J. Agric. Sci. Mansoura Univ., 27 (9): 5769 - 5780, 2002

IMPACT OF CULTIVAR, POLYETHYLENE GLYCOL AND TYPE OF MEDIUM ON GROWTH, DEVELOPMENT AND SOME CHEMICAL CONSTITUENTS OF *MUSA SPP.* CULTURED *IN VITRO*.

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ABSTRACT

Aseptic cultures of banana cultivars (cvs) Maghraby (Mg), Valery (V), Grand Nain (GN) and Hindy (H) were established from shoot tip explants. They were initiated on MS-basal medium supplemented with 3 mg/l benzyl adenine (BA) and 6 g/l agar (starting medium). Explants were transferred at 4 week intervals until the onset of proliferation (about 2 months). Thereafter, 4 subcultures were made on multiplication medium containing BA (5 mg/l). The produced shoots were used to evaluate the *in vitro* responses of tested cultivars to water (osmotic) stress induced by PEG with respect to the type of medium. Shoot survival was gradually delayed by the elevated levels of PEG (≥ 15 g/l), but the response was more pronounced in Mg and V than GN followed by H. Applying PEG (10 and 20 g/l) to the medium progressively reduced the *in vitro* growth and development of shoots, but the magnitude of this reduction was more evident in case of V and Mg than GN followed by H. However, the root growth and development showed a contrary trend under the effect of PEG, but a similar response under the effect of cultivar. At 2 % PEG, medium liquefaction improved the growth and development of both shoots and roots. Moreover, the cultivar H achieved the highest values of these characters, whereas V and Mg had the lowest ones. It was found that, the accumulation of total soluble sugars (TSS) and proline in shoots was positively correlated with water stress induced by PEG and agar, while the reverse was true in case of N, P and K accumulation. Furthermore, the metabolite accumulation (N, P, K, TSS and Proline) in shoots of PEG-treated media was superior in the cultivar H and inferior in Mg. Therefore, it was deduced that: (1) the cultivar H is the most tolerant to water stress, (2) although, the cultivars GN, V and Mg did not survive at 4.5% (W/V) PEG, the cultivar GN appeared to be more tolerant than V and Mg, and (3) the higher levels of metabolite accumulation, especially proline end TSS, enhance the plant tolerance to water stress.

Keywords: Osmotic stress; Micropropagation; Survival; Nitrogen; Phosphorus; Potassium; Sugars; Proline; Banana.

Abbreviations: BA= benzyl adenine; cv(s)= cultivar(s); DW= dry weight; FW= fresh weight; GN= Grand Nain; H= Hindy; K= potassium; Mg= Maghraby; MS= Murashige and Skoog; N= nitrogen; NAA= alpha-naphthalene acetic acid; No or no= number; P= phosphorus; PEG = polyethylene glycol; TSS = total soluble sugars; V= Valery.

INTRODUCTION

Banana *(Musa spp.)*, belongs to the family Musaceae, has become one of the strategic crops in tropical countries due to its high income to the farmers. Banana fruits represent a stable food or a part of the diet for about milliard person all over the world (FAO, 1992). They are seedless, thus the plant is propagated vegetatively or *in vitro*. The *in vitro* propagation is preferable because it allows the production of a large number of virus-free plants in a relatively shorter time and smaller space.

Banana is cultured wherever water is available with respect to the other environmental factors. Recently, it was introduced to desert regions having relatively lower available water. This makes it necessary to investigate the plant response to water stress during its micropropagation and under greenhouse conditions.

Water stress could be induced in the micropropagation media by adding osmotic and/or gelling agents such as polyethylene glycol (PEG) and agar, respectively. The elevated levels of these agents may reduce the *in vitro* growth and development by affecting both water and mineral uptake. This in turn could affect both mobilization of nutrients (e.g., sucrose) and the structural organization or synthesis of metabolites in the cultured tissues (Ebrahim and Ibrahim, 2000; Almansouri *et al.*, 2001). This effect could differ among plant species or even the cultivars of a given species (Dodd and Donovan, 1999).

Despite the enormous studies on micropropagation of banana, data concerning the effect of gelling agents are relatively scarce (e.g., Ebrahim and Ibrahim, 2000), and there is no information about the effect of PEG. Therefore, the objective of this study was to compare the response of four banana cultivars, cultured *in vitro*, to water stress caused by PEG with respect to the type of medium. As a consequence, the most tolerant cultivar was determined.

MATERIALS AND METHODS

Four cultivars of *Musa spp.*, obtained from the experimental farm (El-Kanater El-khayreia, Kalubiya, Egypt) of the Agricultural Development Systems (ADS) Project (Giza, Egypt), served as the source material for shoot tips during the study period (2000-2002). These cultivars included: Maghraby (Mg), Valery (V), Grand Nain (GN) and Hindy (H).

Aseptic cultures were established from shoot tips which were surface- sterilized in 3 % NaOCl solution (contained 0.1 % tween 20 as a wetting agent) for 20 min. Thereafter, the tips were rinsed several times in sterilized distilled water to remove all traces of chlorine. After removal of the outside tissues, apical meristems were vertically cultured for 4 weeks on MS (Murashige and Skoog, 1962)-basal medium supplemented with benzyl adenine (BA, 3 mg/l) and agar (6 g/l). The growing explants were recultured, at 4 weeks interval, on fresh media until the onset of proliferation (2 months, starting stage). In order to obtain sufficient number of explants, the produced

shoots were subcultured four times on solid MS-basal media supplemented with 5 mg/l BA (multiplication stage).

To determine the lethal concentration of polyethylene glycol (PEG) for each cultivar, PEG-6000 was added to starting solid MS-basal media at levels of 0.0, 5, 15, 25, 35 and 45 g/l. Thereafter, the produced shoots were cultured for 4 weeks on the previous media, then the percentage of survival was determined.

PEG (0.0, 10 and 20 g/l) was added to solid multiplication (supplemented with 5 mg/l BA) and rooting (supplemented with 1 mg/l NAA) MS-basal media, then the media were cultured with the produced shoots. After 4 weeks, shoot growth and development were determined in the multiplication media, while root growth and development were determined in the rooting media. The different stages of banana micropropagation are shown in Fig. 1.

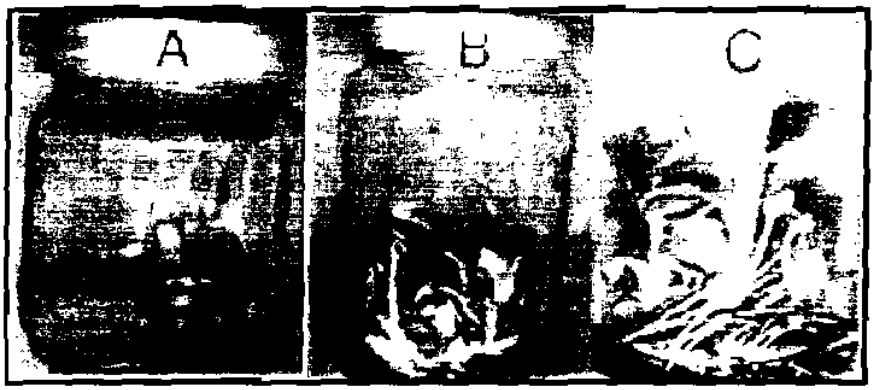

Fig. (1). The different stages of banana micropropagation, (cv. Hindy).

A. Shoot tips were cultured on MS basal medium contained 3 mg/l BA (starting stage).

B. Shoot proliferation on MS basal medium supplemented with 5 mg/l BA (multiplication stage).

C. Root initiation on MS basal medium +1 mg/l NAA (rooting stage).

In order to examine the effect of type of the medium, PEG (20 g/l) was added to multiplication and rooting MS-basal media each as liquid and solid, then the media were cultured with the produced shoots. After 4 weeks, shoot growth and development were determined in the multiplication media, while the root growth and development were determined in the rooting media. Shoot strength (viability and vigor) was calculated as described by Ebrahim and Ibrahim (2000) and presented as follows: (a) negative growth= 1, (b) below average growth= 2, (c) average growth= 3, (d) above average growth= 4, and (e) excellent growth= 5.

The previous experiments indicated that the obtained results of cvs MG and V (especially in Table, 1) were nearly similar with no significant

differences, so, one of them (MG) was selected to run the next experiment with the other cultivars.

To find an explanation for the obtained results, PEG (0.0, 10 and 20 g/l) was added to solid and liquid multiplication MS-basal media. The media were then cultured with the produced shoots of the cultivars Mg, GN and H. After 4 weeks, the obtained shoots were oven-dried and used to determine some chemical constituents. Mixed-acid digestion method was used in preparing the sample solution used for determination of N, P and K (Ebrahim and Aly, 2002). Total-nitrogen content (N) was estimated using the micro-Kjeldahl method (Jacobs, 1958). Phosphorus (P) content was spectrophotometrically determined by molybdenum-blue method (Page, 1982). Potassium (K) was determined according to Allen *et al.*(1974). Sugars were extracted in borate buffer pH 8 (0.1 g DW/5 ml buffer), then total soluble sugars (TSS) were determined by the method adopted by Shaffer and Hartmann (1921). Proline, in ethanol extract, was quantified according to Bates *et al.* (1973).

All media were distributed in 200 ml Pyrex-glass jars (25 ml/jar), solidified with 0.6 % (w/v) agar, autoclaved for 20 min at 121 °C and 1.2 kg/cm^2, then cooled and kept for 4-15 days before use. In all media, pH was adjusted to 5.7 before solidification (adding agar). All cultures were placed in a growth chamber at 25±3 °C and 40 µmol/m^2/s continuous photosynthetic photon flux provided by cool white fluorescent lamps.

All experiments were repeated twice, under controlled conditions, and conducted by using a completely randomized design in factorial arrangement with 4 replicates at least. All data were averaged and statistically analyzed by using two-and three-way analysis of variance (ANOVA). In case of percentages, the original data were arcsine-transformed prior to statistical analysis. The least significant difference (LSD) at the 0.05 level was used to compare between means directly (Steel and Torrie, 1980) or indirectly by the multiple range test of Duncan (Duncan, 1955).

RESULTS AND DISCUSSION

PEG is a hydrophilic alcohol polymer, having a high water solubility, and no toxicity (Fontana *et al.*, 2001). We used it in our study as an inert osmoticum because it is a non-penetrating solute (Almansouri *et al.*, 2001). It forms hydrogen bonds with water decreasing water potential of the culture medium and finally inhibits water and mineral uptake. In this work, water stress was prevailed by adding PEG to the culture medium. Consequently, the experimental explants might be suffering from low water and nutrient uptake (PEG problems). These problems were found to affect all the tested characters described below.

Shoot survival was significantly influenced by PEG level and banana cultivar (Table 1). It was gradually delayed by the elevated levels of PEG (≥ 15 g/l), but the response was more evident in case of the cultivars (cvs) Mg and V than GN followed by H. Although the cv H survived up to 4.5 % PEG, the other cvs did not survive. There is no literature explaining such *in vitro*

responses, but the effect of PEG level might be due to the PEG problems described above, while the effect of banana cv could be explained on basis of the cv ability to accumulate metabolites. Metabolite accumulation, especially N, TSS and proline, amongst the tested cvs was in the order H>GN>Mg (see Table 6). These results served for determining the PEG concentrations used in the other experiments discussed below.

PEG treatments significantly reduced shoot multiplication and biomass accumulation, but the response was more pronounced in the cvs Mg and V than GN followed by H (Table 2). The effect of PEG level might be referred to the adverse effect of PEG on both mobilization of nutrients (e.g., sucrose) and the structural organization or synthesis of metabolites in the cultured tissues (Ebrahim and Ibrahim, 2000. Almansouri *et al.*, 2001). Whereas, the influence of cv could be explained on basis of the cv ability to resist the stress conditions by accumulating the osmotically active metabolites which contribute in the osmotic adjustment, in this regard, Liu *et al.* (2001) revealed a positive correlation between sugar accumulation and salt-tolerance of soybean cultured in *vitro*. In our study, the correlation between the accumulation of TSS and proline (Table 6), and shoot growth and development (Table 2) supports the above interpretations and should be considered for explaining the variation in stress tolerance amongst the tested cvs.

Table (1). Effect of polyethylene glycol (PEG) on percentage of shoot survival of *in vitro* cultured cultivars of banana. Explants were cultured on MS basal medium contained 3 mg/l BA (starting medium) for 4 weeks.

Cultivars	Polyethylene glycol concentration (g/l)						
	0.0	**5**	**15**	**25**	**35**	**45**	**Mean**
Mg	100.0 a	100.0 a	95.0 b	75.0 bc	20.0e	0.0 g	65.0 m
V	100.0 a	100.0 a	90.0 b	80.0 b	20.0 e	0.0 g	65.0 m
GN	100.0 a	100.0 a	100.0 a	90.0 b	50.0 d	0.0 g	73.3 l
H	100.0 a	100.0 a	100.0 a	100.0 a	70.0bc	20.0 e	81.7 k
Mean	**100.0 r**	**100.0 r**	**96.3 r**	**86.3 s**	**40.0 t**	**5.0 u**	

Values followed by the same letter are not significantly different (LSD at 0.05 level).

Increasing PEG concentration in the medium significantly increased both root development and elongation, but the response was more obvious in case of root development. Also, the magnitude of this increase was more marked in the cv H than GN followed by V and Mg (Table 3). These results agree with others obtained, *in vivo*, by Ebrahim *et al.* (1998) and Aly *et al.* (2001). They could be also observed as an adaptive response of roots to alleviate the inhibitory effect of PEG on water and nutrient uptake. Therefore, the cv H appearing to be the most adapted, had the best root growth and development (see Tables 1, 2 and 3).

Table (2). Effect of polyethylene glycol concentration (PEG) on shoot growth and development of *in vitro* cultured cultivars of banana. Explants were cultured on MS basal medium contained 5 mg/l BA (multiplication medium) for 4 weeks.

Treatments		Shoot No / explants	Shoot FW (g)
Cultivars (A)	PEG conc. (g/l) (B)		
Mg	0.0	3.02	0.88
	10.0	1.96	0.73
	20.0	2.12	0.41
V	0.0	3.00	0.86
	10.0	2.01	0.70
	20.0	1.04	0.44
GN	0.0	3.04	0.83
	10.0	2.51	0.74
	20.0	1.57	0.56
H	0.0	3.02	0.85
	10.0	2.62	0.80
	20.0	1.96	0.66
LSD at 0.05 Level for A		0.12	0.04
B		0.11	0.03
A x B		0.20	0.07

The influence of the cv and type of medium on the *in vitro* growth and development of banana, cultured on/in media contained 2 % PEG, is shown in Tables 4 and 5. It is obvious that the magnitude of growth and development of both shoots and roots, amongst the tested cvs, was in the order H>GN>V>Mg (Tables 4 and 5). These results are in line with those recorded in Tables 2 and 3. Therefore, they could be also explained on the same bases described above. Irrespective of cv, the medium liquefaction was found to improve all tested attributes of growth and development of both shoots and roots (Tables 4 and 5). This finding is in accordance with results obtained by Ebrahim (2002) who attributed this response to: (1) a better contact between explants and liquid medium which increases the availability of growth stimulators and the ability for water and nutrient uptake, (2) dilution of any exudates, from explants, in the liquid medium, and/or (3) more adequate aeration in the liquid medium, which enhances growth and development.

In shoots of PEG stressed cvs, metabolite (N, P, K, TSS and proline) accumulation seemed to be in the order H>GN>Mg (Table 6). This arrangement illustrates the variation, amongst cvs, in the mean values of all attributes recorded in the Tables 1-5. It also confirms that the magnitude of the adaptive response is more pronounced in the cv H than GN followed by Mg. Consequently, the cv H seemed to be more tolerant than GN, Mg and V. However, increasing the level of PEG in the culture medium induced a progressive decline in the contents of N,P and K within shoots of tested cvs, whereas TSS and proline contents were significantly improved (Table 6). These results are in accordance with others reported by several authors (Bandurska, 2000; Pushpam and Rangasamy, 2000; Ronde et al., 2000; Aly et al., 2001 and Liu et al., 2001) and could be due to: (1) the influence of PEG

on medium pH which decreases the availability of P absorption (Aly *et al.*, 2001), and/or (2)the PEG problems described above. On the contrary to PEG, medium liquefaction significantly (P<0.01) improved N, P, and K contents.However, the accumulation of TSS and proline was retarded in control explants, but enhanced in the PEG treated ones (Table 6). This finding might be due to the advantages of liquid medium, which were reported by Ebrahim and Ibrahim (2000) and Ebrahim (2002), and described in detail above.

Table (3). Effect of polyethylene glycol concentration (PEG) on root growth and development of *in vitro* cultured cultivars of banana. Explants were cultured on MS basal medium contained 1 mg/l NAA (rooting medium) for 4 weeks.

Treatments		Root No/shoot	Root Length (cm)
Cultivars (A)	PEG conc. (g/l) (B)		
Mg	0.0	3.4	5.2
	10.0	4.2	5 3
	20.0	4.9	5.6
V	0.0	3.6	5.4
	10.0	4.3	5.5
	20.0	5.3	5.8
GN	0.0	3.8	5.6
	10.0	5.0	5.6
	20.0	6.1	5.8
H	0.0	3.9	5.6
	10.0	4.9	5.7
	20.0	6.2	5.9
LSD at 0.05 Level for A		0.39	0.37
B		0.35	0.33
A x B		0.60	NS

NS: non-significant

Table (4). Effect of type of medium on shoot growth and development of *in vitro* cultured cultivars of banana. Explants were cultured on MS basal medium contained 5mg/l BA (multiplication medium) and 20 g/l PEG for 4 weeks.

Treatments		Shoot no/ explants	Shoot FW (g)	Shoot strength	No of leaves/ shoot
Cultivars (A)	Medium (B)				
Mg	Solid	1.12	0.41	1.4	2.8
	Liquid	1.50	0.57	1.8	3.2
V	Solid	1.04	0.44	1 2	2.9
	Liquid	1.57	0.57	1.7	3.2
GN	Solid	1.57	0.56	2.0	3.2
	Liquid	1.93	0.66	2.3	3.8
H	Solid	1.96	0.66	2.7	3.4
	Liquid	2.22	0.71	3.1	4.2
LSD at 0.05 Level for A		0.44	0.06	0.17	0.15
B		**	**	**	**
A x B		NS	NS	NS	NS

NS: non-significant, and ** : significant difference at P < 0.01

Table (5). Effect of type of medium on root growth and development of *in vitro* cultured cultivars of banana. Explants were cultured on MS medium contained 1 mg/l NAA (rooting medium) and 20g/l PEG for 4 weeks.

Treatments		Root no/shoot	Root length (cm)
Cultivars A)	Medium (B)		
Mg	Solid	4.3	5.6
	Liquid	4.9	6.9
V	Solid	4.3	5.8
	Liquid	5.3	7.1
GN	Solid	4.8	5.8
	Liquid	6.1	8.1
H	Solid	4.8	5.9
	Liquid	6.2	8.4
LSD at 0.05 Level for A		0.38	0.49
B		**	**
A x B		NS	0.70

NS : non-significant, and ** : significant difference at P < 0.01.

Concluding remarks

Banana shoot tip explants could be successfully propagated when they are:(1) surface sterilized in 3 % NaOCl solution for 20 min, (2) cultured for 2 months on MS-basal medium supplemented with BA (3 mg/l) and freshly exchanged at 4 weeks interval, (3) multiplicated by subculturing (4 times)on MS-basal medium containing BA (5 mg/l), and (4) rooted on MS-basal medium supplemented with NAA (1 mg/l). The *in vitro* response of the cvs Mg, V, GN and H was influenced by application of PEG and the medium liquefaction, but the magnitude of the response was cultivator dependent. Shoot survival was developmentally delayed by the elevated levels of PEG ($\geq$ 15 g/l), but the response was more evident in Mg and V than GN followed by H. Applying PEG (10 and 20 g/l) to the medium progressively reduced the growth and development of shoot, while the other of root were enhanced. Medium liquefaction alleviated the detrimental effects of PEG in all tested cultivars. It was concluded that the cultivar H is the most tolerant to water stress induced by PEG and agar. Also, GN is more tolerant than V and Mg. This conclusion was attributed to the impact of water stress on nutrient absorption and mobilization which, in turn, affected the accumulation of most chemical constituents in shoots.

Table (6): Effect of polyethylene glycol concentration (PEG) and type of medium on some chemical constituents of shoots of *in vitro* cultured banana. Explants were cultured on MS basal edium contained 5 mg/l BA (multiplication medium) for 4 weeks.

Treatments			N(mg/g DW)	P (mg/g DW)	K (mg/g DW)	TSS* (g/100 g DW)	Proline (g/100 g DW)
Cultivars (A)	PEG conc.(g/l) (B)	Medium (C)					
Mg	0.0	Solid	22.7	10.0	17.1	25 0	0.14
		Liquid	22.9	12.4	18.7	21.9	0.14
	10.0	Solid	18 4	9 8	13.7	24 0	0 20
		Liquid	20.7	11.1	15 0	26.7	0.26
	20.0	Solid	13 9	9.2	10.9	27.1	0.34
		Liquid	15.0	10.2	12.1	29.2	0.49
GN	0.0	Solid	22.0	10.4	17.3	25.4	0 14
		Liquid	24.1	11 9	18 4	23.4	0.11
	10.0	Solid	18 0	9 9	14.9	25.0	0 31
		Liquid	21.6	11.4	16.0	28.2	0.38
	20.0	Solid	16.2	9.4	11.6	31.2	0.44
		Liquid	17.3	10.6	12 8	33.0	0.54
H	0.0	Solid	21.2	10.2	17.8	25.9	0.15
		Liquid	23.4	12.6	18.6	23.5	0.13
	10.0	Solid	19.4	9.9	15.6	27.3	0.34
		Liquid	22.5	11.8	16 4	30.0	0.44
	20.0	Solid	17.8	9.7	12.0	33.0	0.51
		Liquid	19.4	10.7	13.3	35.4	0.62
LSD at 0.05 Level for A			0.73	0.32	0.93	1.36	0.015
		B	0.73	0.32	0.93	1 36	0.015
		C	**	**	**	**	**
		A x B	1.26	NS	NS	1.96	0 053
		A x C	NS	NS	NS	NS	0 024
		B x C	1.03	0.46	NS	NS	0.021
		A x B x C	NS	NS	NS	NS	NS

NS : non – significant, and ** : significant difference at P < 0.01.
* Total soluble sugars

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تأثيرالصنف النباتى والبولى إيثيلين جليكول ونوع الوسط الغذائى على نمو وتمـيز وبعض المكونات الكيميائية لنبات الموز فى مزارع الأنسجة

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أمكن عمل مزارع أنسجة معقمة لنبات الموز أصناف مغربي (Mg) وفاليرى (V) وجرانداتـان (GN) وهندي (H) ، وذلك من الأنسجة الحية للقمم النامية للمجموع الخضري. بدأت الزراعة على البيئـة الصلبة لموراشيجي وسكوج (MS) والمضاف إليها البنزيل أدينين (BA) بمعدل ٣ مجم/لتر والأجار بمعدل ٦ جم/لتر (بيئة البداية) مع استبدال البيئة بأخرى طازجة كل ٤ أسابيع حتى بداية مرحلة التضـاعف (بعـد حوالي شهرين من بداية الزراعة). عندئذ تم تكرار الزراعة لأربع مرات على بيئة تضاعف عبارة عن بيئـة MS صلبة محتوية على BA بمعدل ٥ ملجم/لتر، ثم أخذت الأفرع الخضرية الناتجة من مرحلة التضـاعف واستخدمت لتقييم استجابة أصناف الموز المختبرة للتوتر المائي (الاسموزى) الناتج من إضافة البولي ايثيليـن جليكول (PEG) إلى بيئة الزراعة الصلبة .

بدأت النباتات الخضرية في الذبول تدريجيا عند إضافة PEG إلى البيئة الغذائية لكل الأصنـاف بمعدلات مرتفعة (≤ ١٥جم/لتر)، ولكن كان التأثير أكثر وضوحا في صنفى (MG ,V) عن صنـفGN عن صنف H. عند إضافة PEG إلى بيئة التضاعف الصلبة بمعدل ١٠، ٢٠ جم/لتر أدى ذلك إلى تثبيـط نمو وتميز المجموع الخضري في كل الأصناف، وقد كان ذلك أكثر وضوحا في صنفى (Mg ,V) عـن صنف H. أما إضافة PEG بنفس التركيزات إلى بيئة تجذ ير فقد أدت إلى زيادة نمـو وتمـيز المجمـوع الجذري في كل الأصناف، ولكن كانت الزيادة أكثر وضوحا في صنف H عن صنفة GN عن صنفـى V و Mg.

وعند دراسة تأثير صلابة البيئة، باستخدام بيئة صلبة وسائلة مضافا إلى كل منهما ٢٠جـم/لـتر PEG ، أدت البيئة السائلة إلى تحسن في نمو وتميز كلا من المجموع الخضري والمجموع الجذري. علاوة على ذلك فقد سجلت أعلى القيم لنمو وتميز المجموعين معا في الصنف H، بينما سجلت أقل القيم في صنفى (Mg and V) . وقد وجد أيضا أن هناك علاقة طردية بين التوتر المائي الناتج من المعاملة بـ PEG ومحتويات السكريات الذائبة الكلية (TSS) والبرولين في المجموع الخضري، على عكس ذلـك فقـد أدت الزيادة في التوتر المائي إلى نقص في محتوى المجموع الخضري مـن النيـتروجين (N) والفوسـفور (P) والبوتاسيوم (K). علاوة على ذلك فقد سجلت النباتات الخضرية لصنف H المعاملـة بـ PEG أعلـى محتوى من النواتج الأيضية المختبرة (N, P, K, TSS and Proline,) بينما سجلت مثيلتـها مـن الصنف Mg اقل محتوى من نفس النواتج.

مما سبق نستنتج أن:

(١) الصنف H هي أكثر أصناف الموز المختبرة تحملا للتوتر المائي

(٢) أظهر الصنف GN تحملا أكثر للتوتر المائي عـن الصنفين (Mg and V) إلا أنه لوحـظ ذبـول موت كل النباتات الخضرية لأصناف (Mg, V and GN) عند إضافة PEG إلى البيئة بمعـدل (٤٥جم/لتر)

(٣) زاد تحمل النبات للتوتر المائي بزيادة تراكم النواتج الأيضية خاصة البرولين والـ TSS .