



ROOTING RECOVERY AND CHEMICAL ANALYSIS OF DATE PALM SHOOTLETS AFTER SORBITOL AND MANNITOL SUGARS STRESS

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Abstract

Date palm micropropagation protocols depend on the success of the *In vitro* rooting stage, which in turn enhance the success of acclimatization stage. Regeneration potential after different stress conditions was widely investigated in different plants cultures. In date palm, low shoot clusters elongation and poor roots formation may obstruct the success of rooting stage. The main object of the present study is the enhancement of date palm rooting stage, by studying the recovery capacity of vegetative growth of date palm shoot clusters *cv.* Bartamoda, after 8 weeks of osmotic stress effect of different concentrations of alcohol sugars, sorbitol or mannitol, at (0.0, 0.1, 0.3, and 0.5 M) supplemented in the rooting medium. Also, chemical analysis of the total chlorophyll content of cultured shoot clusters explants during different osmotic stress treatments and, then after the recovery on normal rooting medium were studied. As the same the compatible solutes accumulation such as free amino acids, phenols, proline, sugars (total soluble sugars and reduced sugars) and carotenoids, were studied. Shoot clusters explants which were cultured on rooting medium for 8 weeks with sorbitol sugar at 0.3 M gave the best recovery of shoot elongation and good root system when were returned on normal rooting medium.

Keywords : Osmotic stress, Alcohol sugars, compatible solutes, *Phoenix dactylifera*, Rooting, *In vitro*

Introduction

Phoenix dactylifera plant, well recognized as the date palm tree, is one of the family Arecaceae; in the plant kingdom. The main cultivation area of date palm trees in the Middle East and North African region. Date palm trees have economic, social and environmental importance, mainly date fruits have, a great nutritional value; moreover, the numerous practical benefits of the whole tree (Gantait *et al.*, 2018). Date palm propagation by tissue culture technique became the most promising route for true-to-type plantlets mass production (Bekheet, 2013). Date palm micropropagation process is carried out through two protocols, either indirect somatic embryogenesis (with interference of callus phase) (Fki *et al.*, 2011; Naik, and Al-khayri, 2016; Zayed, 2017) or, direct adventitious shoots (Jazinizadeh *et al.*, 2015; Meziani *et al.*, 2016; Abahmane, 2017). Rooting stage period is determined by increasing in shoots elongation and roots formation. In date palm, the less of shoot clusters elongation and poor roots formation of the multiplication stage may increase the period of rooting stage, which increases the lab-costs. The weakness of date palm plantlets and their roots system considered to be the major problem during the rooting stage (Abul-Soad *et al.*, 2014). Some studies have been focused on the factors affecting shoots elongation and roots formation, including both of type and concentrations of the exogenous growth regulators and basal salts of cultures medium, the carbon source, activated charcoal and light intensity (El-Dawayati, 2000; Abul-Soad *et al.*, 2014; Abahmane, 2013; Mazri, 2014; Mazri, *et al.*, 2016). The main purpose of tissue culture studies in clonal propagation of plants is to obtain high-frequency shoot regeneration, and establishing strong roots system, that is a prerequisite for an efficient transfer to the outside acclimatization stage. Regeneration potential after different pretreatments of stress conditions has been widely investigated for different plants cultures (Haque *et al.*, 2017). It has been found that cultures

incubation for a certain time under a certain adverse condition of physical (temperature, light, aeration) or chemical (pH, salinity, osmotic agents) stress optimize the regeneration processes of *In vitro* plants (Adamczuk *et al.*, 2012). Osmotic stress has been induced in plants cultures by using high-molecular-weight solutes, such as polyethylene glycol (PEG) (Puente-Garza *et al.*, 2017), or high concentrations of sugars, such as sucrose, (Mishra and Singh, 2016), mannitol and sorbitol (Abu-Romman *et al.*, 2012; Lee and Hung 2014; Chen *et al.*, 2016). Sorbitol and mannitol, as alcohol sugars, are acting as osmotic regulators, or as growth retardants by causing osmotic stress in plant culture media (El-Dawayati *et al.*, 2012). Osmotic stress has effects on morphological and physiological growing pattern of plant cultures (Ghaheri, *et al.*, 2015). Stimulation of shoot organogenesis and plantlet regeneration was studied by the addition of sorbitol in callus growth culture of *Triticum aestivum* L. and *Vicia faba*, (Skrzypek *et al.*, 2008; Mishra and Singh, 2016), also, by the addition of mannitol in callus growth culture of *Oryza sativum* (Lee and Huang, 2014). The addition of mannitol to the growth medium of *Bacopa* sp. cultures at high concentration led to an increase in root length and root branches (Tanveer *et al.*, 2010). It has been reported that the metabolic changes which associated with the defense mechanism against osmotic stress could control root growth, cell elongation and cellulose synthesis in later stages of development of plants (Faizal and Geelen, 2013). Plant responses to osmotic stress and water deficit can affect the concentration, composition, and distribution of both primary and secondary metabolites. Plant metabolites, such as amino acids, prolines, phenols and carbohydrates, pigments maintain life processes and facilitate growth (Mundium *et al.*, 2018). This low molecular weight of organic metabolites, known as compatible solutes, which serve a function in cells to lower or balance the osmotic resistance, to maintain the plant's recovery after the stress is released, their

accumulation are varied among plant species during different stress conditions (Wani *et al.*, 2013).

Depending on previous vision, the recovery potential after osmotic stress during rooting stage of date palm plantlets was discussed in this work, where to date, there is no report on using osmotic stress during the *In vitro* rooting stage of date palm to enhance shoot elongation and root formation of shoot clusters. So, the present study was carried out to study the impact of the osmotic stress by using different concentrations of alcohol sugars, such as sorbitol or mannitol, during the *In vitro* rooting stage of date palm shoot clusters *cv.* Bartamoda. Also, the chemical analysis data were studied about the total chlorophyll content, and the compatible solutes accumulation such as free amino acids, phenols, proline, sugars (total soluble sugars and reduced sugars) and carotenoids, during stress and recovery at normal conditions. Mainly, the prerequisite of our study is to obtain good full intact plantlets that can be successfully transferred to the acclimatization stage in a short time.

Materials and Methods

The present study was conducted in the Central Lab. for Date Palm Research and Development, Agriculture Research Center, Giza, Egypt.

Explant material preparation

Healthy female offshoots of date palm *cv.* Bartamoda were obtained from Aswan governorate, Egypt. The primary preparation and sterilization method for shoot tips explants was conducted according to (El-Dawayati and Zayed, 2017). The indirect somatic embryogenesis protocol for date palm micropropagation as recommended by EL-Dawayati *et al.* (2018), which is started with callogenesis stage to obtain embryonic callus and mature somatic embryos differentiation on nutrient medium with 2,4-D (as growth regulator), to be followed by the multiplication stage for the new converted shoots on nutrient media with Naphthalene acetic acid (NAA) and Benzyl amino purine (BA) (as growth regulators); The beginning of the rooting stage is determined by obtaining suitable shoots length with good thickness and integrity of their stems, which to be able to continue their subsequent growth.

Experiment treatments preparation

Proliferated shoots clusters (which were received at the besting of rooting stage as mentioned above) contained 3-4 shoots, at 5-6 cm in length and have good integrity of stems, without any attachment of secondary embryos or primary roots on their base, were collected and cultured on rooting medium supplemented with different concentrations of sorbitol and mannitol sugars (at 0.1, 0.3, 0.5) for 8 weeks. Also, shoots clusters explants were cultured on rooting medium without addition of sorbitol or mannitol sugars concentration as control treatment.

All cultured explants from control and different stress treatments of sorbitol and mannitol sugars were transferred to recover their growth on normal rooting medium for two subcultures with (8 weeks interval).

Rooting medium consists of 3/4 strength of Murashige and Skoog's medium (MS) of the basal nutrient salts (MS) vitamins, 170.0 mg/L Potassium Phosphate Monobasic (KH₂PO₄), 100 mg/L myo-inositol, 2 mg/L Calcium d-pantothenate (Ca.P), 0.4 mg/L thiamine. HCl, 0.1 mg/L

glycine, 0.2 mg/L biotin and 0.2 mg/L arginine. 40 g/L sucrose was added as carbon source. Also, 1.0 mg/L NAA, 1.0 mg/L Indole-3-butyric acid (IBA), 0.4 mg/L Paclobutrazol (Pbz), (as rooting growth regulators). Medium was solidified with 6 g/L agar; with the addition of 1 g/L activated charcoal (AC). The pH of the medium was adjusted at 5.8. Prepared media were distributed (40 ml per vessel) into big culture vessels (250 ml size). All the culture vessels were capped with polypropylene closure and autoclaved for 20 min at 121 °C and 1.1 kg/cm² pressure. All cultures of all studied treatments were incubated at 25±2°C under a photoperiod of 16 h using cool white fluorescent lamps (Toshiba 40 W tubes) irradiate 3000 Lux.

Vegetative growth and chemical analysis data were recorded for all culture explants for two times, at the end of 8 weeks of osmotic stress condition, and after the recovery on normal rooting medium for two subcultures.

Data of vegetative growth were recorded about shoots elongation, roots number, roots length and growth vigor. Growth vigor degree were scored visually, (such as, 0 = no change; 1 = below average; 2 = average; 3 = above average; 4 = high; 5 = very high), following the recommendation of El-Dawayati *et al.* (2018). Data of chemical analysis were recorded about the content of total chlorophyll (a+b) contents and the contents of some biochemical compounds related to stress conditions (compatible solutes) such as, free amino acids, phenols, proline, sugars (total soluble sugars and reduced sugars) and carotenoids. Only, chemical assay of sugars contents (total soluble sugars and reduced sugars) of all culture explants were studied during stress condition for 8 weeks.

Finally, the full intact plantlets from the all treatments were transferred to the acclimatization stage to observe their growth for 3 months in the greenhouse.

Chemical analysis

Biochemical determination were carried out by using (fresh leaves) 1 gm from each sample of each studied treatment.

Determination of plant pigments:

Chlorophyll a, chlorophyll b and carotenes were extracted and evaluated according to wetstein (1957).

Determination of reducing sugars and total soluble sugars were followed by a procedure according to Shales and Shales (1945).

Total phenols were estimated on prepared samples according to Elizabeth and Kelly (2007) and Patel *et al.* (2010).

The amino acids were determined on extracted samples according to Mc.Grath (1972).

The proline was estimated on prepared samples according to Bates *et al.* (1973).

Statistical Analysis

In this study, each treatment was carried out in triplicates and each replicate contained three culture vessels with one sample per culture vessel. The collected data were calculated as the means from three replicates and were analyzed using SPSS statistical software (IBM SPSS Statistics, version 24). The differences between the

treatments were compared using one-way analysis of variance (ANOVA) according to the method of Tamhane (1977), Post hoc LSD test was also performed at $P \leq 0.05$.

Result and Discussion

Data in Table 1 and Table 2, determined the vegetative growth and chemicals analysis of the total chlorophyll content, and the accumulation of the compatible solute of the date palm shoot clusters explants *cv.* Bartamoda, on the rooting medium of different concentrations of sorbitol and mannitol and, the control treatment for 8 weeks under osmotic condition.

Data in Table 1 revealed that shoot clusters explants of control treatment achieved the highest result of shoot elongation, followed by the elongation of shoots clusters explants of sorbitol treatment at 0.1 M and, mannitol treatment at 0.1M, without significant differences among them. Elongation in date palm shoot clusters was suppressed; also, there is no any sign for new roots formation by the presence of high level of osmotic stress in rooting medium supplemented with sorbitol or mannitol at 0.5 M for 8 weeks (Fig.1).

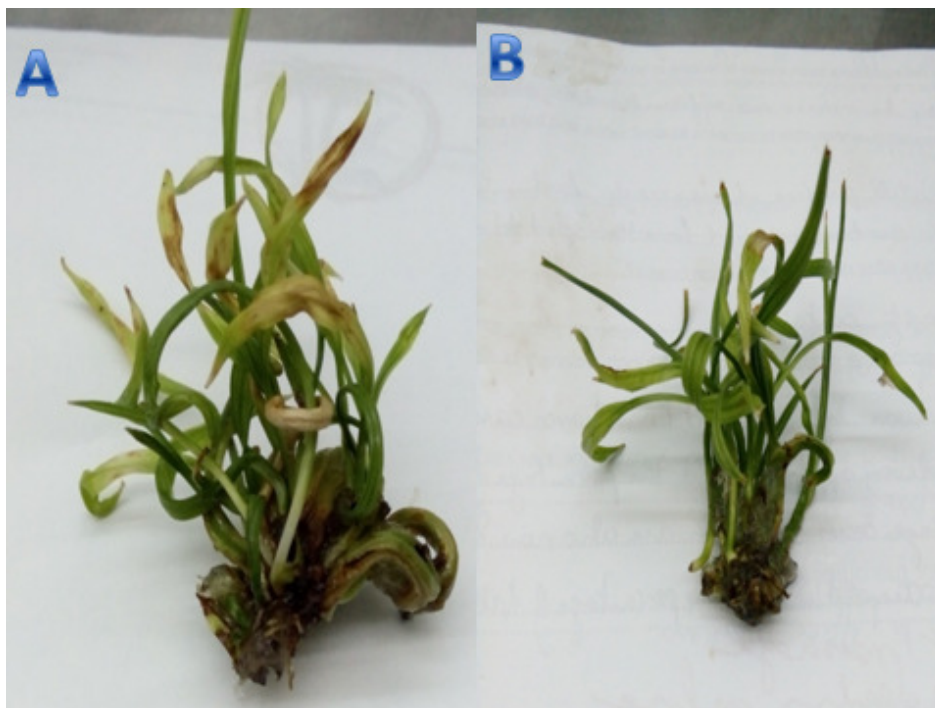


Fig 1: The effect of high level of osmotic stress in rooting medium supplemented with sorbitol and mannitol at 0.5 M for 8 weeks on date palm shoot clusters. The Elongation of shoot clusters was suppressed and, there is no any sign for new roots formation in both treatments and, they showed the lowest results of growth vigor degree. (A): Mannitol at 0.5 M. (B): Sorbitol at 0.5 M.

The highest result of new roots number was recorded by shoot clusters explants on rooting medium of control treatment, followed by the results of new roots number of shoot clusters explants of sorbitol sugar treatments at 0.3 M, 0.1 M and of mannitol sugar treatment at 0.1 M. The elongation value of new developed roots of the cultured shoot clusters explants has not significant differences between the control treatment and the osmotic stress treatments with sorbitol at 0.1, 0.3 M and mannitol at 0.1 M. The increase in concentration of mannitol sugar in rooting medium decreased significantly the growth vigor degree of the cultured shoot clusters explants. Cultured shoot clusters explants on rooting medium supplemented with sorbitol or mannitol sugar at 0.5 M showed the lowest same results of growth vigor degree. Whereas, the best result of growth vigor degree was recorded with shoot clusters explants on control treatment. There is a variety of carbon sources are used in culture media depending upon genotypes and specific stages of growth. Since *In vitro* plant cultures are not fully autotrophic, carbohydrates are supplemented in culture media to maintain the osmotic potential, as well as to serve as energy and carbon sources for developmental processes such as shoot proliferation, root induction as well as embryogenesis, and organogenesis. Mannitol and sorbitol are a six carbon alcoholic sugars that are often used in

laboratories as a medium for inducing osmotic stress in plant and tissue cultures (Yaseen *et al.*, 2013). It has been found that osmotic stress shortened the growth zone and reduced the cell elongation in some species (Royer *et al.*, 2016). Abu-Romman *et al.*, 2012 reported that vegetative and root growth of cucumber microshoots was negatively affected by osmotic stress, each increase in sorbitol sugar in culture medium was accompanied with a significant reduction in the adventitious root. Ghaheri *et al.*, 2015 found in *stevia rebaudiana* that, the more increase in mannitol concentration in the medium, the more decreased in shoot length, internode length, fresh weight of plants and growth rate have occurred. Addition of mannitol to nutrient culture, over a period of 3-4 weeks has an effect on gene expression, morphological and physiological characteristics and biochemical content in plants (Pandey and Chikara, 2014). These findings were paralleled with our presented data of the vegetative growth of cultured explants under osmotic stress condition in comparison with vegetative growth of cultured explants under control condition.

Data in Table 2, the chemical analysis of date palm shoot clusters explants of control treatment, and other different treatments of sorbitol and mannitol in rooting medium, for 8 weeks, showed that, the highest significant

value of chlorophyll content was recorded with shoot clusters explants of control treatment. Where, the lowest value of chlorophyll content was recorded in shoot clusters explants cultured on high concentrations of mannitol sugar, followed by sorbitol sugar at 0.5 M. Chlorophyll content of shoot clusters explants decreased significantly according to an increase of the concentration of sorbitol or mannitol sugars in rooting culture medium.

The same observation was detected in *O. europaea* spp. stressed shoots at sorbitol 0.2 M for 2 months, although the survival rates were 100% , there were growth reduction, and some senescence characteristics and shoots turned light green, suggesting that chlorophyll content was affected, as a result of stress conditions, where the chlorophyll content decreased due to chlorophyll degradation by the activation of chlorophyllases which converts chl b into chl a or be due to chlorophyll synthesis deficiency together with changes of thylakoid membrane structure (Brito *et al.*, 2003).

On the other hand, compatible solutes compounds such as amino acids, phenols, proline, total soluble sugar, reduced sugar and carotenoids, were significantly accumulated in shoot clusters explants of all different concentrations of sorbitol and mannitol treatments, in comparison to the control treatment, of rooting culture medium.

Shoot clusters explants of control treatment recorded the lowest significant value of amino acids, proline, phenols, total soluble sugar and reduced sugars contents. Whereas, the highest significant increase in amino acids, proline, phenols, and total soluble sugar and reduced sugars contents were recorded by date palm shoot clusters explants cultured on mannitol sugar treatment at high concentration at 0.5 M, followed by the results with sorbitol sugar treatment at the same higher concentration at 0.5 M. It can be observed that, the accumulation of amino acids content of date palm shoot clusters explants cultured on sorbitol or mannitol treatment at 0.3 M during 8 weeks, did not much fluctuate in proportion to its content in the shoot clusters explants cultured on control treatment. The increase in phenols and proline contents of date palm shoot clusters explants was positively correlated with the increase in the concentrations of both sorbitol or mannitol sugars in culture rooting medium for 8 weeks. Also, the same trends were observed with the accumulation of total soluble sugar and reduced sugar of date palm shoot clusters explants during 8 weeks of stress condition. It can be observed that all concentrations of mannitol sugar in rooting media had the superiority in increasing proline accumulation in all cultured date palm shoot clusters explants for 8 weeks. The highest value of carotenoids content was recorded in date palm shoot clusters explants cultured on control treatments of rooting medium for 8 weeks. It seems to be that carotenoids accumulation in date palm shoot clusters explants did not has noticeably increased, according to the presence of osmotic stress conditions.

Regarding to the studied compatible solutes accumulation in date palm shoot clusters, during osmotic stress treatments of rooting stage, it was reported that these compounds are accumulated in the cytoplasm and they differ among plant species. Compatible solutes have an important function in cellular osmotic control in response to stress conditions, which play as traps for harmful free radical (He *et al.*, 2018). It was confirmed that, plants exhibit mechanisms

at the molecular and cellular levels during osmotic stress and other stress conditions. The main role of these mechanisms is to overcome the resulting oxidative stresses reactive oxygen species (ROS) which are capable of damaging DNA, proteins, lipids and Chlorophyll. There are mainly enzymatic systems mechanisms, including superoxide dismutase (DOS), catalase, glutathione peroxidase, glutathione reductase, and glucose-6-phosphatedehydrogenase. As well as non-enzymatic mechanisms include low-molecule organic substances, which known as compatipale solutes such as carbohydrates like sugar alcohols (e.g. mannitol, sorbitol, trehalose etc.), free amino acids, proline, carotenoids, as well as numerous phenolic compounds. It was reported that these compounds are accumulated in the cytoplasm and they differ among plant species. Compatible solutes have an important function in cellular osmotic control in response to stress conditions, which play as traps for harmful free radical (He *et al.*, 2018). Stress disturbed amino acid and protein metabolism, lead to accumulation of free amino acids, many studies implicate that amino acids can play wide roles in plants as regulatory and signaling molecules. Since the accumulation of free amino acids under stress conditions indicates their involvement in osmotic adjustment (Rai, 2002; Wedeking *et al.*, 2017). It was indicated that the effect of phenolic acids on growth is a complex process and involved in the metabolism of hormones, protein synthesis, membrane permeability as well as the impact on respiration and oxidative phosphorylation (Vaughan and Ord, 1991). Structure of phenolic compounds, concentration, oxidation-reduction potential and hydrophilicity are factors, which determined their antioxidant properties (Winder *et al.*, 2014).

Proline is one of the most common metabolic responses of compatible solutes accumulated in plants under stress conditions. It contributes to osmotic adjustment, preserves enzymes and other important cellular structures, is assumed to scavenge hydroxyl radicals and is a reserve for carbon and nitrogen immediately after the relief of stress, which enhances tissue recovery (Franco and Melo, 2000; Slama, *et al.*, 2015).

Carbohydrate changes during stress condition are of particular importance because of their direct relationship with physiological processes in plant cell (Kerepesi and Galiba, 2014). High osmotic pressure of medium, is contributing to the stop of cells metabolism and suppression of suspension culture growth of somatic embryogenesis of *Phoenix dactylifera* (Zouine and El-Hadrami, 2004). There are differences between cultivars in the ability to accumulate carbohydrates were evident in control and applied stress conditions (Kerepesi and Galiba, 2014). The total endogenous carbohydrate amount increased in olive shoots grown on media with increasing concentrations of mannitol or sucrose (Rejskova *et al.*, 2007). The role of reducing sugars in the adaptive mechanism is more controversial (Kerepesi and Galiba, 2014). The accumulation of reduced sugars in plant tissue during osmotic conditions has been determined (Kaur *et al.*, 2012). It has been reported that degradation of reduced sugars is more efficient for energy utilization after relief of stress conditions (Rejskova *et al.*, 2007). Our data, showed an evidence of the increase in reducing sugars in proportional to total soluble sugar in all shoot clusters explants of different osmotic treatments. Because *In vitro* grown shoots are basically non-photosynthetic, It can be suggested that the accumulation of

carbohydrates and the composition of those carbohydrates reflected translocation and subsequent metabolism activity and accumulation of sink tissues during stress. In wheat seedlings during osmotic and salt stress the alteration in soluble carbohydrate content showed that the sucrose which translocated to the stem was initially metabolized to monosaccharides, which caused their content to rise, leads to an increase in reducing sugar as indicator of degree of salt and osmotic tolerance (Kerepesi and Galiba, 2014). Munns and Weir (1981) reported that initial changes in osmotic potential were largely due to changes in reducing sugars which supported by the increase in monosaccharides. Moreover, it was found that lower sucrose contents allow an increase in the quantities of reducing sugars (Farrant *et al.*, 1993; Leprince *et al.*, 1993).

Data in Table 3 and Table 4 showed various changes in the vegetative growth and chemicals analysis of the total chlorophyll content, and the accumulation of the compatible solute of the date palm shoot clusters explants *cv.* Bartamoda, of different concentrations of sorbitol and mannitol and, the

control treatment, after the recovery on normal rooting medium for two subcultures.

Date palm shoot clusters explants of different sorbitol and mannitol treatments in rooting medium for 8 weeks, showed various changes in vegetative growth when they returned to recover their growth on normal culture rooting media during two subcultures, comparing to shoot clusters explants of control treatment. Data in Table 3 showed that, the regrowth of shoots and roots of date palm shoots clusters during rooting stage, were sensitive to osmotic stress. Date palm shoots clusters explants cultured for 8 weeks on rooting medium supplemented with sorbitol sugar at 0.3 M exhibited the best higher values of shoot elongation, root number, root length and growth vigor when they returned to recover on normal culture rooting media for two subcultures. These results were followed significantly by vegetative growth recovery of all shoot clusters explants of mannitol sugars treatments at 0.3 M, in comparison with the vegetative growth of unstressed shoot clusters explants of control treatment, as shown in figure 2.

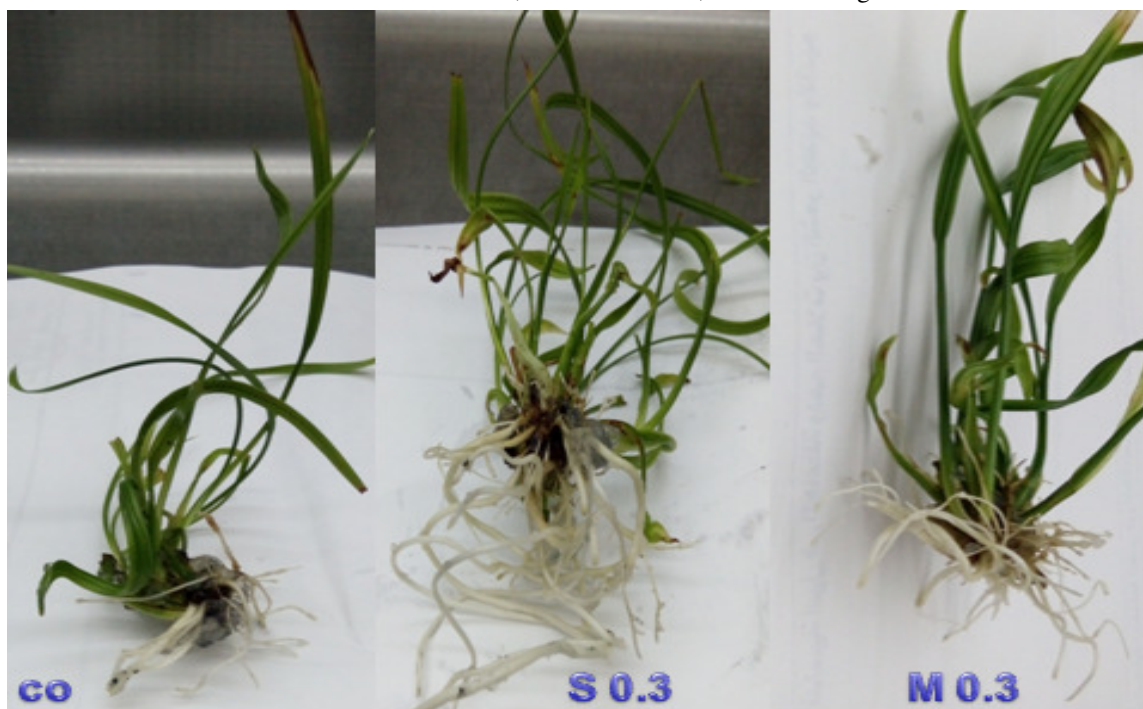


Fig. 2: Vegetative growth and root elongation of the stressed date palm shoots clusters after recovering them on normal rooting media for two subcultures. Co: Control; S 0.3: sorbitol at 0.3 M; M0.3: mannitol at 0.3 M.

Stressed explants of rooting media of sorbitol or mannitol sugars treatment at a high concentration at 0.5 M, had poorly vegetative growth recovery when they returned to culture on normal culture rooting media. On the light of the results obtained it is worth mentioning that, low concentrations of sorbitol and mannitol in growth medium had a little effect on regrowth rates and, a drastic decrease in regrowth rates was shown by high concentrations of, sorbitol and mannitol. But, it was noted that at a certain concentration of sorbitol sugar at 0.3 M, it encourages the recovery of date palm shoots clusters. Many factors affecting the regeneration capacity of the explant, the water content of the explant should be considered as one of the most important factors such as growth regulators and explant type regarding plant tissue culture response. Yildiz *et al.* (2016) reported that high water deficiency in tissue decreased the regeneration capacity of explant significantly. On the other hand, Royer *et al.*, 2016

found that in *Populus* root, following osmotic stress onset, root growth was strongly reduced but recovered rapidly. They confirmed that changes in the environment have a great effect on root growth. The biophysical patterns of root growth have been documented in response to different stress cusses. Studies have focused on comparing root growth, before stress versus the new steady growth rate. It has been shown that cell turgor pressure decreased very rapidly following the onset of osmotic stress, stopping growth, but was restored in growing cells. Growth was then only partially restored, indicating that molecular and physiological rewiring had taken place in addition to physical responses Royer *et al.* (2016). Osmotic stress interfered with growth by changing hormonal status and activating regulatory proteins (Baskin, 2013). According to our results, this may be referred to an adjustment for metabolic stress tolerance at certain concentration of sorbitol sugar during stress period. (Korver

et al., 2018) observed that sorbitol has given more strength to regenerated plant. They reported that stress conditions have major effects on auxin transport and distribution, which expected to have consequences for growth responses in the root.

Data in table 4 showed that the highest significant result of amino acids content was recorded with shoot clusters explants of control treatment on normal rooting medium, followed significantly by amino acids content of shoot clusters explants of sorbitol treatment or mannitol treatment at 0.3 M (without significant differences in between). Stressed shoot clusters explants of mannitol or sorbitol at 0.5 M were the highest in phenols and proline contents after recovery on normal rooting medium. Shoot clusters explants of control treatment was the lowest value in phenols and proline contents. On normal rooting medium, phenols and proline contents of shoot clusters explants increasing by an increase in the concentration of sorbitol or mannitol in rooting media during osmotic stress treatments. Date palm shoot clusters explants of sorbitol treatment or mannitol treatment at 0.3 M, have no a significant difference in proline accumulation values when they recovered on normal rooting medium. However, the data presented here reflect the importance of a physiological analysis of plant response. Obviously, the status of compatible solutes contents of recovered shoot clusters explants could explain our results. Since tissue of the germinating seeds of *Vitis californica* was found to have less total phenolics post-stress recovery (Weidner *et al.*, 2014). Phenolics compounds may be actively inhibit or stimulate growth and development in vegetative parts of plants. Depending on the potential of phenolics reactivity with proteins and enzymes, they are classified as either growth inhibitory or growth stimulatory. There are three forms of phenolic compounds (free, ester- bound and glycoside-bound) were observed to increase during stress conditions. An increase in accumulation of one or more of these forms of phenolic compounds, which depended on plant species, may have adverse effects on cells during stress conditions (Dixon and Paiva 1995; Winder *et al.*, 2001; Winder *et al.*, 2014). Chalker-Scott and Fuchigami (2018) reported that, the stimulatory effects of phenols such as caffeic acid have highly reactive hydroxyl groups in a

conformation that can inactivate IAA-oxidase. Conversely, inducing p-coumaric acid and cinnamic acids are not inimical to IAA-oxidase activity; hence, they are considered to be inhibitory to growth. Also, it has been reported that there is evidence that under different stress there is an increase in phenolic production and their incorporation to cell walls either as suberin and lignin (Chalker-Scott and Fuchigami, 1989).

Earlier studies showed that the mechanism of salinity tolerance in grass pea plants resulted probably from the elevated activity of antioxidant system in the root cells, manifested by increased accumulation of phenolic compounds and peroxidase activity (Piwowarczyk *et al.*, 2016).

On the normal rooting medium for two subcultures shoot clusters explants of control treatment gave the lowest value of chlorophyll content. Nevertheless, Chlorophylls content of shoot clusters explants, after recovery on normal rooting medium showed an increase due to the increase in sugar concentration of sorbitol or mannitol during the previous 8 weeks on rooting medium of different concentrations of sorbitol or mannitol. It is clear from data in table 4 and in table 2, that there is a decrease in contents of the amino acids, phenols, proline, and carotenoids, of date palm shoot clusters explants, after recovery on normal conditions of rooting medium than they were at 8 weeks of stress conditions of sorbitol or mannitol sugars. This observation was in opposite with the accumulation of chlorophylls in shoot clusters explants, which exhibited an increase at normal conditions of rooting medium, for two subcultures than they were at 8 weeks, on rooting medium of stress conditions of sorbitol or mannitol sugars. It can be suggested that the ability to rapidly rebuild plasma membranes to enhance their integrity can be one of the key processes of acclimatization to the emerging stress. Such a process was observed in 'Krab' seedlings. An upward trend in chlorophyll accumulation in seedlings, on higher osmotic stress treatments, indirectly confirmed this hypothesis (Piwowarczyk *et al.*, 2017). Clearly, all received full plantlets of 0.3 M sorbitol treatment exhibited a success in acclimatization stage, with best growth, as shown in Figure 3.



Fig. 3: Date palm plantlets after acclimatization for 3 months in greenhouse; plantlets treated with sorbitol 0.3 M exhibited good vegetative growth (A) and root elongation (B).

Table 1: Vegetative growth of date palm shoots clusters cultured on osmotic stress of different concentrations of sorbitol and mannitol in rooting medium for 8 weeks.

Treat	Shoot L.	Growth vigor	Root No.	Root L.
control	8.22 ^a	4 ^a	8.77 ^a	2.38 ^a
S 0.1	7.77 ^a	3.76 ^b	5.44 ^b	1.71 ^a
S 0.3	6.38 ^b	3.76 ^b	6.22 ^b	1.58 ^a
S 0.5	5.27 ^c	3 ^d	0	0
M 0.1	7.61 ^a	3.55 ^b	5.33 ^b	1.72 ^a
M 0.3	6.37 ^b	3.44 ^c	0.66 ^c	0.1 ^b
M 0.5	5.25 ^c	3 ^d	0	0
LSD at 0.05	1.02	0.22	2.26	0.94

Table 2: Chemical analysis of date palm shoots clusters cultured on osmotic stress of different concentrations of sorbitol and mannitol supplemented in rooting media for 8 weeks.

Treat	amino acids	Phenols	Prolin	Total Sugar	Reduced Sugar	Total chlorophyll	Carotenoids
control	3.459 ^g	48.04 ^g	0.08 ^g	6.357 ^g	5.94 ^g	30.567 ^a	8.657 ^a
S 0.1	7.731 ^c	56.44 ^f	0.119 ^f	6.634 ^e	8.923 ^c	23.357 ^c	8.309 ^d
S 0.3	5.102 ^e	57.11 ^c	0.134 ^d	7.576 ^d	9.319 ^c	22.909 ^d	8.31 ^d
S 0.5	9.892 ^b	65.67 ^b	0.36 ^b	8.507 ^b	9.379 ^b	17.906 ^e	8.41 ^c
M 0.1	7.312 ^d	64.05 ^d	0.125 ^c	6.713 ^f	8.814 ^f	24.181 ^b	8.037 ^f
M 0.3	4.338 ^f	64.47 ^c	0.142 ^c	8.467 ^c	9.211 ^d	13.683 ^f	8.243 ^e
M 0.5	12.8 ^a	68.95 ^a	0.521 ^a	8.557 ^a	9.449 ^a	13.231 ^g	8.483 ^b
LSD at 0.05	0.022	0.081	0.003	0.003	0.003	0.002	0.002

Table 3: Vegetative growth of date palm shoots clusters recovery on normal rooting media for two subcultures after osmotic stress conditions

Treat	Shoot L.	Growth vigor	Root No.	Root L.
control	15.12 ^d	4 ^d	8.66 ^d	4.85 ^d
S 0.1	16.62 ^c	4.33 ^c	9.71 ^c	5.86 ^c
S 0.3	20.98 ^a	5 ^a	12.55 ^a	7.88 ^a
S 0.5	13.33 ^{ef}	3.66 ^f	7.11 ^{ef}	3.43 ^{ef}
M 0.1	13.67 ^c	3.88 ^e	7.55 ^e	3.92 ^e
M 0.3	18.10 ^b	4.88 ^b	10.77 ^b	6.88 ^b
M 0.5	12.22 ^f	3.21 ^g	6.45 ^f	3.11 ^{ef}
LSD at 0.05	1.43	0.09	1.01	0.91

Table 4: Chemical analysis of date palm shoots clusters recovery on normal rooting media for two subcultures. after the osmotic stress of different concentrations of sorbitol and mannitol in rooting medium.

Treat	amino acids	Phenols	Proline	Total chlorophyll	Carotenoids
control	5.447 ^a	83.87 ^g	0.091 ^f	33.267 ^g	5.423 ^a
S 0.1	1.134 ^e	87.16 ^f	0.098 ^c	49.228 ^a	4.309 ^g
S 0.3	3.755 ^b	96.29 ^c	0.121 ^c	49.006 ^b	4.414 ^f
S 0.5	1.602 ^d	98.62 ^b	0.298 ^b	46.122 ^c	4.711 ^e
M 0.1	1.771 ^c	87.63 ^e	0.101 ^d	37.233 ^d	4.722 ^d
M 0.3	3.755 ^b	90.13 ^d	0.122 ^c	34.566 ^e	4.807 ^c
M 0.5	1.035 ^f	115.5 ^a	0.306 ^a	34.132 ^f	4.834 ^b
LSD at 0.05	0.002	0.134	0.002	0.002	0.002

In conclusion, from previous results, using osmotic treatment at 0.3 M sorbitol for 8 weeks during rooting stage enhanced the elongation, roots formation and chlorophyll contents of date palm shoots clusters which, could be transferred to acclimatization stage with high survival rates. Also, our data reflected the importance of a physiological analysis of compatible solutes accumulation as phenols and proline, which have been explained the received results after recovery.

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