} \pm 0.47$	$3.7^{a} \pm 0.47$	$3^{a} \pm 0$	$1^{b} \pm 0$
2	$1^{a} \pm 0$	$1.3^{c} \pm 0.47$	No roots	-
3	$1.3^{a} \pm 0.47$	$5^{bd} \pm 3.6$	No roots	-
4	$1.3^{a} \pm 0.47$	$4.3^{ab} \pm 0.47$	$1^{b} \pm 0$	$0.5^{a} \pm 0$
5	$1^{a} \pm 0$	$2.3^{c} \pm 0.47$	$2^{c} \pm 0$	$0.5^{a} \pm 0$
6	$1.7^{b} \pm 0.47$	$6^{d} \pm 1.4$	No roots	-
F- value	0.850	16	4.756	6.818
Propabilty level (P<)	0.541	0.0001	0.017	0.008

Data (mean ± SD) sharing the same letter in the same column is not significantly different.

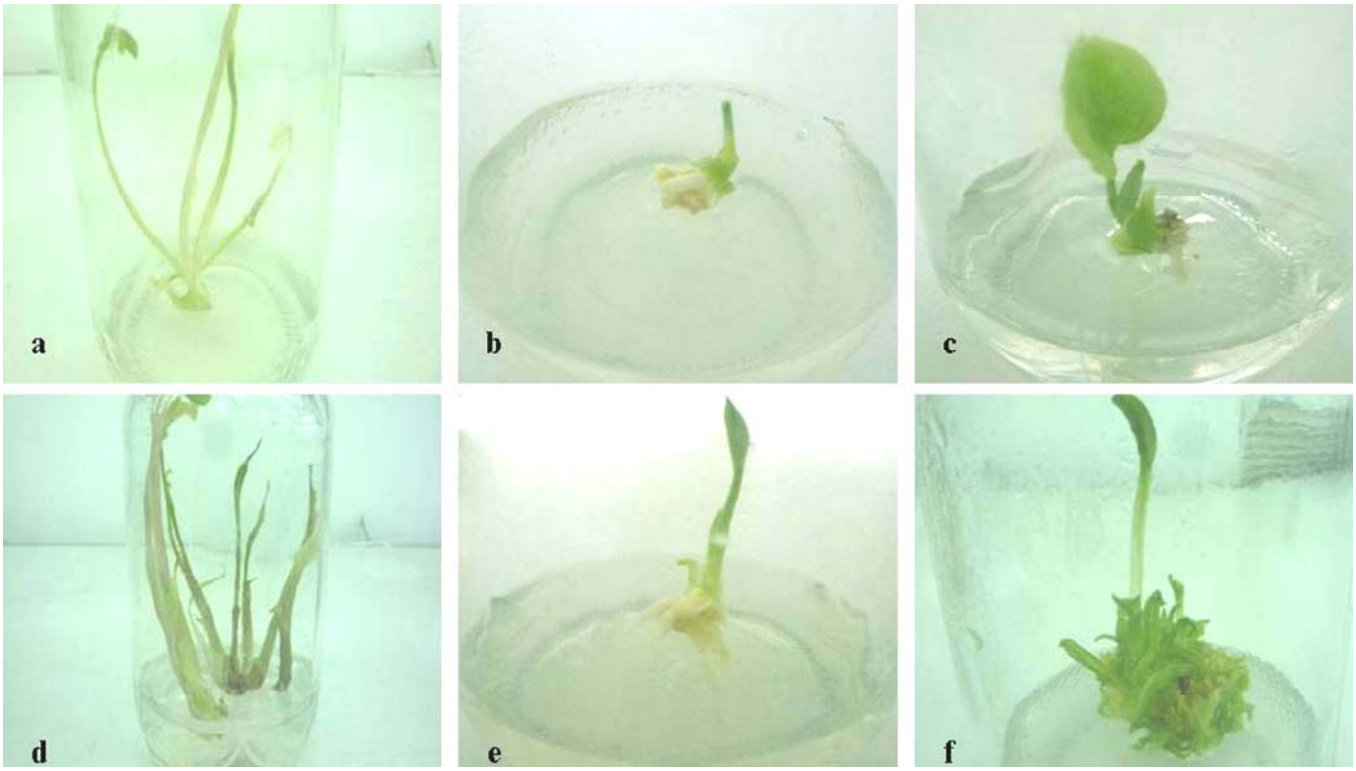

Figure 2. Regeneration of *A. palaestinum* from corms explants cultured on: **(a)** MS-medium with 5 mg/L BA +0.1 mg/L NAA (medium 6), **(b)** MS-medium with 1 mg/L BA + 0.1 mg/L NAA (medium 2), **(c)** MS-medium with 5 mg/L BA + 0.1 mg/L NAA (medium 5), **(d)** MS-medium with 5 mg/L BA + 0.1 mg/L NAA (medium 6), **(e)** Root proliferated on shoots on MS-medium contained 5 mg/L BA (medium 1) and **(f)** multiplication of the regenerated shoots on MS medium supplemented with 0.1 mg/L NAA and 5 mg/L BA.

analysis method described by Casanova et al. (2004).

RESULTS AND DISCUSSION

Tissue culture of *A. palaestinum*

The regeneration of new shoots from primary explants is a prerequisite for any regeneration protocol. In this study, the pre-existing buds started to develop earliest from only the corms and new shoots development was observed within eight weeks while all other plant material (explants) showed no growth response. Data obtained in Table 2 indicates that all the tested media for regeneration produced shoots and medium 6 (5 mg/L BA + 0.1 mg/L NAA) gave the highest shoots number (1.7) and length (6 cm) (Figure 2a and d) while medium 2 (MS + 1 mg/L BA + 0.1 mg/L NAA) gave the lowest shoots number (1) and length (1.3 cm) (Figure 2b and c). From Table 2, it could

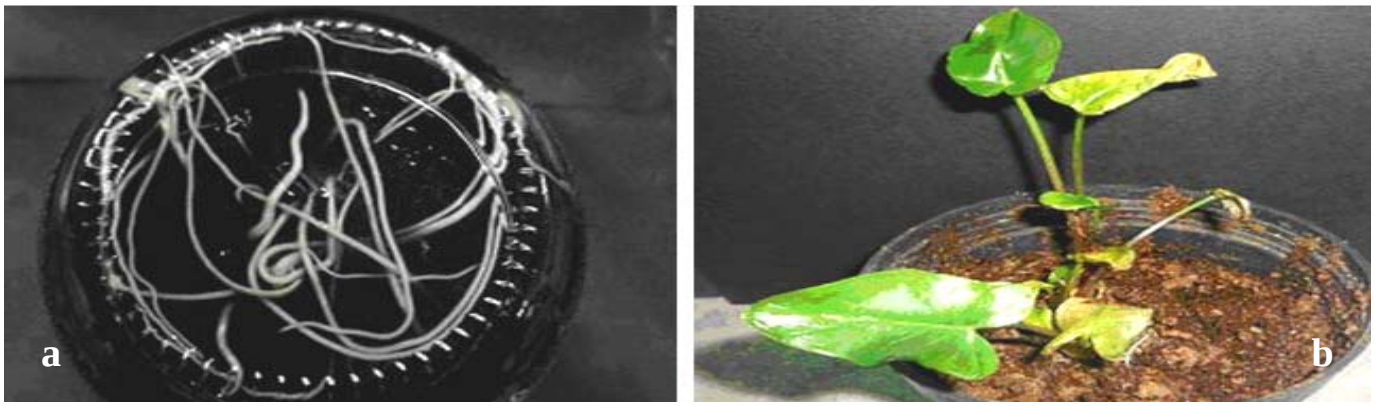

Figure 3. (a) Root formation. (b) Acclimatized complete plantlets of *A. palaestinum.*

also be observed that the highest number and length of roots (3 and 1 cm, respectively) was noticed with medium 1 (MS + 5 mg/L BA) (Figure 2e), whereas, medium 2, 3 and 6 did not show any response for rooting of shoots. The developed shoots were transferred to MS medium supplemented with 0.1 mg/L NAA + 5 mg/L BA and solidified with 2.8 g/L gelrite for multiplication of many long shoots (Figure 2f). The regenerated shoots were cultured on rooting medium that consisted of half strength MS medium + 2 g/L charcoal + 1 mg/L NAA which gave many long roots, its length was about 7 cm (Figure 3a). Rooted plantlets were acclimatized successfully with 95% survival rate (Figure 3b). The regenerated plantlets established in potting mixture were uniform and identical to donor plants with respect to growth characteristics and vegetative morphology.

Our results are in agreement with those of previous studies (Francis et al., 2007; Sivanesan and Jeong, 2007; Samantaray and Maiti, 2008) which showed that combination of cytokinins and auxins triggered the rate of shoot multiplication in various medicinal plants). The type of exogenous cytokinin used in the medium has a marked effect on the frequency of chestnut shoot proliferation (Carmen et al., 2001). In *Plumbago zeylanica,* BA was more effective for shoot bud proliferation than kinetin (Rout et al., 1999). In tomato, NAA showed the most positive effect on induction and elongation of lateral roots such an effect was also observed by Taylor et al*.* (1998). For rooting, activated charcoal may absorb toxic substances in the medium and improving root regeneration and development (Ziv, 1979; Takayama et al*.,* 1980). In this respect, Takayama et al*.* (1980) reported an inhibition of root formation of *Lilium* by BA and that inhibition was completely reversed by the addition of charcoal.

The hardening of *in vitro* raised plantlets is essential for better survival and successful establishment (Deb and Imchen, 2010). In this respect, Shibli et al. (2012) obtained plantlets from somatic embryos of *A. palaestinum* and reported that rooted plants were grown in greenhouse and acclimatized successfully with a 95% survival rate. In the present study, there is no feasible morphological difference among leaves of the *in vitro* raised plants, while some differences in root formation was observed with plantlets grown in media 2, 3 and 6.

Protein profiles

The protein profile system revealed the biochemical variation and evolutionary relationship among the plantlets grown on the six regeneration media and mother plant of *A. palaestinum* were demonstrated in Figure 4. The molecular weights of detected bands for all samples ranged from 7 to 79 KDa. Shoots grown on medium 1 only showed three bands at molecular weights 70, 61 and 46 KDa. There was one band detected at molecular weight 64 KDa in shoots grown on medium 2 and absent from the other samples. Also, band with molecular weight 20 KDa was absent in shoots grown on medium 2 and present in other samples examined. A polypeptide band of molecular weight 37 KDa was detected only in shoots grown in medium 3 and not present in all samples. Moreover, at molecular weight 34 KDa, band was absent in shoots grown on medium 5 and present in other samples examined. Similar protein profiles of mother plant and other plantlets grown in different media were observed at molecular weight 23 KDa. For donor plant, it could be observed that four bands were polymorphic bands at molecular weight of 79, 12, 16 and 13 KDa, respectively.

In the present study, the difference in protein profiles in the examined samples suggests that a real genetic change might have occurred due to the presence of growth regulators in the regeneration media used and these results are in agreement with those of Hendriks and Veries (1995) who detected a group of proteins (54 and 47 KDa) in embryogenic cultures of carrot. Similar finding was also observed by Beckmann et al*.* (1990) who reported that, SDS-PAGE analysis was used in the

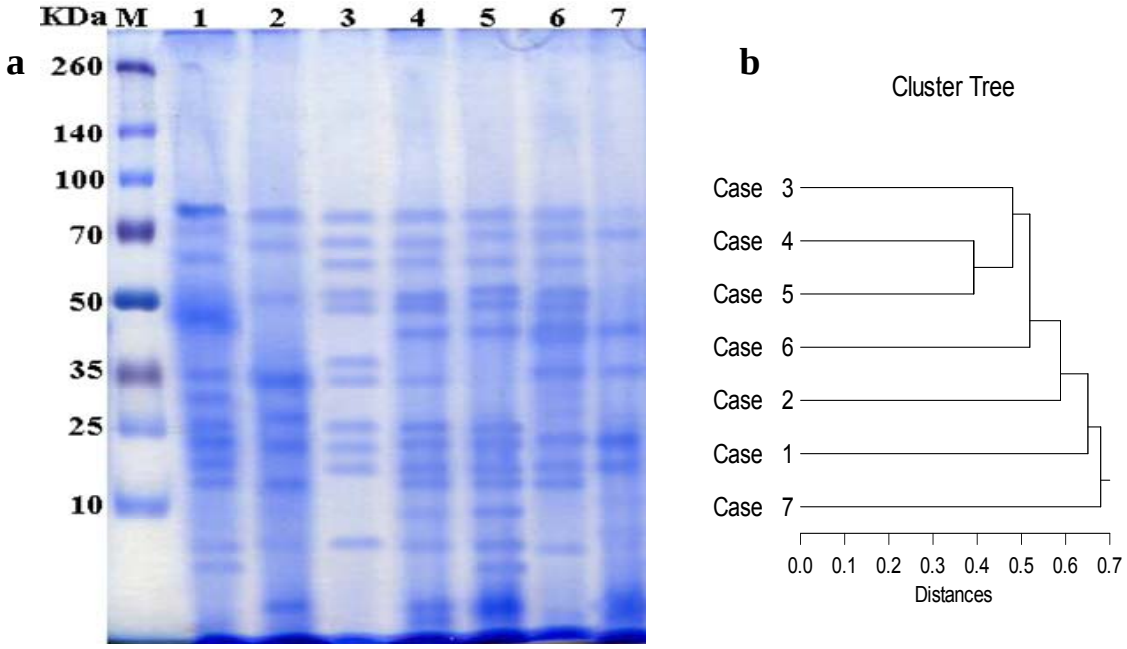

Figure 4. (a) SDS-PAGE of regenerated shoots of different *A. palaestinum in vitro* plants developed on the six tested regeneration media (1: 6) and control mother plant. Lane M refers to low molecular weight standard protein marker. **(b)** Dendrogram for regenerated plants and donor plant constructed from protein analysis data using un-weighted pair-group arithmetic average (UPGMA) and similarity matrices computed according to dice coefficients.

Table 3. Distribution of bands and relative mobilities (RF) of α-esterase of *A. palaestinum in vitro* plants and mother plant.

Primer	5'- Sequence -3'	Size range of the scorable bands (bp)	Total bands	No. of polymorphic bands	Polymorphism (%)
UBC-815	CTCTCTCTCTCTCTCTG	510-248	5	4	80
UBC-818	CACACACACACACACAG	552-317	3	0	0
UBC-824	TCTCTCTCTCTCTCTCG	827-304	7	7	100
UBC-825	ACACACACACACACACT	418-310	4	2	50
UBC-834	AGAGAGAGAGAGAGAGCTT	1324-234	6	2	33.33
UBC-840	GAGAGAGAGAGAGAGAYT	641-247	6	5	83.33
UBC-843	CTCTCTCTCTCTCTCTRA	1190-354	7	7	100
UBC-844	CTCTCTCTCTCTCTCTRC	748-204	8	4	50
UBC-845	CTCTCTCTCTCTCTCTRG	658-200	7	4	57.14
UBC-846	CACACACACACACACART	586-205	7	5	71.42
UBC-850	GTGTGTGTGTGTGTGTYC	756-258	5	4	80
UBC-857	ACACACACACACACACYG	393-191	4	2	50
UBC-864	ATGATGATGATGATGATG	916-315	4	3	75
UBC-873	GACAGACAGACAGACA	616-266	5	4	80
Total			78	53	
Average			5.6	4	65

identification of newly biosynthesized proteins. The obtained α-esterase isozyme banding patterns were typical for mother plant and plantlets raised from tissue culture and no polymorphism could be detected as shown in Table 3. The obtained data shows that band 1 (RF 0.125) was present in all *in vitro* plants and donor plant, while, band 2 (RF 0.173) was absent in all samples except regenerated plant grown in medium 3. However,

Table 4. ISSR amplification products of DNA extracted from *A. palaestinum in vitro* plants and mother plant.

Band number	RF Values	1	2	3	4	5	6	7
1	0.125	+	+	+	+	+	+	+
2	0.173	-	-	+	-	-	-	-
3	0.217	+	+	-	+	+	+	+

1 to 6, the tested regeneration media; 7 mother plant as a control; +, present; -, absent.

band 3 (RF 0.217) was absent from the same medium. In this connection, Saker and Rady (2003) reported that analysis of both esterase and peroxidase isozyme banding patterns does not give any polymorphism in tissue culture raised male and female papaya plants.

ISSR fingerprinting

In order to assess the genetic stability/instability of the regenerated plants, ISSR fingerprinting of the plantlets grown on the six regeneration media and donor mother plant of *A. palaestinum* was carried out. A total number of 78 scorable amplified DNA fragments ranging from 1324 to 191 bp were observed using the 14 ISSR primers, whereas 53 fragments were polymorphic. The 14 primers produced 65% polymorphism. The highest number of polymorphic bands (7) was obtained with primers UBC-824, UBC-843. The lowest number of polymorphic bands (2) was observed with primers UBC-825, UBC-234 and UBC-857 as shown in Table 4.

The highest percentage of polymorphism (100%) was observed with primers UBC-824 and UBC-843 while the lowest percentage of polymorphism (33.33%) was noticed with primer UBC- 834. No polymorphic bands were detected with primer UBC- 818. The obtained results showed that the regenerated plants showed apparent genetic variations when subjected to ISSR analysis, these results are in agreement with those of Hu et al. (2007) who noticed that ISSR primers could produce a high-frequency polymorphism in detection of somaclonal variation in *A. konjac*. Similar findings on genomic variation have been well documented in some other plants (Diwan and Cregan, 1997; Rahman and Rajora, 2001; Kawiak and Lojkowska, 2004). Recently, Biabani et al. (2013) employed 10 ISSR primers to assess genetic diversity among six populations of *Jatropha* from different Asian countries. 143 polymorphic bands were produced and polymorphism ranged between 46.2 and 60.8% between different genotypes.

Cluster analysis was done on the basis of similarity coefficients which ranged from 0-0.6 among the 6 tested regenerated plants and their donor mother plant as shown (Figure 5). The dendrogram constructed from UPGMA cluster analysis of the Dice similarity coefficients was calculated from ISSR data. The dendrogram based on genetic similarities separated the six samples of *A. palaestinum* into two main groups.

The regenerated plant 3 and donor plant 7 was grouped in the first cluster alone, and all other samples were grouped in the second cluster, which was separated into two sub-clusters, the first sub-cluster included *in vitro* plant 1 and the second included the other 3 samples (regenerated plants 4, 5 and 6). The three samples were classified into two sub-clusters, the first included regenerated plant 4 and the second included the other 2 samples sub-cluster.

Phytochemical investigation

Ten (10) compounds were detected and present in the seeds of *A. palaestinum* and its *in vitro* culture samples and identified as: apigenin, apigenin 6,8 di-*C*-glucoside, vitexin, isovitexin, orientin, isoorientin, luteolin 7-glucoside, quercetin, caffeic acid and isoferulic acid. The comparison between the HPLC analysis of seeds, shoots, roots and callus extracts with the identified compounds were summarized in Table 5.

Five compounds, (apigenin, apigenin 6, 8 di-*C*-glucoside, isoorientin, quercetin**,** and caffeic acid) were present in the shoot. Four compounds, (apigenin 6, 8 di-*C*-glucoside, orientin, isoorientin, and quercetin) were detected in the root, while two compounds, (apigenin 6, 8 di-*C*-glucoside and isoorientin) in the callus. Separated flavonoid peaks were initially identified by direct comparison of their retention time with those of standards. Standard solution was then added to the sample and peaks were identified by the increase in their intensity. This procedure was performed separately for each standard.

In general, a profound difference of the compounds was observed between the different analyzed samples. Some compounds are found in the donor plant and not detected in shoots, roots and callus raised from tissue culture. In the present study, five compounds, apigenin, apigenin 6,8 di-*C*-glucoside, isovitexin, quercetin and isoferulic acid were detected in the tested samples for the first time while the other compounds were isolated before by Afifi et al. (1999) and El-Desouky et al., (2007a).

Conclusions

In conclusion, survey of published data and open access patent data base indicated that there is no evidence for

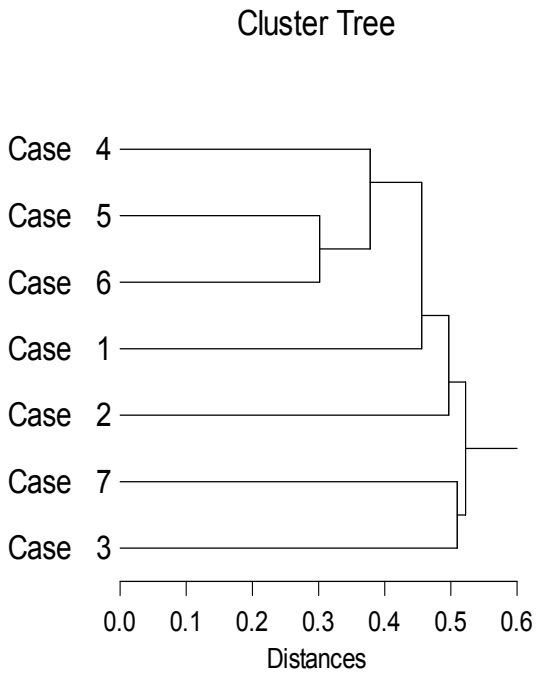

Figure 5. Dendrogram illustrating coefficient similarities among 6 regenerated plants (1:6) and their donor mother plant (7) of *A. palaestinum* based on ISSR data.

Table 5. HPLC analysis of seeds & *in vitro* cultures of *A. palaestinum.*

Compound	Retention time	Seeds	Regenerated Shoots	Roots of regenerated shoots	Callus	Previous work on mother plant
Apigenin	39	+	+	-	-	-
Apigenin 6,8 di-*C*-glucoside	23.26	+	+	+	+	+
Vitexin	26.7	+	-	-	-	+
Isovitexin	27.25	+	-	-	-	-
Orientin	37.1	+	-	+	-	-
Isoorientin	39.4	+	+	+	+	+
Luteolin 7-glucoside	32.9	+	-	-	-	-
Quercetin	3.4	+	+	+	-	-
Caffeic acid	22.3	+	+	-	-	+
Isoferulic acid	14.9	+	-	-	-	-

(+) Present; (-) absent.

preceding trials on protein analysis, DNA fingerprinting and phytochemical investigation of tissue cultures of *A. palaestinum* and only one manuscript on *in vitro* culture of *A. palaestinum* via somatic embryogenesis is published, so there is a huge shortage of information in this plant which we tried to cover in this study by the development of *in vitro* culture protocols and integrated investigations on genetic studies to better understand its genetic diversity, re-establishing and clonalization strategies.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENTS

Financial support of Science and Technology Development Fund (STDF, Egypt), grant no. 4402 is highly appreciated. The authors are thankful to Prof

Ahmed El Oqla, Department of Biological Sciences, Yarmouk University, Jordan, for his cooperation in collecting the plant.

REFERENCES

Aboul-Enein AM, Abu El-Ela, F, Shalaby EA, El-Shemy HA (2012). Traditional medicinal plants research in Egypt: Studies of antioxidant and anticancer activities. J. Med. Plants Res. 6(5):689-703.

Afifi FU, Khalil E, Abdalla S (1999). Effect of isoorientin isolated from *Arum palaestinum* on uterine smooth muscles of rats and guinea pigs. J. Ethnopharmacol. 65:173-177.

Al-Eisawi DM (1982). List of Jordan vascular plants. Mitt. Bot. München. 18:79-182.

Al-Lozi S, Makhadmeh I, Duwayri M, Shibli, R, Migdadi H (2008) Assessment of phenotypic variation of *Arum* species in Jordan. Jordan J. Agric. Sci. 4:367-379.

Beckmann RP, Mizzen LE, Welch WJ (1990). Interaction of Hsp 70 with newly synthesized proteins: implications for protein folding and assembly. Science 248:850-854.

Biabani A, Rafii MY, Saleh GB, Abdul Latif M (2013). Inter- and intra-population genetic variations in *Jatropha curcas* populations revealed by inter-simple sequence repeat molecular markers. Maydica. 58:111-118.

Boyce P (1993). The genus *Arum*. The Royal Botanic Gardens Kew London.

Carmen SJM, Ballester A, Vieitez AM (2001). Effect of Thidiazuron on multiple shoot induction and plant regeneration from cotyledonary nodes of chestnut. J. Hortic. Sci. Biotech. 76:588-595.

Casanova E, Valdes AE, Zuker A, Fernandez B, Vainstein A, Trillas MI, Moysset L (2004). rolC- transgenic carnation plants: adventitious organogenesis and levels of endogenous auxin and cytokinins. Plant Sci. 167:551-560.

Deb CR, Imchen T (2010). An efficient *in vitro* hardening technique of tissue culture raised plants. Biotechnol. 9:79-83.

Diwan N, Cregan PB (1997). Automated sizing of fluorescent labeled simple sequence repeat (SSR) markers to assay genetic variation in soybean. Theor. Appl. Genet. 95:723-733.

El-Desouky SK, Kim KH, Ryu SY, Eweas AF, Gamal-Eldeen AM, Kim Y (2007a). A new pyrrole alkaloid isolated from *Arum palaestinum* Boiss. and its biological activities. Arch. Pharm. Res. 30(8):927-931.

El-Desouky SK, Ryu SY, Kim YK (2007b). Piperazirum, a novel bioactive alkaloid from *Arum palaestinum* Boiss. Tetrahedron Lett. 48(23):4015-4017.

Engelmann F (2004). Plant cryopreservation: Progress and prospects. *In vitro* Cell Dev. Biol. 40:427-433.

Francis SV, Senapati SK, Rout GR (2007). Rapid clonal propagation of *Curculigo orchioides Gaertn* an endangered medicinal plant. *In vitro* Cell Dev. Pl. 43:140-143.

Gottlieb L (1981). Electrophoretic evidence and plant populations. Progr. Phytochem. 7:1-46.

Hamrick JK, Godt MJW (1989). Allozyme diversity in plant species. In: Brown AHD, Clegg MT, Kahler AL, Weir BS (Eds.) Plant Population Genetics, Breeding and Genetic Resources. Sinauer Associates Sunderland pp. 43-63.

Hendriks J, Veries S (1995). Current Issues in Plant Molecular and Cellular Biology. In Terzi M, Cella RR, Falavigna A. (ed) Kluwer Academic Publishers, Netherlands. pp. 359-368.

Hu JB, Gao XX, Xie CH, Liu J (2007). RAPD and ISSR analysis of somaclonal variation among regenerated plants of *Amorphophallus rivieri*. Sci. Agri. Sin. In press.

Johnson M (2007). Somoclonal variation studies on *Phyllanthus amarus* Schum and Thonn. Iranian J. Biotechnol. 5(4):240-245.

Johnson M, Wesely EG, Selvan N, Chalini K (2010). Comparative phytochemical and isoperoxidase studies on leaf and leaves derived callus of *Solanum anguivi* Lam. J. Chem. Pharm. Res. 2 (4):899-906.

Kawiak A, Lojkowska E (2004). Application of RAPD in the determination of genetic fidelity in micropropagation *Drosera* plantlets. *In Vitro* Cell. Dev. Biol.-Plant 40:592-595.

Kite GC, Sharp HJ, Hill PS, Boyce PC (1997). Polyhydroxyalkaloids in the aroid tribes nephthytideae and aglaonemateae: Phytochemical support for an intertribal relationship. Biochem. Syst. Ecol. 25: 757-766.

Laemmli UK (1970). Cleavage of structural proteins during the assembly of the head bacteriophage T4. Nature. 227:680-685.

Makhadmeh I, Al-Lozi S, Duwayri M, Shibli RA, Migdadi H (2010). Assessment of genetic variation in wild *Arum* species from Jordan using Amplified Fragment Length Polymorphism (AFLP) markers. Jordan J. Agric. Sci. 6:224-239.

Mayo S, Bogner J, Boyce PC (1997). The genera of Araceae. Royal Botanical Gardens Kew London UK.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473- 497.

Rahman MH, Rajora OP (2001). Microsatellite DNA somaclonal variation in micropropagated trembling aspen (*Populus tremuloides*). Plant Cell Rep. 20:531-536.

Rout GR, Saxena C, Samantaray S, Das P (1999). Rapid clonal propagation of *Plumbago zeylanica* Linn. Plant Growth Regul. 28:1-4.

Saker MM, Rady MR (2003). Employment of molecular markers for identification of male and female papaya plants. J. Genetic Eng. And Biotechnology (NRC) (1):85-97.

Samantaray S, Maiti S (2008). Rapid plant regeneration and assessment of genetic fidelity of *in vitro* raised plants in *Aloe barbadensis* Mill. Using RAPD markers. Acta Bot. Gallica 155:427-434.

Shibli RA, Duwayri MA, Sawwan JS, Shatnawi MA, Al-Qudah TS (2012). Regeneration via somatic embryogenesis of the endangered wild *Arum* (*Arum palaestinum*). *In vitro* Cell Dev. Biol. 48: 335-340.

Siva R, Krishnamurthy KV (2005). Isozyme diversity in *Cassia auriculata* L. Afr. J. Biotechnol. 4(8):772-775.

Sivanesan I, Jeong BR (2007). Direct shoot regeneration from nodal explants of *Sida cordifolia* Linn. *In vitro* Cell Dev. Pl. 43:436-441.

Smila H, Johnson M, Rajasekarapandian M (2007). Studies on varietal difference, tissue specificity and developmental variation of esterase and peroxidase isozymes in pearl millet (*Pennisetum glacum* (L.) R. Br.). Indian J. Biotechnol. 6:91-99.

Soltis DE, Haufler CHD, Darrow C, Gastony GJ (1983). Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers and staining schedules. Am. Fern J. 73:9-27.

Takayama S, Misawa M (1980). Differentiation in *Lilium* bulbscales grown *in vitro* Effects of activated charcoal, physiological age of bulbs and sucrose concentration of differentiation and scale leaf formation. *In vitro* Physiol. Plant. 48:121-125.

Taylor JLS, Van Staden J (1998). Plant-derived smoke solutions stimulate the growth of *Lycopersicon esculentum* roots *in vitro*. Plant Growth Reg. 26: 77-83.

Weber K, Osborn M (1969). The Reliability of Molecular Weight Determinations by Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis. J. Biol. Chem. 244:4406-4412.

Williams CA, Harbome JB, Mayo SJ (1981). Anthocyaninpigments and leaf flavonoids in the family Araceae. Phytochemistry. 20:217-234.

Yang W, Oliveira AC, Godwin I, Schertz K, Ben netzen JL (1996). Comparison of DNA marker technologies in characterizing plant genome diversity: variability in Chinese sorghums. Crop Sci. 36:1669-1676.

Ziv M (1979). Transplanting *Gladiolus* plants propagated *in vitro*. Sci. Hort. 11:257-26