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EFFECT OF SUGAR CONCENTRATION AND TEMPERATURE ON *IN-VITRO* STORAGE OF SHOOT-TIP EXPLANTS OF GRAND-NAIN BANANA CV.

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ABSTRACT

This work explores the possibility of using *in-vitro* technique to store the explants of banana (*Musa acuminate* cv. Grand-Nain Cavendish subgroup, AAA).The influence of different sugars concentrations and storage temperatures was investigated. Shoot-tip explants of banana Grand-Nain cv. were successfully stored for up to 12 months (without subculturing) on MS medium supplemented with fructose or ribose each at 20, 30 or 40 gl^{-1} or sucrose at 40, 50 or 60 gl^{-1} and stored at 15°C, 17°C and 21°C. The highest survival percentages (100 %) were recorded with all sugar concentrations at 15°C and 17°C, while the 21°C recorded the lowest survival percentage during the storage period (12 months). Data also revealed that the lowest shoot number was recorded with 20 and 30 gl^{-1} ribose and 60 gl^{-1} sucrose at all storage temperatures. The highest shoot length (cm) was recorded with 30 gl^{-1} ribose and 60 gl^{-1} sucrose at 15°C and 17°C. All survived explants were recultured on fresh modified MS medium revealed viability and resumed growth within three week.

Key words: *In-vitro*, Storage, Banana, Sugars, Temperatures.

INTRODUCTION

Banana is one of the most important tropical fruit crops in the world. It is grown in every humid tropical region and constitutes the 4th largest fruit crop of the world and constitutes the 3th largest fruit crop in Egypt following the citrus and grape fruits. Banana is popular due to their typical sweet aromatic taste, large fruits with thick skin and their high yield. Banana production occupies an important share in the total fruit of Egypt. The total area of banana reached 56,422 feddens produced about 875,123 tons with an average of about 17, 29 tons per feddan (Ministry of Agric. A.R.E. 2005).

Fruit tree germplasm is being conserved mainly in the form of a field genebank, which requires much labor money and land. In addition this germplasm is apt to be lost by attack from pathogens, pest and environmental disasters (Ko *et al.,* 1991).Tissue culture systems are aseptic and easily kept free from fungi, bacteria, viruses and insects parasites. An important use of tissue culture at present is the culturing of meristems so as to obtain virus free plants, and the subsequent storage of such plants as pathogene-free stocks by plant breeders. The expense, both in labour and financial terms, of maintaining larg field collection is a further factor contributing to the use of *in vitro* collection. Also in an ideal-tissue culture storage system, genetic erosion is reduced to zero (Dodds, 1991).

Micropropagtion in standard condition is the most widely used tissue culture technique for germplasm conservation. However, in these conditions the frequent transfer makes the technique costly and increases the risk of contamination. Besides, positive selection during subculturing could be a source of genotypic variation. Consequently, it is necessary to reduce the rate of growth so that limited attention is required for plantlets in storage (Dodds, 1991).

Several approaches can be used to achieve germplasm conservation, of which incubation at reduced temperature and low light intensity, modification of culture medium by increasing sugar concentration or decreasing the supply of inorganic nutrients and adding growth retardants to culture medium. Minimal growth storage is a very simple technique that allows storage of plants *in vitro* for period ranging from 6 months to 5 years depending on species. These stored plants can be micropropagated rapidly when desired (Perez-Tornero *et al.,* 1999).

In this concern, Wanas (1992) used modifying sucrose concentration to 2, 4 or 5% for storage of the germplasm of apple rootstock cultures (M9, M26 and MM106). The cultures of M9 and MM106 increased in survival % after 18 months storage at 4%.

Meanwhile, Hassan (1996) showed that clusters of developed buds and shoot tip explants of banana can be stored at 15 °C for at least 12 months with higher survival percentage when compared with survival percentage of explants storage at 27 °C.

This work aimed to determine the effect of different sugars concentrations and temperatures on shoot-tip explants of Grand-Nain banana cv. to extend the subculturing intervals from the normal 3 weeks to much longer period.

MATERIALS AND METHODS

This work was carried out during two successive years (2004-2006) at the laboratory of Virus and Mycoplasma Research Section, Plant Pathology Research Institute, Agriculture Research Center, Giza, Egypt.

Small suckers of about 50-70 cm. in length were carefully cut from mother plants of banana (*Musa acuminate* cv. Grand-Nain Cavendish subgroup, AAA) grown in a private orchard at Al-Behera governorate and the suckers were immediately transferred to the laboratory washed in running tap water for several times. Then tested against banana bunchy top nanavirus (BBTV) and banana mosaic cucumovirus (BMV) using DAS-ELISA according to (Clark and Adam 1977).

The apical parts contained main buds of the suckers were excised then washed in running tap water for about one hour. The isolated explants were soaked under aseptic condition in 70 % Clorox and Tween 20 (0.1%) as surfactant for 20 minuets. Then the explants were rinsed in sterile distilled water for four times each for five minuets to remove all traces of Clorox. The shoot-tip explants (1 cm length x1 cm diameter). free of BBTV and BMV (negative ELISA results) were separately cultured into glass jars (250 ml) filled with 30 ml of Murashige and Skoog (1962) (MS) medium at full strength supplemented with 0.4 mgl^{-1} thiamin-HCl, 100 mgl^{-1} L-arginine 100 mgl^{-1} myo-inositol, 2 mgl^{-1} indole-3 acetic acid (IAA), 5 mgl^{-1} benzyladenine (BA), 160 mgl^{-1} adenine sulphate, 0.2 gl^{-1} charcoal, 2.2

gl^{-1} phytagel as recommended by Ko *et al.*,(1991) plus fructose, ribose at 20, 30 or 40 gl^{-1} and sucrose 40, 50 or 60 gl^{-1}. The cultures supplemented with different sugars concentrations were incubated at three temperature degrees i. e. 15, 17, and 21 °C.

The pH of all prepared media was adjusted to 5.8 with 1 N KOH and the media were autoclaved under 1.5 / b^2 at 121°c for 20 min. and then kept over night at room temperature before culture.

The cultures of all treatments were incubated under photoperiods of 16 hours day and 8 hours night supplied by white fluorescent tubes to provide light intensity of 1000-lux at cultures level.

The data were recorded every 3 months on survival percentage, average shoot number/explants, average shoot length (cm) and up to 12 months.

After storage period (12 months), survived explants were transferred to fresh MS medium supplemented with 30 gl^{-1} sucrose, 5 mg/l BA, 0.1 gl^{-1} myo-inositol and 2.2 gl^{-1} phytagel. After three weeks data were recorded on survival percentage, average shoots number/explants and average shoot length (cm).

The experimental consisted of 27 treatments. Each treatment was represented with six replicates, each with one shoot-tip explants. This factorial experiment was designed in a complete randomize design (C.R.D.), and data bottomed were compared according to method described by Snedecor and Cochran, (1972).

RESULTS AND DISCUSSION

Influence of different sugars concentrations and temperatures during *in-vitr* storage of shoot-tip explants of Grand-Nain banana cv.

a) Survival percentage:

Data in table (1) illustrated that, all sugars concentrations under storage temperatures gave 100 % survival percentage after 3 and 6 months of storage period without significant differences among them. After 9 and 12 months storage period, all sugars concentrations at 15°C and 17°C produced 100 % survival while 21°C recorded the lowest value (83.3 %) survival percentage.

b) Number of shoots per explant:

As shown in table (2). Insignificant differences were noticed among all sugars concentrations and temperatures under study after 3 months. Meanwhile after 6 months, data revealed that the lowest shoot number per explants produced with all ribose and sucrose concentrations (1.00). As for the effect of storage temperatures, data showed that the lowest values for shoot/explants was obtained by 15°C & 17°C. The interaction between the two studied factors recorded that the lowest shoot number/explant produced with all ribose and sucrose concentrations under all storage temperatures. After 9 months of storage data showed that the lowest significant values were recorded by explants stored on MS medium supplemented with 20 or 30 gl^{-1} ribose and sucrose at all concentrations (1.00). Concerning the effect of storage temperature, data showed no significant different at all storage temperature under study. As for the interaction between the two studied factors, the lowest number of shoot/ explants cultured on medium containing 20 or 30 gl^{-1} ribose and sucrose at all concentrations at all storage temperature under study. Also, after 12 months of storage the same trend could be noticed as those of 9 months storage.

C) Average shoots length (cm):

Data in table (3) illustrated that. After 3 months, insignificant differences among all sugars concentrations. As for the effect of storage temperatures, data showed that the highest shoot length was obtained at 21°C and the interaction between the two studied factors showed that, insignificant differences could be noticed among all sugars concentrations under 15°C or 17°C. After 6 months storage, as for the effect of sugars concentrations data showed that the highest shoot lengths were when using 30 or 40 gl^{-1} ribose and 60 gl^{-1} sucrose. The effect of storage temperatures data showed that the highest shoot length was recorded at 21°C and the interaction between the two studied factors showed, that the highest shoot length was recorded by explants stored on MS medium supplemented with 60 gl^{-1} sucrose (3.33 cm) at 21°C. Also, after 9 months and 12 months storage period same trend could be noticed as those of 6 months storage.

Table (1): Effect of different sugar concentrations and temperatures on survival percentage of shoot-tip explants of Grand-Nain banana cv. during *in-vitro* storage.

Sugar \ Temp.		After 3 months			Means	After 6 months			Means	After 9 months			Means	After 12 months			Means
		15°C	17°C	21°C		15°C	17°C	21°C		15°C	17°C	21°C		15°C	17°C	21°C	
Fructose g/l	20	100 a	100 a	100 a	100 A	100 a	100 a	100 a	100 A	100 a	100 a	83.3b	94.4 A	100 a	100 a	83.3b	94.4 A
	30	100 a	100 a	100 a	100 A	100 a	100 a	100 a	100 A	100 a	100 a	83.3b	94.4 A	100 a	100 a	83.3b	94.4 A
	40	100 a	100 a	100 a	100 A	100 a	100 a	100 a	100 A	100 a	100 a	83.3b	94.4 A	100 a	100 a	83.3b	94.4 A
Ribose g/l	20	100 a	100 a	100 a	100 A	100 a	100 a	100 a	100 A	100 a	100 a	83.3b	94.4 A	100 a	100 a	83.3b	94.4 A
	30	100 a	100 a	100 a	100 A	100 a	100 a	100 a	100 A	100 a	100 a	83.3b	94.4 A	100 a	100 a	83.3b	94.4 A
	40	100 a	100 a	100 a	100 A	100 a	100 a	100 a	100 A	100 a	100 a	83.3b	94.4 A	100 a	100 a	83.3b	94.4 A
Sucrose g/l	40	100 a	100 a	100 a	100 A	100 a	100 a	100 a	100 A	100 a	100 a	83.3b	94.4 A	100 a	100 a	83.3b	94.4 A
	50	100 a	100 a	100 a	100 A	100 a	100 a	100 a	100 A	100 a	100 a	83.3b	94.4 A	100 a	100 a	83.3b	94.4 A
	60	100 a	100 a	100 a	100 A	100 a	100 a	100 a	100 A	100 a	100 a	83.3b	94.4 A	100 a	100 a	83.3b	94.4 A
Means		100A`	100A`	100A`		100A`	100A`	100A`		100A`	100A`	83.3B`		100A`	100A`	83.3B`	

Means followed by the same letters are not significantly different from each other at 5% level.

Table (2): Effect of different sugar concentrations and temperatures on average shoot number of shoot-tip explants Grand-Nain banana cv. during *in-vitro* storage.

Sugar \ Temp.		After 3 months			Means	After 6 months			Means	After 9 months			Means	After 12 months			Means
		15°C	17°C	21°C		15°C	17°C	21°C		15°C	17°C	21°C		15°C	17°C	21°C	
Fructose g/l	20	1.00 a	1.00 a	1.00 a	1.00 A	1.33 b	1.33 b	1.50 b	1.39 B	1.50 ab	1.66 a	2.00 a	1.72 A	1.66 ab	1.83 ab	2.00 a	1.83 A
	30	1.00 a	1.00 a	1.00 a	1.00 A	1.50 b	1.50 b	1.83 a	1.61 AB	1.50 ab	1.66 a	2.00 a	1.72 A	1.83 ab	1.83 ab	2.00 a	1.88 A
	40	1.00 a	1.00 a	1.00 a	1.00 A	1.50 b	1.50 b	2.00 a	1.66 A	1.50 ab	1.66 a	2.00 a	1.72 A	1.83 ab	1.83 ab	2.00 a	1.88 A
Ribose g/l	20	1.00 a	1.00 a	1.00 a	1.00A	1.00 c	1.00 c	1.00 c	1.00 C	1.0 b	1.00 b	1.00 b	1.00 B	1.00 c	1.00 c	1.00 c	1.00 C
	30	1.00 a	1.00 a	1.00 a	1.00 A	1.00 c	1.00 c	1.00 c	1.00 C	1.0 b	1.00 b	1.00 b	1.00 B	1.00 c	1.00 c	1.00 c	1.00 C
	40	1.00 a	1.00 a	1.00 a	1.00 A	1.00 c	1.00 c	1.00 c	1.00 C	1.50 ab	1.66 a	1.00 c	1.39 A	1.50 b	1.83 ab	1.00 c	1.44 B
Sucrose g/l	40	1.00 a	1.00 a	1.00 a	1.00 A	1.00 c	1.00 c	1.00 c	1.00 C	1.0 b	1.00 b	1.00 b	1.00 B	1.00 c	1.00 c	1.00 c	1.00 C
	50	1.00 a	1.00 a	1.00 a	1.00 A	1.00 c	1.00 c	1.00 c	1.00 C	1.0 b	1.00 b	1.00 b	1.00 B	1.00 c	1.00 c	1.00 c	1.00 C
	60	1.00 a	1.00 a	1.00 a	1.00 A	1.00 c	1.00 c	1.00 c	1.00 C	1.0 b	1.00 b	1.00 b	1.00 B	1.00 c	1.00 c	1.00 c	1.00 C
Means		1.00A`	1.00A`	1.00A`		1.15A`	1.15A`	1.26A`		1.22A`	1.29A`	1.33A`		1.31A`	1.37A`	1.33A`	

Means followed by the same letters are not significantly different from each other at 5% level.

Table (3): Effect of different sugar concentrations and temperatures on average proliferated shoot length (cm) of shoot-tip explants Grand-Nain banana cv. during *in-vitro* storage.

Sugar \ Temp.		After 3 months			Means	After 6 months			Means	After 9 months			Means	After 12 months			Means
		15°C	17°C	21°C		15°C	17°C	21°C		15°C	17°C	21°C		15°C	17°C	21°C	
Fructose g/l	20	1.00 c	1.00 c	1.66 ab	1.22 A	1.50 bc	1.66 bc	2.33 ab	1.83 AB	2.00 c-f	2.00c-f	2.33b-f	2.11 BC	2.00 gh	2.00 gh	2.33 fg	2.11 EF
	30	1.00 c	1.00 c	2.00 a	1.33 A	1.00 c	1.66 bc	2.33 ab	1.66 B	2.00 c-f	2.00c-f	2.66a-e	2.22 BC	2.00 gh	2.00 gh	2.66 ef	2.22 E
	40	1.00 c	1.00 c	2.00 a	1.33 A	1.00 c	1.00 c	2.66 ab	1.55 B	1.50 ef	1.16 f	2.66a-e	1.77 C	1.50 ij	1.16 ij	3.07 de	1.91 F
Ribose g/l	20	1.00 c	1.00 c	1.33 bc	1.11 A	1.66 bc	2.00 bc	2.00 bc	1.89 AB	2.33 b-f	2.33b-f	3.33abc	2.66 B	2.16 fg	3.66 ef	3.66 c	3.16 D
	30	1.00 c	1.00 c	1.33 bc	1.11 A	2.33 ab	2.33 ab	2.33 ab	2.33 A	3.33abc	3.33abc	3.61 ab	3.42 A	4.66 ab	4.33 b	4.61 ab	4.53 B
	40	1.00 c	1.00 c	1.66 ab	1.22 A	2.33 ab	2.33 ab	2.66 ab	2.42 A	3.33abc	3.33abc	3.83 a	3.50 A	3.33 cd	3.33 cd	4.83 ab	3.83 C
Sucrose g/l	40	1.00 c	1.00 c	1.33 bc	1.11 A	1.00 c	1.00 c	2.00 bc	1.33 B	1.66def	1.33 ef	2.33 b-f	1.77 C	1.66 hi	1.33 ij	3.07 de	2.02 EF
	50	1.00 c	1.00 c	1.33 bc	1.11 A	1.00 c	1.00 c	2.33 ab	1.44 B	1.00 f	1.33 ef	3.07a-d	1.80 C	1.00 j	1.33 ij	3.66 c	2.00 EF
	60	1.00 c	1.00 c	2.00 a	1.33 A	1.66 bc	2.33 ab	3.33 a	2.44 A	3.33abc	3.66 ab	3.66 ab	3.55 A	4.66 ab	5.00 a	5.00 a	4.89 A
Means		1.00 B`	1.00 B`	1.62 A`		1.50 B`	1.70 B`	2.44 A`		2.27 B`	2.27 B`	3.05 A`		2.55 B`	2.68 B`	3.65 A`	

Means followed by the same letters are not significantly different from each other at 5% level.

Influence of different sugars concentrations and temperatures on regeneration capacity (shoot number and length cm) of shoot-tip explants of Grand-Nain banana cv. stored for 12 months when transferred on proliferation medium.

Data in Table (4) showed that, all survived explants were viable and resumed growth after 3 weeks on the new fresh medium and achieved 100 % survival.

Concerning sugars concentrations, data revealed that the highest average shoot number/explants was recorded by explants stored on MS medium supplemented with fructose at 40 gl^{-1} and ribose at 20 gl^{-1} without significant difference between them. While, the lowest value was obtained by ribose 30 gl^{-1} and sucrose 50 gl^{-1}. As for the effect of storage temperatures data showed that the highest number of shoot/explants was obtained by 21°C (3.07). On the other hand, 15°C and 17°C gave the lowest shoot number values (1.00). The interaction between the two studied factors revealed that explants cultured on medium containing fructose at 40 gl^{-1} and stored at 21°C gave the highest shoot number. Meanwhile, the lowest values were obtained at sugars concentrations stored at 15°C and 17°C. Regarding the effect of sugars concentrations on the average proliferated shoot length, data showed that the highest significant average shoot length (4.74 cm) was recorded by sucrose at 60 gl^{-1}. Concerning the effect of storage temperatures it was observed that explants stored at 21°C gave the highest significant value (5.70 cm) on the other hand the lowest value was obtained at 15°C. The interaction between the sugars concentrations and storage temperatures showed significant effect where the highest values were recorded by fructose at 20 and 40 gl^{-1}, ribose 30 and 40 and all sucrose concentrations treatment 21°C. The lowest shoot length (cm) was obtained by fructose at 20 gl^{-1} stored at 15°C.

The obtained results emphasized the effect of sugar concentrations and temperatures on the storage ability of shoot-tip explant Grand-Nain banana cv. cultured *in-vitro*. In the present study, the main target was more conducive to achieve the lowest growth rate and the highest survival percentage by using minimal growth medium and without subculturing for longer period.

Generally, it could be concluded that shoot-tip explants of Grand-Nain banana cv. were successfully storage for 12 months on MS medium supplemented with sucrose at 60 gl^{-1} or ribose at 30 gl^{-1} and

Table (4): The effect of different sugar concentrations and tempera-tures on shoot number and length (cm) of shoot-tip explants Grand-Nain banana cv. after 12 months storage on shooting medium.

Sugar \ Temp.		Shoot number			Means	Shoot length (cm)			Means
		15°C	17°C	21°C		15°C	17°C	21°C	
Fructose g/l	20	1.00 d	1.00 d	2.66 c	1.55 BC	1.33 h	1.66 gh	6.00 a	3.00 D
	30	1.00 d	1.00 d	2.33 c	1.44 C	2.30 e-h	2.76 ef	5.00 ab	3.35 CD
	40	1.00 d	1.00 d	5.66 a	2.55 A	1.43 h	2.53 efg	6.00 a	3.32 CD
Ribose g/l	20	1.00 d	1.00 d	4.33 b	2.11 A	2.53 efg	2.76 ef	4.33 bc	3.21 D
	30	1.00 d	1.00 d	2.00 cd	1.33 C	2.46 efg	3.13 de	6.00 a	3.86 BC
	40	1.00 d	1.00 d	2.33 c	1.44 C	3.00 def	3.33 de	6.00 a	4.11 B
Sucrose g/l	40	1.00 d	1.00 d	3.66 b	1.88 BC	2.00 fgh	2.53 efg	6.00 a	3.51 CD
	50	1.00 d	1.00 d	2.00 cd	1.33 C	3.00 def	3.33 de	6.00 a	4.11 B
	60	1.00 d	1.00 d	2.66 c	1.55 BC	3.90 cd	4.33 bc	6.00 a	4.74 A
Means		1.00 B`	1.00 B`	3.07 A`		2.44 C`	2.93 B`	5.70A`	

Means followed by the same letters are not significantly different from each other at 5% level.

incubated at 15 or 17°C. The stored explants resumed growth and started the regeneration within three weeks after transferring on proliferation medium at 24°C.

Results indicated that the highest survival percentage and the lowest average shoot number were achieved by storage shoot-tip explants of Grand-Nain banana cv. on MS medium supplemented with 60 gl^{-1} sucrose and 30 gl^{-1} ribose. These results are in agreement with finding of Hammatt (1993) who reported that shoot tips from cultured tissues of bird cherry (*Prunus padus L.)* maintained on media with sucrose for 30 months were transferred to medium supplemented with either fructose, glucose, sucrose each at (30 g/l) or sorbitol (20 g/l). He mentioned that heavier cultures and more shoots were obtained with fructose or glucose than with sucrose or sorbitol, while cultures on sorbitol produced few shoots, and this was often hyperhydric. Most axillary shoots on glucose and fructose elongated to give shoots 10 mm in length, while those on sorbitol or sucrose only rarely exceeded 5 mm. Ko *et al.,* (1991) found that all the sugars tested were found to be capable of extending the survival time of banana tissues and ribose was the best followed by sucrose, glucose, fructose and lactose.

Marchal and Folliot (1992) mentioned that accumulation of dry material of banana plants during *in-vitro* growth appears to be linked to the quantity of sucrose concentration to be used during this stage between 70 and 80 mg /l. The use of higher dose had adverse effect on the quality of the plant obtained.

Panis *et al.,* (1996) mentioned that increase in the sucrose levels from 0.1 to 0.75M on MS medium results in a lowered survival rate of banana meristem cultures at 25±2°C under continuous illumination. At 0.6M sucrose, only 17% of the inoculated bud display growth. At 0.75M, no buds regrow and all became brown. It is assumed that the osmotic check at 0.75M is too high to result in blakining, which is a normal reaction of living tissues to stress. 0.4 and 0.5M are especially effective, resulting in a regrowth of 25 and 42.5% respectively. The water content of pregrown meristematic clumps decreases gradually with increasing concentrations of sucrose.

Data also revealed that storage of shoot-tip explants of Grand-Nain banana cv. at 15°C and 17°C resulted the highest survival percentage and the lowest average shoot number. This result's in harmony with those of owned by Hassan (1996) who reported that clusters of developed buds and shoot tip explants of banana can be

stored at 15 °C for at least 12 months with higher survival percentage when compared with survival percentage of explants storage at 27 °C. **Ko *et al.*, (1991)** showed that incubation of tissues on cheesecloth over cotton saturated with 3% ribose solution at 17°C under light is suitable for long-term storage of meristem-tip of Cavendish banana. When these tissues were transferred to fresh MS medium, growth of buds resumed within two weeks.

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تأثير تركيز السكر و درجة الحراره على التخزين المعملى لمنفصلات القمم النامية النباتية لصنف الموز جراندنان

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تهدف هذه الدراسة الى امكانية استخدام تقنية زراعة الأنسجه فى الحفظ المعملى للمنفصلات النباتيه للموز صنف جراندنان. وقد تم دراسة تأثير كل من تركيزات السكريات و درجات حراره التخزين . حيث تخزين منفصلات القمم النامية لصنف الموز جراندنان بنجاح لمدة ١٢ شهر (بدون نقل النموات لبيئه جديدة) على بيئة املاح موراشيجى و سكوج مضاف الها سكر فركتوز او ريبوز بتركيزات ٢٠، ٣٠ او٤٠ جرام/لتر لكل منهما على حده و السكروز بتركيز ٤٠، ٥٠ او ٦٠ جرام/لتر وتم تخزينها على درجات حراره ١٥، ١٧ أو ٢١°م. سجلت اعلى نسبة بقاء للمنفصلات مع كل تركيزات السكريات المستخدمه على درجة حراره ١٥ او ١٧ °م (١٠٠ ٪) بينما درجة ٢١ °م اعطت اقل نسبة بقاء اثناء التخزين لمدة ١٢ شهر مع كل تركيزات السكريات. و اكدت الدراسه ان اقل عدد للبراعم تم الحصول عليه مع البيئات المحتوية على ٢٠ او ٣٠ جرام/لتر ريبوز و ٦٠ جرام/لتر سكروز مع كل درجات الحراره و سجل اعلى طول للبراعم مع ٣٠ جرام/لتر ريبوز و ٦٠ جرام/لتر سكروز على درجة حراره ١٥ و ١٧°م. بعد تخزين المنفصلات النباتية لمدة ١٢ شهر تم نقل المنفصلات الحيه الى بيئه جديده مكونه من بيئة املاح موراشيجى و سكوج المعدله و كانت النموات قادره على استعادة حيويتها خلال ٣ اسابيع.

قام بتحكيم هذا البحث: أ.د. / إبراهيم محمد دسوقى و أ.د. / سمير العجمى