SHORT TERM PRESERVATION FOR FIG (*Ficus carica* cv. Black fig) BY DIFFERENT OSMOTIC STABILIZERS

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Fig (*Ficus carica* L.) is a deciduous tree that belongs to the *Moraceae* family which grows in various diverse environments and soil types. Due to its genomic characteristic in the ability to tolerate water deficit and moderate salinity stress, it is a suitable species for cultivation in semi-arid environments of regions as the Mediterranean and Middle-East, where it is considered as one of the most significant plants crops grown (Metwali *et al*., 2014). In Egypt, fig has a great potential value of use in the agricultural sector due to its biological diversity with wide range of both genotypes and phenotypes (Mars, 2003).The planting of fig (*Ficus carica* L.) occurs primarily with vegetative propagated plants, particularly by the rooting of cuttings or seeds (Mars, 2003; Pasqual and Ferreira, 2007). Due to industrialization and deforestation, many of the species and varieties are currently threatened; in addition to being continuously vulnerable to loss and genetic transmutation because of the absence of safe long-term preservation means as well as acceptable ways for validation.

Hence, the use of tissue culture techniques can be of great interest for germplasm collection, multiplication and storage of recalcitrant and vegetative propagated species. Preservation of plant genetic resources has become extremely important for crop improvement in order to face the increasing depletion of natural resources (Jain, 2012). *Ficus* germplasms are characterized by great diversity since a high number of varieties and accessions has been identified (Mars, 2003). Therefore, establishment of fig germplasm long-term preservation is a major purpose to make fig germplasm available for research. Amongst the different techniques of *in vitro* preservation is the short and medium term conservation. Short –term conservation is applied when there is a need to lengthen the period between subcultures by reducing growth rate. Slow growth is usually achieved by reducing the culture temperature (Moges *et al*., 2003), low light intensity conditions or complete darkness (Wang and Charles, 1991).

Plant cells include a cell wall surrounding their plasma membrane; during tissue culturing, the mechanical barrier of the cell wall is often broken. For this reason osmotic stabilizers are added to avoid

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osmotic stress that has harmful effects on cell metabolism and growth. Low osmotic potentials are usually produced by the addition of various ionic or non-ionic solutes. Non-ionic substances consist of soluble carbohydrates as mannitol, sorbitol, glucose, fructose and sucrose. In general sugar solutions can produce an appropriate osmotic potential (Chawla, 2002). Osmotic potential is generated differently depending on the plant type; therefore finding the appropriate concentration of the osmotic is needed in order to identify the optimum conditions for *in vitro* short-term preservation.

Genetic analysis through molecular markers is a pre-requisite for having deep insight on the genome organization in the wild species. Moreover, the precise number of cultivar and species is still unknown since mislabeling problems are often found (Gao *et al*., 2006). Therefore, it is imperative to establish strategies for the preservation of *Ficus* germplasm and for the identification of its varieties as well. Many DNA based markers are available to identify the varieties and also these markers can be effectively used to answer the phylogenetic relationship between *Ficus* varieties (Salhi- Hannachi *et al*., 2006; Chatti *et al*., 2007). *Ficus* species are represented by a large number of varieties which are facing genetic erosion. Inter-simple Sequence Repeat (ISSR) markers were used to assess the identification of 23 important *Ficus* varieties and to determine the genetic relationships among these species. Out of twenty one ISSR primers tested, five primers produced 116 detectable fragments, out of which 106 were polymorphic across the species/varieties. Each of the five primers produced fingerprint profile unique to each of the species/variety studied and thus could be solely used for their identification. (Rout and Aparajita, 2009). Inter Simple Sequence Repeats (ISSR) is one of the polymerase chain reaction (PCR) varieties that is considered as a fast and inexpensive technique; and is widely used in many applications. The most common use is the detection of genomic instability and mutation. ISSR overcomes several of the limitations encountered by different marker systems and has high reproducibility (Guasmi *et al*., 2006).

The present study was aimed at investigating the use of osmotic stabilizers (mannitol and sorbitol, with concentrations of [40 g/L, 50 g/L and 60 g/L]) at two different temperatures (5°C, 10°C) to determine which osmotic stabilizer, concentration and temperature would display an eminent effect on the *in vitro* fig shoot cultures, in order to achieve optimum media conditions for efficient short -term preservation of fig (*Ficuis carica*). Sucrose was used as the control media at a concentration of (30 g/L).

MATERIALS AND METHODS

The study was carried out in the National Gene Bank and Genetic Resources (NGBGR), Agricultural Research Center, Giza, Egypt, during throughout late 2015 to early 2016.

1. Explant Source

Shoot tip explants from the black fig (*Ficus carica* L.) form Siwa (1 cm long) were taken from in vitro cultures after 1st subculture and separately cultured onto glass tubes (100 x 25 mm), the explant that were used in this experiment were provided by the "National Gene Bank and Genetic Resources (NGBGR), Agricultural Research Center", Giza, Egypt.

2. In vitro conservation

Shoot segments were cut from the explant source in order to develop the shoot tip cultures. The developed cultures were used to determine the effect of two different osmotic agents (sorbitol and mannitol) at two different temperatures (5°C and 10°C). Explants were cultured on full strength MS medium with 0.8% (w/v) agar and different concentration of different osmotic agents. Three concentrations of mannitol and sorbitol, 40 g/L, 50 g/Ll and 60 g/L, were used for the conservation. Explants cultured on medium with 30 g/L sucrose were used as a control. The experiment was arranged as factorial experiment in a completely randomized design of {6 treatments plus the control x 3 conservation periods} using 10 explants for each treatment. The 10 explants were distributed for incubation at two different temperatures (5°C and 10°C). Shoot height, number of branches and were recorded at the end of each conservation period and observed after 30 days intervals for 3 months. The obtained data were statistically analyzed according to Snedecor and Cochran, (1982). Least significant difference (LSD) at $p<0.05$ was employed to estimate the significant of differences among the treatment means.

3. DNA Isolation and ISSR analysis

Genomic DNA extraction was carried out using leaf constituents collected from plantlets of each treatment. DNA was extracted and purified using the DNeasy plant Mini Kit following the manual instructions (QIAGEN, Chatsworth, CA). Molecular fingerprinting of fig based on Inter Simple Sequence Repeats. (ISSRs) was performed according to the procedure given by Sharma *et al*. (1995). ISSR analysis was carried out in a total volume of 50 µl containing 5 µl of 10x buffer, 10 µl of Q solution, 5 µl of 2 mM dNTPs, 80 pmol primer, 0.5 µl hot start taq polymerase and 25 ng DNA. The temperature profile was composed of initial denaturation cycle at 95°C for 25 min followed by 10 touch down cycles of 95°C/30 sec, 65-55°C/1 min, 72°C 90 sec. This was followed by 30 cycles of 95°C, 55°C/ 1min, 72°C/90 sec and then a final extension cycle at 72°C for 7 min. The sequences of the eight ISSR primers (17899-B, 17898-A, 888, 811, 853 BEC, CHR, HAD were synthesized by Pioneer) are presented in (Table 1). Scoring of ISSR data was performed using 1% agarose gel electrophoresis profile, as clear and distinct fragment. The molecular results were analyzed using the Phoretix 1D Pro software from nonlinear Dynamics.

4. Analysis of Reducing Sugars

Weigh out accurately about 1.25 g of pure anhydrous glucose; dissolve in water and make up to (250 cm) in a standard flask. Place some of this solution into a burette. Take (25 cm) of the Fehling's solution and place into a conical flask. Dilute with (25 cm) of distilled water. Boil very gently and slowly add the glucose solution (1cm) 3 at a time into the boiling solution until the blue color has disappeared. Using Fehling's solution can be added to a solution of the sugar whose concentration is known. As the Fehling's solution is added the blue copper (II) ions will be reduced to copper (I) ions. These will precipitate out of solution as red copper (I) oxide. The resulting solution will be colorless. A titration can be carried out to determine an equivalent amount of the sugar to the Fehling's solution. The end point would be when the blue color has just disappeared.

5. Analysis of protein content

With most protein assays, sample protein concentrations are determined by comparing their assay responses to that of a dilution-series of standards whose concentrations are known. Protein samples and standards are processed in the same manner by mixing them with assay reagent and using a spectrophotometer to measure the absorbance. The responses of the standards are used to plot or calculate a standard curve. Absorbance values of unknown samples are then interpolated onto the plot or formula for the standard curve to determine their concentrations, this comparative method for determining the concentration of an "unknown" is conceptually simple and straightforward. However, its implementation in an assay protocol is complicated by pipetting and dilution steps, evaluation of replicates, blank-corrections and other factors. These steps frequently cause confusion with regard to the calculations that are necessary to obtain a final determination.

RESULTS

After three months of fig (*Ficus carica*) conservation incub asated in two temperatures (5°C and 10°C) with the use of different osmotic stabilizers (mannitol and sorbitol, with concentration of [40 g/L, 50 g/L and 60 g/L]) and maintaining a control media (sucrose (30 g/L)), the following observations were made, retrospectively. Observations of minimal change in plant height and branch number were seen, in addition. The osmotic agent sorbitol at 50 g/L had notable results in favor of the short-term conservation of (*Ficus carica*). No genetic alteration was caused by all the treatments as assessed by ISSR analysis.

1. Effect of different mannitol and sorbitol concentrations on shoot height of fig shoot bud culture conserved at two different temperatures through 3 conservation periods

The effect of different concentrations of mannitol and sorbitol, (40g/L, 50 g/L and 60 g/L), on plant shoot height, incubated in (5°C and 10°C) were evaluated at 30 days intervals for a period of

three months. A control medium was used containing 30 g/L of sucrose. The results extracted are presented in (Table 2).The data indicated that after the 1st month, the lowest growth was observed with 40 g/L sorbitol at 5°C and the highest growth was with 50 g/L mannitol at 5°C. During the 2nd month the lowest growth was observed with mannitol 50 g/L at 10°C and the highest growth was with the control media at 10°C. During the 3rd month the lowest growth was observed with sorbitol 50 g/L at 5°C and the highest growth was with sorbitol 50 g/L at 10°C.

2. Effect of different mannitol and sorbitol concentrations on branch number of fig shoot bud culture conserved at two different temperatures through three conservation periods.

The effect of different concentrations of mannitol and sorbitol, (40 g/L, 50 g/L and 60 g/L), on plant branch number, incubated in (5°C and 10°C) were evaluated at 30 days intervals for a period of three months. A control medium was used containing 30 g/L of sucrose. Results extracted are presented in Table (3). The data demonstrated that after the 1st month the lowest growth was detected with 50 g/L sorbitol at 5°C and the highest growth was with control media at 5°C. During the 2nd month the lowest growth was detected with sorbitol 50 g/L at 5°C and the highest growth was with control media at 5°C. After the 3rd month, the lowest growth was observed with sorbitol 50 g/L and sorbitol 60 g/L at 5°C and the highest growth was with sorbitol 40 g/L at 10°C.

3. Protein and sugar contents analysis

After analyzing the soluble sugar contents found in the fig leaves, the data extracted are illustrated in (Table 4). Sorbitol was found to have higher contents of soluble sugars in comparison to mannitol. However, sorbitol with the concentration of 50 g/L displayed the highest values of soluble sugar content. Protein content of the fig leaves was also analyzed and the data extracted are illustrated in (Table 5). The data generally revealed that sorbitol treated leaves had higher protein content in comparison to the ones treated with mannitol in both conservation temperatures. Fig leaves treated with mannitol at 60 g/L in a temperature of 10°C had higher protein content in comparison with ones treated with sorbitol 60 g/L in a temperature of 10°C. However, Fig leaves treated with sorbitol 50 g/L in 5°C had the highest protein content% amongst them all.

4. Genetic stability using ISSR analysis

The effects of growth regulators, growth retardants, photoperiod and cold acclimatization on genetic stability under osmotic stress storage conditions were examined. Assessment of genetic stability was performed by ISSR analysis with DNA extracted from different *in vitro* explants preserved at (5°C and 10°C) in dark storage. Amplification patterns of preserved material were compared with the non-preserved explant. The results showed no differences among control varieties plantlets and storage treatments. So the different treatments were effective for

short- term storage and no genetic alteration was caused by all the treatments as assayed by ISSR analysis.

DISCUSSION

Fig (*Ficus carica*) is constantly inclined towards integrity loss and genetic transmutation due to lack of safe long-term preservation and adequate validation means. *In vitro* preservation of vegetative propagated genetic resources provided an effective conservation system for the guarantee of food supplies. De Oliveira *et al*. (2000) stated that *in vitro* conservation by minimal growth rate of the plantlets can be achieved through adding osmotic stabilizers such as sucrose, sorbitol, or mannitol in culture medium and/or by decreasing the temperature in the conservation chambers .They also reported that several researchers recommend the use of temperatures between 15°C and 21°C in conservation chambers in order to decrease the growth rate. In the present study, after three months of conservation, sorbitol was perceived as the suitable osmotic agent for fig (*Ficus carica*) short-term conservation at a concentration of 50 g/L in 5°C, where our results were supported by the findings of Brundus and Constantirocicia (2012) who conducted a similar study on potato "*Solanum tuberosum"* explant conservation for 17 months, they found that sorbitol inhibited the growth of plantlets and no significant difference occurred in their traits.

Although mannitol showed the lowest growth in shoot height during the 2nd month with concentration of 50 g/L at 10°C, it was not able to sustain a longer conservation period. The result could be explained by similar findings of Sukendah and Cedo (2005) who worked on coconut (*Cocose nuciferal*) embryo explant conservation under cold temperature on different concentrations of mannitol media for a period of three months. They explained that the use of mannitol media for more than 3 months of storage could induce morphological abnormalities in seedling derived subsequently from the stored embryo. .In a study conducted on *Arabidopsis* by Trontin *et al*. (2014), they stated that plant growth is extremely sensitive to mannitol and growth rates drop rapidly when plants are exposed to low concentrations, but this effect levels subside as mannitol levels increase, since mannitol is considered to be an inert slow metabolizing alcoholic sugar (Chawla, 2002). However, as for sorbitol, high concentrations were needed to induce significant growth inhibition. Their findings could possibly explain why our results for shoot growth inhibition during the 2nd month of conversation at 10°C was in favor of mannitol; as the period got longer its osmoticum role was altered especially with the lower temperature.

Our results are in contrast to what was mentioned in the work of Gupta (2001) who mentioned in his study that no successful conservation could be achieved at temperatures below 6°C, and there is a variation in recovery of percentage with different genotypes. Our findings were in favor of the lower conservation tempera-

ture (5°C) rather than (10°C). Jain (2012) gave an elaborative description about *in vitro* conservation's advantages with low temperature manipulation and use of osmotic stabilizers which include disease-free planting material, high plant multiplication rate, all year round plant supply to the cultivators, potential of producing low cost planting material, and preserve the genetic fidelity with molecular markers verifications. Hence, Jain (2012) explanation as well as our ISSR results give support to lower temperatures conservations and dismisses Gupta (2001) notion.

The validation of the efficiency of in vitro conservation is necessary to determine the genetic resultant progeny's status. Bearing in mind the importance of ensuring genetic stability of micro-propagated plants in any conservation program, it is important to choose a technique that does not induce variation. Rahman and Rajora (2001) stated that genetic variations incidence is a serious problem in micro-propagation of crop species because of their unpredictable nature. The variations produced during tissue culture procedures are commonly caused by chromosomal translocations and single gene mutations. Detection through genetic variation analysis can assist in comprehending the molecular basis of various biological phenomena in plants. Phillips *et al*. (1994) mentioned that variations induced in tissue cultured plants are most likely to be revealed in the banding profiles established by different marker systems. In this study, ISSR, markers were used to depict the genetic similarity of the source plant and its offshoots. The ISSR results of the Fig (*Ficus carica*) showed no genetic variation from the plant source. Our results concluded that the molecular marker approach is a useful tool in the evaluation of the genetic stability of *in vitro* propagated plants. Our results are parallel to Kumar *et al*. (2011), who conducted an assessment on genetic fidelity of micro-propagated plants of *Simmondsia chinensis* (Link) Schneider using RAPD and ISSR markers; as well as the research conducted by Tiwari *et al*. (2013), who stated that ISSR markers for indicated axillary bud multiplication can also be used as one of the safest modes for the production of true-to-type plants.

Finally it was concluded that it necessary to define for each species factors such as composition of culture medium, conservation chamber temperature and period of transference to maximize the efficiency of conservation. The conserved material also needs to be monitored concerning its genetic stability and viability. After analyzing the findings; we can conclude that the use of sorbitol (50 g/L) in 5°C is suitable for growth suppression which helped the survival of explants up to three months without any genetic mutations. Therefore, this slow-growth protocol is an effective method for *in vitro* conservation of *Ficus carcica.*

SUMMARY

Fig (*Ficus carica* L.) is a deciduous tree that belongs to the *Moraceae* family, and one of the most suitable species for

cultivation in semi-arid environments found in regions of the Mediterranean and Middle-East; it is considered to be one of the important crop plants grown in Egypt. Many of the species are currently threatened; continuously vulnerable to loss and genetic transmutation due to the absence of safe long-term preservation. *In vitro* preservation of vegetative propagated genetic resources aided in providing an effective conservation system for the guarantee of food supplies. The present study used shoot tip cultures that were obtained from the black fig (*Ficus carica*) from Siwa. Shoot tip explants were cultured on conservation media composed of full strength MS medium with 0.8% (w/v) agar and different concentrations of different osmotic agents. The study investigated the use of osmotic stabilizers (mannitol and sorbitol, with concentration of [40 g/L, 50 g/L and 60 g/L]) in the media at two different temperatures (5°C and 10°C) through a 3 months period in order to determine which osmotic stabilizer, concentration, and temperature would display an eminent effect on the *in vitro* short-term storage of fig shoot cultures. A (30 g/L) concentration of sucrose was used as the control media. Results were in favor of sorbitol (50 g/L) at a temperature of 5°C. In addition Inter Simple Sequence Repeats (ISSR) marker was performed to assess molecular characterization of genetic identity and stability; results illustrated that no genomic instability and mutations were found in the propagated fig (*Ficus carica* L.) cultures.

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Table (1): Primers used for ISSR analysis.

Primer Code	Primer Sequence
P2-17899-B	$(CA)_6$ GG
P3-1517898-A	$(CA)_6$ AC
P15-888	TAC $(CA)_7$
P23-811	$(GA)_8$ C
P24-853	$(TC)_8$ GT
P60-BEC	$(CA)_7$ TC
P61-CHR	$(CA)_7$ GG
P-62HAD	CT $(CCT)_3$ CAC

Table (2): Effect of different concentrations of sorbitol and mannitol on shoot bud height.

Treatments	Shoot height (cm)					
	1st Month		2nd Month		3rd Month	
	5°C	10°C	5°C	10°C	5°C	10°C
1- Control (Sucrose 30 g/L)	2.300 def	3.533 ab	2.467 bcd	3.933 a	2.000 cde	3.100 ab
2. Sorbitol (40 g/L)	1.300 g	3.167 bc	1.567 de	2.967 b	1.833 de	1.700 e
3. Sorbitol (50 g/L)	2.000 fg	2.833 b-e	2.000 cde	2.567 bc	1.533 e	3.633 a
4. Sorbitol (60 g/L)	1.933 fg	2.333 def	2.000 cde	2.400 bcd	2.067 b-e	2.133 b-e
5. Mannitol (40 g/L)	2.367 def	2.067 efg	2.467 bcd	2.700 bc	2.000 cde	2.367 b-e
6. Mannitol (50 g/L)	4.233 a	1.467 g	3.833 a	1.433 e	1.667 e	2.400 b-e
7. Mannitol (60 g/L)	2.933 bcd	2.433 c-f	2.433 bcd	2.300 b-e	2.967 abc	2.800 a-d

Values having the same letter(s) in the same column are not significantly different at P≤0.05

Table (3): Effect of different concentrations of sorbitol and mannitol on branches number.

Treatments	Branches number					
	1st Month		2nd Month		3rd Month	
	5°C	10°C	5°C	10°C	5°C	10°C
1- Control (Sucrose 30 g/L)	3.667 a	1.667 ab	5.000 a	1.667 b	3.333 b	2.000 bcd
2. Sorbitol (40 g/L)	1.000 b	2.333 ab	1.667 b	2.333 b	2.333 bcd	5.000 a
3. Sorbitol (50 g/L)	1.333 ab	1.000 b	2.000 b	----	2.000 bcd	-----
4. Sorbitol (60 g/L)	-----	-----	1.333 b	----	1.000 cd	------
5. Mannitol (40 g/L)	1.667 ab	1.667 ab	2.333 b	1.667 b	2.333 bcd	3.000 b
6. Mannitol (50 g/L)	1.000 b	1.000 b	1.333 b	1.000 b	1.667 bcd	1.667 bcd
7. Mannitol (60 g/L)	2.000 ab	2.333 ab	2.333 b	2.000 b	2.667 bc	2.333 bcd

Values having the same letter(s) in the same column are not significantly different at P≤0.05

Table (4): Soluble sugar content of black fig leaves.

Treatments	Soluble sugar content					
	Glucose		Fructose		Sucrose	
	5°C	10°C	5°C	10°C	5°C	10°C
1- Control (Sucrose 30 g/L)	2.133 a	1.167 a	2.283 b	1.333 ab	2.550 b	3.100 ab
2. Sorbitol (40 g/L)	2.850 a	1.667 a	2.267 b	2.000 ab	1.767 d	1.700 e
3. Sorbitol (50 g/L)	2.917 a	2.667 a	3.200 a	3.333 a	2.883 a	3.633 a
4. Sorbitol (60 g/L)	2.417 a	----	2.200 b	-----	2.100 c	2.133 b-e
5. Mannitol (40 g/L)	2.133 a	1.667 a	2.583 ab	2.000 ab	2.183 c	2.367 b-e
6. Mannitol (50 g/L)	2.217 a	1.000 a	2.633 ab	1.167 ab	2.033 cd	2.400 b-e
7. Mannitol (60 g/L)	2.850 a	2.167 a	2.367 ab	2.167 ab	2.583 ab	2.800 a-d

Values having the same letter(s) in the same column are not significantly different at P≤0.05

Table (5): Protein content% with different concentrations of sorbitol and mannitol.

Treatments	Protein content%	
	Sucrose	
	5°C	10°C
1- Control (Sucrose 30 g/L)	3.333 b	2.000 bcd
2. Sorbitol (40 g/L)	2.333 bcd	2.000 bcd
3. Sorbitol (50 g/L)	5.000 a	-----
4. Sorbitol (60 g/L)	1.000 cd	3.000 b
5. Mannitol (40 g/L)	2.333 bcd	-----
6. Mannitol (50 g/L)	1.667 bcd	1.667 bcd
7. Mannitol (60 g/L)	2.667 bc	2.333 bcd

Values having the same letter(s) in the same column are not significantly different at P≤0.05

Fig. (1): The figure illustrates the source of explant that were used in preservation A. Explant source for sorbitol media; B. Explant source for mannitol media; C. Explant source for control media.

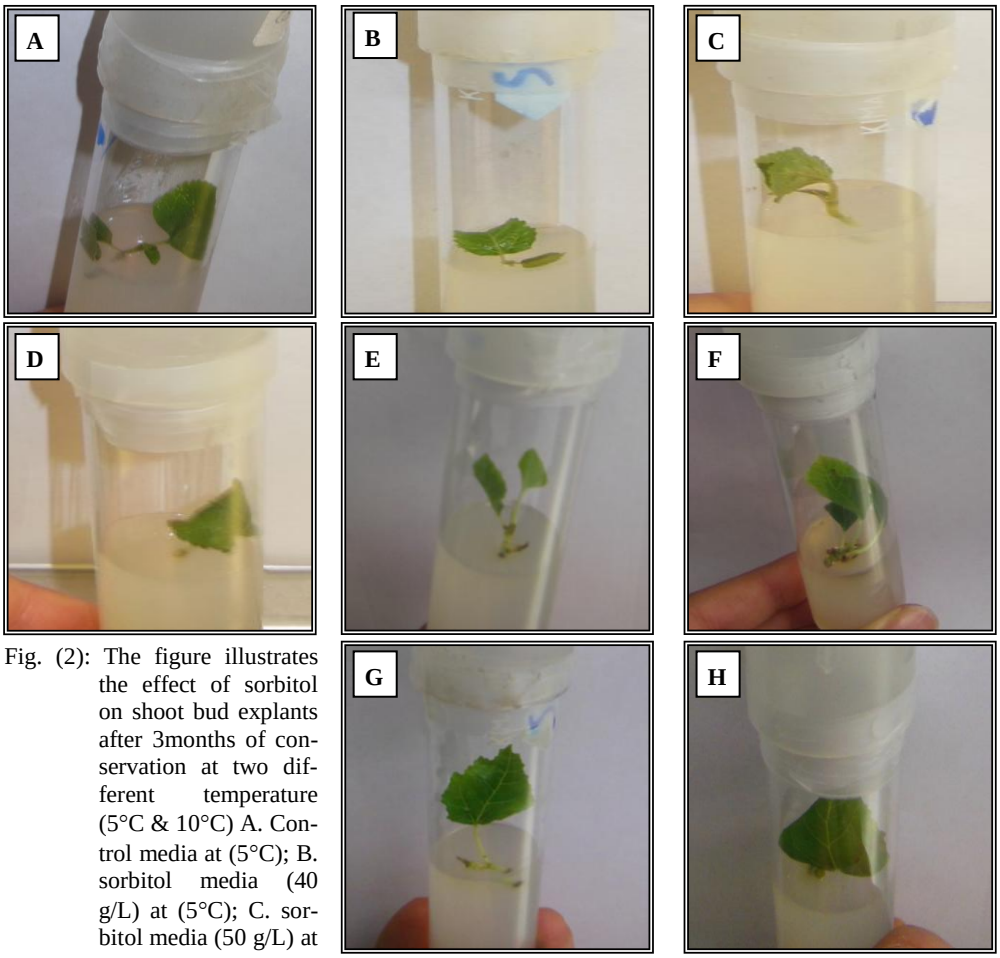

Fig. (2): The figure illustrates the effect of sorbitol on shoot bud explants after 3months of conservation at two different temperature (5°C & 10°C) A. Control media at (5°C); B. sorbitol media (40 g/L) at (5°C); C. sorbitol media (50 g/L) at (5°C); D. sorbitol media (60 g/L) at (5°C); E. Control media at (10°C); F. sorbitol media (40 g/L) at (10°C); G.sorbitol media (50 g/L) at (10°C); H. sorbitol media (60 g/L) at (10°C).

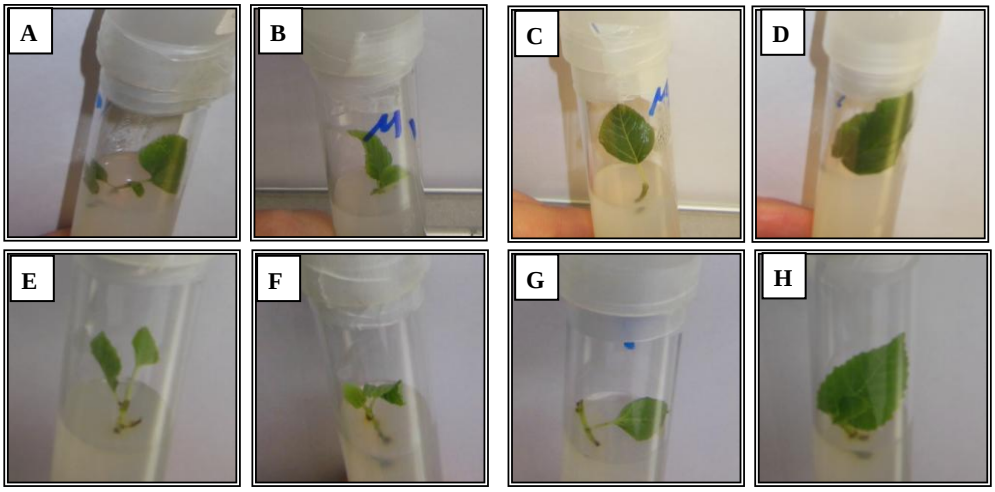

Fig. (3): The figure illustrates the effect of mannitol on shoot tip explants after 3months of conservation at two different temperature (5°C and 10°C) A. Control media at (5°C); B. mannitol media(40 g/L) at (5°C); C. mannitol media (50 g/L) at (5°C); D. mannitol media (60 g/L) at (5°C); E. Control media at (10°C); F, mannitol media (40 g/L) at (10°C); G. mannitol media (50 g/L)(10°C); H. mannitol media (60 g/L) at (10°C).

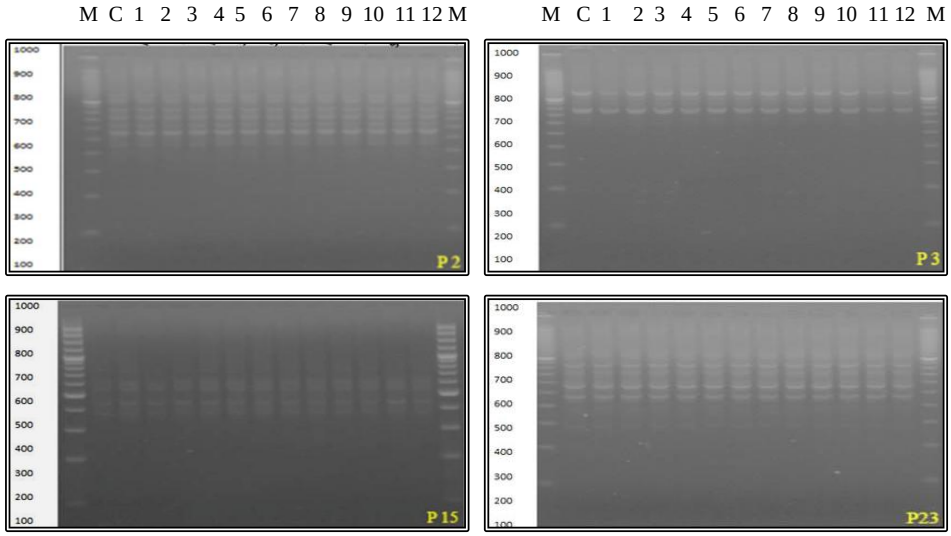

Fig. (4): ISSR profiles of fig (*Ficus carica*) is detected by p2-17899-B primer, p3-1789b-A primer, p15-888 primer, and p23-811 primer.
M= Marker 1= Control 2= sorbitol media (40 g/L) at (5°C); 3= sorbitol media (50 g/L) at (5°C); 4= sorbitol media (60 g/L) at (5°C); 5= sorbitol media (40 g/L) at (10°C); 6= sorbitol media (50 g/L) at (10°C); 7= sorbitol media (60 g/L) at (10°C), 8= mannitol media(40 g/L) at (5°C); 9= mannitol media (50 g/L) at (5°C); 10= mannitol media (60 g/L) at (5°C); 11= mannitol media (40 g/L) at (10°C); 12= mannitol media (50 g/L)(10°C); 13= mannitol media (60 g/L) at (10°C).

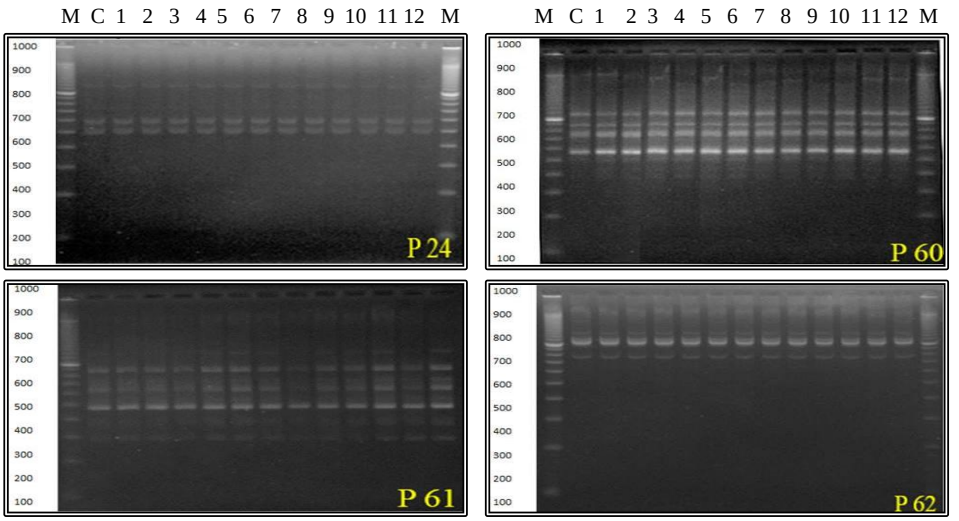

Fig. (5): ISSR profiles of fig (*Ficus carica*) is detected by p24-853 primer, p60-BEC primer, p61-CHR primer, and by p62-HAD primer.

M= Marker 1= Control 2= sorbitol media (40 g/L) at (5°C); 3= sorbitol media (50 g/L) at (5°C); 4= sorbitol media (60 g/L) at (5°C); 5= sorbitol media (40 g/L) at (10°C); 6= sorbitol media (50 g/L) at (10°C); 7= sorbitol media (60 g/L) at (10°C), 8= mannitol media(40 g/L) at (5°C); 9= mannitol media (50 g/L) at (5°C); 10= mannitol media (60 g/L) at (5°C); 11= mannitol media (40 g/L) at (10°C); 12= mannitol media (50 g/L)(10°C); 13= mannitol media (60 g/L) at (10°C).

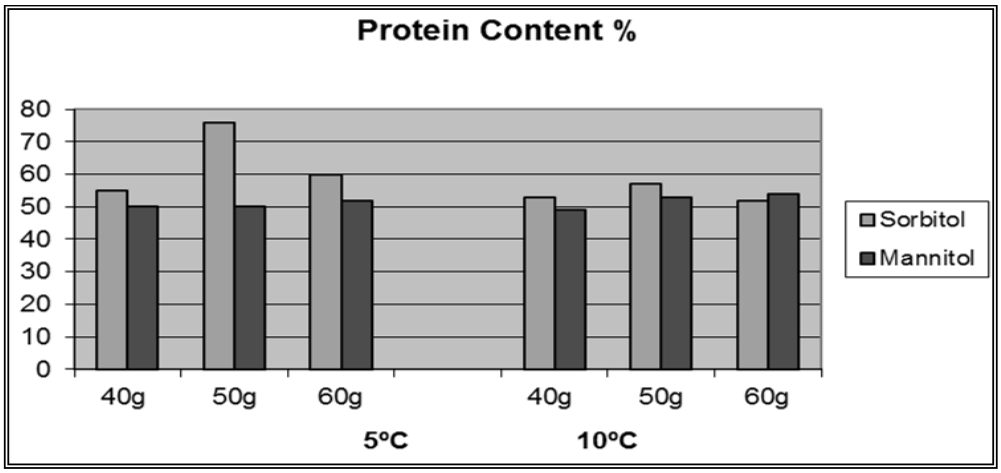

Fig. (6): Protein content %

The figure illustrates the fig leaves protein content % analysis after the 3rd month recovery phase. The treatments used were sorbitol and mannitol with the concentrations of (40 g/L, 50 g/L, and 60 g/L).