



Minimal Media Strength for *In vitro* Conservation of Strawberry (*Fragaria ananassa*) Cultures

Zaid K. Kadhim¹ & Muslim A. A. Abdulhussein^{2*}

¹Department of Horticulture and Land Landscape, College of Agriculture, University of Karbala, Iraq

² Department of Horticulture and Land Landscape, Faculty of Agriculture, University of Kufa, Iraq

*Corresponding author email: muslim.alrubaye@uokufa.edu.iq

Received 15th December 2020; Accepted 1st March 2021; Available online 29 October 2021

Abstract: An experiment for strawberry *Fragaria ananassa* cv. Festival *in vitro* conservation of cultures for nine months was studied. Three MS medium strength (full strength MS, $\frac{3}{4}$ MS, $\frac{1}{2}$ MS, and $\frac{1}{4}$ MS) and the storage period (3, 6, and 9 months) in slowing down or reducing growth. Results showed a significant effect of the medium salt strength, as the full strength treatment was higher by achieved the highest percentage of viability (survival %) and storage characteristics of cultures (N%, P%, carbohydrates%, protein %) and regeneration ability characteristics (shoots and roots number and length). Three-month storage period achieved the highest rate compared to the nine-month storage period, which recorded the lowest average for the studied characteristics. The full strength treatment for three months storage period realized the highest rate compared to treatment $\frac{1}{4}$ strength for the storage period of nine months, which recorded the lowest rate of the studied traits. The application of minimal strength for *in vitro* conservation allows strawberry cultures to remain viable with a slow growth rate and a significant increase in the storage life of the tissues to nine months.

Keywords: *In vitro* conservation, Minimal medium salt, Slow growth storage, Strawberry.

Introduction

Strawberry (*Fragaria ananassa* Duch.) is a perennial herbal fruit plant, with fruits that appreciated for its fragrance, taste and antioxidant capacity resulting from high levels of anthocyanin, and other therapeutic and nutritional properties (Husaini & Neri, 2016). The cultivation of strawberry has spread in a wide range of geographical areas in the world, as it has extended from tropical

and subtropical regions to cold regions, making it adapted to a wide range of climatic conditions as it is grown in about 80 countries (FAO, 2016). In Iraq, strawberry cultivation is still limited and its cultivation is only at the scientific experiment stations, home gardens and some small agricultural areas. The production of strawberry concentrated in the governorates of Mosul, Karbala and Baghdad,

where production in the country has not reached the required level (Franco, 2009).

Strawberry propagates in two ways: the sexual method, in which the seeds used to produce seedlings that are planted in a permanent place or to generate new varieties. This method is not desirable because it produces plants that are genetically different from the parent plant and therefore percentage of seed germination is low, so that they are propagated by vegetative methods. The strawberry plant's infection with some pathogens, especially viral diseases, result in a decrease in production in quantity and quality, so it is preferable to use the tissue culture method to generate plants free from pathogens for commercial production (Rokosa & Mikiciuk, 2017).

The difficulty of storing genetic resources in their traditional forms, especially in the form of seeds or runners, the technology of preserving genetic resources via *In vitro conservation* and super-freezing *Cryoconservation* have been introduced to application with the assistance of tissue propagation of plants which has been proven to be used as a mean of storage. *In vitro conservation* of plants cultures, such as strawberries, used for conservation of genetic resources to avoid the loss of many species and varieties by fungal and viral infections or harmful of environmental factors, the possibility of multiplying it without difficulty in tissues culture, the difficulty of preserving its genetic assets in fields and reserves, as well as the conservation cultures by slowing its growth is important in the field of reproduction by preserving it until needed for plantlets production market (Maryam *et al.*, 2014). One of the storage methods is minimal of nutrient medium by reducing the amount of nutrients. This method has been successfully

used in storing different vegetatively propagated horticultural plants, such as, *Citrus limon* (Samarina *et al.*, 2014), *Ziziphus jujube* Mill (Sota & Kongjika, 2014), and *Prunus webbii* (Sota & Kongjika, 2019).

Because of rare studies on this topic in Iraq, this study aimed to test the possibility of storing strawberry cultures in short- and medium-term storage in laboratories under conditions designed to slow down their growth by reducing of nutrient salts.

Materials & Methods

The study was carried out in the Laboratory of Tissue culture, Faculty of Agriculture, University of Kufa during April 2018 to June 2020. Runner segments and runner tips of Strawberry cv Festival was used as explant. Runner tips of festival cultivar were excised with the help of sharp blade and used as sources of meristems. The shoot tips were washed for 2-3 times with sterile distilled water. Surface sterilization was done by dipping the shoot tips into 3% Clorox (6% Sodium hypochlorate) solution with shaking for 15 minutes followed by 3-5 times washing with sterile distilled water. Surface sterilized runner tips that were carefully cultured on MS (Murashige & Skoog, 1962) medium supplemented with 0.5 mg.l⁻¹ BAP + 0.1 mg.l⁻¹ IBA (Mohammed, 2019) in test tube. The cultures were incubated in a growth chamber under 16/8 h light/dark cycle at 25 ± 1°C under illumination with white fluorescent tubes at an intensity of 1000 lux for 3 weeks. The regenerated shoots were carefully removed from the test tubes and transferred to new media for further multiple shoot induction.

After obtaining the sufficient number of multiply shoots, the experiment was carried out to show the effect of the strength of the

salts of MS nutrient medium (supplemented with 1 mg.l⁻¹ BAP + 0.1 mg.l⁻¹ IBA according to Mohammed, (2019) as shoot multiplication medium) and the periods of conservation on slowing or reducing culture growth. The MS medium was prepared with different concentrations of the strength of its salts (full strength, ¾, ½, ¼), and explants were cultured in the form of a small mass with three shoots in 1 litre glass bottles, contained of 100 ml of MS. Cultures were incubated in the growth chamber with light intensity (1000) lux for a period of three, six and nine months). Then the vitality ratio (survival %), nutrients (N, P, K), protein and carbohydrates in shoots on the basis of criteria described by Reed (1992) was evaluated.

After each conservation period (3, 6 and 9 months) cultures reculture in a medium free of growth regulators for two weeks, and then cultured in the medium of multiplication (previously used) by three shoots per glass bottle containing 30 ml of the MS medium and incubated for one month under the same incubation conditions. The ability of the cultures to multiply was evaluated based on growth indicators, as a number of shoots, length of shoots and number of leaves. Then the shoots were transferred to the rooting medium containing the growth regulator IBA at a concentration of 1.0 mg.l⁻¹ (Mohammed, 2019). The cultures were incubated with the same previous lighting conditions, after four weeks of transferring the number and length of roots was counted.

Chemical analysis

Carbohydrates in cultures were estimated in phenol-sulphuric acid method (Dubois *et al.*, 1956), protein was estimated according to Lowry *et al.* (1951) and contained of

nitrogene, phosphorus, potassium ions estimated using methods described by Kalra (1998).

Experimental design and statistical analysis

The laboratory conservation of the cultures was carried out as a factorial experiment using a complete random design (CRD) with two factors, the first is the strength of MS salts (full strength, ¾, ½, ¼), the second is storage periods (3, 6 and 9 months) and 10 repetitions per treatment (Al Rawi & Khalaf Allah 2000). Data were subjected to statistical analysis and averages differences compared according to the Duncan (multi-border) test at the probability level of 0.05 using the program GenStat (VSN International, 2019).

Results

Cultures vitality

Data in table (1) and fig. (1) explained that there are no significant differences between conservation periods in survival %, as showed in and fig. (1), and shoots content of P%, K%, while there is a significant effect on the percentage of shoots N%, protein, and carbohydrates, where conservation for three months achieved the highest percentage compared to the nine months which achieved the least percentage. Also, there were significant differences between the media with respect to the strength of the salts of the nutrient medium in percentage of vitality, N%, P%, protein, and carbohydrates, as the full-strength treatment achieved the highest percentage compared to the treatment ¼ salt strength, which recorded the lowest percentages, while, there was no significant effect of the salt strength on the percentage of potassium K%.

Table (1): Effect of medium salts strength on the storage characteristics, immediately after laboratory conservation of strawberry cultures.

Treatments		Survival %	N%	P%	K%	Protein %	Carbohydrate %
Conservation periods (month)	months 3	100 a	3.17 a	0.381 a	3.11 a	19.84 a	20.31 a
	months 6	91.7 a	2.51 b	0.336 a	2.76 a	15.71 b	17.23 ab
	months 9	83.3 a	2.04 b	0.259 a	2.43 a	12.73 b	13.69 b
MS Media strength	¼ MS	77.8 b	1.83 b	0.214 b	2.2 a	11.42 b	12.13 c
	½ MS	88.9 ab	2.84 a	0.323 b	3.01 a	17.74 a	19.32 ab
	¾ MS	100 a	2.49 ab	0.295 b	2.71 a	15.58 ab	15.7 bc
	Full Strength	100 a	3.14 a	0.471 a	3.14 a	19.64 a	21.16 a
Conservation periods x MS Media strength	months+ ¼ MS 3	100 a	2.53 ab	0.264 abc	2.73 a	15.8 ab	15.17 abcd
	months+ ½ MS 3	100 a	3.26 a	0.379 abc	3.15 a	20.37 a	22.7 a
	months+ ¾ MS 3	100 a	3.38 a	0.361 abc	3.21 a	21.11 a	20.26 ab
	months+ MS 3 Full Strength	100 a	3.53 a	0.522 a	3.34 a	22.08 a	23.1 a
	months+ ¼ MS 6	66.7 b	1.59 b	0.21 c	2 a	9.92 b	12.28 bcd
	months+ ½ MS 6	100 a	2.78 ab	0.328 Abc	3 a	17.36 ab	18.55 abc
	months+ ¾ MS 6	100 a	2.43 ab	0.292 Abc	2.92 a	15.17 ab	16.38 abcd
	months+ MS 6 Full Strength	100 a	3.26 a	0.515 Ab	3.1 a	20.38 a	21.71 a
	9months+ ¼ MS	66.7 b	1.36 b	0.169 c	1.87 a	8.53 b	8.93 d
	months+ ½ MS 9	66.7 b	2.48 ab	0.261 abc	2.87 a	15.48 ab	16.71 abcd
	months+ ¾ MS 9	100 a	1.67 b	0.231 bc	2.01 a	10.46 b	10.47 cd
	months+ MS 9 Full Strength	100 a	2.63 ab	0.376 abc	2.98 a	16.46 ab	18.67 abc



Fig. (1): Strawberry Cultures survival after nine months of in vitro storage.

In addition, there is a significant effect of the interaction between the media strength and storage periods in the percentage of vitality. Storage periods for six and nine months in $\frac{1}{4}$ MS salt strength achieved the lowest percentage (66.7%) compared to the others interference which achieved (100%), while the intervention treatment (three months + full strength) achieved the highest percentage in which was 3.53%, 0.522%, 3.34%, 22.08%, 23.1% for N%, P%, K%, protein, and carbohydrates respectively.

Regeneration ability after in vitro conservation

Table (2) showed that there are significant differences between the periods of conservation on the number of shoots, length of shoots, number of leaves, and number of roots). Three months period of conservation achieved the highest rate of 4.17 shoots, 3.99 cm, 4.16 leaves, 3.04 roots, respectively, compared to nine months conservation period, which achieved the lowest average of 2.87 shoots, 2.73 cm, 3.06 leaves, and 2.24

roots, respectively, while the duration of conservation did not record any significant difference for the length of the roots.

There were significant differences between the media (table 2) in number of shoots, length of shoots, number of leaves, number of roots, and length of roots) as the full strength treatment gave the highest rate of 4.48 shoots, 4.11 cm, 4.34 leaves, 3.12 roots, 4.36 cm, respectively, compared to the $\frac{1}{4}$ MS salts, which recorded the lowest means (2.67 shoots, 2.47 cm, 2.73 leaves, 1.96 roots, 2.84 cm), respectively.

It is noted from the same table that there is a significant effect of the interaction between the MS strength and storage periods on (number of shoots, length of shoots, number of leaves, number of roots, and length of roots) , in which interaction treatment (three months + full strength) is recording the highest rate of 5.33 shoots, 4.78 cm, 5.0 leaves, 3.33 roots, 5.0 cm respectively, compared to the treatment (9 months + $\frac{1}{4}$ MS), which scored the lowest rate (2.11 shoots, 2.04 cm, 2.29 leaves, 1.56 roots, 2.43 cm) respectively.

Table (2): Effect of the medium strength on regeneration ability after *in vitro* conservation of strawberry cultures.

Treatments		shoots Number	shoots Length (cm)	Leaves Number	Root Number	Root Length (cm)
Conservation periods(month)	months 3	4.17a	3.99a	4.16a	3.04a	4.1a
	months 6	3.47ab	3.5ab	3.5ab	2.7ab	3.6a
	months 9	2.87b	2.73b	3.06b	2.24b	3.23a
MS Media strength	¼ MS	2.67b	2.47b	2.73b	1.96b	2.84b
	½ MS	3.50b	3.60ab	3.77ab	3.05a	3.95ab
	¾ MS	3.37ab	3.37a	3.45ab	2.51ab	3.41ab
	Full Strength	4.48a	4.11 a	4.34 a	3.12 a	4.36 a
Conservation periods x MS Media strength	months+ 3 ¼ MS	3.44 abc	3.14 ab	3.6 ab	2.56 ab	3.38 ab
	months+ ½ 3 MS	3.9 abc	4.03 ab	4 ab	3.16 ab	4.00 ab
	months+ 3 ¾ MS	4 abc	4.03 ab	4.03 ab	3.11 ab	4.00 ab
	months+ 3 MS Full Strength	5.33 a	4.78 a	5.00 a	3.33 a	5.00 a
	months+ 6 ¼ MS	2.44 bc	2.24 b	2.3 b	1.78 ab	2.72 ab
	months+ ½ 6 MS	3.44 abc	3.76 ab	3.83 ab	3.11 ab	4.07 ab
	months+ 6 ¾ MS	3.56 abc	3.98 ab	3.67 ab	2.78 ab	3.42 ab
	months+ 6 MS Full Strength	4.44 ab	4.03 ab	4.21 ab	3.15 ab	4.18 ab
	9months+ ¼ MS	2.11 c	2.04 b	2.29 b	1.56 b	2.43 b
	months+ ½ 9 MS	3.15 bc	3.27 ab	3.48 ab	2.89 ab	3.79 ab
	months+ ¾ 9 MS	2.56 bc	2.09 b	2.65 B	1.63 b	2.8 ab
	months+ 9 MS Full Strength	3.67 abc	3.53 ab	3.82 ab	2.89 ab	3.89 ab

Discussion

The results of tables (1 and 2) showed a significant effect of the concentration of medium MS salts on the percentage of cultures viability and regeneration ability (shoots multiplication and rooting), these results agreed with what Ahmed *et al.* (2010), Samarina *et al.* (2014) and Sota & Kongjika (2019) who found that the survival % and shoots multiply decreases with increasing conservation time, but it remained survival.

The medium nutritional status is one of the most important factors that affect the growth of the cultures grown *in vitro*, because of its importance in providing the nutrients necessary for the growth and development of cultures, since the explants depending on what the medium provides of nutrients (Vinay & Afroz, 2015). so, the components of the nutrient medium and its physical state is very important and must be determined due to its importance for in plant growth in a specific stage and its subsequent effect later stages of micropropagation. The Medium salt strength is one of the factors most often evaluated in studies related to the *in vitro* conservation of various plant species (Huang *et al.*, 2014). Several modifications can be made in the MS medium to reduce the growth of plant parts preserved in the laboratory. This involves reducing plant metabolism by increasing the laboratory period of plant growth, without altering the genetic make-up, plant quality, and its ability to multiply and produce new plantlets after storage (Kamińska *et al.*, 2016).

Our results showed that the conservation period has a significant effect on the percentage of cultures vitality in the laboratory to maintain the ability to multiply and rooting, as this effect

varied according to the strength of medium, as it was noticed that the conservation period of 3 and 6 months had good results, if compared to the 9-month conservation period, and with the increase in the conservation period to 9 months, the viability percentage and ability to multiply and rooting decreased. These results are in line with what Trejgell *et al.* (2015) found when storing *Senecio macrophyllus* shoots *in vitro* for three and six months.

Conclusion

Strawberry Cultures can be *in vitro* conserved successfully for nine months with minimal salt strength of MS medium.

Acknowledgements

The authors are very much grateful to staff of plant tissue culture Lab., Faculty of Agriculture, University of Kufa for the financial support to this study.

Orcids

M. A. Abdulhussein: <https://orcid.org/0000-0002-9813-0764>

References

- Ahmed, M., Anjum, M. A., Shah, A.H., & Hamid, A. (2010). *In vitro* conservation of *Pyrus germplasm* with minimal growth using different temperature regimes. *Pakistan Journal of Botany*, 42, 1639-1650. [http://www.pakbs.org/pjbot/PDFs/42\(3\)/PJB42\(3\)1639.pdf](http://www.pakbs.org/pjbot/PDFs/42(3)/PJB42(3)1639.pdf)
- Al- Rawi, K. M., & Khalaf Allah A. A. M. (2000). *Design and Analysis of Agricultural Experiments*. Books House for Printing and Publishing. University of Mosul. 484 pp. (in Arabic).
- Dubois, M. K., Crilles, K. A., Hamiltor, J. K., Rebers, D. A., & Smith, F. (1956). Colorimetric method for determination of sugars and substance. *Analytical Chemistry*, 28, 350-365. <https://doi.org/10.1021/ac60111a017>

- FAO, (2016). *Food and Agriculture Organization of the United Nations*. FAOSTAT agriculture data. <http://faostat3.fao.org/download/Q/QC/E>. Accessed May 2016.
- Franco, S., (2009). *The Production of Strawberries in Iraq*. Ministry of Agriculture, Iraq. pp. 1-10. U.S. Agency for International development (USAID).
- Huang, H. P., Wang, J., Huang, L. Q., Gao, S. L., Huang, P., & Wang, D. L. (2014). Germplasm preservation *in vitro* of *Polygonum multiflorum* Thunb. *Pharmacognosy Magazine*, 10, 179–184. <http://www.phcog.com/text.asp?2014/10/38/179/131083>
- Husaini, A. M., & Neri, D. (2016). *Strawberry: Growth, development and diseases*. CABI Publishing, 315pp. <https://doi.org/10.1079/9781780646633.0000>
- Kamińska, M., Skrzypek, E., Wilmowicz, E. E., Tretyn, A., & Trejgell, A. (2016). Effect of light conditions and ABA on cold storage and post-storage propagation of *Taraxacum pieninicum*. *Plant Cell, Tissue and Organ Culture*, 127, 25-34. <https://doi.org/10.1007/s11240-016-1026-1>
- Kalra, Y. P., (1998). *Handbook of Reference Methods for Plant Analysis*. Soil and Plant Analysis Council, Inc., CRC Press. FL., 287pp. https://plantstress.com/wp-content/uploads/2020/03/Handbook_of_Reference_Methods_for_Plant_Analysis-1998.pdf
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with Folin phenol reagent. *Journal of Biological Chemistry*, 193, 295-297. [https://www.jbc.org/article/S0021-9258\(19\)52451-6/pdf](https://www.jbc.org/article/S0021-9258(19)52451-6/pdf)
- Maryam, A., Tariq, A., Chuadhary, S., Azmat, R., Javed, S., & Khanam, S. (2014). A review: Role of tissue culture (*in vitro*) techniques in the conservation of rare and endangered species. *Pacific Journal of Life Sciences*, 2, 93-103. <https://www.academia.edu/7259757>
- Mohammed, M. H. (2019). *Response of strawberry plant *Fragaria ananassa* Duch to light quality and some growth stimulators in vitro*. M. Sc. Thesis. Faculty of Agriculture, University of Kufa, 111pp. (in Arabic).
- Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum*, 15, 473- 497. <https://doi.org/10.1111/j.1399-3054.1962.tb08052.x>
- Reed, B. M. (1992). A cold storage of strawberries *in vitro*: A comparison of three storage systems. *Fruit Varieties Journal*, 46, 98–102. <https://europepmc.org/article/agr/ind92031926>
- Rokosa, M., & Mikiciuk, M. (2017). *In vitro* regeneration of *Fragaria* plants. *Acta Scientiarum Polonorum, Hortorum Cultus*, 16, 145–158. http://www.hortorumcultus.actapol.net/volume16/issue5/16_5_145.pdf
- Samarina, L. S., Choudhary, R., Kolomiets, T. M., Abilfazova, Y. S., & Saran, P. L. (2014). *In vitro* Conservation Technique for Russian *Citrus limon*. *Agricultural Research*, 3, 279-283. <https://www.tib.eu/en/search/id/BLSE%3ARN363052056>
- Sota, V., & Kongjika, E. (2014). Slow growth *in vitro* conservation of *Zizyphus jujuba* Mill. *Agriculture & Forestry*, 60, 27-37. <http://89.188.43.75/agricultforest/20140218-03%20SOTA%20and%20KONGJIKA.pdf>.
- Sota, V., & Kongjika, E. (2019). Micropropagation and *in vitro* conservation via slow growth techniques of *Prunus webbii* (Spach) Vierh: An endangered plant species in Albania. *International Journal of Agricultural and Biosystems Engineering*, 13, 11, 280-283. <https://doi.org/10.5281/zenodo.3566425>
- Trejgell, A., Kaminska, M., & Tretyn, A. (2015). *In vitro* slow growth storage of *Senecio macrophyllus* shoots. *Acta Physiologiae Plantarum*, 37, 234. <https://doi.org/10.1007/s11738-015-1983-8>.
- Vinay, S. & Afroz, A. (2015). *Plant Tissue Culture*. I K. International Publishing House. 378pp. <https://www.waterstones.com/book/plant-tissue-culture/vinay-sharma/afroz-alam/9789384588441>
- VSN International. (2019). GenStat for Windows 12th Edition. VSN International, Hemel Hempstead, UK.

تقليل أملاح الوسط الغذائي لخرن زروعات نبات الفراولة خارج الحسم الحي

زيد خليل كاظم¹ و مسلم عبدعلي عبد الحسين²

¹ قسم البستنة وهندسة الحدائق، كلية الزراعة، جامعة كربلاء، العراق

² قسم البستنة وهندسة الحدائق، كلية الزراعة، جامعة الكوفة، العراق

المستخلص: أجريت التجربة لدراسة تأثير الخزن المختبري لزروعات نبات الفراولة لمدة 9,6,3 أشهر المزروعة في الوسط الغذائي MS ذو قوه املاح مختلفة (قوه كامله, 3١4 القوه, 1١2 القوه, 1١4 القوه). أظهرت النتائج وجود تأثير معنوي لقوة الوسط في تقليل النمو، اذ أظهرت القوة الكاملة تفوقا بإعطائها أعلى نسبة بقاء وحيوية ومحتوى من N , P والكربوهيدرات والبروتين في الفروع والقابلية على التوليد (استعادة التضاعف والتجدير) المتمثلة في عدد الأفرع والجذور وإطوالها. كما ان الخزن المختبري لمدة 3 اشهر قد أعطى أفضل النتائج للصفات المدروسة وأبدت معاملة الخزن لمدة 3 اشهر في وسط كامل القوة تفوقا على المعاملات الاخرى في حين أعطت معاملة الزراعة في 1١4 قوه الوسط لمدة 9 أشهر اقل القيم للصفات المدروسة. ان الزروعات النسيجية للفراولة يمكن خزنها مختبريا بنجاح لمدة 9 أشهر دون تأثير حيويتها وقدرتها على التضاعف والتجدير.

الكلمات المفتاحية: نبات الفراولة، الخزن المختبري للزروعات النسيجية، تقليل املاح الوسط، الخزن بتقليل النمو خارج الجسم الحي.