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# A New Aspect for *In vitro* Propagation of Jerusalem Artichoke and Molecular Assessment Using RAPD, ISSR and SCoT Marker Techniques

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JERUSALEM artichoke is an important crop for a wide range of agricultural, medicinal and industrial purposes. Its tubers are rich in inulin and it is cultivated for food and animal feed. The current study aimed to develop an applicable protocol for micropropagation of three cultivars of Jerusalem artichoke (i.e., Balady, Fuza and Alba) using stem node explants. These explants were cultured on ½ MS medium augmented with different concentrations of kanamycin or cefotaxime. The lowest contamination correlated with highest survival percentages were recorded for ½ MS supplemented with 62.5mg $L^{-1}$ cefotaxime. For shoot multiplication, the maximum number of shoots (11) were obtained for Alba cultivar cultured on MS fortified with 1mg $L^{-1}$ BA + 0.1mg $L^{-1}$ NAA + 50mg $L^{-1}$ nano selenium. For rooting *in vitro*, the maximum values of rooting percent, root numbers/plantlet and length of roots were observed with ½ MS + 2mg $L^{-1}$ IBA + 0.1mg $L^{-1}$ NAA + 0.5mg $L^{-1}$ KIN. Resulted *in vitro* regenerated plantlets, were acclimatized on perlite and peat moss mixture (1:1), which gave the highest percent of survival (100%) for Alba followed by Fuza and Balady (80 and 60%, respectively). Moreover, the molecular characterization based on RAPD, ISSR and SCoT techniques was carried out. The polymorphic percentages among *in vivo* shoots of the three cultivars recorded 38.09, 42.3 and 34.61%, respectively; 61.53, 67.8 and 50% between *in vivo* shoots, *in vitro* regenerates and stem derived calli of the three cultivars. The dendrogram analysis of combined techniques showed high similarity between Balady and Fuza followed by Alba.

**Keywords:** Asteraceae, *Helianthus tuberosus*, Markers, Molecular assessment, Stem nodes.

## Introduction

Jerusalem artichoke (*Helianthus tuberosus* L.) is a perennial plant known as sunroot, sunchoke, earth apple or topinambur and belongs to family Asteraceae. It is native to Eastern North America and closely related to sunflower (*Helianthus annuus* L.). Its tubers are rich in inulin (Monti et al., 2005; Tassoni et al., 2010), a valuable source of fructose which is frequently prescribed for diabetics (Rani, 1997). J. artichoke is exploited for a wide range of uses or technologies where it is cultivated as a forage and vegetable crop, or a source of inulin which is fundamental for food, medical and industrial purposes (Kaszás et al., 2018) as well as for bioethanol production (Long et al., 2016; Paixão et al., 2018; Kotsanopoulos et al., 2019). J. artichoke has been intensively investigated recently due to its advantageous characteristics including the high tolerance to different stresses such as drought (Puangbut et al., 2017), salinity (Fang et al., 2018; Luo et al., 2018), waterlogging (Yan et al., 2018) and the high productivity and elevated growth rate with minimal to zero fertilizer requirements (Kotsanopoulos et al., 2019). Traditionally, the tubers, stem cuttings, rhizomes and transplants emerged from sprouted tubers could be used for vegetative propagation of J. artichoke but the seeds for breeding (Kays & Nottingham, 2008).

Despite its importance, there are rare published

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literatures on *in vitro* propagation of J. artichoke due to one or more greatest challenges *(in vitro* hyper-hydricity; Abdalla et al., 2014), the long period of dormancy (about 4– 6 months; Kays & Nottingham, 2008) and high contamination rate (Abdalla et al., 2014). The previous few studies have reported that the *in vitro* propagated J. artichoke could be achieved on a simple nutrient medium by repeated subcultures from axillary meristems of *in vivo* growing plants (Gamburg et al., 1999); somatic embryogenesis (El Mostafa et al., 2008); *in vitro* regeneration (Karadag et al., 2013). The *in vitro* plantlets provided stem node segments for micro-tubers formation (Gamburg et al., 1999). Therefore, there is an urgent need to address the *in vitro* propagation of J. artichoke due to its obvious advantages as providing pathogen-free plant materials, massive vegetative propagules and production of secondary metabolites. Moreover, germplasm conservation and production of transgenic plants can also be achieved (Kays & Nottingham, 2008).

In the last decades, genetic stability of regenerated plantlets has been estimated by phenotypical, cytological, phytochemical and protein-based markers. Further, more advanced techniques based on DNA molecular markers have been employed to validate the genetic stability of *in vitro* derived plantlets (Chavan et al., 2015), due to reliable, reproducible and environmental independent results which are generated by molecular marker techniques. Therefore, a considerable literature has grown up around the theme of the assessments of genetic structure and genetic relatedness in plant germplasm (Hamblin et al., 2007; Ingvarsson & Street, 2011). Genetic homogeneity of regenerated plantlets was successfully tested using Random Amplified Polymorphic DNA (RAPD) and Inter-Simple Sequence Repeat (ISSR). Recent developments in the field of plant molecular analysis have gained considerable interest in using gene targeted markers like Start Codon Targeted (SCoT) and CAAT Box Derived Polymorphism (CBDP) (Asthana et al., 2011; Rai et al., 2012; Sharma et al., 2019). RAPD and ISSR techniques depend on uncoding regions of genome whereas SCoT which is a reliable and simple dominant marker is correlated to functional genes and their corresponding traits as well (Xiong et al., 2011).

The specific objective of this study was to develop a cost effective and efficient *in vitro* propagation protocol for three cultivars of J. artichoke; Balady, Fuza and Alba. Moreover, molecular characterization using RAPD, ISSR and SCoT techniques was employed to confirm the genetic fidelity of the regenerated plantlets.

## Materials and Methods

This study was carried out at Plant Biotechnology Department, Genetic Engineering and Biotechnology Research Division, National Research Centre, Egypt in cooperation with Horticulture Department, Faculty of Agriculture, Ain Shams University, during 2016 and 2017.

*Plant materials*

Tubers of Jerusalem artichoke Balady were obtained from Agricultural Research Center, Giza; whereas, tubers of Fuza obtained from Ismailia and the tubers of Alba were obtained from Debrecen University, Hungary. The tubers of the three cultivars were cultivated in May 2015 in the experimental farm of faculty of Agriculture, Cairo University and harvested in December 2015. A part of these tubers has been stored in soil *in situ* and covered with their dry stalks until April 2016. In May 2016, mulching of stalks totally removed and the soil was irrigated regularly until the new shoots were produced. Shoots (about 3-4 months old) were collected mainly in September 2016 as a source of stem node explants.

*Establishment of aseptic cultures*

The shoots were cut into stem nodes (~3cm each) containing buds. Then, these explants were washed using water containing antiseptic substance followed by running tap water for one hour followed by immersion in 70% ethanol for 30sec. The explants were surface sterilized under air laminar flow followed by four washes using sterile distilled water. Further, stem nodes were primed in 30% of commercial Clorox (sodium hypochlorite 5.25%) containing two drops of tween 20 and shaked well for 15 min then washed using sterile distilled water four times. The explants were then immersed in 0.02% mercury chloride for few seconds, then washed using sterile distilled water four times. The sterilized explants were cultured on the following media as follows:

$S_1$= ½ MS without antibiotic
$S_2$= ½ MS + 50mg $L^{-1}$ kanamycin
$S_3$= ½ MS + 100mg $L^{-1}$ kanamycin
$S_4$= ½ MS + 200mg $L^{-1}$ kanamycin
$S_5$= ½ MS + 62.5mg $L^{-1}$ cefotaxime

$S_6$= ½ MS + 125mg $L^{-1}$ cefotaxime
$S_7$= ½ MS + 250mg $L^{-1}$ cefotaxime

All media were fortified with 30g $L^{-1}$ sucrose and 7g $L^{-1}$ agar. 0.1N KOH/ HCl were used to adjust the pH of media to 5.8. The media were distributed into 300ml glass jars, where each jar contained 50ml and sterilized using the autoclave for 15min at 121ºC and 1.2kg $cm^{-2}$. The antibiotic (kanamycin or cefotaxime) was added by filtration using 0.2-µm Acrodisc syringe filters (USA). The cultures were incubated under dark conditions for one week at 26±1ºC then incubated under light intensity of 1500 lux provided by fluorescent lamps for 16hrs photoperiod. Five replicates (jars) for each treatment were used and every replicate contained two stem nodes. After two weeks of culture, the contamination (%) and survival (%) were estimated.

*Shoot multiplication*

Stem node explants derived from *in vitro* plantlets (6 weeks old) were cultured on the following media:

$M_1$= MS basal (free growth regulators)

$M_2$= MS + 1mg $L^{-1}$ BA + 0.1 mg $L^{-1}$ NAA

$M_3$= MS + 1mg $L^{-1}$ BA + 0.1 mg $L^{-1}$ NAA + 50 mg $L^{-1}$ nano-Selenium

Number of shootlets/explant, shootlet length (cm) and number of leaves/shootlet were recorded after five weeks from culture.

*In vitro rooting*

The multiplicative shootlets of Jerusalem artichoke were separated and cultured on the following media:

$R_1$= ½ MS basal (free growth regulators)
$R_2$= ½ MS + 2mg $L^{-1}$ NAA
$R_3$= ½ MS + 2mg $L^{-1}$ NAA + 0.1 mg $L^{-1}$ IBA
$R_4$= ½ MS + 0.1mg $L^{-1}$ NAA + 2 mg $L^{-1}$ IBA + 0.5 mg $L^{-1}$ KIN

Five weeks from culture on rooting media, number of roots, rooting percent and length of roots (cm) were estimated.

*Acclimatization*

In vitro derived plantlets were washed from the medium residues and cultured into three different compositions of growing media, i.e., peat moss, perlite: peat moss (1:1 v/v) and perlite: peat moss (1:2 v/v). Survival (%) and plant length (cm) after one month of acclimatization were recorded.

*Molecular assessment using RAPD, ISSR and SCoT based markers*

DNA isolation procedure was performed according to the described method by DNeasy Plant Mini Kit (QIAGEN). The names of primers and their nucleotide sequences used for RAPD, ISSR and SCoT techniques are listed in Table 1.

**TABLE 1. Names of primers and their nucleotide sequences used for RAPD, ISSR and SCoT techniques.**

| | Name | Sequence |
|---|---|---|
| **RAPD** | OP- B9 | 5´ TGG GGG ACT C 3` |
| | OP-C9 | 5´ CTC ACC GTC C 3` |
| | OP-C13 | 5` AAG CCT CGT G 3` |
| | OP-K1 | 5´ TGC CGA GCT G 3` |
| | OP-K3 | 5 CCC TAC CGA C 3` |
| **ISSR** | 44A | 5 CTC TCT CTC TCT CTC TTG 3` |
| | 44B | 5` CTC TCT CTC TCT CTC TGC 3` |
| | HB-9 | 5`CAC CAC CAC GC 3` |
| | HB-12 | 5`CAC CAC CAC GC 3` |
| | HB-15 | 5´ GTG GTGGTG GC 3` |
| **SCoT** | SCoT 2 | ACC ATG GCT ACC ACC GGC |
| | SCoT 3 | ACG ACA TGG CGA CCC ACA |
| | SCoT 4 | ACC ATG GCT ACC ACC GCA |
| | SCoT 9 | ACA ATG GCT ACC ACT ACC |
| | SCoT 10 | ACA ATG GCT ACC ACC AGC |

*Data analysis*

The Program of Gel works ID advanced software UVP-England was applied to measure the similarity matrices. The relationships among genotypes as resulted from dendrogram were determined using SPSS windows (Version 10, 1983) program. According to Yang & Quiros (1993), the plot of phenogram and pairwise difference matrix among cultivars were calculated using DICE computer package (Dice, 1945).

*Statistical analysis*

All stages of the experiment were set up in factorial experiments in five replicates. All data were analyzed using Duncan's multiple range test at 0.05 level through statistical analysis software (CoHort 2004, USA) according to Snedecor & Corchan (1980) to verify the differences between means of treatments.

## Results and Discussion

*Establishment of aseptic cultures*

The lowest contamination (0%) and the highest survival (100%) was recorded with $S_5$ (½ MS + 62.5mg $L^{-1}$ cefotaxime) followed by $S_3$ (½ MS + 100mg $L^{-1}$ kanamycin) for all cultivars and no significant differences were found between them (Table 2; Fig. 1, photos 4 and 5). Antibiotics are used in plant tissue culture to prevent, control, or eliminate persistent bacterial contamination where surface sterilants alone are not effective (Bunn & Tan, 2002). Cefotaxime is a semisynthetic analog of cephalosporin, the third-generation antibiotic secreted by fungi from the genus *Cephalosporum.* As mentioned in the literature review, cephalosporin antibiotics are effective at relatively low concentrations; they have a rather wide spectrum of biological activity and minimum toxicity on eukaryotes (Dias & Dolgikh, 1997). A previous study has noted the stimulatory effect of some antibiotics, cefotaxime in particular, on callus induction, growth and morphogenesis of maize (Danilova & Dolgikh, 2004). They found that cefotaxime did not affect the induction frequency and growth of the embryogenic callus but enhanced its morphogenesis into regenerated plantlets.

*Shoot multiplication*

The highest significant value for number of shootlets/explant (11) was recorded for Alba cultivar cultured on MS + 1mg $L^{-1}$ BA + 0.1mg $L^{-1}$ NAA + 50mg $L^{-1}$ nano-Se compared to the other values followed by Fuza on the same medium. While the minimum number of shootlets/explant (2), was noticed for Balady cultured on MS basal medium with significant differences with the other values. Moreover, the maximum number of leaves/ shootlet was achieved on the same medium for Fuza without significant differences with the other cultivars. On the other hand, MS basal medium was the best medium for shootlet length for all cultivars without significant differences among them (Table 3; Fig. 1, photo 6).

**TABLE 2. Impact of different antibiotic treatments on contamination and survival percent after two weeks from culturing stem nodes of Jerusalem artichoke cultivars during 2016 – 2017.**

| Treatments | Contamination (%) | | | Survival (%) | | |
|---|---|---|---|---|---|---|
| | Balady | Fuza | Alba | Balady | Fuza | Alba |
| $S_1$= ½ MS without anti. | 100c | 100c | 100c | 0.0f | 0.0f | 0.0f |
| $S_2$= ½ MS + 50mg $L^{-1}$ kan. | 50b | 50b | 50b | 25d | 26.3d | 28d |
| $S_3$= ½ MS + 100mg $L^{-1}$ kan. | 0a | 0a | 0a | 90ab | 91.5ab | 94.0a |
| $S_4$= ½ MS + 200mg $L^{-1}$ kan. | 0a | 0a | 0a | 72c | 74.4c | 75.3c |
| $S_5$= ½ MS + 62.5mg $L^{-1}$ cef. | 0a | 0a | 0a | 100a | 100a | 100a |
| $S_6$= ½ MS + 125mg $L^{-1}$ cef. | 0a | 0a | 0a | 80bc | 80bc | 80bc |
| $S_7$= ½ MS + 250mg $L^{-1}$ cef. | 0a | 0a | 0a | 10.2ef | 12.2e | 14.2e |

- Each treatment was the mean of 5 replicates and each jar contains 2 explants.
- Values followed by the same letters were not significantly different by Duncan's test at 0.05 level.
- Note: Stem nodes were taken from *in vivo* shoots (3-4 months old).
- Antibiotic, anti; kanamycin (kan.) and cefotaxime (cef.).

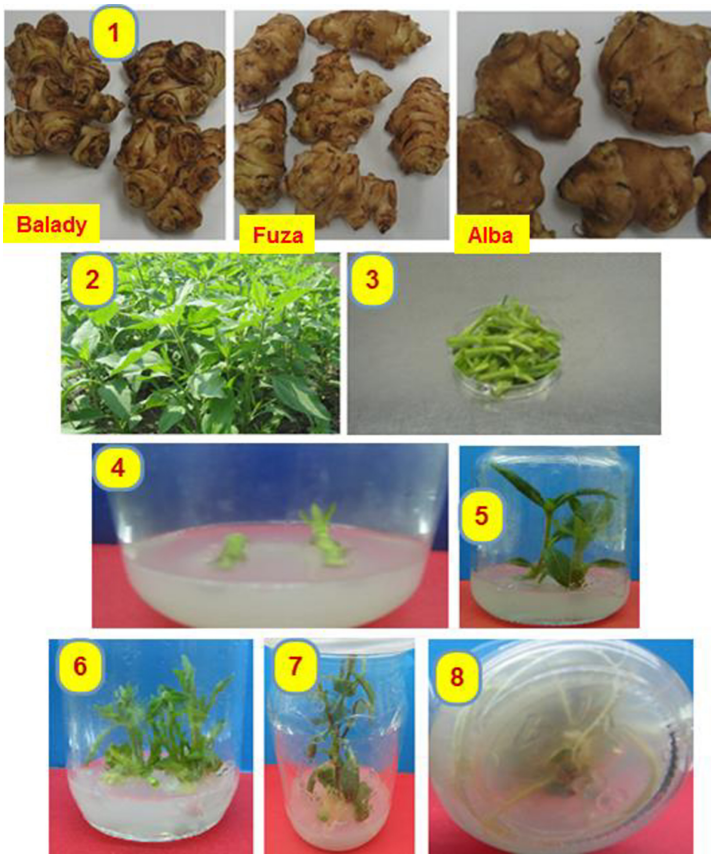



**Fig. 1. Micropropagation of Jerusalem artichoke cultivars. Balady, Fuza and Alba (photo 1); *In vivo* plants 3-4 months old as a source of stem node explants (photo 2); Stem node explants (photo 3); Establishment of aseptic cultures from stem nodes (photos 4 and 5); Shoot multiplication (photo 6); *In vitro* rooting (photo 7); Rooted plant (photo 8).**

**TABLE 3. Effect of supplementation of MS medium with BA and NAA with/without Nano-Se on No. of shootlets produced from stem node explant, shootlet length (cm) and No. of leaves/shootlet of Jerusalem artichoke cultivars after 5 weeks of culture and incubated at 26± 1ºC and 16/8hrs photoperiod.**

| Treatments | Balady | Fuza | Alba |
|---|---|---|---|
| **No. of shootlets/explant** | | | |
| $M_1$= MS basal (free growth regulators) | 2h | 3g | 4f |
| $M_2$= MS + 1mg $L^{-1}$ BA + 0.1mg $L^{-1}$ NAA | 4f | 6d | 7c |
| $M_3$= MS + 1mg $L^{-1}$ BA + 0.1mg $L^{-1}$ NAA + 50mg $L^{-1}$ Nano-Se | 5e | 8b | 11a |
| **Shootlet length** | | | |
| $M_1$= MS basal (free growth regulators) | 9.38a | 9.00a | 9.38a |
| $M_2$= MS + 1mg $L^{-1}$ BA + 0.1mg $L^{-1}$ NAA | 2.25c | 2.00c | 2.25c |
| $M_3$= MS + 1mg $L^{-1}$ BA + 0.1mg $L^{-1}$ NAA + 50mg $L^{-1}$ Nano-Se | 4.20bc | 3.0bc | 4.20bc |
| **No. of leaves/shootlet** | | | |
| $M_1$= MS basal (free growth regulators) | 5.83d | 6.0cd | 6.63abcd |
| $M_2$= MS + 1mg $L^{-1}$ BA + 0.1mg $L^{-1}$ NAA | 6.50bcd | 6.7abc | 7.00ab |
| $M_3$= MS + 1mg $L^{-1}$ BA + 0.1mg $L^{-1}$ NAA + 50mg $L^{-1}$ Nano-Se | 7.00ab | 7.42a | 6.90ab |

- Each treatment was the mean of 5 replicates and each Jar contains 2 explants.
- Values followed by the same letters were not significantly different by Duncan multiple test at 0.05 level

Several reports have shown that *in vitro* regeneration could be considered one of the most common plant tissue culture techniques for overcoming the environmental conditions. One interesting finding is plant growth regulators (PGRs) which are crucial substances for shoot multiplication in several plant species. In most culture media, cytokinins are important and critical plant growth regulators (Bekircan et al., 2018; Sandhu et al., 2018). It was found that axillary shoot multiplication of sea oats (*Uniola paniculata* L.) could be enhanced by adding benzyl adenine (BA) (Valero-Aracama et al., 2010). This result seems to be consistent with other researches which found that supplementation of MS medium with 2mg $L^{-1}$ BAP gave the best result for frequency of shoot formation of *Rumex pictus* Forssk (El-Shafey et al., 2019).

Recently, Sandhu et al. (2018) reported that the multiplication rate of shoot in different bamboo species could be improved by the combination of auxin and cytokinins. Similarly, positive and enhancing effect of naphthalene acetic acid (NAA) and BA combinations on shoot multiplication rate was recorded by many researchers such as Somashekar et al. (2008) on *Pseudoxytenanthera stocksii*, Agnihotri et al. (2009) on *Dendrocalamus hamiltonii*, Bisht et al. (2010) on *Gigantochloa atroviolacea* and Beena et al. (2012) on *Bambusa pallida*. On the other hand, Shahin & De Klerk **(**2020) confirmed the enhancement potential of fluridone and imazalil, as new regulator substances, in combination with benzylaminopurine on *in vitro* shoot branching of *Zantedeschia sprengeri* (Paco).

Selenium nanoparticles have wide applications in the fields of medicine, microelectronic, agriculture and animal husbandry, because of its biological and industrial properties. In recent years, there has been an increasing amount of literature on nanotechnology and its applications in the field of plant tissue culture (Álvarez et al., 2019; Abdel-Wahab et al., 2020). It has been noted that callus production and *in vitro* rooting of tobacco could be stimulated by high concentrations of selenium nanoparticles (265–532µM), where recent evidence suggests that selenium nanoparticles can improve the growth, morphogenesis of tobacco and inhibit the vitrification of tobacco plantlets without any change in the chlorophyll content (Shoeibi et al., 2017).

*In vitro rooting*

Rooting percentages were significantly differed among treatments but there were no differences among cultivars under the same treatment (Fig. 1, photos 7 and 8). The maximum rooting formation (100%) was recorded on $R_4$ (½ MS + 0.1mg $L^{-1}$ NAA + 2mg $L^{-1}$ IBA + 0.5mg $L^{-1}$ KIN) for all cultivars, whereas the minimum rate of rooting (40%) was obtained with $R_1$ (½ MS free growth regulators) (Table 4). There were significant differences for root length and roots number among treatments and cultivars under the same treatment (Table 4). The medium (R4) produced the longest root lengths (10.3, 5.4 and 5.1cm) for Alba, Fuza and Balady, respectively. On the other hand, the minimum root lengths (1.4, 2.0 and 2.3cm) were achieved on $R_1$ for Balady, Fuza and Alba, respectively. The same direction of root length was recorded for number of roots, where the best result was observed on $R_4$ for Alba followed by Fuza and Balady (6.0, 4.8 and 4.5, respectively). However, the lowest number of roots formation (2.0, 2.2 and 2.4) was obtained on $R_1$ for Balady, Fuza and Alba, respectively. It is concluded that $R_4$ achieved the highest values for rooting percentage, roots length and roots number, respectively for all cultivars and the Alba was the best. Contrary, Karadag et al. (2013) noticed that root formation of tuberous plants was enhanced using gibberellic acid. Where, the regenerated plantlets of J. artichoke were rooted efficiently on MS medium containing 1.0mg $L^{-1}$ gibberellic acid.

Root initiation and development of regenerated shootlets are required for the successful micropropagation protocol. Healthy and developed roots enable plantlets to acclimatize worthily (Dewir et al., 2016). Many studies reported that high rates of root induction can be resulted on half strength MS medium, *Pongamia pinnata* (Sujatha et al., 2008), cassia plants (Agrawal & Sardar, 2007; Parveen & Shahzad, 2010; Parveen et al., 2010) and *Albizia lebbek* (Perveen et al., 2011).

*Acclimatization*

*In vitro* propagation offers intensive and rapid regeneration, pathogen free and true to type planting material regardless of the weather conditions and the season. However, the application of this technology in large scale has been faced by main constraints represented in low survival of *in vitro* regenerated plantlets under *ex vitro* acclimatization conditions. So, the commercial production of *in vitro* propagated plants needs to be low cost with high survival

rates (Chandra et al., 2010). In this context, the obtained results clearly indicated that rooted shootlets were successfully acclimatized on the mixture of peat moss and perlite (1:1) for all cultivars with significant differences between them and with high survival percentage reaching to 100% for Alba (Table 5). Also, the maximum plant height for all cultivars was recorded with the same mixture and the Alba was the longest (10.6cm).

**TABLE 4. Effect of MS medium fortified with different concentrations of IBA and NAA on rooting (%), roots length (cm) and number of roots after 5 weeks of culture.**

| Treatments | Balady | Fuza | Alba |
|---|---|---|---|
| **Rooting (%)** | | | |
| $R_1$= ½ MS basal (free growth regulators) | 40d | 40d | 40d |
| $R_2$= ½ MS + 2mg $L^{-1}$ NAA | 60c | 60c | 60c |
| $R_3$= ½ MS + 2mg $L^{-1}$ NAA + 0.1mg $L^{-1}$ IBA | 80b | 80b | 80b |
| $R_4$= ½ MS + 2mg $L^{-1}$ IBA + 0.1mg $L^{-1}$ NAA + 0.5mg $L^{-1}$ KIN | 100a | 100a | 100a |
| **Roots length (cm)** | | | |
| $R_1$= ½ MS basal (free growth regulators) | 1.40l | 2.00k | 2.30j |
| $R_2$= ½ MS + 2mg $L^{-1}$ NAA | 2.60i | 2.70h | 2.90g |
| $R_3$= ½ MS + 2mg $L^{-1}$ NAA + 0.1mg $L^{-1}$ IBA | 3.00f | 3.50e | 4.20d |
| $R_4$= ½ MS + 2mg $L^{-1}$ IBA + 0.1mg $L^{-1}$ NAA + 0.5mg $L^{-1}$ KIN | 5.10c | 5.40b | 10.30a |
| **Number of roots** | | | |
| $R_1$= ½ MS basal (free growth regulators) | 2.00l | 2.20k | 2.40j |
| $R_2$= ½ MS + 2mg $L^{-1}$ NAA | 2.60i | 3.00h | 3.10g |
| $R_3$= ½ MS + 2mg $L^{-1}$ NAA +0.1mg $L^{-1}$ IBA | 3.20f | 3.60e | 4.00d |
| $R_4$= ½ MS + 2mg $L^{-1}$ IBA + 0.1mg $L^{-1}$ NAA + 0.5mg $L^{-1}$ KIN | 4.50c | 4.80b | 6.00a |

- Shoots of Jerusalem artichoke of different cultivars were cultured and incubated under light condition (16/8) at 26± 1ºC.
- Each treatment was the average of 5 replicates and each jar contains one shootlet.
- Values followed by the same letters were not significantly different by Duncan's test at 0.05 level.

**TABLE 5. Influence of peat moss and perlite mixture on survival (%) and plant length (cm) of *in vitro* derived plantlet of Jerusalem artichoke cultivars.**

| Treatments | Survival (%) | | | Plant length (cm) | | |
|---|---|---|---|---|---|---|
| | Balady | Fuza | Alba | Balady | Fuza | Alba |
| Peat moss | 20e | 40d | 60c | 4.35i | 5.0h | 5.8g |
| Peat moss: perlite (1:1) | 60c | 80b | 100a | 7.0e | 8.0c | 10.6a |
| Peat moss: perlite (2:1) | 40d | 60c | 80b | 6.25f | 7.5d | 8.7b |

- Each treatment was the average of 5 replicates and each pot contains one shootlet.
- Values followed by the same letters were not significantly different by Duncan's test at 0.05 level.

*Molecular assessment using RAPD, ISSR and SCoT based markers*

As shown in Table 6 and Fig. 2, RAPD banding profiles produced by using OP-B9; OP-C9; OP-C13; OP-K1 and OP-K3 primers in the examined samples of Jerusalem artichoke cultivars; Balady (lane1 *in vivo* shoots; lane 2 *in vitro* regenerates and lane 3 stem derived callus); Fuza (lane 4 *in vivo* shoots; lane 5 *in vitro* regenerates and lane 6 stem derived callus) and Alba (lane7 *in vivo* shoots; lane 8 *in vitro* regenerates and lane 9 stem derived callus) showed that OP-B9 primer presented 10 bands giving 4 monomorphic and 6 polymorphic bands with 60% polymorphism among *in vivo* and *in vitro* regenerates and calli cultures for the three cultivars. While, OP-C9 primer showed 6 bands resulted into 2 monomorphic and 4 polymorphic band produced 66.66% polymorphism. However, OP-C13 primer showed 3 bands resulted into 2 monomorphic and

1 polymorphic band with 33.33% polymorphism. While, OP-K1 primer gave 4 bands resulted into 1 monomorphic and 3 polymorphic band with 75% polymorphism. Using OP-K3 primer recorded 3 bands resulted into 1 monomorphic and 2 polymorphic bands with 66.66% polymorphism. Further, the total polymorphism after applying the RAPD technique was 61.53% between the *in vivo* and *in vitro* cultures (shootlets and callus) of the three cultivars under study. While, applying of RAPD technique for molecular distinguish among the three *in vivo* cultivars of Jerusalem artichoke Balady (lane 1); Fuza (lane 4) and Alba (lane 7) based DNA fingerprints (Table 7 and Fig. 3); OP-B9 primer presented 7 bands giving 6 monomorphic, 1 polymorphic and 1 unique band with 14.28% polymorphism while, OP-C9 primer showed 6 bands resulted 3 monomorphic, 3 polymorphic and 3 unique bands produced 50% polymorphism. However, OP-C13 primer showed 3 bands resulted into 2 monomorphic, 1 polymorphic and 1 unique band with 33.33% polymorphism. While, OP-K1 primer gave 3 bands resulted into 1 monomorphic, 2 polymorphic and 2 unique bands with 66.66% polymorphism. Using OP-K3 primer recorded 2 bands resulted into 1 each of monomorphic, polymorphic and unique bands with 50% polymorphism. Furthermore, the total polymorphism after applying the RAPD method among the *in vivo* cultivars under study recorded 38.09%. Moreover, the dendrogram of RAPD analysis for three cultivars was carried out (Fig. 4). the dendrogram analysis showed high similarity between Balady and Fuza (100%), while low similarity was recorded between Alba and the other cultivars (0.35%). It can be concluded that primers OP-B9 and OP-K3 can be used to distinguish among *in vitro* derived cultures from the three cultivars of Jerusalem artichoke. However, OP-K1 can be used for *in vivo* cultivars of Jerusalem artichoke. Balady and Fuza have a 100% similarity, however, the similarity percentage between Alba compared to either of Balady or Fuza was 0.35% only.

**TABLE 6. Polymorphism percent detected by the five primers of RAPD, ISSR and SCoT techniques for *in vivo* and *in vitro* derived cultures of Jerusalem artichoke cultivars.**

| Primer Name | Total band | Monomorphic band | Polymorphic band | Polymorphism % |
|---|---|---|---|---|
| **RAPD** | | | | |
| OP-B9 | 10 | 4 | 6 | 60% |
| OP-C9 | 6 | 2 | 4 | 66.66% |
| OP-C13 | 3 | 2 | 1 | 33.33% |
| OP-K1 | 4 | 1 | 3 | 75% |
| OP-K3 | 3 | 1 | 2 | 66.66% |
| Total | 26 | 10 | 16 | 61.53% |
| **ISSR** | | | | |
| 44A | 4 | 3 | 1 | 25% |
| 44B | 4 | 1 | 3 | 75% |
| HB-9 | 7 | 5 | 2 | 28.5% |
| Hb-12 | 6 | 1 | 5 | 83.3% |
| HB-15 | 7 | 2 | 5 | 71.4% |
| Total | 28 | 9 | 19 | 67.8% |
| **SCoT** | | | | |
| SCoT 2 | 6 | 3 | 3 | 50% |
| SCoT 3 | 4 | 2 | 2 | 50% |
| SCoT 4 | 7 | 5 | 2 | 28.5% |
| SCoT 9 | 5 | 2 | 3 | 60% |
| SCoT 10 | 4 | 1 | 3 | 75% |
| Total | 26 | 13 | 13 | 50% |

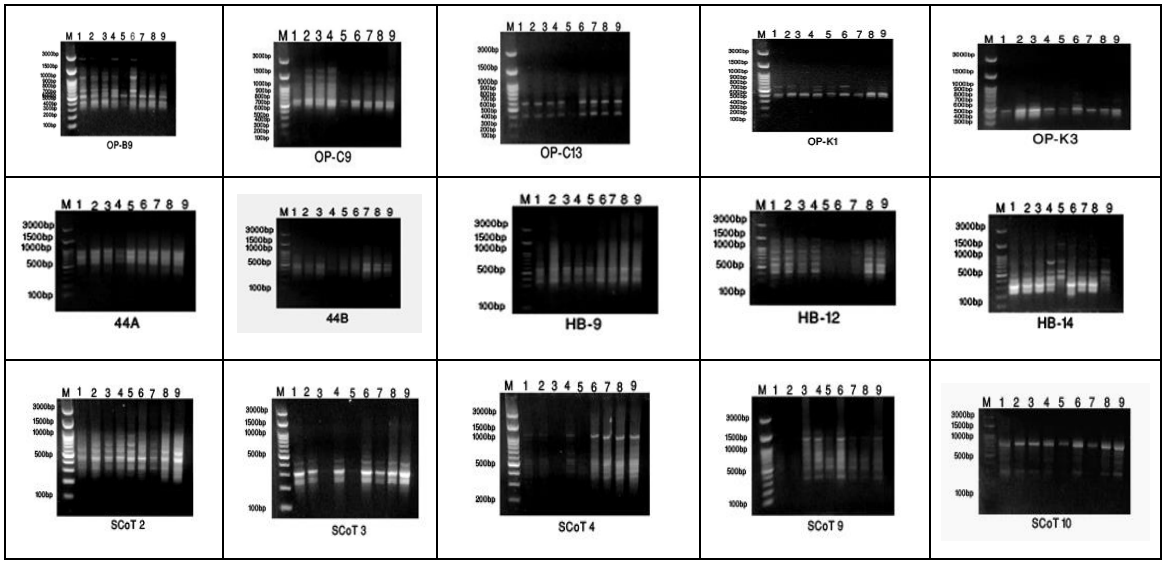



**Fig. 2. RAPD, ISSR and SCoT amplification electrophoretic profiles of Jerusalem artichoke cultivars; Balady (lane1 *in vivo* shoots; lane 2 *in vitro* regenerates and lane 3 stem derived callus); Fuza (lane 4 *in vivo* shoots; lane 5 *in vitro* regenerates and lane 6 stem derived callus) and Alba (lane7 *in vivo* shoots; lane 8 *in vitro* regenerates and lane 9 stem derived callus) [M= DNA marker (1Kb ladder); Row 1 RAPD, Row 2 ISSR and Row 3 SCoT].**

**TABLE 7. Polymorphism percent detected by the five primers of RAPD, ISSR and SCoT techniques for *in vivo* shoots of Jerusalem artichoke cultivars.**

| Primer Name | Total Band | Monomorphic Band | Polymorphic Band | Unique Band | Polymorphism % |
|---|---|---|---|---|---|
| **RAPD** | | | | | |
| OP-B9 | 7 | 6 | 1 | 1 | 14.28% |
| OP-C9 | 6 | 3 | 3 | 3 | 50% |
| OP-C13 | 3 | 2 | 1 | 1 | 33.33% |
| OP-K1 | 3 | 1 | 2 | 2 | 66.66% |
| OP-K3 | 2 | 1 | 1 | 1 | 50% |
| Total | 21 | 13 | 8 | 8 | 38.09% |
| **ISSR** | | | | | |
| 44A | 4 | 3 | 1 | 1 | 25% |
| 44B | 4 | 1 | 3 | 3 | 75% |
| HB-9 | 7 | 5 | 2 | 2 | 28.5% |
| HB-12 | 6 | 2 | 4 | 4 | 66.66% |
| HB-15 | 5 | 4 | 1 | 1 | 20% |
| Total | 26 | 15 | 11 | 11 | 42.3% |
| **SCoT** | | | | | |
| SCoT 2 | 6 | 3 | 3 | 2 | 50% |
| SCoT 3 | 4 | 4 | - | - | - |
| SCoT 4 | 7 | 6 | 1 | 1 | 14.28% |
| SCoT 9 | 5 | 2 | 3 | 3 | 60% |
| SCoT 10 | 4 | 2 | 2 | 2 | 50% |
| Total | 26 | 17 | 9 | 8 | 34.61% |

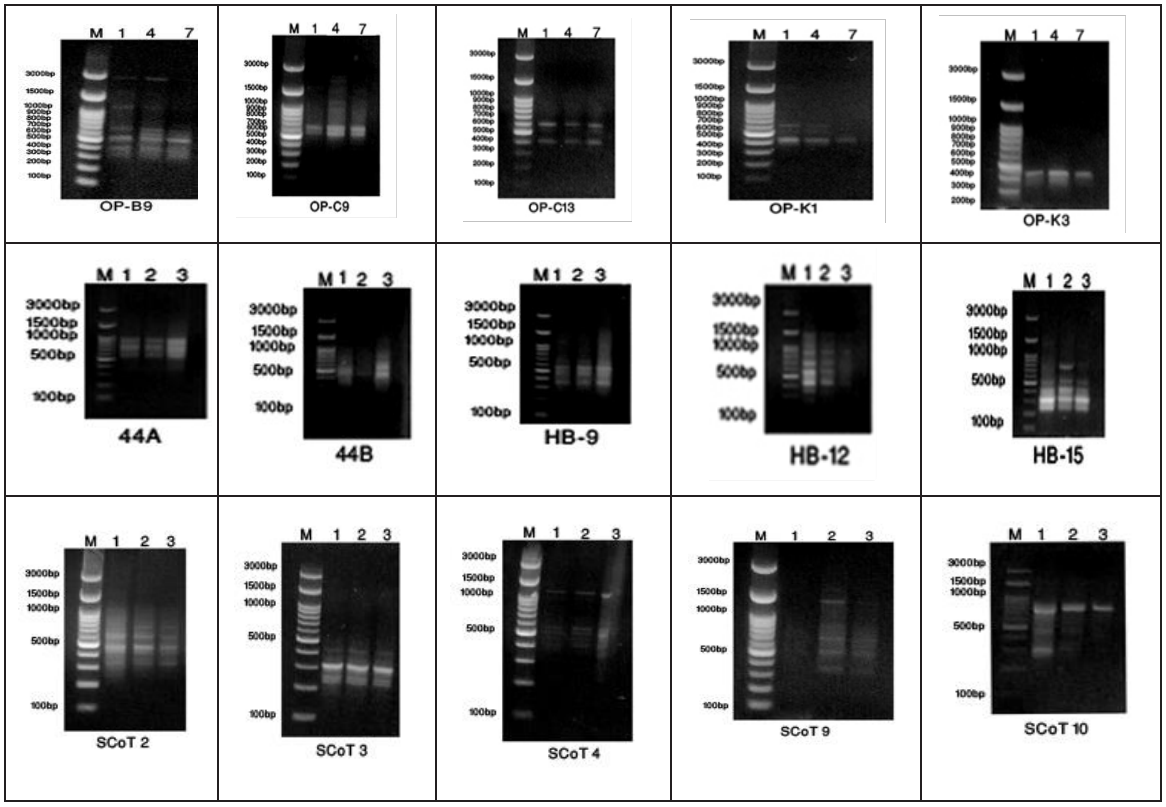
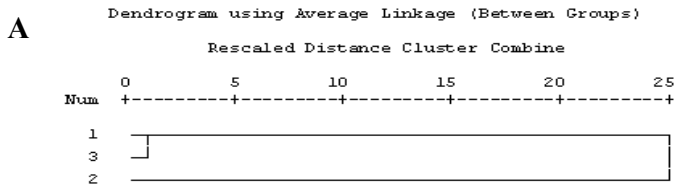



**Fig. 3. RAPD, ISSR and SCoT amplification electrophoretic profiles of Jerusalem artichoke cultivars; lane1 *in vivo* shoots of Balady; lane 2 or 4 *in vivo* shoots of Fuza and lane 3 or 7 *in vivo* shoots of Alba [M= DNA marker (1Kb ladder); Row 1 RAPD, Row 2 ISSR and Row 3 SCoT].**

ISSR banding profiles produced by the five primers; 44A, 44B, HB-9, HB-12 and HB-15 in the three cultivars of Jerusalem artichoke are illustrated in Table 6 and Fig. 2; Balady (lane1 *in vivo* shoots; lane 2 *in vitro* regenerates and lane 3 stem derived callus); Fuza (lane 4 *in vivo* shoots; lane 5 *in vitro* regenerates and lane 6 stem derived callus) and Alba (lane7 *in vivo* shoots; lane 8 *in vitro* regenerates and lane 9 stem derived callus). 44A primer presents 4 bands giving 3 monomorphic and 1 polymorphic band with 25 polymorphic percentages. However, 44 B primer showed 4 bands resulted into 1 monomorphic and 3 polymorphic bands produced 75 polymorphic percentage. While, HB-9 primer showed 7 bands resulted into 5 monomorphic and 2 polymorphic bands with 28.5% polymorphic. While, HB-12 primer gave 6 bands resulted into 1 monomorphic and 5 polymorphic bands with 83.3 polymorphic percentage. Using HB-15 primer recorded 7 bands resulted into 2 monomorphic and 5 polymorphic bands with 71.4 polymorphic percentage. Furthermore, the total polymorphic percentage after applying the ISSR technique was 67.8% between the *in vivo* cultivars and *in vitro* derived cultures (shoots or callus) of the three cultivars of Jerusalem artichoke under investigation. Regarding the application of ISSR technique for molecular distinguish among the three *in vivo* cultivars of Jerusalem artichoke Balady (lane 1); Fuza (lane 4) and Alba (lane 7) based DNA fingerprints (Table 7 and Fig. 3); 44A primer presents 4 bands giving 3 monomorphic, 1 polymorphic and 1 unique band with 25 polymorphism percentage. While, 44 B primer showed 4 bands resulted 1 monomorphic, 3 polymorphic and 3 unique bands produced 75 polymorphism percentage. However, HP-9 primer showed 7 bands resulted into 5 monomorphic, 2 polymorphic and 2 unique bands with 28.5 polymorphism percentage. While, HP-12 primer gave 6 bands resulted into 2 monomorphic, 4 polymorphic and 4 unique bands with 66.66% polymorphism percentage. Using HP-14 primer recorded 5 bands resulted into 4 monomorphic, and 1 each of polymorphic and unique bands with 20 polymorphism percentage. Furthermore, the total polymorphism percentage 42.3 after applying of ISSR technique among the *in vivo* shoots of the three cultivars under investigation.

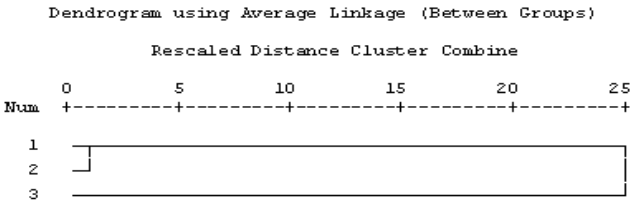
**A**




**Dendrogram of RAPD analysis for three cultivars**

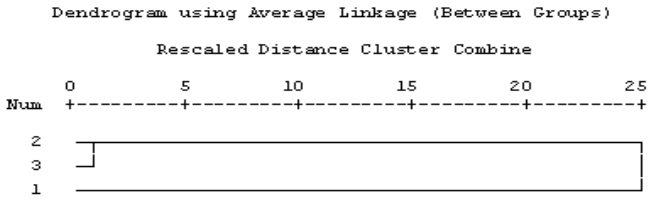
**B**

| | 1 | 2 | 3 |
|---|---|---|---|
| 1 | 1.0 | | |
| 2 | 1.00 | 1.0 | |
| 3 | 0.35 | 1.00 | 1.0 |



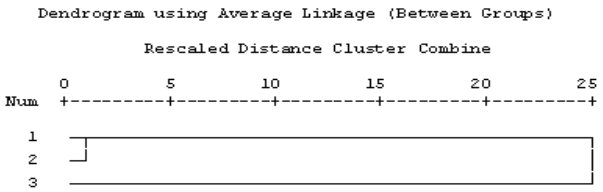

**Dendrogram of ISSR analysis for three cultivars**

| | 1 | 2 | 3 |
|---|---|---|---|
| 1 | 1.0 | | |
| 2 | 0.00 | 1.0 | |
| 3 | 0.33 | 1.00 | 1.0 |



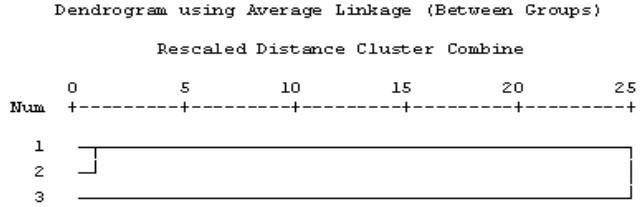

**Dendrogram of SCoT analysis for three cultivars**

| | 1 | 2 | 3 |
|---|---|---|---|
| 1 | 1.0 | | |
| 2 | 0.00 | 1.0 | |
| 3 | 1.00 | 0.00 | 1.0 |




**Dendrogram combination of RAPD and ISSR analysis for three cultivars**

| | 1 | 2 | 3 |
|---|---|---|---|
| 1 | 1.0 | | |
| 2 | 0.00 | 1.0 | |
| 3 | 0.71 | 1.00 | 1.0 |




**Dendrogram combination of RAPD, ISSR and SCoT analysis for three cultivars**

| | 1 | 2 | 3 |
|---|---|---|---|
| 1 | 1.0 | | |
| 2 | 0.00 | 1.0 | |
| 3 | 1.00 | 0.75 | 1.0 |

**Fig. 4. Dendrogram of RAPD, ISSR and SCoT analysis for Jerusalem artichoke; Balady (1), Fuza (2) and Alba (3) column (A) and similarity index of the three cultivars column (B)**

Moreover, the dendrogram of ISSR analysis for three cultivars was carried out. As shown in Fig. 4. Dendogram analysis showed high similarity between Balady and Fuza (100%), while low similarity was recorded between Alba and the other cultivars (0.33%). The dendrogram of combination RAPD and ISSR analysis for J. artichoke; Balady, Fuza and Alba cultivars and similarity index of the three cultivars are shown in Fig. 4. Dendogram analysis showed high similarity between Balady and Fuza (100%), while similarity between Alba and the other cultivars was 0.71 (%). It can be concluded that using primers HP-12 could be used for distinguishing among *in vitro* derived cultures of J. artichoke. However, 44 –B primer can be used for distinguishing between *in vivo* cultivars of Jerusalem artichoke. The cultivars Balady and Fuza have similarity reaching to 100%. However, the similarity percentage between Alba compared to either of Balady or Fuza cultivars was 0.33% only. However, combination of RAPD and ISSR showed 0.71% of similarity between Alba compared to Balady and Fuza. For SCoT analysis, the results represented in Table 6 and 7, Figs. 3 and 4 as mentioned for RAPD and ISSR techniques.

An efficient protocol for plant regeneration of Jerusalem artichoke was described in the current study and genetic fidelity analysis of regenerates using RAPD-PCR, ISSR and SCoT techniques was confirmed as well. DNA molecular markers could be considered the most successful method to estimate the variability within genomic structure of the regenerates (Kumar et al., 2015). The last two decades have seen a growing trend towards applying RAPD technology to identify the somaclonal variations (Al-Zahin et al., 1999; DeVerno et al., 1999). Recent developments in RAPD technique have led to a renewed interest in identification of the genetic fidelity of regenerated plantlets (Qin et al., 2006; Devarumath et al., 2007; Thiyagarajan & Venkatachalam, 2012).

The results presented that five random primers were screened only 61.53% among *in vivo* and *in vitro* cultures of the three cultivars and 38.9% polymorphism between *in vivo* cultivars. Balady and Fuza have similarity reached to 100%. However, the similarity percentage between Alba compared to either Balady or Fuza cultivars was 0.35% only. For several crops, ISSR technique could be used in fingerprinting and diversity studies due to the high efficiency of its primers. These primers have the ability to generate easily, non-expensive and are a powerful tool for discovering the polymorphisms (Joshi et al., 2000; Qian et al., 2001). Moreover, Hou et al. (2005) documented that ISSR was more efficient than RAPD in detecting genetic diversity among barley accessions. In the same context, it is reported that population structure and genetic diversity of some crops (i.e., tea, coffee and lentil) were identified successfully using ISSR (Girma et al., 2010). The obtained results clearly showed that polymorphism among *in vivo* and *in vitro* cultures was 67.8% and among different cultivars was 42.3%. The combination of RAPD and ISSR showed 0.71% similarity between Alba compared to Balady or Fuza. Whereas, SCoT method recorded less total polymorphism (50% between *in vivo* shoots, *in vitro shoots* and stem derived callus of the three cultivars) while it was 34.61% among the mother plant of the three cultivars. SCoT achieved high similarity between Balady and Fuza followed by Alba.

## Conclusion

Jerusalem artichoke is a promising and an important crop for the industrial and medicinal sectors. This crop has several distinguished benefits starting from its high content of inulin, phytoremediation and reaching to its suitable use for biorefinery applications. Those three mentined cultivars of Jerusalem artichoke were *in vitro* investigated, the molecular characterization of regenerates using RAPD, ISSR and SCoT techniques were evaluated as well. An efficient and successful *in vitro* propagation protocol was established for commercial production of studied cultivars of Jerusalem artichoke using stem node cultures. High multiplication rate (11, 8 and 5 for Alba, Fuza and Balady, respectively) was achieved as compared to few previous studies. As far as we know, this is the first work dealing with the DNA fingerprinting and genetic fidelity assessment of *in vivo* and *in vitro* regenerates derived from stem node explants of Jerusalem artichoke.

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*Conflicts of interest:* The authors declare that there is no conflict of interest.

*Author contribution:* This study was designed and implemented by authors, where all authors contributed in writing the manuscript, interpreting information presented and have read and agreed to the version of the manuscript.

*Ethical approval:* This article does not contain any studies with human participants or animals performed by any of the authors.

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# إتجاه جديد للإكثار المعملى للطرطوفة والتقييم الجزيئى باستخدام تقنيات ISSR, RAPD وSCoT

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تعتبر الطرطوفة محصولا هامًا لمدى واسع من الأغراض الزراعية والطبية والصناعية. درناتها غنية بالإنيولين وتزرع للغذاء وعلف للحيوان. هدفت الدراسة الحالية إلى تطوير بروتوكول قابل للتطبيق للإكثار المعملى لثلاثة أصناف من الطرطوفة وهى بلدى وفيوزا وألبا باستخدام منفصلات العقل الساقية. تمت زراعة هذه المنفصلات النباتية على بيئة مواشيج وسكوج نصف قوة معززة بتركيزات مختلفة من الكانامايسين أو السيفوتاكسيم. تم تسجيل أقل تلوث مرتبط بأعلى نسب بقاء لبيئة مواشيج وسكوج نصف قوة مزودة بـ 62.5 مجم/لتر سيفوتاكسيم. لتضاعف الأفرع، تم الحصول على أقصى عدد للأفرع (11) للصنف ألبا المنزرع على بيئة مواشيج وسكوج المزودة بـ 1 مجم/لتر بنزيل أدنين + 0.1 مجم/لتر نفثالين حمض الخليك + 50 مجم/لتر نانوسيلينيوم. للتجذير فى المعمل، لوحظت القيم القصوى لنسبة التجذير، طول الجذور/نبتة، عدد الجذور مع بيئة مواشيج وسكوج نصف قوة + 2 مجم/لتر إندول حمض البيوتريك +0.1 مجم/لتر نفثالين حمض الخليك + 0.5 مجم/لتر كينتين. تم أقلمة النباتات الناتجة فى المعمل على مخلوط من البتموس والبيرليت بنسبة 1:1 والتى أعطت أعلى نسبة بقاء (100%) للصنف ألبا يليها فيوزا وبلدى (80 و 60% ، على التوالى). علاوة على ذلك، تم إجراء التوصيف الجزيئي على أساس تقنيات RAPD ، ISSR ، SCoT. سجلت النسب المئوية لمتعددة الأشكال المظهرية بين أفرع النباتات الأم للأصناف الثلاثة 38.09 ، 42.3 ، 34.61% على التوالى؛ 61.53 ، 67.8 ، 50% بين أفرع النباتات الأم والنباتات الناتجة فى المعمل وكالس الساق للأصناف الثلاثة. أظهر تحليل القرابة للتقنيات مجمعة تشابهًا عاليًا بين الصنف بلدى وفيوزا يليهم ألبا.