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Article

Effect of sucrose in strawberry micro-propagation using platform bioreactor under temporary immersion system

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ABSTRACT:
The study was carried out in the tissue culture laboratories at the College of Agriculture / University of Kufa during the period from April 2020 to October 2021 where the aim was to assess the efficiency of micropropagation of strawberries by adding different concentrations of sucrose (30, 40 and 50 $g.L^{-1}$) to the nutrient medium in combination with different cultivation systems: Semi-solid media system and temporary immersion system using the Plantform bioreactor. The results showed that both the highest concentration of sucrose and the culture system under the medium immersion, individually or combined, were the highest and significantly effective in increasing the rate of number of shoots (runners), leaf content of macro and minor elements, total soluble sugars, plant pigments and fresh weight of biomass. Generally, the highest values were recorded in the shoots grown in liquid media supplied with 50 $g.L^{-1}$ sucrose under temporary immersion in the PlantForm bioreactor compared with those grown in semi-solid media.
Keywords: Fragaria × ananassa, micro-propagation, plant nutrition, tissue culture, PlantForm bioreactor

INTRODUCTION

Strawberry (Fragaria ananassa Duch.)) is one of the most widely distributed small fruit plants worldwide. Strawberries are propagated by dividing one-year-old, free-viral plants into varieties that do not produce runners or produce few. Or by runners, the most common method of propagating strawberries at a commercial level [1]. In general, the tissue culture technique has been widely used in the propagation of plants by vegetative progeny and the field of plant breeding because of its importance in increasing production and obtaining the best quality fruits [2].

To avoid the continuous immersion of the planted parts in the liquid medium and to increase the ventilation around it, the liquid culture system was resorted to under the temporary immersion system (TIS). Several bioreactors have been used for this purpose, including the Plantform bioreactor employed successfully in the commercial propagation of many economically important plants [3].

The plant parts grown outside the living body need the nutrient medium to provide nutrients and a source of carbon[4]. Therefore, all studies agreed on adding sucrose to the nutrient medium as a carbon source, whether grown in a liquid or

solid medium[5]. In recent years, some researchers have resorted to increasing the concentrations of sucrose used in the culture media to increase the energy supplied to the plant part and thus increase the growth and doubling of plantations for several plants, most of which were under the temporary immersion regime[6,7,8,9,10].

By reviewing the available sources, it was found that there were few studies that included studying the effect of cultivation systems under temporary immersion on the propagation of strawberries. Therefore, the research was conducted to test the efficiency of the temporary immersion system using a Platform bioreactor in the propagation of strawberry cultivar Rubygem by interfering with different concentrations of sucrose in the nutrient medium and evaluating the effect of this method on the number and quality of the produced branches in the multiplication stage

MATERIALS AND METHODS

The study was carried out in the Department of Horticulture and Landscaping - College of Agriculture, University of Kufa's tissue culture laboratories from April 2020 to October 2021. The runners were taken from the strawberry plants' cv. Rubygem and the leaves were removed, the tops of the runners with a length of 1 cm were separated and sterilized, and the growing top was excised. The experiment included using the cultivation system (cultivation in semi-solid medium or culture in liquid medium with the temporary immersion system TIS) and adding sucrose to the medium at 30, 40 and 50 gm.L^{-1} concentrations. In order to determine their effect on shoots multiplication of strawberry, ruby gem. The prepared nutritional media (MS) of 4.43 g.L^{-1} supplemented with inositol Myo100 mg.L^{-1} with the addition of 0.75 mg.L^{-1} BAP (with a steady supply of 0.5 mg.L-1 IBA and 10 mg.L^{-1} silver nitrate), was used in plantform bioreactor temporary immersion system containers. As for the semi-solid medium, 7g.L^{-1} of the inert substance agar was added to it for the purpose of hardening the food medium. Branches were cultured 6 branches per bioreactor container containing 300 ml of nutrient medium. A branch was cultured in each glass vial containing 30 ml of semi-solid nutrient medium with 10 repetitions (each container corresponds to 10 semi-solid media culture bottles), and each treatment was repeated 3 times. The cultures were incubated in the growing room at 23-25°C with light for 16:8 hours light: dark, for 6 weeks. After the incubation period, data on the traits under study were taken.

Measurements and data analysis

The experiment measurements included the number of shoots (plant part branch^{-1}), shoot length (cm), the weight of the fresh mass (measured by the weight of the fresh mass of the plants in the container for each treatment and the rate was calculated based on the shoots multiplication rate for each plant part). The leaves content of chlorophyll and total carotene (mg.100gm-1 fresh weight) was also calculated using a UV-visible Spectrophotometer. And leaf content of total soluble sugars (mg.100gm-1 fresh weight) by phenol-sulfuric acid method Modification of Phenol Sulfuric Acid Colorimetric Method (PSACM) according to Dubois et al. (1956)[11] by UV-Visible Spe- ctrophotometer at a wavelength of 490 nm. The data were analyzed using the statistical analysis system (Genstat12th VSN International GenStat 12.1 (2009)[12] with a Windows computer operating system. An Analysis of Variance (ANOVA) was conducted, and means were compared using the Least Significant Difference (LSD) test at a probability level of 0.05

RESULTS

The results (Table) showed that the highest values for the number of shoots, shoot length and fresh biomass weight were higher with the increased sucrose

concentration. The effect of the temporary immersion medium was also significant with respect to the characteristics, number of shoots, fresh biomass weight (Table 1), leaf content of chlorophyll, carotene and total soluble solids (Table 2). While the solid medium had the highest effect in the case of branch length. The interaction treatments differed significantly according to the concentration of sucrose and the different mediums used.

Treatments	No. of shoots				Shoot's length (Cm)				Fresh biomass weight(g)			
	Sucrose conc. ($g.L^{-1}$)			Average	Sucrose conc. ($g.L^{-1}$)			Average	Sucrose conc. ($g.L^{-1}$)			Average
Culture system	30	**40**	**50**		30	**40**	**50**		30	**40**	**50**	
semi-solid medium	9.50	9.15	12.55	**10.40**	1.67	1.84	2.62	**2.04**	10.87	13.14	17.22	**13.743**
liquid medium under temporary immersion	10.45	13.75	16.00	**13.40**	1.38	1.78	2.24	**1.80**	13.62	15.30	25.06	**17.993**
Average	**9.97**	**11.45**	**14.27**		**1.525**	**1.81**	**2.43**		**12.24**	**14.22**	**21.14**	
L.S.D. ($P \leq 0.05$)	Culture	S. conc.	Inter.		Culture	S. conc.	Inter.		Culture	S. conc.	Inter.	
	1.21	2.11	2.98		0.081	0.125	0.278		1.24	1.96	2.78	

Table1. Effect of cultivation system and sucrose concentration on vegetative characteristics of micro-propagated strawberry after 8 weeks of cultivation in nutrient media (when 10 mg/L AgNO3 + 0.1 mg/L IBA fixed)

Treatments	Leaf content of total chlorophyll ($mg.g^{-1}$ FW)				Leaf content of carotene ($mg.g^{-1}$ FW)				Leaf content of total soluble sugars ($mg.g^{-1}$ DW)			
	Sucrose conc. ($g.L^{-1}$)			Average	Sucrose conc. ($g.L^{-1}$)			Average	Sucrose conc. ($g.L^{-1}$)			Average
Culture system	30	**40**	**50**		30	**40**	**50**		30	**40**	**50**	
Semi-solid medium	42.6	63.1	81.8	**62.50**	10.30	12.0	12.30	**11.53**	0.396	0.410	1.276	**0.694**
Liquid medium under temporary immersion	53.1	76.3	89.9	**73.10**	10.10	15.50	17.40	**14.33**	0.468	1.808	2.203	**1.493**
Average	**47.85**	**69.70**	**85.85**		**10.20**	**13.75**	**15.35**		**0.432**	**1.109**	**1.740**	
L.S.D. ($P \leq 0.05$)	Culture	S. conc.	Inter.		Culture	S. conc.	Inter.		Culture	S. conc.	Inter.	
	6.24	9.96	16.78		1.26	2.03	3.18		0.74	0.87	1.67	

Table2. Effect of cultivation system and sucrose concentration on leaf nutrient content of micro-propagated strawberry after 8 weeks of cultivation in nutrient media (when 10 mg/L AgNO3 + 0.1 mg/L IBA fixed)

DISCUSSION

The findings showed significant differences between sucrose concentrations in all the traits under study, as all the values recorded were at their highest levels in the treatment of 50 g.l-1 sucrose, with significant differences from other sucrose concentrations. In general, the interaction of cultivation treatment under temporary immersion and 50 g.l-1 sucrose was superior in recording the highest rate for all studied traits except for the shoot length, which recorded the highest rate at the same sucrose concentration on the solid medium, which did not differ significantly from the treatment of temporary immersion medium.

The results indicate the effect of the cultivation system and sucrose concentrations in the superiority of the vegetative characteristics of the plants grown under the temporary immersion system over those grown under the liquid culture system. Air periodically and get rid of the accumulated gases. The absorption of nutrients and hormones over the entire surface of the plant part ensures maximum growth. This led to an increase in the efficiency of the activity of the planted part in drawing water and nutrients, and then the growth and development of plants, which was reflected in an increase in the live mass and dry mass due to the increase in the number of shoots[13]. These results agreed with former studies in Rubus[14], Chrysanthemum[15], olives[16], in date palm[17], and potatoes[18,19], from observing an increase in branch number and lived mass in the Plantform system compared to semi-solid media.

The nutrient medium is one of the most important factors affecting the growth of plant parts grown outside the living body. The medium provides the nutrients necessary for the growth and development of the transplanted part since the transplanted cells and tissues live in a state of throwing completely dependent on what is provided by the nutrient medium[5]. Therefore, serious attention to the components and physical state of the medium is important and must be determined precisely to calculate the requirements of the implanted portion of each element of the medium very carefully [4].

It was also noted that sucrose in the food media significantly affected the characteristics of the number of branches and the fresh weight of the live mass. This may be due to the importance of adding sucrose to the nutrient medium for direct branch growth and doubling, as well as the increase in the number of branches formed by increasing the concentration added to reach the optimum concentration (50 $g.L^{-1}$), which is higher than the standard sucrose concentration for MS medium (30 g). It is known that adding sucrose to the nutrient medium for plant tissue culture aims to provide the cultivated plant part with the energy necessary for growth and development, leading to the inhibition of chlorophyll formation[20]. Plant parts grown ex vivo lose their ability to photosynthesize to a large extent. They cannot build the sugar glucose, the carbon source prepared for energy. The sucrose added to the food medium partially decomposes into its components (glucose, fructose and sometimes levulose) upon sterilization (Autoclave). Then, it is completely degraded in tissues and plant cells by the enzyme invertase found in the cell wall or through the reverse reaction of the sucrose synthase enzyme in the cytoplasm. The cells absorb glucose first from the nutrient medium and then fructose[21].

Thorpe (1978)[22] indicated that glucose metabolic processes increase during the formation of primary meristems. And that sucrose is the carbon source of energy

during the processes of morphogenesis in cells grown outside the vivo. Increasing the sucrose concentration in the nutrient media over the standard limit (30 $g.L^{-1}$) for MS medium increased the rate of cross-branch formation of the cultured tissues. This is due to an increase in the energy supply rates of the cultured cells and tissues, or indirectly, as the increase in sucrose concentrations in the nutrient medium increases the activity of nitrate ions $NO3^-$ and ammonium $NH4^+$. As well as increasing the effectiveness of cytokinin in stimulating cell division[23]. These results agree with what Abdullah et al. (2013)[24] and Mohaseb et al. (2014)[25] found that the addition of sucrose in the food media at a concentration higher than 30 g/L had a significant effect in stimulating the formation of transverse shoots on strawberry plants in semi-solid media and Salix viminalis and Castanea [10,26] cultured in a similar environment.

As for the increase in plant pigments (total chlorophyll and carotenoids), it may be because the liquid media with the temporary immersion system allow gas exchange and ventilation, as well as an increase in the availability of nutrients and sucrose, which is used by the tissue to build sugars. Cultivation with temporary immersion systems to prevent nutrient deposition and allow optimal growth of plant tissues. The content of the liquid medium is of the major and minor elements in a more ready way, and its entry into the plant tissues is responsible for the accumulation of carbohydrates and the continuation of the growth and formation of the branches. The availability of carbohydrates in the form of sucrose in the culture medium can cause an increase in the internal levels of sugars in the plant tissue[27]. The increase also occurred due to the role of the contents of the medium of nutrients (nitrogen, iron, zinc and manganese in particular) in the plant and its impact on many vital activities inside the plant. This is reflected in the amount of manufactured compounds in the leaves, including vegetable pigments and carbohydrates. Iron is important in building chlorophyll and forming many compounds, such as cytochrome and ferredoxin, that are important in photosynthesis and activate some enzymes in the shoots[28].

CONCLUSION

The results showed that the liquid media under the temporary immersion system using Plantform Bioreactor was the best for the growth of strawberry plantlets. The medium led to a clear increase in runners' plantlets and their contents of carbohydrates and some vegetative pigments. The sucrose required for culturing in liquid media under the Plantform Bioreactor temporary immersion system was higher than the concentration required for culturing in semi-solid media. It appears that the physical condition of the nutrient medium had a significant impact on the efficiency of utilizing nutrients and growth regulators by the cultured tissues, as it was shown that the liquid medium under the temporary immersion system was superior to the semi-solid medium.

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