Available online at http://bjas.bajas.edu.iq
https://doi.org/10.37077/25200860.2022.35.2.17
College of Agriculture, University of Basrah

Basrah Journal of Agricultural Sciences

ISSN 1814 – 5868 Basrah J. Agric. Sci. 35(2), 240-247, 2022 E-ISSN: 2520-0860

Impact of Different Sucrose Concentrations on Shoot Multiplication of Papaya (*Carica papaya* L.) Cultured *in vitro*

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Received 21st April 2022, Accepted 21st August 2022, ; Available online 22nd December 2022

Abstract: Papaya is a heterozygous plant commonly cultivated by seed but, unfortunately, they are not true to type. Moreover, the hybrid seed varieties like the Red Lady are very highly expensive. Hence, tissue culture techniques offer an alternative method to produce a million clones within a short period and a reasonable price. Thus, the current study aimed to optimize the shoot multiplication rate of papaya (*Carica papaya* L. cv. Red Lady) *in vitro*. Five concentrations of sucrose (10, 20, 30, 40, and 50 $g.L^{-1}$) were applied for the papaya shoot proliferation. Results demonstrated that the 30 $g.L^{-1}$ sucrose was significantly superior in the rate of shoot numbers (4.1 shoots. $explant^{-1}$), shoot length (0.90 cm), (2.7 leaves. $shoot^{-1}$), leaf area (1.40 cm^2) and fresh weight (0.192 g) in compared with other sucrose treatments. Whereas, the 40 $g.L^{-1}$ sucrose treatment was significantly superior in dry weight of shoot compared to the other treatments, which recorded 0.058 g. While the treatment of 10 $g.L^{-1}$ sucrose recorded the lowest values in shoot numbers, length, and dry weight. Current study conclude that the 30 $g.L^{-1}$ sucrose is the best concentration treatment that must be used in papaya micropropagation, where it gives the maximum rate of shoot numbers and other morphological traits.

Keywords: 6-benzylaminopurine, Explant, , *In vitro*, Shoot multiplication, Tissue culture.

Introduction

Papaya (*Carica papaya* L.) belongs to the Caricaceae family. It is considered one of the most important tropical and sub-tropical fruits that is aboriginal to southern Mexico and South America. Brazil, Nigeria, Indonesia, Malaysia and India are the most predominant productive locations for this fruit (Burns *et al*., 2022). Papaya is a very popular fruit because is rich in ascorbic acid and provitamin A (Patel *et al*., 2013). Papaya fruit also contains proteolytic enzymes, which are used in the medicine industries (Baeza *et al*., 1990). Papaya is traditionally propagated *via* seed in different commercial orchards (Fernando *et al*., 2001). But this mean of propagation is unfavorable because the produced offspring lack similarities with their mother plant (Tsai *et al*., 2009). Also, this method of propagation leads to the spread of diseases in papaya farms, especially viral diseases (Al-Shara *et al*., 2018). Papaya is dioeciously plant and rarely monoecism, where %50 of the progeny will be males and 50% will be females. Moreover, it is not possible to determine sex during the juvenile stage (Wu *et al*., 2012). All above reasons makes the seeds of desirable characteristics varieties of papaya very expensive for the

growers (Schmildt *et al*., 2015). However, this problem has been overcome by the technique of tissue culture using explants of female or hybrid papaya seedlings. (Ragavendran & Natarajan, 2017). Nowadays, papaya micropropagation has become crucial for the propagation of certain species and also for genetic applications (Lai *et al*., 2000). The growth and propagation of shoots *in vitro* relies on a various factors among which is the sugar kinds and concentrations in the culture medium (Haque *et al*., 2003). The cultures of cells, tissues, and organs are not fully autotrophic; consequently, the medium needs to incorporate sugars to help development and growth. (Yaseen *et al*., 2013). A few examinations have shown that sucrose is the most favored sugar utilized in culture media; since it is the most considered normal sugar in the phloem sap of plants (Fuentes *et al*., 2000; Ahmad *et al*., 2007). A few investigations have been centered on the results of sucrose added to tissue cultural media for shoot multiplication (Haque *et al*., 2003; Kabir *et al*., 2007; Kanth *et al*., 2017). Therefore, this study is planned to assess the impact of sucrose concentrations in further developing shoot multiplication of papaya grown *in vitro*.

Materials & Methods

The experiment was accomplished at the Plant Tissue Culture Laboratory of the Fadak Agricultural Company, Basrah, Iraq.

Surface sterilization of explants

The explants (axillary shoot) were excised with a sharp blade from 3-month-old hybrid papaya seedlings cv. Red Lady (Plate 1, A and B). Explants were sterilized with 0.01% f mercuric chloride ($HgCl_2$) containing with few drops of Tween 20 for ten minutes. After that, explants were rinsed multiple times with sterile distilled water.

Initiation stage

Initiation medium was prepared from Murashige and Skoog inorganic salts (MS) (Murashige & Skoog, 1962) and containing 30 $g.L^{-1}$ sucrose and supplemented with 0.5 $mg.L^{-1}$ benzyl adenine (BAP) and 0.5 $mg.L^{-1}$ α, naphthalene acetic acid (NAA), , and solidified with 8 $g.L^{-1}$ agar (Plate 1, C). The pH of the medium was adjusted to 5.8 prior autoclaving. Medium was autoclaved under pressure at15 lb $inch^{-2}$ and a temperature of 121°C for 20 minutes. Sterile axillary buds were inoculated to this medium and incubated at 25°C with 16 hours light: 8 hours dark. The illumination was provided by LED white light (30 Watt and 10000 Kelvin).

Impact of various sucrose concentrations on shoot multiplication

For optimization of papaya shoot proliferation *in vitro*, four concentrations of sucrose (10, 20, 30, 40, and 50 $g.L^{-1}$) in the MS culture medium were examined. The culture media mentioned above was supplemented with 0.5 $mg.L^{-1}$ BAP, 0.1 mg. L^{-1} NAA, 100mg. L^{-1} myo-inositol, and 8 $g.L^{-1}$.agar. . The shoot cultures were incubated in the same conditions mentioned previously. Data was recorded on (i) Shoot number, (ii) Shoot length (cm), (iii) Leaves number per shoot, (iv), Leaf area (cm^2), and (v) Shoot fresh and dry weight (g).

Statistical analysis

The experiments were set up according to a randomized complete block design (CRD) with three replications that had 10 cultures per treatment. Data analysis was performed by analysis of variance. Comparison between treatment means was performed using the Least Significant Difference (LSD) test at the 1% probability level (Snedecor & Cochran, 1989).

Results

Influence of different sucrose concentrations on shoot proliferation

The results reveal that the addition of different concentrations of sucrose to multiplication medium affected the total shoot numbers. However, the treatment of 30 $g.L^{-1}$ sucrose recorded significant superiority in shoot numbers (4.1shoots. $explant^{-1}$) in comparison with other treatments. Whereas, sucrose concentration higher and lower than 30$g.L^{-1}$ had an inhibitory effect. Where the lowest shoot numbers recorded (1.3 and 1.5) on medium containing 10 and 50 $g.L^{-1}$ of sucrose (Fig. 1) and Plate 1 (D, E and F). Nevertheless, cultures showed the following gradient in the total shoot numbers according to the sucrose concentrations: 30g>40g>20g>50g>10g.

Results also demonstrated (Fig. 2), that there are no significant differences between the means of sucrose treatments in terms of shoot length. Where, the treatment of 10 $g.L^{-1}$ sucrose recorded the lowest mean shoot length reach (0.67cm). While, the length of the shoot rising with increasing sucrose concentration, where the highest shoot length (0.90 cm) was recorded on medium containing 30 $g.L^{-1}$ sucrose. On the contrary, the length of the shoot decreased by increasing the concentration of sucrose to 40 and 50 $g.L^{-1}$, where the shoots length amounted 0.84 and 0.71 cm, respectively.

Similarly, the highest mean number of leaves per shoot was recorded in treatment 30$g.L^{-1}$ sucrose, which reached 2.7 leaves (Fig. 3). While a declined in leaf numbers was recorded in two treatments 10 and 50 $g.L^{-1}$ sucrose which amounted (1.7 and 1.8 leaves $shoot^{-1}$), respectively. Whereas, no significant differences in response were recorded in 20 and 40 $g.L^{-1}$ sucrose, where leaves number reached 2.0 and 2.4 leaves $shoot^{-1}$, respectively. Study also found that the treatment of 30 $g.L^{-1}$ sucrose stimulating the highest response to leaf area leaf area was amounted 1.40 cm^2. While the treatment of 50 $g.L^{-1}$ sucrose recorded the lowest mean leaf area, get to 0.58 cm^2. On the other hand, this treatment did not differ significantly from the treatment of 10 $g.L^{-1}$ sucrose, which recorded 0.67 cm^2 (Fig. 4).

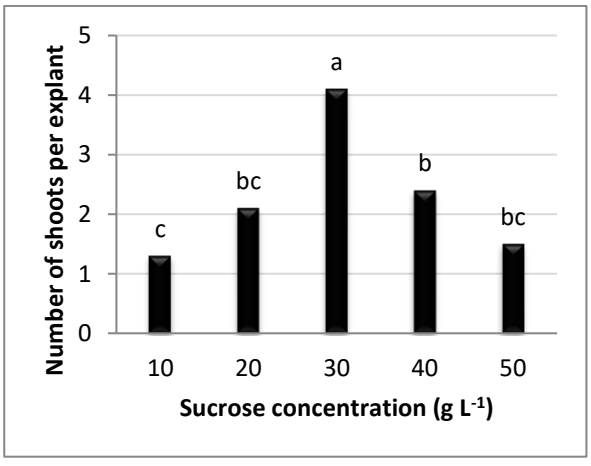

Fig. (1): Impact of different concentrations of sucrose on the number of shoots per explant.

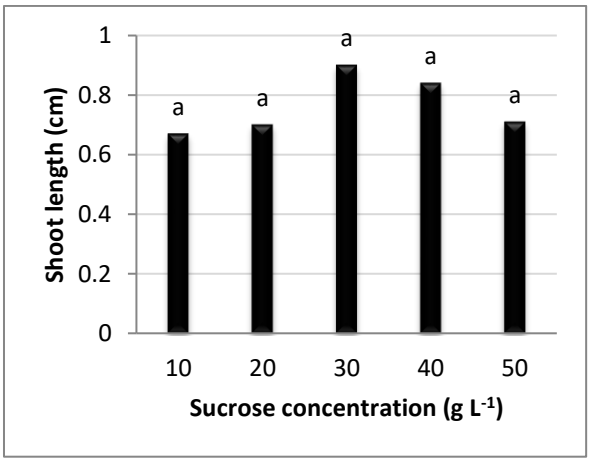

Fig. (2): Influence of various concentrations of sucrose on the shoot length (cm).

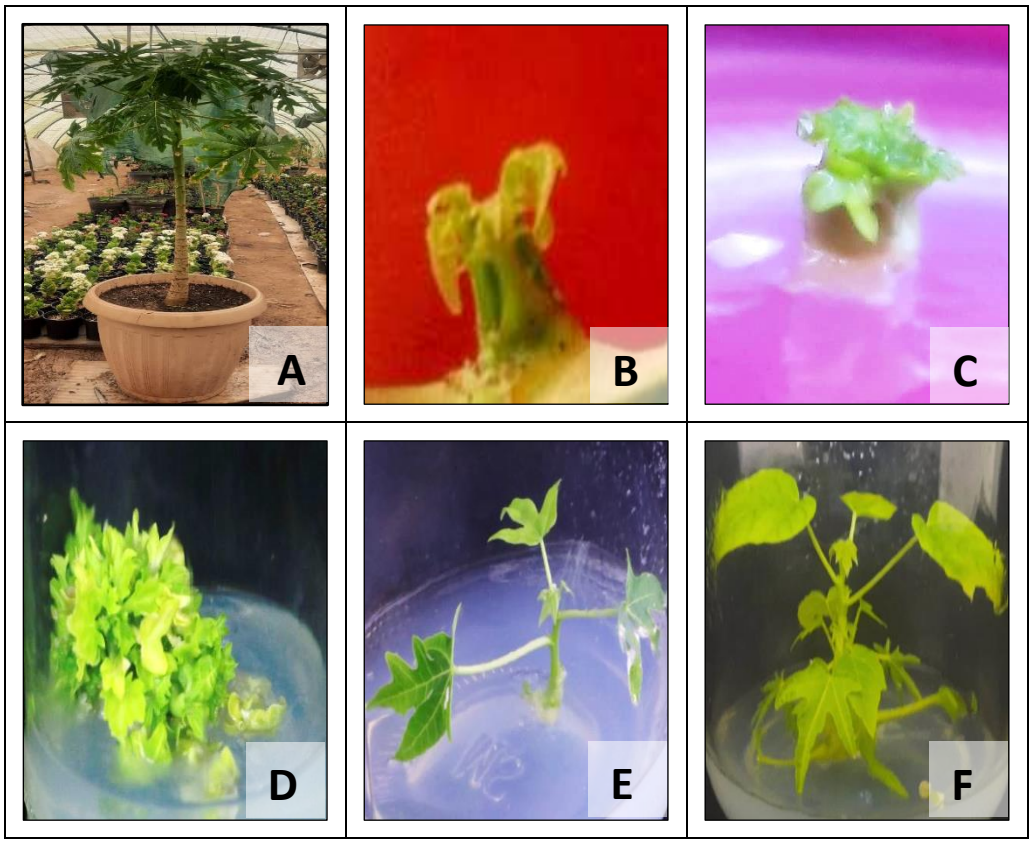

Plate 1: Shoot proliferation by using lateral buds of papaya (*Carica papaya* L.); A-Hybrid papaya seedling, B-Lateral bud (explant), C-Lateral bud was cultured on MS medium, D-Shoot multiplication, E and F-Separated shoots.

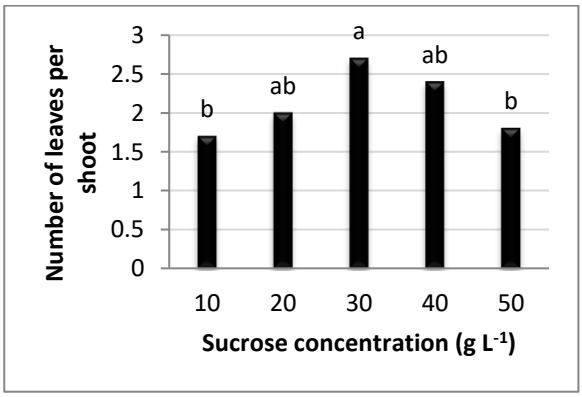

Fig. (3): Effect of different concentrations of sucrose on the number of leaves per shoot.

Similarly, present study revealed that the concentration of sucrose (30 $g.L^{-1}$) has a positive effect on mean of shoot's fresh weight, where it reached 0.192g. While the treatment of 40 $g.L^{-1}$ sucrose recorded the lowest mean fresh weight of the shoots, which amounted 0.136 g. On the contrary, results demonstrated that the shoot's dry weight increased with an increase in the concentration of sucrose added to the MS medium. Where, highest value of a shoot's dry weight (0.058 g) was noted on MS medium enriched with 40 $g.L^{-1}$ sucrose, while the treatment of 10 $g.L^{-1}$ sucrose recorded the lowest shoot's dry weight reached to 0.042 g (Fig. 5).

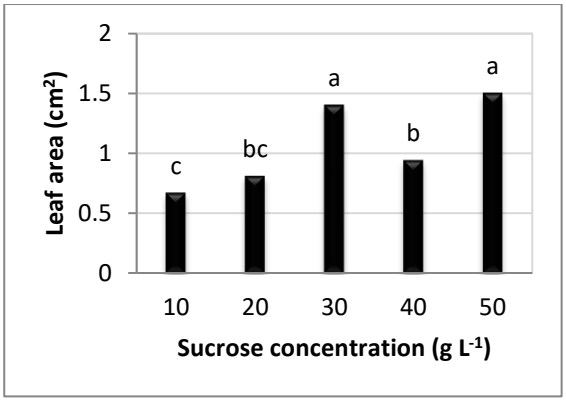

Fig. (4): Effect of different concentrations of sucrose on the leaf area (cm^2).

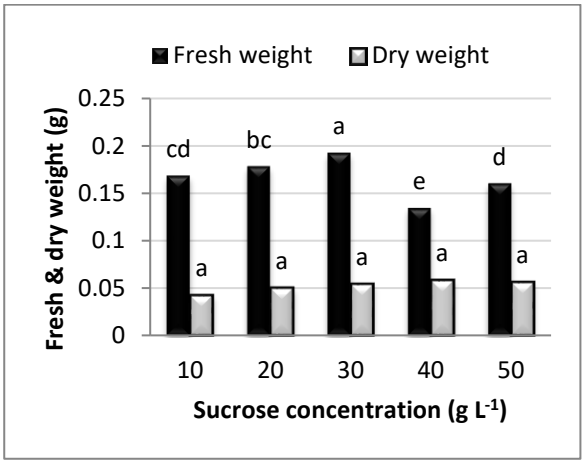

Fig. (5): Effect of different concentrations of sucrose on the fresh and dry weight (g).

Discussion

In general, Sucrose is the energy-rich organic compound necessary for the process of aerobic respiration to release the high energy needed for cell division and enlargement in plant tissues, which leads to their growth, development, and formation of shoots (Taiz *et al*., 2010). Therefore, tissue culture studies focus on getting the best sugar concentration in micropropagation systems of plants (Mendoza & Kaeppler, 2002). However, using a high concentration of sucrose in the plant tissue culture system may have a beneficial effect, where it increases the growth and development of micropropagated plants like potato. But on the contrary, sucrose concentration may have inhibitory effects on micropropagated plants caused by an excessive osmotic contribution or by the toxicity of the carbon source (Ślesak *et al*., 2004). The results of the current study stipulated that *in vitro* papaya shoot proliferation was affected by sugar concentrations in the multiplication medium. Generally, 30 $g.L^{-1}$ sucrose is the optimum level to stimulate healthier and more vigorous axillary buds in the formation and proliferation medium. According to Javed & Ikram, (2008) the optimal concentration of sucrose in a medium must meet the energy requirements for cell division and differentiation without any negative osmotic effect on shoot formation and multiplication. In agreement with the present study, the use of 30 $g.L^{-1}$ sucrose in micropropagation media has been vastly reported by Hazarika *et al*. (2000), Kabir *et al*. (2007) and Patel *et al*. (2013).

On the contrary, high sucrose concentration had an inhibitory effect on the number of shoots, shoot length, number of leaves per shoot, and the leaf area. This may be due to the decrease in the osmotic capacity of cells and their exposure to stress (Kadota *et al*., 2001; Jain & Babbar, 2003; Silva, 2004; Ahmad *et al*., 2007). Also, high concentrations of sucrose lead to an increase in the concentration of injurious phenols and inhibition of growth in plant tissues (Hilae & Te-chato, 2005; Yildiz *et al*., 2007; Yaseen *et al*., 2009).

Conclusion

The better explant used for papaya shoot proliferation is axillary buds. While the optimal sucrose concentration suitable for shoot multiplication system in papaya is 30g L^{-1}. Increasing the concentration of sucrose above the optimum level leads to a decrease in the number and length of papaya shoots, number of leaves per shoot, and leaf area.

Contributions of authors

E.E.A: Collect samples and data from a micropropagation experiment conducted in the laboratory.

M.A.I.: Preparing the research plan and contributing to the practical part of the experiment in the laboratory, writing the manuscript, evaluating it, and statistical analysis of the experiment data.

A.M.J: Contribute to preparing the plan for the manuscript and reviewing the results of the experiment.

Acknowledgments

We dedicate thanks and appreciation to the manager of the Tissue Culture Laboratory at Fadak Private Company, Basrah, Iraq.

Conflicts of interest

The authors declare that they have no conflict of interests.

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تأثير تراكيز مختلفة من السكروز في إخلاف أفرع البابايا *Carica papaya* L. بتقنية الزراعة خارج الجسم الحي

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الملخص: البابايا عبارة عن نبات متغاير الزيجوت تزرع عادة بالبذور، لكنها تكون مغايرة للشجرة الام. علاوة على ذلك، فإن أصناف البذور المهجنة مثل Red Lady تكون باهظة الثمن للغاية. وقد قدمت الزراعة النسيجية طريقة بديلة لإنتاج مليون نسخة خلال فترة قصيرة وبسعر معقول. وهدفت الدراسة الحالية لتحسين معدل تكاثر السيقان او البراعم في البابايا (*Carica papaya* L.) صنف Red Lady خارج الجسم الحي. تم تطبيق خمسة تراكيز من السكروز (10 و 20 و 30 و 40 و 50 غم. لتر$^{-1}$) لاخلاف براعم البابايا. أوضحت النتائج أن تركيز السكروز 30 غم. لتر$^{-1}$ سكروز كان متفوقًا بشكل ملحوظ في معدل أعداد الفروع (4.1 برعم. جزء نباتي $^{-1}$) ومعدل طول الساق (0.90 سم) وعدد الاوراق (2.7 ورقة. ساق$^{-1}$) و مساحة الورقة (1.40 سم^2) والوزن الطازج (0.192 غم) بالمقارنة مع معاملات السكروز الأخرى. في حين تفوقت معاملة السكروز بجرعة 40 غم لتر$^{-1}$ معنويا في الوزن الجاف للساق مقارنة بالمعاملات الأخرى، التي سجلت 0.058 غم. بينما سجلت معاملة 10 غم. لتر$^{-1}$ سكروز أقل القيم في عدد الفروع والطول والوزن الجاف. وخلصت الدراسة الحالية إلى أن تركيز السكروز 30غم. لتر$^{-1}$ هو افضل تركيز والذي يجب استخدامه في التكاثر الدقيق للبابايا، كونه اعطى أقصى معدل لأعداد الفروع والسمات المورفولوجية الأخرى.

الكلمات المفتاحية: 6-بنزيل أمينوبيورين، خارج الجسم الحي *In vitro*، جزء نباتي، تضاعف الأفرع، زراعة الأنسجة.