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In vitro slow growth storage of date palm *Phoinex dactylifera* cv. Gondelah using somatic embryo and the shoot cultures

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ABSTRACT

In vitro germplasm conservation is one of the main axes in the date palm *(Phoinex dactylifera)* micropropagation protocol. In the present study, a set of slow growth storage treatments planned by using the manipulation of the MS salts strength, besides adding the abscisic acid (ABA) growth retardant, to the storage culture media of tow regenerative *in vitro* germplasm materials, i.e., the somatic embryo and the shoot cultures, of the date palm Gondelah cultivar. The investigated study conducted by observing the growth ability under the conservation conditions, the survival and the recovery growth, after the conservation period for 18 months at 15 ˚C of the minimal growth conditions, the results revealed that half-MS strength medium, supplemented with ABA growth retardant at 8 mg/l, was the best to sustain the slow growth storage and the recovery of the date palm somatic embryo clusters explants. On the other hand, the full-MS medium supplemented with ABA growth retardant at 8 mg/l was most suitable for the *in vitro* preservation and the recovery of the shoot clusters of the date palm, Gondelah cultivar. Where, all recovered explants could regenerate as full intact plantlets, and successfully transferred to the acclimatization stage, to be available for the commercial or research purposes.

***Keywords*:** Abscisic acid, *in vitro*, MS salts strength*, Phoinex dactylifera*, Slow growth storage, Somatic embryos, Survival percentage.

Introduction

Plant tissue culture techniques offer a large production of the genetically identical progeny of many important economic crop varieties (Gulzar *et al.,* 2020). Moreover, advanced approaches using *in vitro* culture, were developed to preserve the valuable genetic materials of commercial and threatened plant varieties (Rajasekharan and Wani, 2020). During the last few decades, science researches have intensively pay attention to the *in vitro* storage of plant genetic resources, which can be recovered as desired after different periods ranging from short or-medium term by using slow growth conditions storage (Hammond *et al.,* 2019), or to a long term by using cryopreservation under ultra-low temperature at -196 ˚C (Shahzad *et al.,* 2017 and Rajasekharan and Wani 2020). It seems to be that, *in vitro* germplasm could addressed the most important obstacles of open filed preservation, by enabling safely and determined spaces of maintenance of different explants stocks (Engelmann, 2011and Hammond *et al.,* 2019). *In vitro* conservation also, reduces the risk of contamination and genetic instability generated, due to the continuous subculturing process, during plants micropropagation protocols, and, it facilitates the international exchange of plants stocks (Cruz-Cruz *et al.,* 2013 and Holobiuc *et al.,* 2018). Storage under the slow growth conditions is the most common and reliable approach for preservation of genetic materials for short or medium-term duration, (Rajasekharan, and Wani, 2020). That is not problematic or needed a highly technical (Chauhan *et al.,* 2019). It is well documented that, *in vitro* storage by minimal growth conditions has successfully achievement with many micropropagated plant species (Hassan and Bekheet, 2008; Pan *et al.,* 2014 and Rahayu *et al.,* 2015). Mainly, Conservation under slow-growth conditions has the possibility to prolong the regeneration cycle of the explants materials from a few weeks to a number of months, by manipulating in the appropriate physical or/and chemical growth conditions of conserved cultures (Engelmann, 2011 and Hammond *et al.,* 2019).

Reduction of growth temperature is the key factor of conservation under slow-growth conditions, commonly ranging from 5-20°C, depended on the origin region of the conserved explant (Shahzad *et*

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al., 2017). The modification in optimal composition of culture media is also an important factor, which can be achieved by, the reduction in nutrient salt strength (Mancilla-Álvarez *et al.,* 2019), addition of the growth retardants, such as abscisic acid (Banasiak and Snyman, 2017), increasing the osmotic potential by adding the osmotic agents (Nasiruddin and Islam, 2018) and reducing the oxygen content inside cultures containers (de Lacerda *et al.,* 2020).

The date palm tree considered to be the most important horticulture crop in the Middle East and Northern Africa countries. It possesses high economic value, not only for the high nutrition value of the sweet fruits but also for the other multi-purposes of the whole tree. The date palm is highly rich in gene diversity, where more than about 5000 cultivars exist globally. The date palm propagation, mainly, depends on the traditional method, by using the offshoots. (Gantait *et al.,* 2018). However, the micropropagation technique through tissue culture protocols of date palm cultivars, whether by somatic embryogenesis (Zayed, 2017) or organogenesis (Bekheet, 2013 and Ali and Abdulzhara, 2018), has an important role in the date palm propagation, and contributed in spreading cultivars of date palm worldwide.

The important date palm cultivars are facing crucial problems such as, lethal infection with bayoud or red palm weevil infestation, besides rapid soil and genetic erosion, water shortage and environmental pollution (Abul-Soad *et al.,* 2017). Thus, increasing the date palm trees population, and developing the germplasm conservation strategies are urgently needed. Until now date palm *in vitro* conservation did not have enough researches studies. The most studies of date palm *in vitro* conservation storage under minimal growth conditions were carried out on Egyptian cultivars (Bekheet *et al.,* 2002, 2005; Hassan, 2002; El-Dawayati, 2008; El-Ashry *et al.,* 2013 and El-Bahr *et al.,* 2016) or on Arabian cultivars growing in Egypt (El-Dawayati *et al.,* 2018a). The studies demonstrated that, the low incubation temperature of date palm conservation, gave the most sufficient results for ensuring high potential of survival and recovery of *in vitro* conserved explants (Gantait et al. 2018), using variety of explants forms, like callus cultures (Bekheet *et al.,* 2002; Diab *et al.,* 2014 and EL Baher *et al.,* 2016), shoot tips (El-Dawayati *et al.,* 2013), somatic embryos (Hassan, 2002; Bekeet *et al.,* 2005 and El-Dawayati et al. 2018a) and the proliferated shoots cultures (Bekheet *et al.,* 2002).

However, the slow growth condition storage, by modification in components of cultures media of the date palm applied, by a number of treatments such as, adding osmotic agents like (high concentrations of sucrose, sorbitol and mannitol), adding growth retardant such as the abscisic acid (ABA) and/or altering the nutrient salts strength of MS (Hassan, 2002; El-Baher *et al.,* 2016 and El-Dawayati, 2017). In general, all *in vitro* conservation studies, conducted to recognize the optimal protocol for each plant varieties, since there is no standard protocol for all plants genotypes. The presented work aims to study the *in vitro* conservation, and the regrowth recovery, of the conserved somatic embryo, and shoot cultures, of date palm explants, of the dry cultivar Gondelah, under the altering of MS salts strength and growth retardant i.e., abscisic acid (ABA), at 15 ˚C of minimal growth conditions.

Materials and Methods

Selected healthy offshoots, (about 3-5 kg in weight and 70-80- cm in height), of well-known, Gondelah dry cultivar of the date palm trees growing in Aswan governorate, Egypt, were used, as a source of plant materials. All preparation and sterilization methods, of shoot tips, followed as recommended by (Zayed, 2017).

1. Conservation of plant materials:

The study was in progress with two types of explants, (somatic embryo clusters and shoot clusters), collected through a successful protocol series, of date palm micropropagation, by indirect somatic embryogenesis, described by (Zayed, 2017).

1. The somatic embryo cluster consist of (8-10) of secondary embryos.
2. The shoot cluster consists of (4- 5 proliferated green shoots) at 5-7 cm in height. Any adjacent secondary embryos or adventitious roots at the shoot clusters bases were removed.

The studied treatments of the slow growth conditions:

To standardize the studied treatments for inducing minimal growth conditions of the conserved explants cultures, the nutrient basal media of MS of Murashjge and Skoog (1962) was used in

combination with the different concentrations (0.0, 4 and 8 mg/l) of abscisic acid (ABA) as growth retardant as follow:

1. 0.0 ABA +full MS
2. 4 mg/l ABA + full-MS
3. 8 mg/l ABA + full-MS,
4. 0.0 ABA + 1/2-MS
5. 4 mg/l ABA + 1\2-MS
6. 8 mg/l ABA + 1\2-MS

Conservation media for all studied treatments was, supplemented with 90 g/L sucrose and the other basic components of the date palm multiplication medium, which consists of, 0.05 mg/l Benzyladenine (BA), 0.1 mg/l Naphthaleneacetic acid (NAA), 100 mg/l myo- inositol, 170 mg/l $KH_2PO_4.2H_2O$, and 4 mg/l thiamine HCl. pH of all media were adjusted to 5.8 prior to agar addition at 8 g/l. All media were autoclaved at 121°C for 20 min. The culture jars of each conservation treatment were conserved under complete darkness at 15 ˚C for 18 months.

2. Recorded data

2.1. The growth ability degree during conservation period: -

The growth ability, of the conserved explants on the different studied conservation treatments was evaluated at the end of conservation period, by visually observation as degree 1----Poor, 2--- Fair, 3---Good, 4---v. good and 5---Excellent, as followed recommendation by (El-Dawayati *et al.,* 2018b), The conserved explants degrees from (1-2), they are dry and browning tissues with a few reactions to develop new growth (secondary embryos or shoots). Conserved explants degrees at (3), they are mostly had the similar appearance as they had been originally conserved, with moderates browning degrees and had few reactions to develop new growth (secondary embryos or shoots). Conserved explants rated from (4-5), they are highly reacted under storage conditions by developing new growth (secondary embryos or shoots).

2.2. The Survival percentages

The survival percentage was determined after 4 weeks from transferring the conserved explants to the recovery medium under the normal growth conditions. The recovery medium was the same components of the multiplication medium of date palm, (mentioned above), but sucrose was added at 40 g/l. The pH of all media were adjusted to 5.8 prior to agar addition at 6 g/L. All media were, autoclaved at 121 ˚C for 20 min. The recovered cultures were incubated under normal growth conditions at 27 ± 1°C under 16:8-h light/dark. The survival percentages of the conserved explants, of different studied conservation treatments, evaluated visually, on the basis of suggestion by (Reed, 1992): that, the conserved explants with heavy browning, drying and without new growth appearance considered as unsurvived.

$$Survival\ percentage = \frac{No.\ of\ survived\ conserved\ explants}{Total\ No.\ of\ conserved\ explants} \times 100$$

3. The growth recovery

All survived explants of the somatic embryo and the shoot cultures obtained from the all different studied conservation treatments were transferred to resume their recovery growth, on recovery media, under the normal growth conditions for three subcultures (8 weeks' intervals) to evaluate their performance after the conservation period.

3.1. The growth recovery of the conserved somatic embryo clusters

Data collected about the regeneration parameters, were the new secondary somatic embryo formation, the converted shoot number and the converted shoot length, of the conserved somatic embryo, which obtained from the different studied conservation treatments, when returned on the recovery media, for three subcultures under the normal growth conditions, after the conservation period. Also the subsequent developing growth of the regenerated shootlets from all conservation treatments, observed until the full plantlets obtained, and can be transferred to the acclimatization stage.

3.2. The growth recovery of the conserved shoot clusters

Data collected about the regeneration parameters were the new secondary somatic embryo formation, the converted shoot number, the converted shoot length, and the developing root system, of

the conserved shoot clusters, which developed from the different studied conservation treatments, when returned on the recovery medium, for three subcultures under the normal growth conditions, after the conservation period. The recovery data for the evaluation of the new secondary embryo formation and the developing root system, recorded visually by degree as above-mentioned. The subsequent elongation of the regenerated shootlets from all conservation treatments, observed until the full plantlets obtained, and can be successfully established in acclimatization stage.

For conservation treatments, each treatment of each conserved explants consisted of 4 replicates and each replicate consisted of 7 culture jars, with one conserved explant/jar, total 28 conserved explants for each conservation treatment. Whereas, for each treatment, during the recovery conditions, all recorded data, analyzed by four replicates in each conservation treatment, and each replicate consisted of five culture jars with one explant/jar, total 20 recovered explants of (somatic embryo cluster or shoot cluster).

4. Establishment of acclimatization stage

All developed rooted plantlets of all treatments were firstly collected and transferred to liquid pre-acclimatization medium, which composed of 1/4 MS medium supplemented with10 g/l sucrose and 6 g/l polyethylene glycol 8000 (PEG) for about 8-10 weeks. Then the full Plantlets with well-established shoot and root system were washed with tap water, and carefully transferred to plant on small pots filled of prepared soil ratios as peatmos: vermiculate: sand, 1: 1: 1. The humidity was maintained initially by covering the pots with transparent polythene bags. The successful growth of acclimatized plantlets was observed for 3 months during acclimatization stage.

Statistical analysis

Data were expressed as mean ± standard error (SE) and analyzed by ANOVA. To examine the significant differences among the treatments, (Duncan, 1955) multiple range test at ($P<0.05$), performed.

Results and Discussion

The present data reveal the effect of the MS salts strength, with the addition of the ABA, during the *in vitro* storage of the explants under the slow growth conditions. The results discussed about the studied parameters of the growth ability degree, the survival percentage of the conserved explants for both of the somatic embryo and the shoot clusters of the date palm Gondelah cultivar under conservation conditions at 15 ˚C for 18 months. Then the growth recovery parameters were evaluated, when the conserved explants, returned to resume their developing under normal growth conditions for three subcultures after the conservation period.

1. Effect of the MS strength in combination with the abscisic acid on the conservation and the growth recovery of somatic embryo

1.1. The Growth ability degree of the conserved somatic embryo during 18 months at 15 ˚C:

The results in Figure (1) illustrate that, the highest growth ability degree obtained when somatic embryo clusters of the date palm conserved on the full-MS or half- MS of conservation media, and free of ABA, (without significant difference in between). The visually observation during the conservation conditions clearly revealed that, somatic embryo conserved on both of full-MS or half-MS and free of ABA concentrations, recorded the maximum signs of growth and development (like producing new differentiated secondary embryos and converted shoots, un tabulated data), in comparison to those conserved on full-MS or half- MS media supplemented with ABA. It can be also, observed from the result in Figure (1) that, the conserved somatic embryo clusters cultured on half- MS salts strength conservation media and supplemented with ABA at 4 mg/ l or 8 mg/l treatments showed the lowest growth ability degree under conservation conditions for 18 months. Concerning the manipulation of MS salts strength in conservation of somatic embryo, the recorded data exhibited a significant effect of half MS salts strength reduction on the reducing the growth ability degree of conserved explants, under the storage conditions for 18 months. The results also showed that the presence of ABA in the conservation media of the conserved somatic embryo significantly affected on the growth ability degree, under storage condition at 15 ˚C, for 18 months. However, there was no significant differences observed

concerning the increasing of ABA concentration from 4 mg/l to 8 mg/l the growth ability degree of the conserved embryo clusters, under storage condition for 18 months.

In this respect, many different studies on plants slow growth storage, indicated that using of some chemical compounds, may help to slow down the growth of the *in vitro* cultures, by acting directly to promote organ dormancy, to minimize cellular metabolism, or to prevent cell nuclear division and making cells more tolerance to low temperature. This may enable cultures to be storage for longer period at low temperature (George, 1993). Kamin´ska *et al.,* (2016) reported that, the addition of ABA growth retardant to storage medium increased several folds the level of the indigenous ABA in plant tissues, which resulted in a reduction in the visual rating under conservation conditions. In this context there are strategies of plants germplasm conservation used growth inhibitors abscisic acid ABA to delay the rate of growth and development of *in vitro* cultures (Bello-Bello *et al.,* 2015 and Huang *et al.,* 2014). According to the obtained results, the same finding was observed by (El-Dawayati, 2008), when the date palm callus conserved at 1ow temperature (5 ˚C or 15 ˚C) on full-MS supplemented with ABA for 12 months, where all recorded growth signs of developing friable callus, embryonic callus and differentiated somatic embryos, during conservation conditions, significantly decreased comparing to those of the callus explants conserved on full-MS of conservation medium free of ABA. However, some studies recommended utilization of media with salt concentration reduction to half than the usual level for the conservation of germplasm, as a means of slowdown the plant growth. (Cordeiro *et al.,* 2014).

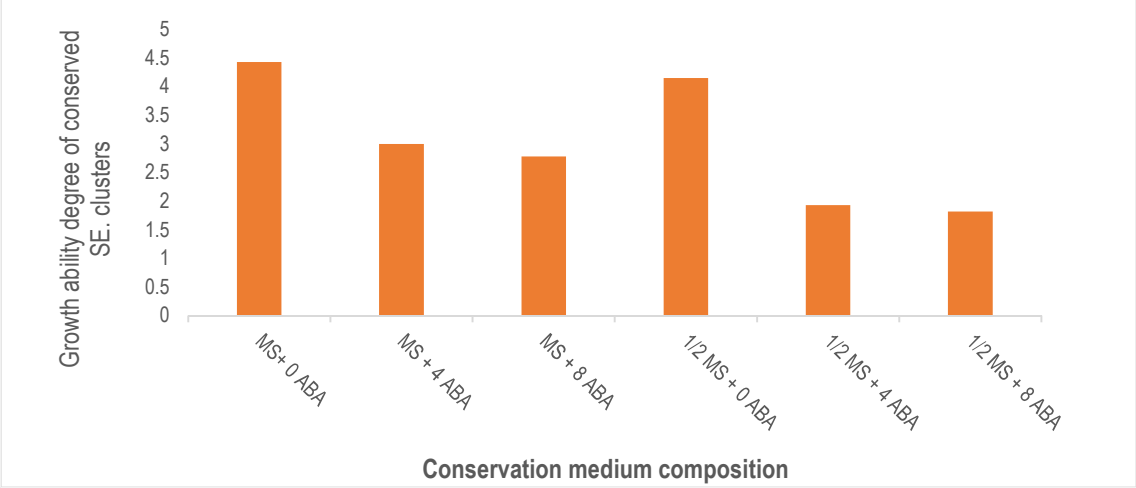

Fig. 1: Effect of MS strength, with ABA on the growth ability degree of conserved somatic embryo of date palm Gondelah cv. under conservation conditions at 15 C˚ for 18 months

1.2. The Survival percentage of the conserved somatic embryo after conservation period:

All the conserved somatic embryo clusters of date palm of all studied treatments were transferred to recovery medium, in order to evaluate their survival percentages, under the normal growth conditions, after 18 months of conservation conditions at 15 ˚C. Survival percentages of the conserved somatic embryos of date palm Gondelah cultivar are illustrated in Table (1). Data revealed that there were no significant differences among studied treatments in relation to the effect of the interaction between the MS strengths and ABA concentrations on the survival percentage, when returned to the normal growth conditions for 4 weeks, on the recovery medium. In this study, the MS strength did not significant effect on the survival percentage of the conserved somatic embryo, on recovery medium, after 18 months of the conservation period at 15 ˚C. Regardless of MS strength, the presence of ABA concentrations significantly affected on the survival percentage of the conserved somatic embryo, after 18 months of the conservation period at 15 ˚C, and increasing the ABA concentrations in the conservation medium to 8 mg/l reduced the survival percentage of the conserved somatic embryo, on recovery medium. On the other hand, the mean value of the survival percentage of the conserved somatic embryo on medium contained ABA at 4 mg/l did not significant decreased comparing to the survival percentage on ABA- Free medium.

Table 1: Effect of MS strength, with ABA on the survival percentage of conserved somatic embryo of date palm Gondelah cultivar after 4 weeks culturing on normal growth conditions

MS salt Strength	ABA mg/l	Conserved SE. clusters Survival %
1MS	0	92.86
	4	89.28
	8	85.71
Mean (A)		**89.28**
1/2MS	0	96.43
	4	92.86
	8	89.28
Mean (A)		**92.85**
	Mean (B)	
94.64 a	**91.07ab**	**87.48 b**

Different small letters within a column indicate statistically significant differences by Duncan's multiple range test ($P<0.05$)

In this study, the MS strength did not give significant effect on the survival percentage of the conserved somatic embryo, on recovery medium, after 18 months of the conservation period at 15 ˚C. similar finding was reported by (Cordeiro, 2014) who found that the basal medium (MS, ½MS, WPM, and ½WPM) did not affect the slow-growth storage of *M. moricandiana* under the tested conditions of slow growth storage. Also the presented results indicate that there is an appropriate concentration of the ABA for sustaining the survival percentage which is in line with Huang *et al.,* (2014) who found that the addition of ABA at effective concentrations 2 - 5 mg/l, did not decrease the survival rate of *in vitro* conserved buds of *Polygonum multiflorum* Thunb. Jarret and Gawel (1991) suggested that to identify an optimal ABA concentration, some consideration must be given, for each genotype or group of genotype. However, the identification of nontoxic levels of ABA concentration, which only suppressing the total growth for each plant, may result in increased explants viability after removal from ABA.

1.3. The growth recovery of the conserved somatic embryo for three subcultures:

In this study the result in Figure (2) exhibited that, the highest value of the new secondary embryos formation on recovery media was recorded by the somatic embryos conserved on 1/2-MS medium and supplemented with ABA at 8 mg/l treatment, after18 months of conservation period, followed by 1/2-MS medium with ABA 4 mg/l. Where, the lowest value of new secondary embryos formation that regenerated from the conserved somatic embryo was recorded by using the full-MS medium without ABA.

Fig. 2: Effect of MS strength medium, with the ABA on the new secondary embryo formation of conserved somatic embryo of date palm Gondelah cv after 3 subcultures on the normal growth conditions

It is clear that the reduction of MS salts strength medium gave a significant effect on the new secondary embryos formation which recovered from the conserved somatic embryos, under the normal growth

conditions. Also the addition of ABA in the conservation media showed a significant effect on the regenerated new secondary embryos formation. The increasing of the ABA concentration from 0.0 mg/l to 8 mg/l in the conservation medium, significantly increased the regeneration of the new secondary embryos that recovered, when returned to normal growth conditions, after the conservation period at 15 ˚C. The presented results are in agreement with Sukendah and Cedo (2005) who found that MS medium at half-strength was better medium for coconut embryos embryo storage than the full-strength medium. And Hassan *et al.,* (2016) who appointed that the inclusion of ABA in conservation media of date palm somatic embryos of Sewy cultivars, for 6, 8 and 10 months without sub culturing, significantly increased the recovery percentages of new regenerated secondary embryos compared to the control treatment.

Data presented in Table (2) revealed that the highest number of converted shoots regenerated from the conserved somatic embryos on recovery medium, after 18 months was recorded on 1/2-MS medium supplemented with ABA at 8 mg/l, followed by 1/2-MS medium supplemented with ABA at 4 mg/l. The explored data here indicated that, shoots conserved on both of full-MS or 1/2-MS medium without ABA, gave the lowest significant values of the regenerated number of shoots, when the conserved explants returned to recover under normal growth conditions. This is can be explained by the highest growth ability degree of those conserved somatic embryo clusters during conservation period Figure (1), which may lead to the poor recovery under normal growth conditions. Data analysis in Table (2) showed that the salt strength of MS medium has a significant effect on the number of converted shoots that recovered from the conserved somatic embryos, since conserved somatic embryos on 1/2MS medium highly increased the regeneration of converted shoots on recovery medium after conservation period. On the other hand, Data in Table (2) also showed that the highest value of shoots length of regenerated explants on recovery medium after conservation period, obtained when somatic embryos clusters conserved on full-MS medium, supplemented with ABA at 8 mg/l for 18 months. It is clear from data in Table (2) showed that the salt strength of MS medium has a significant effect on the length of the converted shoots that recovered from the conserved somatic embryos when they returned to resume their growth under normal growth conditions, where somatic embryos conserved on full-MS media treatments showed the highest significant mean values of the shoots length that regenerated on recovery medium under normal growth conditions, after 18 months of the conservation period at 15 ˚C. The results in Table (2) obviously indicated that the presence of ABA as growth retardant in the conservation media of somatic embryos has a significant effect on both of shoot number and length of converted shoots that regenerated from the conserved somatic embryos, when they returned to resume their growth under the normal growth conditions for three subcultures, after the conservation period. And it could be observed from the results in Table (2) that, increasing the ABA concentration from 0 to 8 mg/L significantly increased the number of converted shoots, that recovered from the conserved somatic embryos, when they returned to resume their growth, under normal growth conditions.

Table 2: Effect of MS strength medium, with the ABA on the converted shoot number and length of conserved somatic embryo of date palm cv Gondelah after three subcultures on the normal growth conditions.

MS salt Strength	ABA conc. mg1-1	Converted Sh. No.	Converted Sh. L
	0	17.6 d	4.97 de
1MS	4	20.65 cd	6.37 b
	8	26.05 c	7.15 a
Mean (A)		**21.4 b**	**6.163 a**
	0	19.55 d	4.8e
1/2MS	4	34.85 b	4.52 d
	8	40.45 a	4.57 c
Mean (A)		**31.61 a**	**4.63 b**
	Mean (B)		
18.57 c	**27.75 b**	**33.25 a**	**(Sh. No.)**
4.88 c	**5.445 b**	**5.86 a**	**(Sh. L)**

Different small letters within a column indicate statistically significant differences by Duncan's multiple range test ($P<0.05$)

It is worth to mention that, regenerated shoots from converted somatic embryo clusters of conservation treatment of half salts strength MS supplemented with ABA at 8 mg/l exhibited fast

development to full healthy plantlets, that can be transferred to acclimatization stage, Figure (6). In general, the regenerated shoots that recovered from conserved somatic embryos clusters resumed their development to full plantlets, which indicates that somatic embryo cluster of date palm is a recommended explant for germplasm conservation of date palm. On the light of presented results, the similar finding by Hwida and Abd El-Kader (2012) who found that provided the conservation media of *Balanitis aegyptiaca* with ABA at 10 mg/l raised means of shootlets number of explant on recovery medium provided the conservation media of *Balanitis aegyptiaca* with ABA at 10 mg/l raised means of shootlets number of explant on recovery medium. In contrast, in contrast, Kamińska *et al.,* (2016) found that, the addition of ABA to storage medium, of *Taraxacum pieninicum* at 10 ˚C, resulted in an increase in the endogenous ABA levels, which negatively affected the propagation of shoots during regrowth, where on medium without ABA allowed good regrowth after 9 months.

2. Effect of MS strength in combination with the abscisic acid on the conservation and the growth recovery of the shoot cultures

2.1. The Growth ability degree of the conserved shoots during 18 months at 15 ˚C:

The revealed results in Figure (4) showed that the highest growth ability degree during the conservation conditions of conserved shoots were registered on full-MS or half-MS media and free of ABA. The visually observation, obviously showed maximum growth and development of the conserved shoots on full-MS or half- MS media and without ABA, comparing to the few growth signs of shoot conserved on full-MS or half- MS media and supplemented with ABA. On the other hand, the growth ability of conserved shoots on half-MS medium, implemented with ABA concentrations at 4 mg/l or, 8 mg/l recorded the lowest values (without significant difference in between). Regarding to the effect of MS salt strength of conservation media, on the growth ability degree of conserved shoots, the recorded data indicated that full- MS medium significantly increased the growth ability degree values comparing to those on half- MS of medium.

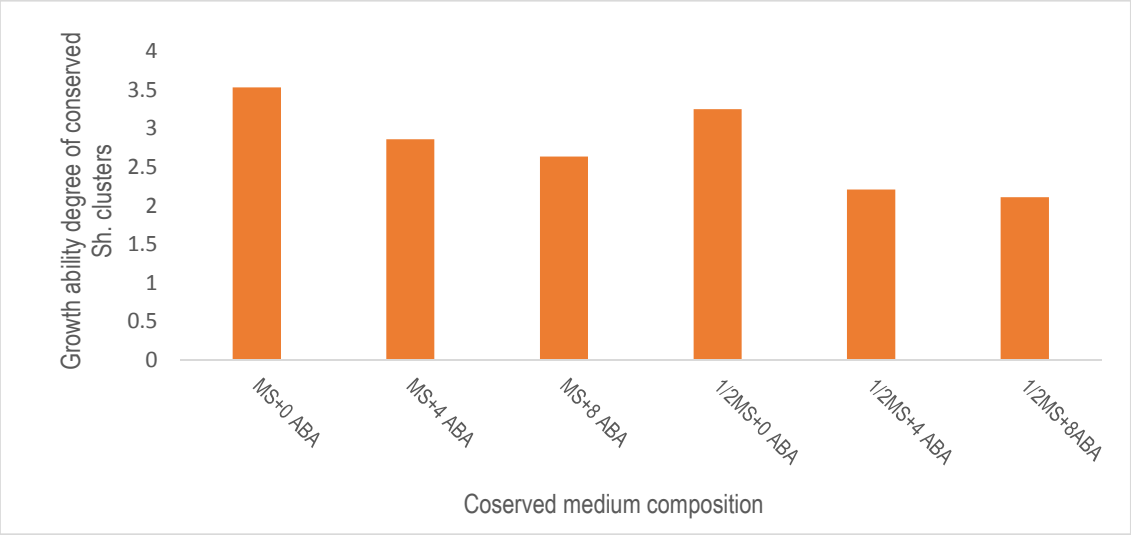

Fig. 3: Effect of MS strength medium, with ABA on the growth ability degree of conserved shoot of date palm Gondelah cultivar under conservation conditions at 15 ˚C for 18 months

Also, the presence of ABA in conservation media had a significant effect on the growth ability degree of the conserved shoots under storage condition at 15˚C for 18 months. In this concern, (Gopal *et al.* 2004) reported that, the abscisic acid generally acts as an endogenous growth retardant and has been used for growth reduction of *in vitro* cultures. The application of *in vitro* conservation techniques allows cultures to remain viable with a slow growth rate and a significant increase in the storage life of the tissues (Cruz-Cruz *et al.,* 2013). It was suggested that ABA could alter the carbohydrate metabolism of plant cells (Silva and Scherwinski-Pereira 2011). Similar finding in agreement with presented results, Srivastava *et al.,* (2013) found that ABA significantly decrease growth of *Glycyrrhiza glabra* shoots on conservation media when compared with controls. Meanwhile, Pan *et al.,* (2014) showed that growth of grape shoots significantly declined when ABA was added to the storage media, and also in the conservation cultures of *Thymbra spicata* L. var. spicata microshoots (Tahtamouni *et al.,* 2016). But on

the opposites, El- Dawayati *et al.,* (2013) indicated that the presence of ABA did not affect significantly on the growth of the conserved shoot tip explants of the date palm Zaghlool cultivar.

2.2. The Survival percentage of the conserved shoots after conservation period:

All conserved shoot clusters of all studied treatments, were transferred to culture on recovery medium, in order to evaluate their survival percentages, under the normal growth conditions, after 18 months of conservation conditions at 15 ˚C.

In relation to the effect of the MS strength media in addition of the ABA during the conservation conditions, the results in Table (3) showed that, the conserved shoots of cultured on full-MS medium and free of ABA, totally recorded 100% of survival percentage, when returned to culture on recovery medium, followed by the survival percentage of the conserved shoots on full-MS media supplemented with ABA at 4 mg/l or 8 mg/l, but without significant difference among them. Data in Table (3) showed that the survival percentages of the conserved shoots significantly affected by the MS salts strength of conservation medium, where the highest mean of survival percentages recorded with shoots conserved on full MS strength media for 18 months.

Table 3: Effect of MS strength medium, with ABA on the survival percentage of conserved shoots of date palm Gondelah cv, after 4 weeks culturing on normal growth conditions

MS salt strength	ABA mg/l	Conserved. Sh. clusters. Survival %
1MS	0	100 a
	4	96.43 a
	8	92.86 a
Mean (A)		**96.43 a**
1/2MS	0	92.86 a
	4	82.14 ab
	8	75.00 b
Mean (A)		**83.33 b**
	Mean (B)	
96.43 a	**89.2 ab**	**83.93 b**

Different small letters within a column indicate statistically significant differences by Duncan's multiple range test ($P<0.05$).

In respect to presented findings similar result was obtained as 100% of survival percentage of callus explants of date palm, conserved on MS medium and ABA-free, at 15 ˚C, for 6 or12 months (El-Dawayati, 2008). Also, in the *in vitro* conservation of *Laelia anceps*, the highest percentage of survival (90%) was observed for conserved shoots on full MS media supplemented with ABA (Ramírez-Mosqueda *et al.,* 2019). Using ABA in conservation medium of *Dillenia indica* increased the storage period for 9 months with high survival rate and 100% regeneration ability of the conserved shootlets (Abd el-kader *et al.,* 2019). Similar finding was obtained by (Kolomiets *et al.,* 2016) who found that addition of ABA to half MS medium decreased the survival rate of *Campanula sclerophylla* after conservation period at 7 ˚C. Similar to this work result, Westcott *et al.,* (1977) found that the survival rate of potato cultures was enhanced at all tested concentration levels of ABA, but the most successful results obtained when ABA added at 5 and 10 mg/l. Silva and Scherwinski-Pereira (2011) reported that in conservation media of *P. aduncum and P. hispidinervum* shoots, ABA affected the survival of shoots at 0, 0.5 and 1.0 mg/l ABA was 100% .Pan *et al.,* (2014) reported that, the addition of ABA 0.5 - 3 mg/l increased the survival rate of grape shoots compared to control after 10 months of conservation.

According to the presented results, the highest mean of survival percentages recorded with date palm shoots conserved on full MS strength media for 18 months.

Similar finding in this respect, Pan *et al.,* (2014) found that the full MS medium was the optimal medium for high survival percentages of Chinese wild grape (*Vitis heyneana* Roem. & Schult) at different studied storage period. On the opposite to the presented results it was reported in *Vanilla species* shoots conserved on half strength of MS medium, the highest survival percentage of plantlets on recovery medium Bello-Bello *et al.,* 2015. Also, Rahayu *et al.,* (2015) found that, the decreasing of nutrition concentration in conservation medium of *Carica pubescens* epicotyls increased the survival percentages on recovery medium. It can be concluded that the optimal nutrient medium, for *in vitro* storage, differs from one species, or even cultivar to other.

On the light of presented results in Table (3), conserved shoots of all treatments exhibited good survival percentages during 18 months of slow growth conditions at 15 ˚C, which confirmed with Cordeiro *et al.,* (2014) who reported that requirement ratio of plant survival under slow-growth conditions should be greater than or equal to 50% to pass to the recovery stage, assuming that conserved explants grown under conditions permitting ≥ 50% survival would gave better results in the recovery conditions, since low survival ratio under the slow growth conditions might provide some problems in the explants maintenance and, consequently, more difficulty in their recovery under normal growth conditions.

2.3. The growth recovery of the conserved shoot for three subcultures:

In this study results of Figure (4) showed that, the presence of ABA in the conservation media of date palm shoots for 18 months at 15 ˚C was significantly affected on the values of new secondary embryos, that developed on the recovery medium. The highest significant value of new secondary embryos formation on recovery medium was recorded with shoots conserved on full-MS medium supplemented with ABA at 8 mg /l. Whereas the shoots conserved on 1/2-MS medium supplemented with ABA at 8 mg/l, for 18 months recorded the lowest value of the new secondary embryos formation, when returned to culture for three subcultures under the normal growth conditions. Meanwhile, the increasing of the ABA concentration from 4 mg/l to 8 mg/l in the conservation medium of the shoots significantly increased the formation of the new secondary embryos values, that regenerated when the conserved shoots returned to normal growth conditions, after the conservation period. Also, obtained results confirmed that the MS strength medium has a significant effect on the values of new secondary embryos, that recovered from the conserved shoot, when returned to culture on recovery medium, for three subcultures, under the normal growth conditions, after conservation period for 18 months at 15 ˚C Also, data revealed that, the MS strength medium has a significant effect on the values of new secondary embryos that recovered from the conserved shoot clusters of date palm explants, when returned to culture on recovery medium for three subcultures under the normal growth conditions, after conservation period for 18 months at 15 ˚C. In this respect Al-Baba *et al.,* (2018) reported that, the presence of ABA growth retardant in the storage MS media of *Ziziphora tenuior* reduced all evaluated growth parameters, and maintained their recovery after storage period.

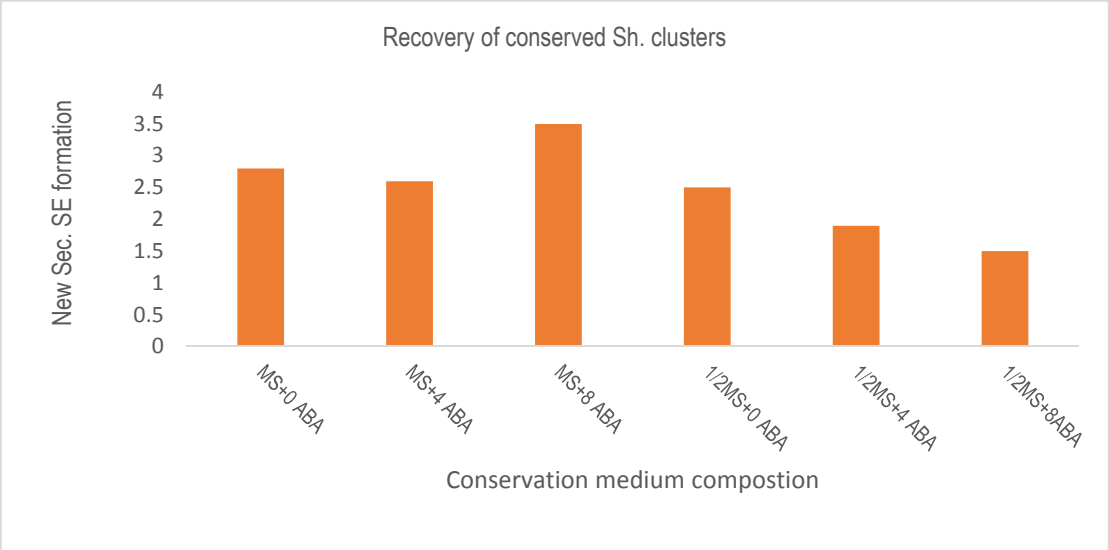

Fig. 4: Effect of MS strength medium, with the ABA on the new secondary embryo formation of conserved shoots of date palm Gondelah cv after 3 subcultures on the normal growth conditions

From results in the Table (4), the highest values of recovered shoots number and length, that developed from the conserved shoots on recovery medium for three subcultures after 18 months of conservation period were recorded on full-MS medium supplemented with ABA at 8 mg/l. The revealed data here showed that conserved shoots on full MS strength media or 1/2 MS strength media and free of ABA, gave the lowest significant values of the recovered shoots number and length when returned to culture on recovery medium for three subcultures after 18 months of conservation period. Data also

indicated that the MS strength medium had no significant effect on the number of the recovered shoots that developed from conserved shoots when returned to resume their growth under normal growth conditions. On the other hand, data showed that MS salt strength media has a significant effect on the length of conserved shoots after18 months of the conservation period. Where, the full-MS media treatments showed the highest significant mean value of shoots length of conserved shoots when returned to culture on recovery medium for three subcultures after18 months. It is obviously observed from data in Table (4) that, presence of ABA concentrations in the conservation media of the date palm shoot clusters has a significant effect on their shoots number and length recovery, under the normal growth conditions, for three subcultures, after the conservation period for 18 months. However, increasing of ABA concentration in conservation media, significantly increased the number of recovered shoots under normal growth conditions for three subcultures after18 months. In accordance with the present result, Tregjell *et al.,* (2015) found in *Senecio macrophyllus* cultures, the addition of ABA to storage medium stimulated survival and high proliferation of shoots during regrowth in optimal conditions in comparison to shoots stored on medium without ABA. Kolomiets *et al.,* (2016) found that, the addition of 5 mg/l ABA alone or in combination with 3 g/l sorbitol in conservation culture media of *Campanula sclerophylla*, caused no significant effect on shoot number comparing to the control treatment after 9 months of conservation period at 7 ˚C. However, Al- Baba *et al.,* (2018) found that the recovery of conserved microshoots of *Ziziphora tenuior* response to ABA application in terms of the number of newly developed shoots showed no significant differences among values to the control treatment and ABA concentrations of storage media but the application of ABA negatively affected on the shoot height recovery at all levels compared to the control (without ABA). On the opposite, Silva and Scherwinski-Pereira (2011) found that the addition of ABA in conservation media of *P. aduncum* and *P. hispidinervum* shoots, affected on decreasing the shoot number and length during the growth recovery conditions.

Table 4: Effect of MS strength medium, with the ABA on the recovered shoot number and length of conserved shoots of date palm Gondelah cultivar after 3 subcultures on the normal growth conditions.

MS salts strength	ABA mg/l	Sh. No	Sh. L
1MS	0	12.2 d	11.08 c
	4	18.1 b	13.88 a
	8	24.55 a	14.5 a
Mean (A)		**18.28**	**13.15 a**
1/2MS	0	10 d	10.85 d
	4	15.11c	11.75 b
	8	20.7 b	11.25 c
Mean (A)		**15.27**	**11.28 b**
	Mean (B)		
11.1 c	**16.06 b**	**22.62 a**	**Sh.No**
10.96 b	**12.81 a**	**12.87 a**	**Sh.L**

Different small letters within a column indicate statistically significant differences by Duncan's multiple range test ($P<0.05$)

The results in Figure (5) showed that conserved shoots, on full-MS strength media, supplemented with 8 mg/l, for 18 months, showed the highest degree of the new roots system, when returned to resume their normal growth, for three subcultures, after the conservation period. Whereas, shoots conserved on the full-MS strength medium, or 1/2-MS strength medium, and free of ABA for 18 months recorded the lowest degree of the new roots system, when return to culture on recovery medium, for three subcultures, after conservation period. In this study, data showed that salts strength of MS medium, was not significantly affected on the new roots system of the conserved shoots when returned to resume their growth, under normal growth conditions for three subcultures, after 18 months of conservation period. But the presence of ABA in the conservation medium has a significant effect on the new root system, that developed from the conserved shoots cultured on the recovery medium, for three subcultures, after 18 months of conservation period. Al- Baba et al. (2018) found that complete roots recovery percentages obtained in all ABA stored microshoots of *Ziziphora tenuior*, one month after transferring to normal growth conditions. In general, unexpectedly, high survival rates of the date palm

shoot clusters explants, during the slow-growth conditions, did not ensure their speedy and complete development during the recovery conditions, Cordeiro *et al.,* (2014) Indicated that in addition to high survival rates, the explants require a minimum multiplication rate for recovery; where the time during slow-growth did not affect their regenerative potential. From the presented results the best growth recovery achieved for the conserved shoot clusters of date palm, obtained on optimized conservation medium composition of full MS salts strength with the addition of ABA at 8 mg\l, where strong recovered full plantlets successfully transferred to acclimatization stage Figure (7).

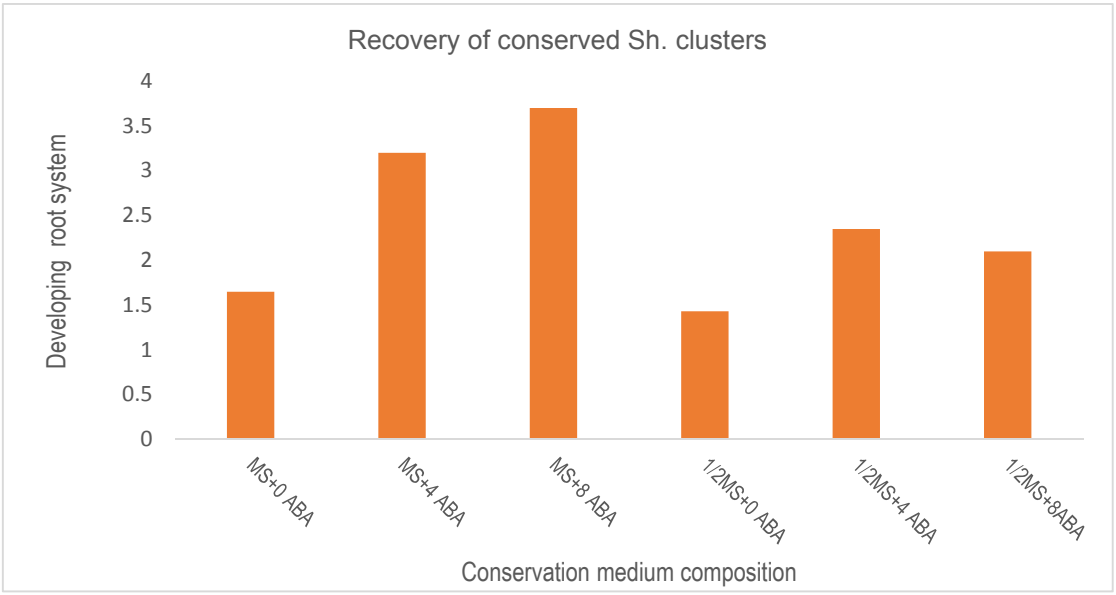

Fig. 5: Effect of MS strength medium, with the ABA on the new roots system of conserved shoots of date palm Gondelah cv after 3 subcultures on the normal growth conditions

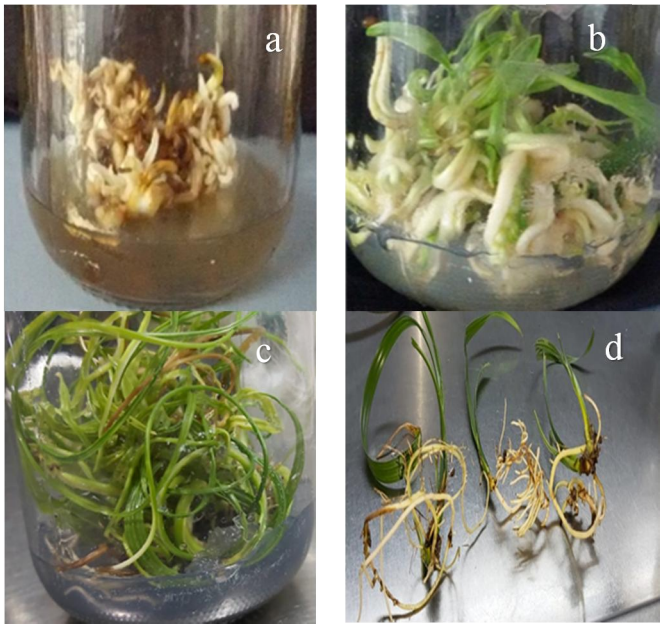

Fig. 6: The conserved somatic embryo cluster of date palm Gondelah cv. on the half MS strength medium supplemented with ABA at 8 mg/l for 18 months at 15 ˚C (a) exhibited the best converted shoots regeneration when returned to culture on the recovery medium under the normal growth conditions after conservation period (b, c) and the subsequent full healthy plantlets (d) received, that can be transferred to the acclimatization stage.

Fig. 7: The conserved shoot clusters of date palm Gondelah cv. on the full MS strength medium supplemented with ABA at 8 mg/l for 18 months of the conservation period at 15 ˚C (a), exhibited the best shoots regeneration when returned to culture on the recovery medium under the normal growth conditions after conservation period (b, c) and subsequent full healthy plantlets obtained (d) Then successfully transferred to acclimatization stage (e).

Conclusion

This study showed that the slow growth conditions by manipulation of the nutrient salts strength of the conservation media with the addition of the abscisic acid growth retardants, have a significant role on the *in vitro* conservation of somatic embryos and shoots cultures of the date palm, Gondelah cultivar for 18 months at 15 ˚C. From the obtained results it could be concluded that half-MS strength medium, supplemented with ABA at 8 mg/l, is the best to sustain the slow growth storage and the recovery of somatic embryo clusters. Whereas, the full-MS strength medium supplemented with the ABA at 8 mg/l was most suitable for *in vitro* preservation of shoot clusters, moreover, all recovered explants could regenerate, and successfully transferred to the acclimatization stage. From this study it is seems to be that, it is important for successful slow-growth storage of date palm cultivars, to study the vital capacities of different sources of germplasm materials, and their potential for the stable regrowth, after the conservation period, since different germplasm materials for the same genotype, may differ in the proper protocol for the *in vitro* conservation. Meanwhile, the successful of a certain protocol of slow growth storage depends on the maintenance of the cultural survival during extended storage period beside the rapid regrowth after reverting to the standard and normal growth conditions. In general, for the date palm sustainable development, intensive studies, are needed for different strategies of germplasm conservation of all valuable cultivars.

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