

RESEARCH ARTICLE

Effect of Exchange of Gases for Tissue Culture Vessels to Produce the Meristem of *Solanum tuberosum* in vitro

Malek Hubaish Walli¹, Haider Mahmood Jasim² and Faiq Hassan Ali³

¹AL- Muthanna University, College of Nursing/Iraq.

^{2,3}AL- Muthanna University, College of Education for Pure Sciences/Iraq.

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ABSTRACT

The microenvironment in plant tissue culture vessels has a significant effect on the growth and development of plantlets in vitro. The studies have indicated the gas exchange between outside and inside air can effect on microenvironment of culture vessels, therefore, the experiment was conducted to determine the effect of gas exchange on the production of the fabric tissue to micropropagation of *Solanum tuberosum* and the possibility of storing the fabric to a long time without drought to the culture. In the experiment, two factors were used. The first factor is the volume of gas exchange of the diameter of the filter (1 cm, 1.5 cm, 2 cm, 2.5 cm), and the second factor used four varieties of *Solanum tuberosum* (eastma, red scarlet, arora, violet).

Keywords: Gas exchange, Micropropagation and *Solanum tuberosum*, Tissue culture.

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INTRODUCTION

It was thought that the growth of plants in vitro depends on the composition of nutrients in media. Therefore efforts are made to improve the composition of the growing medium.¹ The studies and research have shown that the aerial environment in the tissue culture vessel varies significantly from the surrounding external environment in the laboratory. The process of aeration of a tissue culture vessel can be affected by different environmental factors such as light and temperature, relative humidity, etc.² Therefore, the main objective of the experiment is to find a filter design that is more efficient to achieve optimal ventilation than is currently used, screw caps, aluminium foil, transparent films such as standard plastic cover, polypropylene, etc.^{1,3}

Carbon dioxide gas in a tissue culture vessels

Carbon dioxide concentration is low during the photoperiod and is high during the dark period in a tissue culture vessel containing chlorophyll tissues. A CO₂ concentration depends on the aeration efficiency of the vessels and the photosynthetic efficiency of the tissues. In contrast, the concentrations of ethylene and water vapor (relative humidity) are high in the vessel than those of the surrounding outer environment.²

Oxygen gas in a tissue culture vessels

The nature of cell differentiation in culture may be regulated by the available oxygen concentration. Dalton and Street found chloroplast development in suspension cultures to be favored

by low partial pressures of oxygen and high CO₂ concentrations (5–10%). Ethylene, naturally produced by the cultured cells.⁴

Ethylene gas in a tissue culture vessels

In 1934, R.Gone announced the presence of ethylene gas within plant tissues during the growth stages of high-end plants. Both Park and Burke have announced that the amount of ethylene is high in meristem. The concentration of ethylene and water vapor (relative humidity) is higher in agricultural vessels compared to the surrounding external environment concentrations during the dark or light period. Ethylene concentrations remain high in the agricultural vessels regardless of the type of tissue used in the vessels. The adverse effect of the high concentration of ethylene gas in tissue culture vessels causes an increase in cell growth horizontally in plant stem and morphological abnormalities of leaves.⁵

MATERIALS AND METHODS

In the experiment, the complete randomized design was used as a two-factor in an experiment with three replicates per treatment. The first factor in the experiment was four potato varieties (estima, violet, aurora, red scarlett), The second factor used filter with 0.5 pour diameter¹⁷ for four diameters of gas exchange p (1 cm, 1.5 cm, 2 cm, 2.5 cm), plant parts such as capillary and axillary buds of plants produced from secondary agriculture were then planted in 250 mL flasks⁶ after preparation of the nutrition medium⁷ in the laboratory after adding 0.2 mL of Kinetin to the medium⁸ in each flask was

put 100 mL of nutrition medium and closed the flasks using a cotton filter with a disc shape and a tape of polyethylene. Flasks were weighed to install the first measurement to see the rate of water loss from the media, then The vessels were placed in the growth chamber at a constant temperature of 25–27 °C through a temperature scale and a relative humidity of 45% by used a humidity meter placed in the growth chamber. The weights of the flasks were read weekly using a sensitive balance; the height of the plant was measured in each experimental unit using the ruler and the number of leaves on the stalk. Plants of each experimental unit were dried in an oven to extract the wet weight and dry weight for each treatment. The ethylene concentrations in the culture vessels were determined by removing 500 L samples of gas from the culture vessels and analyzing by means of gas chromatography (PYE Unicam, UK). To measure the relative humidity, the humidity probe (Vaisala HMP 31 UT, Finland) was inserted into the headspace of the culture vessels. Growth (fresh and dry mass) of shoots and roots were measured on Day 31. The data were collected and statistically analyzed using the MESTAT-C program.

RESULTS AND DISCUSSION:

Effect of the gas exchange volume of the filter on the studied characters:

The results of the statistical analysis of the data of the experiment and the characteristics studied and showed in Table 1 there is a correlation between the volume of gaseous exchange and the amount of water loss from the culture media per week, also found that the high more gas exchange volume that due to high loss of the water amount and this found in the level (2.5 cm) and it is gave (9.64 G/week) and showed more significant on the levels (1, 1.5, 2 cm), which is identical to what many researchers have said in their research and publications

that the moisture within the vessels has a significant role in the speed of the dry of the middle and the destruction of cultivated plants.^{9,10}

The level (2.5 cm = 10.65 cm, 2 cm = 10.9 cm) showed no significant between them in the height of the plant, but both levels (2.5 cm, 2 cm) showed more significant with the two levels (1.5 cm), while the number of leaves showed the level (1.5 cm = 103.92 leaf/plant) to be more significant at the levels (1 cm, 2.5 cm) but both levels (1.5 cm, 2 cm) did not show any significant differences at the level of 0.01, the proportion of moisture in vessels containing growing buds also depends on the growth and growth of the left area because the leaves absorb the radiation and usually show temperatures higher than the temperature of the ambient air thus increasing the transpiration.⁴ The emitted temperature has an effect on the increase in the production of ethanol in plant tissues.¹ The researchers also stated that ethyl gas has a dampening effect on the growth of the buds of the dicot plants, and that high concentration causes morphological abnormalities of the plant in the stem and leaves.^{6,7} As for the soft weight of the plant of water and fibre where the weight was higher at (2 cm) (27.06 g) and better than the two levels (1 cm, 1.5 cm) but with a level (2.5 cm) there was no significant difference at the level of 0.01, the weight of the plant after drying which represents the weight of fibre, was The level (2.5 cm) is more weight where it reached (1.5 g/plant) and there was no significant difference with the levels (2 cm, 1.5 cm) but the level (1 cm) was the least weight where (1.26 g/plant), through those results that the physical properties of vessels and lids or closures had an effect on the growth of plants⁸ it is certain that the light inside of the vessels, moisture and ethyl gas role in the length of the baskets and the number of leaves and the weight of the material dry addition, the number of plants in the vessel and the shape of the vessel also has an effect.

Table 1: Showed loss of water per week (gm)

		1 cm	1.5 cm	2 cm	2.5 cm
Loss.w.weekly(gm)	Estima	5.53	7.83	8.87	9.57
	Violet	5.33	7.93	9.17	10.03
	Aurora	4.5	7.33	9.27	9.5
	Red Scarlett	6.87	7.83	9.33	9.7

Table 2: Showed (A) plant weight.b.drying. (B) plant weight.a.drying (C) height of plants

		1 cm	1.5 cm	2 cm	2.5 cm
A)Plant.w.b.drying(gm)	Estima	17.1	23.93	24.67	24.97
	Violet	16.6	25.23	27.53	28.6
	Aurora	11.74	18.87	26	28.43
	Red Scarlett	21.03	27.93	28.33	28.33
LSD 0.01	Diater filter x Varieties = 3.231				
B)Plant.w.a.drying(gm)	Estima	1.27	1.47	1.47	1.5
	Violet	1.07	1.43	1.5	1.5
	Aurora	1.43	1.5	1.5	1.5
	Red Scarlett	1.27	1.5	1.5	1.5
LSD 0.01	Diater filter x Varieties = 0.1414				
C)Height of plants (cm)	Estima	8.22	10.37	9.9	9.87
	Violet	9.5	10.8	11	11
	Aurora	5.03	7.53	11.37	10.4
	Red Scarlett	10.1	11.17	11.33	11.33

Table 3: Showed (A) Filter effect on studied qualities. (B) Effects of spices on qualities studied

A	1 cm	1.5 cm	2 cm	2.5 cm	LSD.01
Loss.w.weekly(gm)	5.56	7.73	9.22	9.64	0.3446
Plant.w.b.drying(gm)	16.62	24.25	27.06	26.9	1.615
Plant.w.a.drying(gm)	1.26	1.48	1.49	1.5	0.0707
Height of plants (cm)	8.21	9.97	10.9	10.65	0.5268
Number of leaves	63	103.92	97.25	94.75	7.036
B	Estima	Violet	Aurora	Red Scarlett	LSD .01
Loss.w.weekly(gm)	7.95	8.12	7.65	8.43	0.3446
Plant.w.b.drying(gm)	22.67	24.49	21.26	26.41	1.615
Plant.w.a.drying(gm)	1.43	1.38	1.48	1.44	0.0707
Height of plants (cm)	9.59	10.58	8.58	10.98	0.5268
Number of leaves	90.42	91.67	84.42	92.42	7.036

The genetic qualities of the varieties played a significant role in influencing the studied qualities, the Table 3 showed the superiority of the item Scarlett response to the item (estmaa, violet, aroura) for the studied qualities (amount of water loss, soft weight, plant height, number of leaves) and due to several factors including genetic factors Morphological and physiological, genetic maps of tubers and wild species have revealed that at least eleven genes are responsible for the role of genes in morphological and environmental conditions.^{11,12} Also, the stage of plant growth has a role in photosynthesis and increasing the rate of leaching and gaseous exchange, where¹³ The photosynthesis and photometric representation in the third paper compared to the fifth paper was higher efficiency. The statistical analysis and the average table and the overlap between the volume of gaseous exchange and the genetic qualities of the species at the level significant differences at $p \leq 0.01$. it was found that the volume of gaseous exchange (2.5 cm) is the most lost water in the experiment and in the class (violet) 10.03 g/week. The volume of gaseous exchange and the genetic characteristics of the varieties has a significant effect on the production of the meristem where the size (1.5 cm) and for the species (red Scarlett) more significant about (108 leaf/plant), that the amount of water in the middle and the concentration of gases resulting from metabolism as ethylene affect the development and growth of the plant. While for the effect of overlap between factors high the plant, the size (2 cm) and the species (Arora) were both the most lengthy of the rest of the varieties, but the integrity distortions in the level (1 cm) and the increase in the length of the coefficients from the normal limit of the treatment (2.5 cm) appeared, the results of the statistical analysis of the weight of dry plants and to know the amount of fibers and dry substances in the plant has emerged that there are no more differences significant between the levels (1.5, 2, 2.5 cm) but the level (1 cm) gave clear more differences significant between the varieties and this shows that the effect of the volume of gaseous exchange on the weight of the dry material is significant differences. While for the soft weight of the plant, the overlap between the varieties and the volume of the gas exchange was clear where the level (2.5 cm) and the violet species were more weight (28.6 g/plant).

CONCLUSION

Volume (1.5–2) cm for gaseous exchange in the culture vessels to keep the plants stored and cultivated for as long as possible, reached more than three months and prevented the drying of the culture medium during that period at a temperature (25–27 m) and relative humidity within the laboratory (45%). The best volume of gaseous exchange for the production of the meristematic tissue of the potato plant is (1.5 cm) gave the largest number of meristematic tissue in the apical and intermediate buds during the three-month trial period. Researchers in this field may expand the study and search for ideal filters (caps) that can control the amount of gases inside and out of the vessels not only in the field of agricultural production but in the field of microbiology, plant breeding and algae in the glass vessels.

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