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USING GROWTH RETARDANTS FOR PRESERVATION OF DATE PALM SOMATIC EMBRYOS

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ABSTRACT

The purpose of this study is to devise a simple and reliable method for *in vitro* storage of date palm cultures through slowing growth by reducing temperature and growth retardants. Direct somatic embryos of Date palm *(Phoenix dactylifera* L.) cv. Bartamuda were preserved *in vitro* in modified MS medium supplemented with growth retardants Abscisic acid (ABA), Paclobutrazol (PBZ), Ancymidol at 1, 2, 4 mg/L or Chlorocholine chloride (CCC) at 0.25, 0.5,1,4 mg/L.

Somatic embryos cultured on medium without growth retardants were used as a control. All treatments were incubated under 27 or 18 °C to study their effects on survival percentage and chemical contents after 18 months. Preserved somatic embryos were transferred to normal conditions and cultured on fresh culture medium without growth retardants where they grown for 4weeks. The survival percentage of embryos was lowest when embryos were previously cultured on medium without growth retardants and when were incubated under 27°C. Somatic embryos preserved on media with 2mg/L CCC or ABA had a higher survival percentages.

Somatic embryos preserved on 2mg/L CCC had a higher survived percentage, higher significant values of total sugar, non reducing sugar, L-proline and lowest significant value of total phenols. Preserved embryos were successfully complete their growth and developments in subsequent stages *in vitro* and were transferred to green house with higher survival percentage and normal growth.

Keywords: *Phoenix dactylifera*, conservation, growth retardants.

INTRODUCION

Date palm *(Phoenix dactylifera* L.) is a monocotyledonous diocios tree species of major economic importance in the arid regions of the Middle East and North Africa (AL-Khayri, 2002). Fruit tree germplasm is being conserved mainly in the form of a field gene bank, which requires much labor money and land. In addition, this germplasm is apt to be lost by attack from pathogens, pests and environmental disasters (Ko *et al.*, 1991).

The main objective of the minimal growth procedure is to extend the subculturing interval from the normal 2 to 6 week to too much longer period (e.g., 3 to 12 months). Several approaches can be used to achieve this goal, of which incubation at reduced temperature and low light intensity (or in darkness), 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 very simple technique that allows storage of plant *in vitro* for periods ranging from 6 months to 5 years depending on species. These stored plant can be micro propagated rapidly when desired (Perez-Tornero *et al.*, 1999).

Very little research has so far been carried out on the germplasm storage of dates. Date seed may be held in common storage at moderate

temperatures for at least 5-6years. Longer storage results in loss of date viability. However, because date seeds are so heterogeneous, seed storage is obviously not the ideal method for preserving germplasm reference stocks. Tissue culture and crypreservation techniques may prove the most effective (Watanabe and Pehu, 1997).

This paper describes a system for preservation of date palm somatic embryos using under 27 or 18 °C reduced growth retardants (ABA, Paclobutrazol, Ancymidol or CCC). In addition, the estimation of reduced and non-reduced, total sugars, total phenols and proline content of successfully preserved date palm after the preservation period is described.

MATERIALS AND METHODS

The present study was carried out through 2007-2009 at the Central Laboratory of Date Palm Research and Development Agric. Res. Center, Giza, Egypt.

2.1. Plant materials

Direct somatic embryos of date palm *(Phoenix dactylifera* L.) cv. Bartamuda as described by (Hassan, 2007) were used as a source for explants.

2.2. Preservation culture media and temperature storage

- MS medium (Murashig and Skoog, 1962) + 0.1mg/L NAA (naphthalene acetic acid) + 0.05mg/L BA (Benzyl adenine) + 30gm/l sucrose+1gm/L A.C (activated charcoal) with different supplementation of growth retardants [Abscisic acid (ABA), Paclobutrazol (PBZ) and ancymidol) at the concentrations (0.0, 1.0, 2.0 and 4.0 mg/L) and Chlorocholine chloride (CCC)] at the concentrations (0.0, 0.25, 0.50, 1.0 and 2.0 mg/L) were used to preserve somatic embryos of date palm for 18 months at normal temperature (27°C) or reduced temperature (18°C). Each treatment contained 3replicates (5jar in each replicate), which were incubated in 16hr illumination of 500 Lux (white fluorescent lamps). The medium of each treatment was distributed into small jars (150 mL) at rate of 40 mL/jar and were autoclaved for 20 min. at 121°C (1.2 Kg/cm²). All culture jars were incubated in 16hr under illumination of 500 Lux (white fluorescent lamps).

2.3. Data collection and Statistical analysis

After preservation period, all remained clusters in every treatment were transferred to fresh culture medium with 0.1mg/L NAA + 0.05mg/L BA and incubated under normal condition to estimate the plant recovery and survival percentages.

Treatments with higher survival percentage were statistically analysed with each other to compare survival percentages, total reduced and non-reduced sugar, total phenols content (mg/g fwt.) and proline content (mg/g dwt.).

All experiments were arranged in a complete randomized design and data were analyzed according to the methods described by Snedecor and Cochran (1980). The averages were compared using the least significant difference (L.S.D.) test.

2.4. Chemical analysis

-Total sugars: Dry samples of leaves (0.1g) were analysis according to Dubois *et al.*, (1956) to account total sugars.

-Total phenols: The fresh samples of the plant leaves were collected and extracted as described by A.O.A.C. (1980) to estimate total phenols.

-Proline content: Dry samples of leaves (0.5g) were used to stimulate praline content according to Bates *et al.* (1973).

RESULTS

Data in Table (1) indicate survival percentage after 18 months for the somatic embryos of date palm under different temperatures and ABA concentrations. The highest significant percentage was achieved when somatic embryos preserved under 18°C compared with 27°C (39.99 and 26.66, respectively), while the most effective concentration of ABA was 2 mg/L to achieve the highest significant survival percentage (59.99).

Interaction between temperature and ABA was found to be most suitable to preserve somatic embryos for 18 months with higher survival percentage, i.e., 73.33% somatic embryos preserved on medium with 2 mg/L ABA and incubated under 18°C showed good and healthy growth when transferred to normal conditions (27°C+ medium without ABA) for one month.

Table (1): Effect of ABA concentrations on survived percentage of preserved direct somatic embryogenesis of date palm (cv. Bartamuda) incubated under 27 or 18°C after 18 months

Concentration (mg/L)	18°C	27°C	Mean
0.0	13.33	2.22	7.78
1.0	33.33	26.66	29.99
2.0	73.33	46.66	59.99
4.0	40.00	31.11	35.55
Mean	39.99	26.66	

L.S.D. at 0.05
Concentration = 8.124 Temperature = 5.637
Interaction: Concentration × temperature = 11.48

Data in Table (2) show that the effect of PBZ concentrations on survival percentage of date palm somatic embryos conserved under 18 or 27°C after 18 months. It's clearly that 18°C was suitable for somatic embryos preservation than 27°C (42.21 and 19.99, respectively), while the most favorable concentration of PBZ in this respect was 1mg/L and increased the concentration of PBZ from 1 to 4 mg/L preserved medium decreased the survival percentage from 46.66 to 29.99.

The interaction between temperature and PBZ showed that preservation of somatic embryos on medium with 1mg/L PBZ and incubated at 18°C produced the highest value of survival percentage after 18 months.

The effect of growth retardants ancymidol on survival percentage of date palm somatic embryos incubated under 18 or 27°C for 18months are

recorded in Table (3). Reduction the temperature to 18°C produced the highest significant survival percentage when somatic embryos were transferred to normal growth, while only 13.33% from all preserved embryos on 27°C were successfully grow after they transferred to fresh medium (34.99 and 13.33, respectively).

Table (2): Effect of PBZ concentrations on survived percentage of preserved direct somatic embryogenesis of date palm (cv. Bartamuda) incubated under 27 or 18°C after 18 months

Concentration (mg/L)	18°C	27°C	Mean
0.0	13.33	2.22	7.78
1.0	66.66	26.66	46.66
2.0	53.33	26.66	39.99
4.0	35.55	24.44	29.99
Mean	42.21	19.99	

L.S.D. at 0.05
Concentration = 8.56 Temperature = 6.05
Interaction: Concentration × temperature = 12.01

Table (3): Effect of ancymidol concentrations on survived percentage of preserved direct somatic embryogenesis of date palm (cv. Bartamuda) incubated under 27 or 18°C after 18 months

Concentration (mg/L)	18°C	27°C	Mean
0.0	13.33	2.22	7.77
1.0	33.33	4.44	21.11
2.0	40.00	13.33	26.67
4.0	53.33	33.33	43.33
Mean	34.99	13.33	

L.S.D. at 0.05
Concentration = 3.45 Temperature = 2.44
Interaction: Concentration × temperature = 4.88

Concentrations of ancymidol play a distinct role in this respect. Increasing the concentration of ancymidol in preserving medium from 1to 4 mg/L increased significantly the survival percentage from 21.11 to 43.33. Data of interaction between temperature and ancymidol concentrations showed that somatic embryos of date palm were successfully preserved for 18 month with higher survival percentage when ancymidol was added to preserve medium at 4 mg/L and incubated under 18°C (53.33).

Survival percentages of date palm somatic embryos preserved on CCC and incubated under 18 or 27°C are present in Table (4). The addition of CCC to preservation medium at 2 mg/L or 0.5 mg/L produced the highest significant survival percentages of date palm somatic embryos (57.88 and 56.66, respectively) without significant differences. Only 7.78% of all preserved embryos were successfully grown when transferred to normal growth condition after 18 months of conserved period.

Table (4): Effect of CCC concentrations on survived percentage of preserved direct somatic embryogenesis of date palm (cv. Bartamuda) incubated under 27 or 18°C after 18months

Concentration (mg/L)	18°C	27°C	Mean
0.0	13.33	2.22	7.78
0.25	33.33	53.33	43.33
0.5	53.33	60.00	56.66
1.0	60.00	20.00	40.00
2.0	86.66	28.88	57.88
Mean	49.33	32.88	

L.S.D. at 0.05
Concentration = 6.87 Temperature= 4.34
Interaction: concentration × temperature = 9.71

The highest significant survival percentage was obtained when incubation was done on 18°C compared with 27°C (normal temperature). Transferring of date palm preserved somatic embryos at 2 mg/L CCC and incubated at 18°C to normal conditions after 18 months produced the highest significant survival percentage (86.66) and preserved embryos appeared as in good healthy growth.

A comparative study on the effect of superior treatments in preservation of date palm somatic embryos is existed in Table (5). Data revealed that the highest significant percentage survival and plant recovery after transferring to normal conditions were obtained when date palm somatic embryos were preserved on culture media supplemented with 2 mg/L CCC or 2 mg/L ABA (86.66 or 73.33% respectively) without any significant difference between both of them, while no significant differences could be observed between the addition of 2 mg/L ABA, 1 mg/L PBZ or 1mg/L CCC to the preservation medium (73.33, 66.66 and 60, respectively).

Data in the same Table about the effect of the superior treatments on chemical analysis showed the following:

Phenols:

The highest significant value of phenols was observed when somatic embryos were preserved on medium with 2 mg/L ABA and the lowest significant one was that supplemented with 2 mg/L CCC.

Table (5): Effect of superior treatments on survival percentages and chemical analysis of date palm preserved somatic embryos (cv. Bartamuda) after 18months of preservation at 18°C

Concentration (mg/L)	Survival percentage	Av. phenols (mg/g fwt)	Av. total sugars (mg/g dwt.)	Av. reducing sugars (mg/g dwt.)	Av. Non-reducing sugars (mg/g dwt.)	Av.proline (mg/g dwt.)
2 CCC	86.66	0.298	0.369	0.102	0.267	1.482
2 ABA	73.33	0.440	0.359	0.190	0.169	1.260
1PBZ	66.66	0.334	0.293	0.111	0.182	1.120
1 CCC	60.00	0.376	0.273	0.184	0.089	0.645
L.S.D. at 0.05	15.73	0.044	0.004	0.056	0.025	0.051

Total sugars:

Analysis of preserved somatic embryos reflected that preservation of embryos on medium with 2 mg/L CCC had a highest significant value of total sugar followed by that of 2 mg/L ABA, while the lowest significant value was resulted with medium with 1mg/L CCC, which produced the lowest significant value of survival percentage.

Reducing sugars:

Somatic embryos preserved on 2 mg/L ABA or 1mg/L enriched with reducing sugar without significant differences in-between while the lowest significant values were observed 2mg/L CCC or 1mg/L PBZ.

Non-reducing sugars:

As observed with survival percentage, the highest significant value of non reducing sugars was noticed with somatic embryos preserved on 2 mg/L CCC, which gave the highest significant survival percentage. In contrast the lowest one was observed with 1mg/L CCC, which produced the lowest significant survival percentage.

L-proline:

Same Table indicates that L-proline concentrations were increased significantly with increase in survival percentage and the lowest significant value of this amino acid was noticed with somatic embryos preserved on medium supplemented with 1mg/L CCC, which produced the lowest significant survival percentage.

It can be concluded that somatic embryos preserved on medium supplemented with 2mg/L CCC remind viable after 18 months on 18°C with higher survival percentage and they also enriched with total sugars, non-reducing sugars, L-proline and had a lowest significant value of phenols.

DISCUSSION

Protoplasts, single cells, and organized tissues (meristems, somatic embryos) can be stored (Bajaj, 1983). There are two methods of long-term storing *in vitro* plant cultures: slowing down or suspending growth. Slow growth is achieved by modifying the culture medium or reducing temperature (Withers, 1991).

Preservation using low teampeature

Storage under low temperature is one of the major tissue culture techniques used for preservation of genetic resources (Moges *et al.*, 2003). Elimination of disease problems and reduction of genetic modification as well as low labor and space requirements are major achievements of cold preservation of plant material (Hopkins, 1995 and Reed *et al.*, 1998). Under such condition, accumulation of unsaturated lipids on the cell membrane would cases cell membrane thickening and retards cell division and elongation (Engelmann, 1997). Data under dissection showed that reducing the temperature of preservation to18°C achieved higher survival percentage than 27°C. In this respect, meristem cultures of some root tuper crops and shoot-tips of banana (*Nusa* spp.) were preserved for up to 18months at 18°C (Wang and Charles, 1991). Also, (Wannas, 1992) reported that 85% of apple

shoot cultures remained alive after 18 months of *in vitro* storage at 8 and 4°C, respectively, in the dark. Bekheet (2007) stated that somatic embryos and undifferentiated culture of date palm cv. Zaghlool were successfully stored for 12 months at low temperature (5°C) in the dark with relatively high percentage (50%) of cultures remained viable without serious signs of senescence.

Explants of date palm stored at 18°C showed more survival percentage compared with those stored at 27°C (Hassan, 2002).

Lopez-Delgado and Scott (1998) reported that required intervals between subcultures of micro plants of three cv. of potato were shorter in warm growth room 26.6 ± 1.5°C.

Preservation using osmoticum

Results under discussion showed that all concentrations of growth retardants increased survival percentage of preserved somatic embryos of date palm compared with control treatments specially CCC, ABA at 2 mg/L and PBZ at 1mg/L.

Osmotic agents are materials that reduce the water potential of cells. The addition of osmotica to the culture has been proved to be efficient in reducing growth and increasing the storage life of many *in vitro* grown tissues of different plant species (Wilson *et al.*, 2000). High levels of osmotic agents in the medium would act against the creation of a critical turgor pressure, which must be established before cell expansion can occur. This stress condition will inhibit both callus growth and shoot formation (Brown *et al.*, 1979).

There a report on long-term effects of growth retardants on the growth and development of plants obtained in vitro from media with these substanas. Tri-butyl-2, 4 dichlorbenzylphosphonium chloride (Phosphon D), malic hydrazide, succinic acid-2, 2-dimethyl hydrazide (B-995), CCC and ancymidol were reported to be good materials to lengthen the storage life *in vitro* grown tissues (Dodds and Roberts, 1985).

ABA plays a principal role in stress signal transduction. The signal transduction pathway has been shown to modulate many physiological processes in plants induction of dormancy, embryo maturation, inhibition of guard-cell opening and adaptation to environmental stress (Zeevaart and Creelman, 1988 and Davies and Jones, 1991).

Hamed and Badr El-Den (2005) reported that added PBZ or CCC to the conserved media of Ananas gave the highest significant survival percentage when shoots transferred to fresh medium and incubated under normal conditions. They also, found that the addition of growth retardant at any concentrations used (1, 2 or 3 mg/L) increased significantly the survival percentages compared without addition.

Pinto *et al.* (2005) reported that PBZ drench at 0.5, 0.75 or 1.0 mg/pot of Zinnia resulted in shorter plants with shorter interned.

Eliasson *et al.* (1994) included PBZ 0.15-0.6 $mg.dm^{-3}$ to in vitro rooting of *Pyrus serotina* and found that a significant effect on reduction of shoot was higher when treated with flurprimidol or PBZ at 5$mg.dm^{-3}$. *Tibouchina urvilleana* formed very small leaves (Kozak, 2006).

PBZ reduces vegetative growth in plants, since it inhibits gibberellins biosynthesis (Shortening in axis and node interval) (Wheaton, 1989).

Pieniazek *et al.* (1978) reported that the addition of CCC, ABA, Alar and Athyrel to the medium supplemented with 5% sorbitol inhibited growth of apple callus.

REFERENCES

A.O.A.C. (1980). Association of Official Agriculture Chemists. Official Method of Analysis. Washington D.C., USA. 832p.

Al-Khayri; J. M. (2002). Growth, proline accumulation, and ion content in sodium chloride-stressed callus of date palm. *In vitro*: Cell Develop. Biol., 38:79-82.

Bajaj; Y. P. S. (1983). Cryopreservation and international exchange of germplasm. In:Sen, S. K., Giles, K. L. (ed.): Plant Cell Culture in Crop Improvement. Pp. 19-41. Plenum Press, New York, USA.

Bates; L. S.; Waldern, R. P. and Tear, I. D. (1973). Rapid determination of free proline under water stress studies. Plant and Soil. 39: 205-207.

Bekheet; S. A. (2007). *In vitro*: Mid-term storage of date palm germplasm by slow growth and encapsulated somatic embryos. Egypt. J. Agric. Res., 85(1B): 549-558.

Brown; D. C. W.; Leung, D.W. M. and Thorpe T. A. (1979). Osmotic requirements for shoot formation in tobacco callus. Phys. Planta. 46:36-41.

Davies; W. J. and Jones H. G. (1991). Abscisic acid: physiology and biochemistry. Bios, Oxford. Pp 39-52.

Dodds; J. H. and Roberts L. W. (1985). Experiments in plant tissue culture. 2nd ed, Cambridge University Press, pp:172-179.

Dubois; M., Smith F., Gilles K. A, Hamilton J. K. and Rebers P. A. (1956). Colorimetric method for determination of sugars and related substances. Annal. Chem., 28(3):350-356.

Eliasson; M. K., Beyl C. A. and Barker P. A. (1994). *In vitro*: Responses and acclimatization of *Prunus serotina* with paclobutrazol. J. Plant Growth Regulation. 13:137-142.

Engelmann; F. (1997). *In vitro*: Conservation methods, in Biotechnology and Plant Genetic Resources: Conservation and Use. B. V. Ford-Lloyd, J. H. Newburry and J. A. Callow (eds.), CABI, Wellirgford. pp: 119-162.

Hamed; A. H. and Bader El-Den A. M. (2005). *In vitro:* C onservation of pineapple (*Ananas comosus*) under minimal growth conditions. 3rd Conference Recent Technologies in Agriculture, Cairo University. 482-492.

Hassan; M. M. (2002). *In vitro*: Studies on somatic embryogenesis conservation of date palm. Ph.D. Thesis, Faculty of Agriculture, Cairo University.

Hassan; M. M. (2007). Induction of direct somatic embryogenesis of date palm (*Phoenix dactylifrea* L.) using ABA. Egyptian Journal of Genetic and Cytology. 36(1): 93-104.

Hopkins; W. G. (1995). Introduction to plant physiology. John Wily & Sons. Inc., New York, USA. pp 1-20.

Ko; W. H.; Hwang, S. C. and Ku, F. M. (1991). A new technique for storage of meristem-tip cultures of 'Cavendish' banana. Plant Cell, Tiss. Org. Cult., 25(3):179-183.

Kozak; D. (2006). The effect of growth retardants applied *in vitro* on the acclimatization and growth of *Tibouchina urvilleana* cogn. *In vivo*. Acta Sci. Pol., Hortorum Cullus. 5(1): 65-70.

Lopez-Delgado; H. L. and Scott, I. M. (1998). Storage of potato microplants in vitro in the presence of acetyl salicylic acid. Plant Cell Tiss. Org. Cult., 54:145-152.

Moges; A. D.; Karam, N. S. and Shibli, R. A. (2003). Slow growth *in vitro* preservation of African violet (*Saintpaulia ionantha* Wendl.) shoot-tips. Advanced HortScience. 17:1-8.

Murashige; T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco cultures. Physiol. Plant. 15:473-497.

Perez-Tornero; O; Ortin-Parraga, F.; Egea, J. and Burgos, L. (1999). Medium-term storage of apricot shoot-tips *in vitro* by *in vitro* minimal growth method. HortScience. 34(7): 1277-1278.

Pieniazek; J; Holubowicz, T.; Machnik, B. and Kasprzyk, K. (1978). Apple stem callus frost tolerance and growth modification by adding sorbitol and some growth regulators to the medium. Acta Horticulture. 81:91-95.

Pinto; A. C. R; Rodrigues, T. J. D.; Leite, I. C. and Barbosa, J. C. (2005). Effect of growth retardants on development and ornamental quality of potted lilliput (*Zinnia alegans*) Jacq. Sci. Agric., 62(4): 337-345.

Sendecor; G. W. and Cochran, W. G. (1980). "Statistical Methods". Oxford and J. B. H. Publishing Co., 6th Edition.

Reed; B. M., Paynter C. L.; Denoma, J. and Chang Y. (1998). Techniques for medium-and long-term storage of pear (*Pyrus* L.) genetic resources. Plant Genetic Resources Newsletter. 115:1-5.

Wang; P.J. and Charles, A. (1991). Micropropagation through meristem culture. In Bajaj, Y.P.S.eds. The Biotechnology in Agriculture and Forestry. High-Tech. and propagation, vol.17, Springer-Verlag Berlin Heidelberg. pp:32-52.

Wannas; W. H. (1992). *In vitro*: Storage of proliferated apple root-stock shoot-tip cultures. Ann.Agric..Sci. Ain Shams Univ., 37:501-510.

Watanabe; K. N. and Pehu, E. (1997). The application of biotechnology to date palm culture. Plant Biotechnology and Plant Genetic Resources for Sustainability and Productivity. Chapter (14)., (ed.) R. G. Lande Company.

Wheaton; T. A. (1989). Triazole bioregulators reduce internode length and inner branch angle of citrus. Acta Hort. (Growth Regulators in the Fruit Production): Penticton, Canda. 239:277-280.

Wilson; S. B., Rajapakse, N. C. and Young, R. E. (2000). Media composition and light effect storability and post storage recovery of micropropagated hosta plantlets. HortScience. 35:1159-1162.

Withers; L. A. (1991). Biotechnology and plant genetic resources conservation. In: Paroda, R. S., Arora, R. K. (ed.): Plant Genetic Resources, Conservation and Management. Concepts and Approaches. Pp.273-297. International Board for Plant Genetic Resources. New Delhi, India.

Zeevaart; J. A. D. and Creelman R. A. (1988). Metabolism and physiology of abscisic acid. Annu. Rev. Plant physiol. Plant Mol. Biol., 39:349-373.

استخدام مثبطات النمو لحفظ أجنة نخيل البلح

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تهدف هذه الدراسة لحفظ الأجنة الخضرية المباشرة لنخيل البلح عن طريق تقليل النمو باستخدام درجة الحرارة المنخفضة (١٨° م) واضافة مثبطات النمو ثم حفظ الأجنة الخضرية بزراعتها على بيئة(MS) المضاف اليها أيى من حمض الابسيسك، الباكلوبترازول، الأنسيميدول بتركيزات ١، ٢، ٤مجم/لتر وكذلك باضافة الســيكوسيل بتركيزات ٠,٢٥، ٠,٥،١، ٢مجم/لتر وقد تم زراعة الأجنة الجسمية على بيئة خالية من مثبطات النمو للمقارنة وتم حفظ الزراعات على درجتي حرارة ٢٧ ، ١٨° م لمدة ١٨شهر.

وقد تم نقل الأجنة التى تم حفظها الى بيئات طازجة خالية من مثبطات النمو وتحضينها فى درجة حرارة ٢٧°م لمدة ٤أســابيع لتقدير مدى حيويتها وأظهرت الدراسة أن أقل نسبة نجاح للأجنة نتجت بزراعتها على البيئات الخالية من مثبطات النمو والمحضنة على ٢٧° م بينما أعطت المعاملة المضاف اليها ٢مجم/لتر سيكوسيل أعلى نسبة نجاح وأعلى محتوى من السكريات الكلية والغير مختزلة والبرولين وأقل محتوى من الفينولات بفرق معنوى مقارنة بباقى المعاملات محل الدراسة وقد أكملت الأجنة المحفوظة باقى مراحل نموها المتتالية داخل المعمل وتم نقلها بنجاح للصوبة مع ارتفاع نسبة نباتات الأقلمة ومظهر النمو الطبيعى.