J. Agric. Sci. Mansoura Univ., 32 (8): 6735 - 6743, 2007

'pEFFECT OF MEDIUM COMPOSITION ON THE HYPERHYDRICITY OF GLOBE ARTICHOKE *IN VITRO*

El-Shabasi, M.S.S.
Hort. Res. Inst., Agric. Res. Centre, Giza, Egypt.

ABSTRACT

Hyperhydricity or vitrescence was a frequent problem during the micropropagation of globe artichoke and it was influenced by medium composition, and type and concentration of cytokinins used in culture medium. Using a multiplication medium containing half-strength of NH_4NO_3 AND KNO_3 and doubly weights of calcium chloride and magnesium sulphate specified for Murashige and Skoog (MS) medium recorded the lowest value of hyperhydric cultures percentage. In addition, this medium caused significant increases in leaves dry matter % as well as chlorophyll (a) and (b) content. Supplementation of MS medium with 2.0 mg/l kinetin + 1.0 mg/l IAA was the optimum for shoot proliferation with relatively low hyperhydricity.

INTRODUCTION

Hyperhydricity or vitrification is a physiological malformation affecting tissue culture generated plants. This malformation is associated with excessive hydration and poor lignifications and results in poor regeneration of plants (Ueno *et al*., 1998). Hyperhydricity is a disorder of tissue cultured plants where leaves become translucent and stems swollen, distorted and brittle. These plants cannot survive for transplanting to greenhouse conditions. Hyperhydricity occurs as a result of leaf lacunae containing water rather than air (Schloupf *et al*., 1995). Paques (1991) mentioned that under some cultural conditions, highly abnormal tissues and organs may be detected after a period of culture. Shoots or plantlets become difficult to propagate *in vitro*, their capacity for survival may be severely reduced and many may perish in the culture vessel, or upon transfer to the external environment. Shetty *et al*. (1996a) stated that the prevention of hyperhydricity was characterized by high chlorophyll and reduced water content in oregano shoots. Onisei *et al*. (1988) mentioned that globe artichoke plantlets were difficult to establish in soil due to leaf vitrification and poor rooting.

In this respect, glassiness, translucency, vitrescence, succulence and waterlogging are also terms used when describing this physiological disorder (George, 1993).

Medium composition is considered one of the most important factors which influence hyperhydricity. The hyperhydric condition often occurs in some species on full strength MS salts, but not on media with less concentrated macrountrients. Shoot of *Salix babylonica* became hyperhydric on MS salts, while those on Knop's macronutrients were always normal in appearance (Beauchesne, 1981). Furthermore, Ziv and Ariel (1992) added that the best growth of *Dianthus caryophyllus* shoots occurred on MS salts when the levels of nitrogenous compounds were adjusted to provide 40 mM NO^-_3 but only 6 mM HN^+_4. Also, Rodriguez *et al*. (1996) reported that shoot proliferation of *Agave victoria - reginae* was obtained, and hyperhydricity was eliminated on a modified MS medium (with NH_4NO_3 reduced to 5 mM)).

Chauvin and Salesses (1988) stated that hyperhydricity of chestnut can be prevented by culture on a modified MS medium containing half strength of nitrates. Moreover, Rodriguez *et al*. (1991) used only half MS level of nitrates in combination with double weights of calcium chloride and magnesium sulphate for reducing the frequency of hyperhydric shoots in *Pyrus communis*.

In shoot cultures, the induction of hyperhydricity can be influenced by the type and concentration of the growth regulators used. In many species, it is especially likely to be induced by high levels of cytokinin, while lower rates induce the proliferation of normal shoots (George, 1993). The cytokinin BA has been especially noted to induce hyperhydricity when used at high concentration (Hosoki and Tahara, 1993). Symptoms of hyperhydricity can sometimes be prevented by culture on, or transfer to, a medium containing a cytokinin other than BA *e.g.,* kinetin (Dencso, 1987; Llorente *et al*., 1996). The induction of hyperhydricity is not confined to BA alone, and high rates of other cytokinins may also induce the symptoms. More than 3 mg/l 2iP caused severe vitrescence of papaya shoots (Kataoka and Inoue, 1992).

The objective of this investigation was to study different factors which influence hyperhydricity in order to enhance the *in vitro* derived shoots of globe artichoke with less hyperhydricity.

MATERIALS AND METHODS

This work was carried out during 2006 in the tissue culture laboratory, Horticulture Research Institute (HRI), Agriculture Research Centre, Ministry of Agriculture and Land Reclamation, Giza, Egypt.

Plant material:

Globe artickoke plants (*Cynara scolymus* L, cv. Herious) grown in Kaha Vegetable Research Farm, HRI, Kalubia Governorate were used as plant material. Offshoots were excised from selected and vigorously growing plants in March according to Bekheet (1992). The basal parts of these offshoots were removed to a length till 5 cm, the outer leaves were also removed, and the inner leaves were shortened. Shoot tips were surrounded by 2-3 pairs of leaves and shortened to 2-3 cm in length. Shoot tip explants were washed under running tap water for 1hr. Explants were than superficial disinfected by 70 % ethanol for 5 sec. followed by immersion in 30% (v/v) commercial bleach containing 5.25% sodium hypochlorite plus Tween 20 (2 drops/100 ml) for 20 min with agitation. Explants were then rinsed three times using sterile distilled water and trimmed to 3-4 mm in length before inoculation on the nutrient media.

This study included two experiments:

Experiment (1)

This experiment aimed to investigate the effects of nitrogenous compounds and other macronutrients in multiplication medium on the reduction of hyperhydric percentage of the cultures. In this experiment the microelements of Murashige and Skoog (1962) as well as five compositions of macronutrients were subjected as follows:

1. Macronutrients of MS medium (MS1) used as control.

2. Macronutrients of MS, but the levels of nitrogenous compounds were adjusted to provide 40 mM NO^-_3 (3437.67 mg/l KNO_3) and only 6 mM NH^+_4 (480.28 mg/l NH_4NO_3) (MS2).
3. Macronutrients of MS, but ammonium nitrate and potassium nitrate used at half-strenght ($^1/_2$ x NH_4NO_3 & KNO_3) (MS3).
4. Macronutrients of MS3, while the weights of calcium chloride and magnesium sulphate were doubled (MS4).
5. Macrountrients of Knop's, 1865 (MS5).

The weights of macronutrients and the concentration of ions NO^-_3, NH^+_4 and total N used in these five compositions are listed in Table (1).

Table (1): The five compositions of macronutrients and the ions concentrations added to micronutrients and vitamins of MS medium.

Macronutrients	mg/l				
	MS1	**MS2**	**MS3**	**MS4**	**MS5**
KNO_3	1900	3437.67	950	950	250
NH_4NO_3	1650	480.28	825	825	-
$CaCl_2.2H_2O$	440	440	440	880	-
$MgSO_4.7H_2O$	370	370	370	740	250
KH_2PO_4	170	170	170	170	250
$Ca(NO_3)_2.4H_2O$	-	-	-	-	1000
Ions			**mM/l**		
NO^-_3	39.40	40	19.70	19.70	10.94
NH^+_4	20.61	6	10.305	10.305	-
Total N	60.01	46	30.005	30.005	10.94

All culture media used were supplemented with 30 g/l sucrose, 7 g/l agar, 50 mg/l $NaH_2PO_4.2H_2O$, 20 mg/l adenine hemisulfate, 3mg/l kinetin and 0.3 mg/l IAA. Prepared media (50 ml) were dispensed into 400 ml glass jars, and autoclaved at 121°C for 20 min. Shoot tip explants were individually placed in culture jars containing the various multiplication media. All cultures were incubated at 25±2°C and 1000 Lux (16 hr/day) provided by white fluorescent lamps. Each treatment consisted of at least 30 culture jars. Data were recorded after 6 weeks of culturing as percentage of cultures which gave hyperhydric (vitrified) shoots, number of shoots / explant, average shoot weight (g), leaves dry matter (%) and photosynthetic pigments (mg/100g fresh weight of leaves) which were determined according to A.O.A.C. (1990).

Experiment (2)

This experiment aimed to investigate the effect of type and concentration of cytokinin added to multiplication medium on reducing the hyperhydric percentage of cultures.

Two cytokinins were used in this experiment *i.e*., kinetin at 1 and 2 mg/l as well as dimethylallylamino purine (2iP) at 1 and 2 mg/l. The solid MS medium supplemented with 50 mg/l $NaH_2PO_4.2H_2O$, 20 mg/l adenine hemisulfate and 1 mg/l IAA was used in this part of study. Normal shoots (non-hyperhydric shoots) obtained in the previous experiment were taken and individually subcultured in 400 ml culture jars containing 50 ml of the various four media. Each treatment consisted of at least 30 culture jars. Data were

recorded after 6 weeks of culturing as percentage of cultures which gave hyperhydric shoots, fresh weight of cultures, number of shoots / explant and average shoot weight (g).

Treatments of the two experiments were arranged in a complete randomize design. The obtained data were subjected to the analysis of variance according to Snedecor and Cochran (1980). The differences between various treatment means were tested by least significant difference (L.S.D.).

RESULTS AND DISCUSSION

Experiment (1) :

The effect of nitrogenous compounds and other macronutrients on the shoot growth of *Cynara scolymus*, cultured for 6 weeks on multiplication medium, is shown in Tables (2 and 3).

Table (2) : Effect of nitrogenous compounds and other macronutrients on hyperhydric (%), number of shoots/explant and average fresh weight of shoot (g), after 6 weeks of culture.

Culture medium[k]	Percentage of cultures which gave hyperhydric shoots	No. of shoots / explant	Average fresh weight of shoot (g)
MS1	43.75	14.87	0.207
MS2	43.32	14.62	0.221
MS3	36.65	11.25	0.232
MS4	24.57	11.11	0.316
MS5	53.75	12.00	0.183
L.S.D. 5%	16.05	N.S.	N.S.

[K]MS1 = Macronutrients of MS medium, *i.e.* control; MS2= Macronutrients of MS, but No_3^- at 40 mM and NH^+_4 at 6 mM; MS3= Macronutrients of MS, but NH_4NO_3 and KNO_3 at half - strength; MS4= Macronutrients of MS3, but calcium chloride and magnesium sulphate at double weights; MS5 = Macronutrients of Knop's, 1865.

Table (3) : Effect of nitrogenous compounds and other macronutrients on leaves dry matter (%) and photosynthetic pigments, after 6 weeks of culture.

Culture medium[k]	Leaves dry matter (%)	Photosynthetic pigments (mg/100g fresh weight of leaves)		
		Chlorophyll (a)	Chlorophyll (b)	Carotenoides
MS1	5.82	22.99	6.32	13.49
MS2	7.04	19.97	4.21	11.67
MS3	8.44	24.53	7.03	15.53
MS4	8.00	27.59	7.03	16.64
MS5	5.89	22.70	6.36	13.25
L.S.D. 5%	2.07	4.74	1.91	N.S.

[K]MS1 = Macronutrients of MS medium, *i.e.* control; MS2= Macronutrients of MS, but No_3^- at 40 mM and NH^+_4 at 6 mM; MS3= Macronutrients of MS, but NH_4NO_3 and KNO_3 at half - strength; MS4= Macronutrients of MS3, but calcium chloride and magnesium sulphate at double weights; MS5 = Macronutrients of Knop's, 1865.

It is obvious that the MS4 multiplication medium which containing half-strength of NH_4NO_3 and KNO_3 and doubly weights of calcium chloride and magnesium sulphate specified for MS medium recorded the lowest value of hyperhydric percentage. By other words, MS4 medium had the highest percentage of cultures which gave normal shoots. On the contrary, the MS5 medium which containing Knop's macronutrients showed the highest percentage of hyperhydric cultures. Data also, revealed that shoot number per explant and average fresh weight of shoot were not significantly affected by these treatments. In this connection, Brutti, *et al*. (2000) established globe artichoke shoot culture on culture medium containing MS mineral salts modified by 50% reduction in NH_4NO_3 and KNO3. In addition, Rodriguez *et al*. (1991) added only one half of the weight of nitrates specified for MS medium to each litre of medium, while the weights of calcium chloride and magnesium sulphate were doubled. This approach (in combination with 2-phased layers of medium) was successful for reducing the frequency of hyperhydric shoots in *Pyrus communis.* Also, Chauvin and Salesses (1988) noted that hyperhydricity of chestnut which occurred on MS medium was prevented by using macronutrients which contained only half the MS level of nitrates. In this connection, Brand (1993) used MS or Woody Plant (WP) media for shoot tip culture of *Amelanchier arborea*. He noted that hyperhydricity and tissue nitrate concentration also increased in response to increasing ammonium nitrate in the medium. Since hyperhydricity was shown to be both positively and negatively correlated with increases in tissue nitrate content, it is unlikely that tissue nitrate level alone directly affects hyperhydricity. Le Dily *et al*. (1993) suggested that the deficient structure of cell walls in hyperhydric tissues of *Beta vulgaris* may permit uncontrolled uptake of NH^+_4 ions. However NH^+_4 is taken up rapidly, even by normal tissues, and can lead to changes in the way of other ions absorbetion. Daguin and Letouzé (1985) stated that gradually increasing ammonium to the normal level in MS medium induces a parallel development glassiness and hypolignification in *Salix babylonica*. In quince, elevated levels of sucrose, up to 4.5% together with a double dose of $MgSO_4$ decreased vitrification (Orlikowska, 1987). While the current results are not agree with those of Beauchesne (1981) who reported that shoots of *Salix babylonica* became hyperhydric on MS salts, while those on Knop's macronutrients were always normal in appearance. Also, the findings concerning reducing the level of HN^+_4 in the medium to 6 mM are not in accordance with those of Ziv and Ariel (1992) on carnation and Rodriguez *et al*. (1996) on *Agave victoria - reginae*.

Concerning the leaves dry matter percentage, it is obvious from data presented in Table (3) that the highest percentage of leaves dry matter was obtained when the shoot tip explants were cultured on MS3 multiplication medium which contained half-strength of NH_4NO_3 and KNO_3 or on MS4 medium which the same as MS3, but in combination with double weights of calcium chloride and magnesium sulphate. By other words, using these two culture media caused decreasing in leaves water content. The superiority of MS3 and MS4 in increasing the dry matter of leaves could be attributed to their influences in reducing the hyperhydricity. In this connection, Orlikowska and Olszewski (1993) stated that total hyperhydricity (vitrification) of apple

rootstock P60 shoots was observed on a medium solidified with the agar which contained the least Ca and Mg and produced high water availability. Also, the results obtained can be supported by the findings of Ueno *et al*. (1998) on raspberry, Olmos *et al*. (1997) on carnation and Shetty *et al*. (1996 a and b) on oregano (*Origanum vulgare*) who noted that a higher water content was observed in hyperhydrated leaves.

With regared to plant photosynthetic pigments, data presented in Table (3) cleared that chlorophyll (a) and chlorophyll (b) content were significantly affected by medium composition treatments. The highest chlorophyll (a) and (b) content were detected in plantlets grown on MS4 medium. Such data also revealed that carotenoides content was not significantly affected by these treatments. The superiority of MS4 in increasing leaves chlorophyll (a) and (b) content could be related to its composition which contained double weight of magnesium sulphate as compared to Murashige and Skoog-medium. The most well known role of Mg is its occurrence at the centre of the chlorophyll molecule (Mengel and Kirkby, 1979). In this connection, Shetty *et al.* (1996 a and b) mentioned that the prevention of hyperhydricity was characterized by high chlorophyll content in oregano shoots. Also, Righetti (1996) noted that the chlorophyll was increased in unhyperhydrated shoots of cherry (*Prunus avium*) cultured *in vitro*.

From the previously mentioned results, it could be suggested that lowering the concentration of the ammonium ion (NH^+_4) to 6 mM (as in MS2), or eliminating it from the medium (as in MS5) were not efficient to prevent or reduce hyperhydric in globe artichoke cultures. However, reducing total nitrogen to 30 mM ($^1/_2$ x NH_4NO_3 & KNO_3), but the proportion of nitrate to ammonium ions was maintained at 1.91, in combination with double weights of calcium chloride and magnesium sulphate (as in MS4) appear to be most suitable.

Experiment (2):

The effect of type and concentration of cytokinin on the *in vitro* shoot growth of globe artichoke, cultured for 6 weeks on multiplication medium, is shown in Table (4). It is clear that hyperhydric cultures percentage, fresh weight of culture and shoot number per explant were significantly affected by cytokinin treatments. However, fresh weight of shoot was not significantly affected by these treatments. The lowest percentage of hyperhydric cultures was noticed when the shoot tip explants were cultured on multiplication medium containing 1.0 mg/l kinetin. The highest values of fresh weight of culture and shoot number per explant were resulted from using multiplication medium supplemented with 2.0 mg/l kinetin. In this connection, Nin *et al*. (1994) stated that hyperhydricity decreased in shoot tip cultures of *Artemisia absinthium* when the cytokinin (BA) rate was reduced to 0.88 μM. On the other hand, Marino *et al*. (1993) added that proliferation of apricots was proportional to BA concentration. However, 8.8 μM BA was often associated with hyperhydricity. Also, in *Salvia leucantha* shoot cultures Hosoki and Tahara (1993) found that hyperhydric shoots percentage raised from 10.7 to 47 when the level of cytokinin (BA) raised from 0.1 to 1.0 mg/l. In melon,

shoots placed on an agar-solidified medium becoming hyperhydric when the cytokinin (BA) level was raised from 0.1 to 0.7 mg/l (Leshem *et al*., 1988).

Table (4) : Effect of two concentrations of kinetin and 2iP on hyperhydric (%), fresh weight (g), number of shoots / explant and average fresh weight of shoot (g), after 6 weeks of culture.

Treatments	Percentage of cultures which gave hyperhydric shoots	Fresh weight of culture (g)	No. of shoots / explant	Average fresh weight of shoot (g)
Kinetin (1.0 mg/l)	33.33	2.686	4.38	0.612
Kinetin (2.0 mg/l)	35.99	3.460	6.39	0.556
2iP (1.0 mg/l)	34.33	1.041	1.75	0.626
2iP (2.0 mg/l)	50.50	1.999	3.27	0.625
L.S.D. 5%	11.92	1.280	2.29	N.S.

Moreover, Dencso (1987) mentioned that many glassy shoots in *Gerbera jamesonii* cultures were found when 1-10 mg/l BA was added to encourage shoot proliferation, but very few if 1 mg/l kinetin was used instead. In jojoba, shoot multiplication rate was significantly greater in BA (1.0 mg/l) than kinetin (1.0 mg/l) treatment, but the presence of BA caused light hyperhydricity in 30% of explants (Llorente *et al*., 1996). Kataoka and Inoue (1992) noted that more than 3 mg/l 2iP caused severe vitrescence of papaya shoots.

Finally, from the above mentioned results, it could be suggested that, supplementation of MS medium with 2.0 mg/l kinetin + 1.0 mg/l IAA enhanced the *in vitro* derived shoots of globe artichoke with less vetrification.

REFERENCES

A.O.A.C. (1990). Association of Official Analytical Chemists. Official Methods of Analysis (15th Ed.) Washington, D.C., U.S.A.

Beauchesne, G. (1981). Les milieux mineraux utilisés en culture *in vitro* et leur incidence sur 1' apparition de boutures d'aspect pathologique. Compt. Rend. Acad. Agic. Paris 67 : 69-74. In : Plant Propagation by Tissue Culture. Part 2 : In Practice (ed., E.F. George) Exegetics Ltd., England. pp. 662.

Bekheet, S.A. (1992). Studies on Propagation of Globe Artichoke Through Tissue Culture Technique. M. Sc. Thesis, Fac. Agric., Ain shams Univ., Egypt.

Brand, M.H. (1993). Agar and ammonium nitrate influence hyperhydricity, tissue nitrate and total nitrogen content of serviceberry (*Amelanchier orborea*) shoots *in vitro*. Plant Cell, Tissue and Organ Culture, 35: 203-209.

Brutti, C.; Apostolo, N.M.; Ferrarotti, S.A.; Llorente, B. E. and Krymkiewicz, N. (2000). Micropropagation of *Cynara scolymus* L. employing cyclodextrins to promote rhizogenesis. Scientia Hort., 83: 1-10.

Chauvin, J. E. and Salesses, G. (1988). Advances in chestnut micropropagation (*Castanea* SP.). Acta Hort., 277 : 340-345.

Daguin, F. and Letouzé, R. (1985). Relations entre hypolignification et état vitreux chez *salix babylonica* en culture *in vitro*. Role de la nutrition ammoniacale. Can. J. Bot., 63: 324-326 (c.f. Agricola DATA BASE Internet).

Dencso, I. (1987). Factors influencing vitrification of carnation and conifers. Acta Hort., 212: 167-176.

George, E.F. (1993). Plant Propagation by Tissue Culture. Part 2 : In Practice. Exegetics Ltd., England. pp. 654-669.

Hosoki, T. and Tahara, Y. (1993). *In vitro* propagation of *Salvia leucantha* Cav. HortScience, 28 :226.

Kataoka, I. and Inoue, H. (1992). Factors influencing *ex vitro* rooting of tissue cultured papaya shoots. Acta Hort., 321 : 589-597.

Knop, W. (1865). Quantitative Untersuchungen über die Ernahrungsprozesse der Pflanzen. Landwirtsch. Vers. Stn. 7, 93-107. In: Plant Culture Media. Vol.1: Formulations and Uses (ed., E. F. George, D. J. M. Puttock and H. J. George) Exegetics Ltd., England. pp251.

Le Dily, F.; Hault, C.; Gaspar, T. and Billard, J.P. (1993). Does altered nitrogen metabolism and H_2O_2 accumulation explain the vitrified status of the fully habituated callus of *Beta vulgaris* (L.) . Plant Cell, Tissue and Organ Culture, 35: 69-74.

Leshem, B.; Shaley, D.P. and Izhar, S. (1988). Cytokinin as an inducer of vitrification in melon. Ann. Bot., 61: 255-260.

Llorente, B.; Apostolo, N.; Princen, L.H. and Rossi, C. (1996). Micropropagation of jojoba : effect of hormonal and clonal variation at the multiplication stage. Proceedings of the Ninth international conference on jojoba and its uses and of the Third international conference on new industrial crops and products, Catamarca, Argentina, 25-30 September 1994. 50-52 (c.f. Agricola DATA BASE Internet).

Marino, G.; Bertazza, G.; Magnanini, E. and Altan, A.D. (1993). Comparative effects of sorbitol and sucrose as main carbon energy sources in micropropagation of apricot. Plant Cell, Tissue and Organ Culture, 34: 235-244.

Mengel, K. and Kirkby, E.A. (1979). Principles of Plant Nutrition, International Potash Institute, Switzerland, p. 593.

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

Nin, S.; Schiff, S.; Bennici, A. and Magherini, R. (1994). *In vitro* propagation of *Artemisia absinthium* L. Advances in Horticultural Science, 8: 145-147.

Olmos, E.; Piqueras, A.; Martines – Solano, J.R. and Hellin, E. (1997). The subcellular localization of peroxidase and the implication of oxidative stress in leaves of regenerated carnation plants. Plant Science Limerick, 130: 97-105.

Onisei, T.; Toth, E.T. and Amariei, D. (1988). Callus induction and plant regeneration of *Cynara scolymus* L..Revue Roumaine de Biologie, Biologie Vegetale,33:115-119(c.f. Agricola DATA BASE Internet).

Orlikowska, T. and Olszewski, T. (1993). Influence of agar brands on the multiplication of apple rootstock and on chemical and physical attributes of the media. J. Fruit and Ornamental Plant Res., 1: 115-125.

Orlikowska, T. (1987). Vitrification problem in the *in vitro* culture of fruit tree rootstocks. Acta Hort., 212 : 239-244.

Paques, M. (1991). Vitrification and micropropagation : causes, remedies and prospects. Acta Hort., 289 : 283-290.

Righetti, B. (1996). Chlorophyll, ethylene and biomass determination in *Prunus avium* cv. Victoria cultivated *in vitro* under different atmospheric conditions. J. Hort. Sci., 71 : 249-255.

Rodriguez, G. B.; Gutierrez, M.A. and Acosta, D. B. (1996). Somatic embryogenesis of *Agave victoria – reginae* Moore. Plant Cell, Tissue and Organ Culture, 46 : 85-87.

Rodriguez, R.; Diaz-Sala, C.; Cuozzo, L. and Ancora, G. (1991). Pear *in vitro* propagation using a double-phase culture system. HortScience, 26: 62-64.

Schloupf, R.M.; Barringer, S.A. and Splittstoesser, W.E. (1995). A review of hyperhydricity (vitrification) in tissue culture. Plant Growth Regulator Society of America Quarterly, 23: 149-158.

Shetty, K.; Carpenter, T.L.; Curtis, O.F. and Potter, T.L. (1996a). Reduction of hyperhydricity in tissue cultures of oregano (*Origanum vulgare*) by extracellular polysaccharide isolated from *Pseudomonas* spp. Plant Science Limerick, 120: 175-183.

Shetty, K.; Curtis, O.F. and Levin, R. E. (1996b). Specific interaction of mucoid strains of *Pseudomonas* spp. With oregano (*Origanum vulgare*) clones and the relationship to prevention of hyperhydricity in tissue culture. J. of Plant Physiology, 149: 605-611.

Snedecor, G.W. and Cochran, W.G. (1980). *Statistical Method*. 7th Ed., Iowa State Univ. Press, Ames Iowa, U.S.A.

Ueno, K. I.; Cheplick, S. and Shetty, K. (1998). Reduced hyperhydricity and enhanced growth of tissue culture-generated raspberry (*Rubus* sp.) clonal lines by *Pseudomonas* sp. isolated from oregano. Process Biochemistry, 33: 441- 445.

Ziv, M. and Ariel, T. (1992). On the relation between vitrification and stomatal cell wall deformity in carnation. Acta Hort., 314 : 121-129.

تأثير تركيب البيئة على ظاهرة البلل الشديد بالماء في مزارع أنسجة الخرشوف

محمد صفوت سعد الشباسي

معهد بحوث البساتين – مركز البحوث الزراعية – الجيزة – مصر

البلل الشديد بالماء أو المظهر الزجاجي مشكلة تتكرر أثناء الإكثار السريع للخرشوف وهذه الظاهرة تتأثر بالبيئة وبنوع وتركيز السيتوكينينات المستخدمة. استخدام بيئة التضاعف المحتوية على نصف تركيز نترات الأمونيوم ونترات البوتاسيوم وضعف تركيز كلوريد الكالسيوم وكبريتات المغنسيوم المنصوص عليها في بيئة موراشيج وسكوج هذه البيئة سجلت أقل نسبة مئوية من المزارع المبتلة. علاوة على ذلك، هذه البيئة أدت إلى زيادة معنوية في النسبة المئوية للمادة الجافة بالأوراق وكذلك محتوى الأوراق من كلوروفيل (أ)، (ب). إضافة ٢,٠ ملليجرام/ لتر كينتين + ١,٠ ملليجرام/ لتر أندول حمض الخليك أدى للحصول على أفضل تكاثر للأفرع مع انخفاض نسبي للبلل الشديد بالماء (المظهر الزجاجي).