# *IN VITRO* PROPAGATION AND CONSERVATION OF JERUSALEM ARTICHOKE

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The aim of this study is to preserve Jerusalem artichoke or tartufi (*Helianthus tuberosus* L.), due to the long time and risks of conservation in the open field. Shoot tip with single node explants were preserved for 3, 6 and 9 months on Murashige and Skoog (MS) medium fortified with three types of sugars (sucrose, sorbitol and mannitol) at 20, 30 and 40 g/l under full darkness at 15ºC. Medium with 30 g/l sucrose showed the highest survival percentage (100%) and was more effective with all periods of storage. The lowest survival percentage (22.22%) was recorded by mannitol (20 and 30 g/l) after 9 months period. The results showed that the highest increase in shoot length reached 11.5 cm with explants conserved on medium with 40 g/l sucrose for 9 months. On the other hand, no increase in shoots length has been recorded after 3 or 6 months of storage with sorbitol and mannitol treatments. Shoot number was also significantly induced only with sucrose, the highest mean value (4.0) was recorded with 40 g/l after 9 months. Sorbitol and mannitol caused complete absence of induced shoots/explant (1.0) with all concentrations. The highest mean values of roots length (2.17 cm) and number (7.0) were recorded by sucrose at 30 and 40 g/l after 9 months of storage. Complete absences of rooting was achieved with sorbitol and mannitol treatments after 3 or 6 months of storage. For *in vitro* recovery after storage, explants were cultured on MS medium supplemented with 0.5 mg/l benzyl aminopurine (BAP) and 0.125 mg/l naphthalene acetic acid (NAA) and gave the highest shoot length (16.5 cm) with sorbitol at 30 g/l, shoot number (1.33) with sucrose at 20 and 40 g/l, root number (7.00) with sucrose at 20 and 40 g/l and root length (18.33 cm) with mannitol at 30 g/l.

**Keywords**: tartufi, *in vitro* preservation, shoot tip, slow growth, sugars

## INTRODUCTION

Jerusalem artichoke or tartufi (*Helianthus tuberosus* L.) belongs to Asteraceae family, native to North America. Jerusalem artichoke has good cultivation, production under arid and semi-arid areas, characterized by good freezing and draught tolerance, high resistance to pests and plant diseases (Margaritis et al., 1981; Bajpai and Margaritis, 1982; Bajpai and Bajpai, 1991; Nakamura et al., 1996 and Guo et al., 2013). Jerusalem artichoke is cultivated as a valuable edible plant with strong medicinal properties. Consumption of tubers regulates blood pressure, helps normalize the blood sugar level and the functioning of the digestive system, protects the liver and kidneys, facilitates the absorption of iron, calcium, and magnesium and helps removing alcohol from blood. Tubers have cleansing properties, help eliminating heavy metals and organic toxins, improve the immune system, so they can be helpful in all kinds of infections. Moreover, Jerusalem artichoke can be used for bioethanol production (Chen et al., 2011). The increased oil price for nonrenewable oil resources has stimulated worldwide interest in the utilization of fermentation ethanol as a potential liquid fuel, and it could potentially also apply for soil remediation via removal of heavy metal pollutants (Margaritis and Bajpai, 1983).

Jerusalem artichoke can be efficiently propagated *in vitro* on relatively simple nutrient medium through repeated subcultures. Only one bud of two per node produces a shoot suitable for the next subculture. Tissue culture prevents the loss of plant genetic resources. *In vitro* conservation of plants based on tissue culture techniques enables storage of explants in the short, medium, or long terms. Jerusalem artichoke has been predominantly micropropagated through the culturing of tuber tissue explants. Thus, tissue culture enables germplasm of Jerusalem artichoke to be preserved in biodiversity conservation programs (Abdalla et al., 2014).

When using slow growth method, plants metabolic activity can be reduced through changes in the growth medium components (organic and inorganic nutrients, osmotic regulators, and growth retardants) (Rodrigues et al., 2018). Slow growth is one of the most successful *in vitro* techniques for medium-term germplasm conservation enabling plant storage for a prolonged period without plant manipulation, while maintaining plant resources readily available (Carlos et al., 2013 and Peng et al., 2015). Review on Jerusalem artichoke reported the success of mid-term preservation by reducing temperature and long-term cryopreservation, but slow growth storage using osmotic regulators of *in vitro* cultures has not been published (Sá et al., 2011). Several species of economic and medicinal importance had been conserved using the *in vitro* slow growth conservation technique, especially those which are vegetatively propagated (Sá et al., 2011 and Kamińska et al., 2016), because they don't perform photosynthesis activity. Plants that are *in vitro* preserved need a carbon source to supply

their energetic demands for ensuring their development. In this aspect, an ideal osmotic agent would be non-toxic and non-penetrating, reducing the osmotic potential of the medium (Bündig, 2016). Also, sorbitol and mannitol play a similar role, however sorbitol has more beneficial effects than mannitol according to Khan et al. (2009). It is necessary to expand the research since there is no standard conservation protocol that can be used for all species because the effects can vary according to the species, explant type and genotype among other aspects (Sá et al., 2011).

Therefore, the objective of the present investigation was to induce slow-growth preservation of Jerusalem artichoke shoot tip explants conserved at 15°C under complete darkness using different concentrations of osmotic agents (sucrose, sorbitol and mannitol).

## MATERIALS AND METHODS

The present study was carried out during the period from 2021 to 2022 at Tissue Culture Unit, Department of Genetic Resources, Desert Research Centre, Cairo, Egypt and Plant and Cryopreservation Laboratory, National Gene Bank, Agricultural Research Center, Giza, Egypt.

### 1. Culture Medium

Murashige and Skoog (MS) micro and macro-elements medium (1962) were used in the present study (Sigma-Aldrich, Germany). The pH of the medium was adjusted to 5.7 using 1.0 M HCl or 1.0 M NaOH. Phytagel at 3% (w/v) was added after pH adjustment. Media were dispensed into glass jars and sterilized for 20 min at 121°C.

### 2. Explants

Tubers of Jerusalem artichoke were brushed and washed under running water to remove mud and dirt, then kept in paper bags at 24°C until small sprouts appeared. Buds were excised and rinsed in distilled water, dipped in 70% ethanol for one min and stirred in 0.1% mercuric chloride for 10 min. To overcome phenols formation, buds were put in an antioxidant-sterilized solution (100 mg/l ascorbic acid and 150 mg/l citric acid) for 10 min. Finally, sprouts were rinsed with sterile distilled water three times.

### 3. *In Vitro* Propagation

Sterilized 0.3-0.5 mm meristems were placed in culture tubes (25x150 mm) containing 10 ml of solid starting MS medium supplemented with 2.0 mg/l D-calcium pantothenate, 0.1 mg/l gibberellic acid and 30 g/l sucrose, then the medium was solidified with 3.5 g/l phytagel for the initiation stage. Cultures were incubated at 20±2°C in darkness for 24 h for 3-4 days, then under 16 h (2000-lux, daily fluorescent tubes) for 2-4 weeks to produce *in vitro* plantlets. For mass multiplication of shoots, nodal cuttings with 1-2 nodes were cultured on solid MS medium with combinations of benzyl aminopurine (BAP) at 0.0, 0.25, 0.5 mg/l and naphthalene acetic acid (NAA)

at 0.0, 0.125, 0.25 mg/l to find the optimal combination for multiplication. Cultures were incubated for 3-4 weeks at 20±2°C under a 16 h photoperiod using cool white, fluorescent lights (50-60 μE/$m^2$/s light intensity). Survival percentage, shoots number, shoot length (cm), leaf number, rooting percentage, roots number and root length (cm) were recorded for each micropropagule.

**4. *In Vitro* Preservation**

Shoot tips with single node explants were placed in culture tubes containing 10 ml MS medium supplemented with different levels of sucrose, sorbitol and mannitol at 20, 30 and 40 g/l. Two explants per culture tube and six culture tubes were considered as a treatment. Cultures were stored for 3, 6 and 9 months in an incubator at full darkness (Eyela LT1-1200-Japan) at 15°C. Survival percentage, shoot number per propagule, shoot length (cm), root number per propagule and root length (cm) were recorded for each conservation period.

**5. *In Vitro* Recovering After Preservation**

*In vitro* preserved plantlets were evaluated for recovering. Plantlets were subcultured on the optimal multiplication medium; cultures were incubated for 6-8 weeks at 22±2°C under a 16 h photoperiod using cool white, fluorescent lights (50-60 μE/$m^2$/s light intensity). Survival percentage, shoot number per propagule, shoots length (cm), root number per propagule and root length (cm) were recorded for each micropropagule.

**6. Statistical Analysis**

Data of experiments were statistically analyzed as completely randomized design with four replicates. The recorded data were analyzed statistically using analysis of variance technique (ANOVA) and by multiple range tests (Steel et al., 1997). Duncan's Multiple Range Test as described by Duncan (1955) and Least Significant Difference (LSD) at $p \leq 0.05$ level of confidence were employed to compare the differences among means.

## RESULTS

**1. *In Vitro* Propagation**

Plantlets obtained through meristem culture of Jerusalem artichoke were multiplied on MS medium with nine combinations of growth regulators of BAP (0.0, 0.25, 0.5 mg/l) and NAA (0.0, 0.125, 0.25 mg/l). Data in Table (1) reveal that the survival percentage was 100% with all treatments. Medium supplemented with 0.5 mg/l BAP achieved the highest mean values of both length (15 cm) and number (7) of shoots and leaves number (14.67). Maximum percentage of rooted plantlets (100%) was recorded with NAA at 0.125 or 0.25 mg/l. The highest length of roots (4.67 cm) was obtained with BAP at 0.5 mg/l plus NAA (0.125 or 0.25 mg/l). Also, the highest number of roots (4.33) was recorded with 0.5 mg/l BAP and 0.25 mg/l NAA.

**Table (1).** Effect of different growth regulators on survival %, shoot length (cm), shoot number, leaves number, root length (cm) and root numbers of *in vitro* propagated Jerusalem artichoke.

| BAP conc. (mg/l) | NAA conc. (mg/l) | Survival % | Shoot length (cm) | Shoot no./ propagule | Leaves no./ propagule | Rooting % | Root length (cm) | Root no./ propagule |
|---|---|---|---|---|---|---|---|---|
| **0.00** | **0.000** | 100 | 8.00d | 3.00c | 11.00bc | 0.00d | 0.00c | 0.00d |
| **0.00** | **0.125** | 100 | 9.33d | 4.00bcd | 11.00bc | 100.00a | 4.66a | 3.00bc |
| **0.00** | **0.250** | 100 | 9.67dc | 4.33bc | 12.66ab | 100.00a | 2.33b | 3.67ab |
| **0.25** | **0.000** | 100 | 14.0b | 6.33a | 14.61a | 0.00d | 0.00c | 0.00d |
| **0.25** | **0.125** | 100 | 14.33b | 4.67bc | 10.00c | 33.33c | 2.83b | 3.67ab |
| **0.25** | **0.250** | 100 | 13.00b | 5.00b | 9.00c | 55.00b | 3.83a | 3.00bc |
| **0.50** | **0.000** | 100 | 15.00a | 7.00a | 14.67a | 58.33b | 0.64c | 2.33c |
| **0.50** | **0.125** | 100 | 12.00c | 4.33bc | 9.00c | 25.00c | 4.67a | 3.67ab |
| **0.50** | **0.250** | 100 | 13.67b | 3.67cd | 10.67bc | 26.67c | 4.67a | 4.33a |

Means followed by the same letter (s) in each column are not significantly different from each other at 5% level.

**2. *In Vitro* Preservation**

Jerusalem artichoke shoot tip with single node explants were preserved on MS medium supplemented with different levels of sucrose, sorbitol and mannitol at 20, 30 and 40 g/l for 3, 6 and 9 months, at 15°C in complete darkness. Preservation vegetative parameters are shown in Tables (2-7) and Fig. (1). For survival percentage, data in Table (2) demonstrate that survival percentage of shoot tip explants was decreased gradually as the conservation period increased without significant differences among 3 and 6 months (81.48 and 74.08%), respectively. Also, increasing the conservation period from 6 to 9 months gradually decreased the survival percentage from 74.08 to 55.56% without significant differences among them. Concerning the effect of different sugar concentrations, results showed that 100% was achieved with sucrose at 30 g/l. While the lowest significant value of survival percentage (48.15%) was achieved on conservation media with mannitol at 30 and 40 g/l. Regarding the effect of interaction between conservation periods and different sugar concentrations, results revealed that sucrose at 30 g/l showed 100% survial with all conservation periods. Meanwhile, the lowest survival percentage (22.22%) was recorded on conservation media with mannitol at 20 or 30 g/l for 9 months.

Results in Table (3) indicate the effect of different sugar concentrations and different preservation period on shoot length (cm) of shoot tip explants conserved at 15°C under complete darkness. The effect of different periods on explants preserved for 3 or 6 months was the lowest, insignificant shoot lengths reached 0.89 and 1.31 cm, respectively, which increased significantly reaching 4.29 cm after 9 months. Regarding the effect of different sugar concentrations, results showed that the highest shoot

length reached 6.22 cm with sucrose at 40 g/l. While sorbitol at 40 g/l and mannitol at 30 or 40 g/l showed the lowest mean value of shoot length (0.38, 0.43 and 0.23 cm, respectively) as a positive impact during storage. Concerning the effect of interaction between different conservation periods and sugar concentrations, results showed that increasing sucrose concentrations from 20 to 40 g/l at the end of the conservation period (9 months) increased the mean value of shoot length from 7.33 to 11.5 cm with significant differences among all treatments. Meanwhile, for explants cultured on media with sorbitol or mannitol treatments for 3 or 6 months, the shoot length was remained unchanged (0.0 cm). Shoot length on the other treatments under came in-between.

Concerning shoot number, Table (4) clarifies that 1.0 new shoot/explant was recorded after 3 or 6 months of preservation. While shoot number reached 1.59 after 9 months. Referring to the effect of different sugar concentrations, sucrose achieved the highest mean values of shoot number/ explant (1.33, 1.44 and 2.00) when explants cultured on media containing 20, 30 and 40 g/l, respectively, directly with increasing concentrations. On the other hand, a complete absence of induced shoots (1.0) was shown with sorbitol and mannitol at all concentrations. Regarding the interaction between storage periods and sugars concentrations, the highest mean value of shoot number (4.0) was recorded with sucrose at 40 g/l after 9 months of storage. While no shoots/ explant (1.0) was recoded with either sorbitol or mannitol at all concentrations during preservation periods and all concentrations of sucrose for 3 or 6 months.

Concerning roots number/explant, data in Table (5) indicate that conservation media with different sugar concentrations did not produce any roots at 3 or 6 months of storage. While, after 9 months of storage, the number of roots/explant was increased to 2.22 with significant differences among all conservation periods. Concerning to the effect of different sugar concentrations, results clearly showed that conservation medium with 40 g/l sucrose showed the highest number of roots (2.34). Explants conserved on media with all concentrations of sorbitol or medium with 40 g/l mannitol, showed the lowest number of roots (0.34), in addition to the media with 20 or 30 g/l mannitol (0.23) with no significant differences among them. Regarding the effect of interaction between conservation periods and different sugar concentrations, results revealed that medium with 30 g/l sucrose after 9 months of storage gave the highest mean nubmer of roots (7.0) with significant differences among all treatments.

**Table (2).** Effect of different sugar concentrations and preservation periods on the survival percentage of Jerusalem artichoke explants conserved at 15℃ under complete darkness.

| Treatment | Conc. (g/l) | Survival % Period (months) | | | Mean |
|---|---|---|---|---|---|
| | | 3 | 6 | 9 | |
| **Sucrose** | **20** | 88.89 b | 88.89 b | 88.89 b | 88.89 B |
| **Sucrose** | **30** | 100.00 a | 100.00 a | 100.00 a | 100.00 A |
| **Sucrose** | **40** | 88.89 b | 77.78 c | 77.78 c | 81.48 BC |
| **Sorbitol** | **20** | 77.78 c | 77.78 c | 55.56 e | 70.37 CDE |
| **Sorbitol** | **30** | 77.78 c | 66.67 d | 44.44 f | 62.96 DE |
| **Sorbitol** | **40** | 88.89 b | 77.78 c | 55.56 e | 74.08 CD |
| **Mannitol** | **20** | 66.67 d | 55.56 e | 22.22 h | 48.15 F |
| **Mannitol** | **30** | 66.67 d | 55.56 e | 22.22 h | 48.15 F |
| **Mannitol** | **40** | 77.78 c | 66.67 d | 33.33 g | 59.26 E |
| **Mean** | | 81.48 A | 74.08 AB | 55.56 B | |

Means followed by the same letter (s) in each column are not significantly different from each other at 5% level.

**Table (3).** Effect of different sugar concentrations and preservation periods (month) on shoot length (cm) of Jerusalem artichoke explants conserved at 15℃ under complete darkness.

| Treatment | Conc. (g/l) | Shoot length (cm) Period (months) | | | Mean |
|---|---|---|---|---|---|
| | | 3 | 6 | 9 | |
| **Sucrose** | **20** | 1.84 h | 3.73 e | 7.33 c | 4.30 C |
| **Sucrose** | **30** | 3.00 f | 4.00 d | 8.50 b | 5.17 B |
| **Sucrose** | **40** | 3.17 f | 4.00 d | 11.5 a | 6.22 A |
| **Sorbitol** | **20** | 0.00 k | 0.00 k | 3.00 f | 1.01 D |
| **Sorbitol** | **30** | 0.00 k | 0.00 k | 2.57 g | 0.86 D |
| **Sorbitol** | **40** | 0.00 k | 0.00 k | 1.13 i | 0.38 E |
| **Mannitol** | **20** | 0.00 k | 0.00 k | 2.60 g | 0.87 D |
| **Mannitol** | **30** | 0.00 k | 0.00 k | 1.27 i | 0.43 E |
| **Mannitol** | **40** | 0.00 k | 0.00 k | 0.67 j | 0.23 E |
| **Mean** | | 0.89 B | 1.31 B | 4.29 A | |

Means followed by the same letter (s) in each column are not significantly different from each other at 5% level.

**Table (4).** Effect of different sugar concentrations and preservation periods on shoot number of Jerusalem artichoke explants conserved at 15°C under complete darkness.

| Treatment | Conc. (g/l) | Shoot no./explant Period (months) | | | Mean |
|---|---|---|---|---|---|
| | | 3 | 6 | 9 | |
| **Sucrose** | **20** | 1.00 d | 1.00 d | 2.00 c | 1.33 B |
| **Sucrose** | **30** | 1.00 d | 1.00 d | 2.33 b | 1.44 B |
| **Sucrose** | **40** | 1.00 d | 1.00 d | 4.00 a | 2.00 A |
| **Sorbitol** | **20** | 1.00 d | 1.00 d | 1.00 d | 1.00 C |
| **Sorbitol** | **30** | 1.00 d | 1.00 d | 1.00 d | 1.00 C |
| **Sorbitol** | **40** | 1.00 d | 1.00 d | 1.00 d | 1.00 C |
| **Mannitol** | **20** | 1.00 d | 1.00 d | 1.00 d | 1.00 C |
| **Mannitol** | **30** | 1.00 d | 1.00 d | 1.00 d | 1.00 C |
| **Mannitol** | **40** | 1.00 d | 1.00 d | 1.00 d | 1.00 C |
| **Mean** | | 1.00 B | 1.00 B | 1.59 A | |

Means followed by the same letter (s) in each column are not significantly different from each other at 5% level.

**Table (5).** Effect of different sugar concentrations and preservation periods on root number of Jerusalem artichoke explants conserved at 15°C under complete darkness.

| Treatment | Conc.(g/l) | Root no./explant Period (months) | | | Mean |
|---|---|---|---|---|---|
| | | 3 | 6 | 9 | |
| **Sucrose** | **20** | 0 e | 0 e | 4.00 b | 1.34 B |
| **Sucrose** | **30** | 0 e | 0 e | 7.00 a | 2.34 A |
| **Sucrose** | **40** | 0 e | 0 e | 3.67 c | 1.23 B |
| **Sorbitol** | **20** | 0 e | 0 e | 1.00 d | 0.34 C |
| **Sorbitol** | **30** | 0 e | 0 e | 1.00 d | 0.34 C |
| **Sorbitol** | **40** | 0 e | 0 e | 1.00 d | 0.34 C |
| **Mannitol** | **20** | 0 e | 0 e | 0.67 d | 0.23 C |
| **Mannitol** | **30** | 0 e | 0 e | 0.67 d | 0.23 C |
| **Mannitol** | **40** | 0 e | 0 e | 1.00 d | 0.34 C |
| **Mean** | | 0 B | 0 B | 2.22 A | |

Means followed by the same letter (s) in each column are not significantly different from each other at 5% level.

Root length results are represented in Table (6). Regarding the effect of conservation periods, 9 months period achieved the highest mean value of root length (0.74 cm) with significant differences among all conservation periods. Concerning the effect of different sugar concentrations, results showed that sucrose at 40 g/l gave the highest root length (0.73 cm) with significant differences among all sorbitol or mannitol treatments. The

interaction between conservation periods and different sugar concentrations revealed that medium with 40 g/l sucrose after 9 months of storage gave the highest mean value of root length (2.17 cm) with significant differences among all treatments.

**3. *In Vitro* Recovery of Survival Plantlets After Preservation**

Survived explants of Jerusalem artichoke conserved on full strength MS medium supplemented with different osmotic agents (sucrose, sorbitol and mannitol) at different concentrations were taken at the end of conservation periods (9 months) and cultured in 250 mm jars filled with 25 ml of recovery medium (normal growth medium), consisted of full-strength MS medium supplemented with BAP at 0.5 mg/l and NAA at 0.125 mg/l. The cultures were incubated under normal growth conditions.

**Table (6).** Effect of different sugar concentrations and preservation periods on root length of Jerusalem artichoke explants conserved at 15°C under complete darkness.

| Treatment | Conc. (g/l) | Root length (cm) Period (months) | | | Mean |
|---|---|---|---|---|---|
| | | 3 | 6 | 9 | |
| **Sucrose** | **20** | 0 g | 0 g | 1.83 b | 0.62 AB |
| **Sucrose** | **30** | 0 g | 0 g | 1.50 c | 0.51 B |
| **Sucrose** | **40** | 0 g | 0 g | 2.17 a | 0.73 A |
| **Sorbitol** | **20** | 0 g | 0 g | 0.50 d | 0.17 C |
| **Sorbitol** | **30** | 0 g | 0 g | 0.30 e | 0.11 C |
| **Sorbitol** | **40** | 0 g | 0 g | 0.10 fg | 0.04 C |
| **Mannitol** | **20** | 0 g | 0 g | 0.14 f | 0.05 C |
| **Mannitol** | **30** | 0 g | 0 g | 0.07 fg | 0.03 C |
| **Mannitol** | **40** | 0 g | 0 g | 0.10 fg | 0.04 C |
| **Mean** | | 0 B | 0 B | 0.74 A | |

Means followed by the same letter (s) in each column are not significantly different from each other at 5% level.

Data presented in Table (7) and Fig. (2) show the effect of different sugar concentrations on micropropagation measurements of *in vitro* preserved explants after 9 months. Regarding the shoot length, the highest length (16.50 cm) was recorded with sorbitol at 30 g/l and the lowest (10.00 cm) was achieved with 20 g/l sucrose. For shoot number, results illustrated that the highest mean value (1.33) was recorded with sucrose at 20 or 40 g/l without significant differences among sugar types and concentrations. Also, sucrose produced the highest mean value of roots number (7.00 at 20 or 40 g/l) without significant differences among them. Regarding root length, mannitol at 30 g/l recorded the highest mean value (18.33 cm) and the lowest value (9.16 cm) was recorded with sucrose at 20 g/l.

## DISCUSSION

In mid-term preservation by slow growth, the reduction of osmotic potential of the culture medium can explain the action of the osmotic agents by reducing the water potential and availability of water and nutrients in the medium inducing slower growth (Huang et al., 2014; El-Bahr, 2016 and Rodrigues et al., 2018). Mannitol and sorbitol are main osmotic regulators used to limit the *in vitro* growth of explants by inducing osmotic stress, which modify the hydric potential of the culture medium (Silva and Scherwinski-Pereira, 2011). Sucrose, sorbitol and mannitol act by removing the excess intracellular water by osmotic gradient, causing the culture growth to occur more slowly and making its conservation possible (Arrigoni-Blank, 2014).

**Table (7).** Effect of different sugar concentrations on shoot and root length and number on recovered explants of Jerusalem artichoke after storage for 9 months at 15°C under complete darkness.

| Treatments | Conc. (g/l) | Shoot length (cm) | Shoot no./ propagule | Root length (cm) | Root no./ propagule |
|---|---|---|---|---|---|
| **Sucrose** | **20** | 10.00 d | 1.33 a | 9.16 d | 7.00 a |
| **Sucrose** | **30** | 12.50 c | 1.00 a | 12.50 c | 6.33 a |
| **Sucrose** | **40** | 16.00 ab | 1.33 a | 15.16 b | 7.00 a |
| **Sorbitol** | **20** | 13.83 bc | 1.00 a | 18.00 a | 6.00 a |
| **Sorbitol** | **30** | 16.50 a | 1.00 a | 16.33 ab | 5.66 a |
| **Sorbitol** | **40** | 15.66 ab | 1.00 a | 16.33 ab | 5.33 a |
| **Mannitol** | **20** | 15.00 ab | 1.00 a | 16.67 ab | 5.66 a |
| **Mannitol** | **30** | 15.00 ab | 1.00 a | 18.33 a | 5.66 a |
| **Mannitol** | **40** | 14.83 ab | 1.00 a | 15.17 b | 6.00 a |

Means followed by the same letter (s) in each column are not significantly different from each other at 5% level.

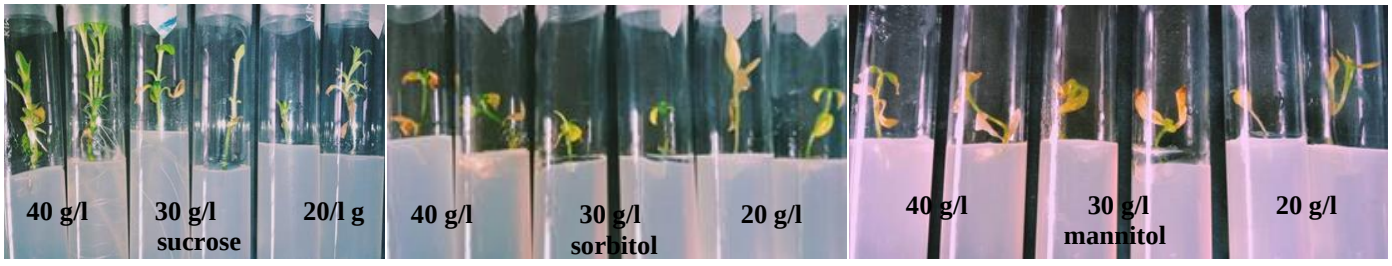


**Fig. (1).** Shoot tip with single node explants of Jerusalem artichoke preserved on different levels of sucrose, sorbitol, and mannitol after 9 months of storage.

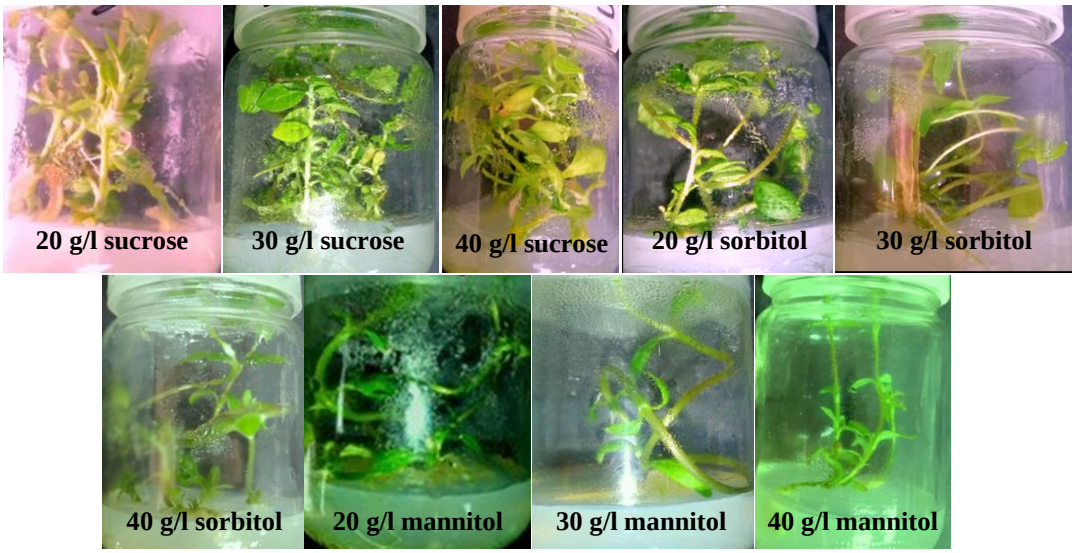


**Fig. (2).** Survived Jerusalem artichoke plantlets recovered on normal growth medium, after being conserved on full strength MS medium supplemented with different levels of sucrose, sorbitol, and mannitol after 9 months of storage.

The results regarding the survival percentage showed that it was decreased significantly by increasing period or sugars type and concentration gradually and the lowest values were achieved by mannitol. These results agree with El-Homosany and Noor El-Deen (2019), who studied the effect of different conservation periods from 2, 4, 6, 8 to 10 months on shoot tip explants of Paulownia (*Paulownia tomentosa*) under different osmotic regulators; sucrose or sorbitol alone or in combinations and they indicated that survival percentage decreased gradually with increasing the conservation period.

The results of the present study indicated that, the addition of sorbitol or mannitol reduced all growth parameters (survival percentage, number and length (cm) of shoots or roots) after 6 months of storage and recorded no increase of shoots or roots number and length with media containing sorbitol or mannitol treatments. These results agree with Shatnawi et al. (2011), who reported that sucrose dose at 3 to 9% (w/v) recorded the maximum survival rate (94.6%) compared to sorbitol or mannitol in *Stevia rebaudiana*. The same results were reported by Sá et al. (2011), who observed a similar response of adding mannitol to the medium that negatively affected the survival percentage of mangaba microplants. Also, Silva and Scherwinski-Pereira (2011) found that most culture media supplemented with sorbitol and

mannitol produced low survival rates in studies with *Piper aduncum* and *Piper hispidinervum.* Similarty, Huang et al. (2014) found that a greater reduction in the shoots of *Polygonum multiflorum* was observed in the presence of higher sucrose, sorbitol and mannitol concentrations. In the same context, Tecla et al. (2019) explained that, with increasing concentrations of the osmotic agents (mannitol or sorbitol) alone in culture medium, a significant reduction in the number of green leaves per plant, declined length of the aerial part, decreased length of the main root and reduced growth frequency were observed with *Poincianella pyramidalis.*

## CONCLUSION

To our knowledge, this study of mid-term conservation of Jerusalem artichoke under osmotic agents is the first. Based on the results of this study, it can be concluded that MS medium supplemented with sorbitol at 20 or 40 g/l can slow the explant growth and recommended for the storage of Jerusalem artichoke explants with survival percentage of 77.78% without changing for 6 months at 15˚C under complete darkness.

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# الإكثار الدقيق والحفظ المعملي لنبات الطرطوفة

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هدف هذه الدراسة هو الحفاظ على نباتات الطرطوفة، وذلك بسبب الوقت الطويل ومخاطر حفظها في الحقول المفتوحة. تم الحفاظ على القمم النامية ذات العقدة الواحدة لمدة ٣ و٦ و٩ أشهر في بيئة موراشيج وسكوج (MS) مضافًا إليها ثلاثة أنواع من السكريات (السكروز والسوربيتول والمانيتول) بتركيز ٢٠ و٣٠ و٤٠ جرام/لتر وحضنت على درجة ١٥ مئوية في إظلام كامل. بالنسبة للنسبة المئوية للبقاء، أظهرت البيئة التي تحتوي على ٣٠ جرام/لتر سكروز أعلى نسبة بقاء وأكثر فاعلية مع جميع فترات التخزين (١٠٠٪). بينما سجلت أقل نسبة بقاء (٢٢.٢٢٪) مع المانيتول عند ٢٠ أو ٣٠ جرام/لتر) بعد ٩ أشهر من التخزين. بالنسبة لطول الساق، أوضحت النتائج أن أعلى متوسط زيادة في طول الساق بلغ ١١.٥ سم مع النباتات المحفوظة في بيئة تحتوي على ٤٠ جرام/لتر سكروز لمدة ٩ أشهر. من ناحية أخرى، لم يتم تسجيل أي زيادة في طول الفروع بعد ٣ أو ٦ أشهر من التخزين مع معاملات السوربيتول والمانيتول. كما أظهر عدد الأفرع زيادة بشكل معنوي فقط مع إضافة السكروز، وسجلت أعلى القيم المتوسطة (٤) مع ٤٠ جرام/لتر بعد ٩ أشهر من الحفظ بينما حقق السوربيتول والمانيتول غياب تام لإنتاج أفرع جديدة (١) مع جميع التركيزات. تم تسجيل أعلى قيم متوسطة لطول الجذور (٢.١٧ سم) وعددها ٧ مع السكروز عند تركيز ٣٠ و٤٠ جرام/لتر بعد ٩ أشهر من الحفظ. كما كان هناك غياب تام للجذور في النباتات باستخدام معاملات السوربيتول والمانيتول بعد ٣ أو ٦ أشهر من الحفظ. بالنسبة لاستعادة النمو بعد الحفظ، تمت زراعة المنفصلات النباتية على بيئة موراشيج وسكوج تحتوي على ٠.٥ مليجرام/لتر بنزيل أمينوبيورين و٠.١٢٥ مليجرام/لتر إندول حمض الخليك وأعطت أعلى طول (١٦.٥ سم) مع السوربيتول عند ٣٠ جرام/لتر، وكان عدد الأفرع الكلي ١.٣٣ باستخدام السكروز عند ٢٠ أو ٤٠ جرام/لتر) وعدد الجذور ٧ مع السكروز عند ٢٠ أو ٤٠ جرام/لتر وطول الجذر ١٨.٣٣ سم مع المانيتول عند ٣٠ جرام/لتر.