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Impact of polyethylene glycol-induced water stress on growth and development of shoot tip cultures from different banana (*Musa* spp.) cultivars

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Abstract

Shoot tip explants of the Egyptian banana cultivars Maghraby, Valery, Grand Nain and Hindy were tested for their tolerance to water stress. Shoot survival, shoot growth and root growth stimulation in presence of polyethylene glycol (PEG) was strongest in cultivar Hindy followed by Grand Nain, Maghraby and Valery. The accumulation of soluble sugars and proline in shoots was positively correlated with the applied polyethylene glycol concentration, while the reverse was true for N, P and K content. The cultivar Hindy exhibited higher metabolite accumulation response and cultivar Maghraby the least. The effects were most clear on liquid medium whereas solid (agar) medium exerted some additional effects increasing the osmotic stress at low PEG concentrations and alleviating the PEG effect at high PEG concentrations. In conclusion, the cultivar Hindy appeared to be the most tolerant to water stress because of strong accumulation of compatible solutes and greater stimulation of root development.

Keywords: Banana (*Musa* spp. L.), medium (solid/liquid), micropropagation, osmotic stress, polyethylene glycol (PEG), proline, sugars

Introduction

Banana (*Musa* spp.) fruits represent a staple food for about one billion people all over the world (FAO, 1992). It has become one of the strategic crops in tropical countries due to its high income potential for the local farmers. Since they are seedless, the plant has to be propagated vegetatively or *in vitro*. The *in vitro* propagation is preferable in modern breeding because it allows the production of a large number of virus-free plants in a relatively short time and in a small space (Cronauer and Krikorian,1984, 1985; Khalil *et al.,* 2002; Sagi *et al.,*1995).

Banana is cultivated in tropical climates wherever a steady year-round water supply is available. Recently it was introduced into tropical regions which have relatively low water availability. This initiated the investigation of banana plant response to water stress during micropropagation and under greenhouse conditions. Water stress can be induced in the micropropagation media by adding a compatible osmoticum such as polyethylene glycol (PEG). Despite the numerous studies on micropropagation of banana, there is no information about the effect of polyethylene glycol on regenerating or regenerated banana tip explants. Since the drought stress tolerance differs among plant species and the cultivars of a given species (Dodd and Donovan, 1999), the *in vitro* response of four banana cultivars which are important for Egyptian horticulture, to polyethylene glycol-induced water stress was studied.

Materials and methods

Plant material: Four cultivars of *Musa* spp. obtained from the experimental farm El-Kanater El-Khayreia, Kalubiya of the Agricultural Development Systems Project (Giza, Egypt), served as the source material for shoot tips during the study period (2000-2002). The cultivars were Maghraby, Valery, Grand Nain, which belong to the semi-dwarf Cavendish group and Hindy is a strain of dwarf Cavendish. All are of triploid *Acuminata* type.

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 Murashige-Skoog basal medium (Murashige and Skoog, 1962) supplemented with benzyladenine (3 mg/l) and agar (6 g/l). The growing explants were recultured, at 4 week intervals, on fresh media until the onset of proliferation (ca. 2 months). This culture period was called starting phase. In order to obtain sufficient number of explants, the produced shoots were subcultured four times on solid Murashige-Skoog basal media supplemented with benzyladenine (5 mg/l) in a so-called multiplication phase. The obtained plantlets were used for the following experiments (Fig.1).

Incubation with polyethylene glycol: To determine the lethal concentration of polyethylene glycol for each cultivar, PEG-6000 was added to solid basal media at levels of 0, 5, 15, 25, 35 and 45 g/l. The produced shoots were cultured for 4 weeks on these media and the percentage of survival was determined.

Polyethylene glycol (0, 10 and 20 g/l) was added to basal media which were either supplemented with benzyladeneine (5 mg/l) to produce shoots or α-naphthalene acetic acid (1 mg/l) to produce roots. After 4 weeks, shoot or root growth and development were determined. This procedure was applied in liquid and in solid media (0.6 % agar). Shoot strength (or vigour) was determined according to Pottino (1981) on a rating scale: 1 = no growth, 2 = below average, 3 = average, 4 = above average, 5 = excellent.

Analytical methods: The metabolites were determined from oven-dried shoots. 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 were determined by the method adopted by Shaffer and Hartmann (1921). Proline, was quantified in ethanol extract according to Bates *et al.* (1973).

All media were filled into 200 ml Pyrex-glass jars (25 ml/jar), autoclaved for 20 min at 121°C and 1.2 kg/cm^2 pressure, then cooled and kept for 4-15 days before use. In all media, pH was adjusted to 5.7 (before adding agar). Growth was in a growth chamber at 25±3 °C and 40 µmol m^{-2}s^{-1} continuous photosynthetic photon flux provided by cool white fluorescent lamps.

Statistical analysis: All experiments were repeated twice and conducted by using a completely randomised design in factorial arrangement with at least 4 replicates. All data were averaged and statistically analysed 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

Polyethylene glycol is a hydrophilic alcohol polymer with high water solubility and low toxicity (Fontana *et al.*, 2001). We used it in our study as an inert non-penetrating osmoticum (Almansouri *et al.*, 2001). Explant survival in the multiplication phase was significantly decreased by high PEG levels (≥ 15 g/l) (Fig. 2), but the inhibition was more evident in case of the cultivars Maghraby and Valery than Grand Nain and Hindy. The latter survived partly up to 45 g PEG /l.

PEG treatments reduced shoot multiplication and biomass per shoot, especially in the cultivars Maghraby and Valery and less in Grand Nain and Hindy (Table 1). Increasing PEG concentration in the rooting medium increased both root number and, slightly, root length. This increase was highest in Hindy, followed by Grand Nain, Valery and Maghraby (Table 1). This may be interpreted as an adaptive response of roots to alleviate the reduced water availability. Thus cultivar Hindy appeared to be the most adapted to water shortage.

The comparison of shoot and root growth at 20 g/l PEG on solid (agar) medium and in liquid medium revealed that growth was better in liquid medium (Table 2). This finding agrees with previous results by Ebrahim (2004) with Calla and Ebrahim and Ibrahim (2000) on Maranta, who attributed this response to a better availability for growth substances and nutrients and better aeration in liquid media. Also agar may add an osmotic effect in addition to PEG. The cultivar tolerance to PEG with respect to growth was again greatest in the order Hindy>Grand Nain>Valery>Maghraby (Table 2).

From the growth response of the 4 banana cultivars it was obvious that cultivar Hindy could adapt best to water stress, followed by Grand Nain and then Maghraby and Valery (with the latter two virtually identical). The analysis of cell contents should show whether the stress tolerance was correlated with accumulation of nutrient salts or production of compatible osmotica, especially soluble sugars and proline. N, P and K content of shoots decreased with increasing levels of PEG, somewhat less in cultivar Hindy than in Grand Nain or Maghraby (Fig. 3). On solid medium the N, P and K contents were marginally but consistently lower than in liquid medium.

A different, complex picture emerged for sugars and proline. Explants of all cultivars in liquid medium exhibited increasing levels of sugars and proline with increasing PEG concentrations. On solid medium there was the same trend, but the difference between no PEG treatment and 10 g/l PEG was small or absent. In addition the level of sugars and proline were higher on solid medium than in liquid medium when PEG was absent, but lower

Table 1. Shoot growth, shoot development, root growth and root development of *in vitro* cultured banana as affected by the cultivar and PEG concentrat□
supple□
acid (1 mg/l, rooting medium) (Mean ±SD)

Cultivar	PEG (g/l)	Shoots/explant	Shoot fresh weight (g)	Roots/shoot	Root length (cm)
	0	3.02 ± 0.13	0.88 ± 0.04	3.4 ± 0.12	5.2 ± 0.18
Maghraby	10	1.96 ± 0.04	0.73 ± 0.04	4.2 ± 0.17	5.3 ± 0.14
	20	2.12 ± 0.05	0.41 ± 0.01	4.9 ± 0.20	5.6 ± 0.12
	0	3.00 ± 0.11	0.86 ± 0.04	3.6 ± 0.16	5.4 ± 0.18
Valery	10	2.01 ± 0.09	0.70 ± 0.05	4.3 ± 0.18	5.5 ± 0.14
	20	1.04 ± 0.05	0.44 ± 0.02	5.3 ± 0.24	5.8 ± 0.19
	0	3.04 ± 0.04	0.83 ± 0.03	3.8 ± 0.10	5.6 ± 0.26
Grand Nain	10	2.51 ± 0.10	0.74 ± 0.05	5.0 ± 0.18	5.6 ± 0.18
	20	1.57 ± 0.12	0.56 ± 0.02	6.1 ± 0.26	5.8 ± 0.14
	0	3.02 ± 0.12	0.85 ± 0.05	3.9 ± 0.14	5.6 ±0.18
Hindy	10	2.62 ± 0.13	0.80 ± 0.03	4.9 ± 0.18	5.7 ± 0.18
	20	1.96 ± 0.06	0.66 ± 0.03	6.2 ± 0.25	5.9 ± 0.19

Table 2. Shoot growth, shoot development, root growth and root development of *in vitro* cultured banana in presence of 20 g/l PEG on solid and liquid medium. Explants were cultured with 20 g/l PEG for 4 weeks on Murashige-Skoog basal medium containing benzyladenine (5 mg/l, multiplication medium) or on Murashige-Skoog basal medium containing naphthyl acetic acid (1 mg/l, rooting medium) (Mean ±SD)

Cultivar	Medium	Shoots/ explant	Shoot frresh weight (g)	Shoot vigour	Leaves/ shoot	Roots/ shoot	Root length (cm)
Maghraby	Solid	1.12 ±0.04	0.41±0.02	1.4 ± 0.05	2.8 ± 0.14	4.3 ± 0.19	5.6 ± 0.24
	Liquid	1.50 ±0.05	0.57 ±0.02	1.8 ± 0.06	3.2 ± 0.17	4.9 ± 0.21	6.9 ± 0.28
Valery	Solid	1.04 ±0.04	0.44 ±0.02	1.2 ± 0.04	2.9 ± 0.11	4.3 ± 0.18	5.8 ± 0.26
	Liquid	1.57 ±0.08	0.57 ±0.03	1.7 ± 0.07	3.2 ± 0.12	5.3 ± 0.24	7.1 ± 0.32
Grand Nain	Solid	1.57 ±0.06	0.56 ±0.03	2.0 ± 0.07	3.2 ± 0.14	4.8 ±0.22	5.8 ±0.24
	Liquid	1.93 ±0.04	0.66 ±0.04	2.3 ±0.08	3.8 ± 0.14	6.1 ± 0.25	8.1 ± 0.33
Hindy	Solid	1.96 ±0.04	0.66 ±0.05	2.7 ± 0.09	3.4 ± 0.11	4.8 ± 0.21	5.9 ± 0.22
	Liquid	2.22 ±0.08	0.71 ±0.04	3.1 ± 0.06	4.2 ± 0.15	6.2 ± 0.25	8.4 ± 0.32

Fig. 1. Regenerating banana plantlets from meristem tip culture. Successive stages of micropropagation of *Musa* spp. (cultivar. Hindy): (A) shoot tips were cultured on Murashige-Skoog basal medium containing benzyladenine (3 mg/l, starting stage), (B) shoot explants were proliferated on basal medium supplemented with benzyladenine (5 mg/l, multiplication stage), and (C) shoot explants were rooted on basal medium containing naphthyl acetic acid (1 mg /l, rooting stage).

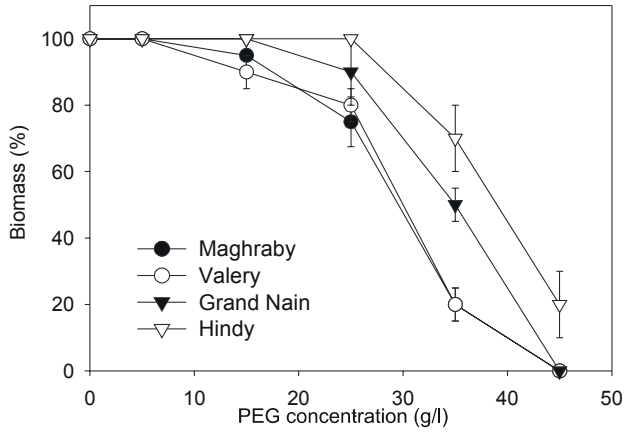

Fig. 2. Percentage of shoot survival of *in vitro* cultured banana cultivars in presence of PEG. Explants were cultured for 4 weeks on Murashige-Skoog basal medium supplemented with benzyladenine (3 mg/l, starting medium). (The results for Maghraby and Valery coincide at high PEG-concentrations) (Mean ±SD).

on solid medium than in liquid medium when PEG was present (Fig. 4). The different banana cultivars showed the same order in compatible solute content as in tolerance towards PEG, namely cultivar Hindy containing the highest levels of nutrients and compatible solutes followed by cultivar Grand Nain and then Maghraby.

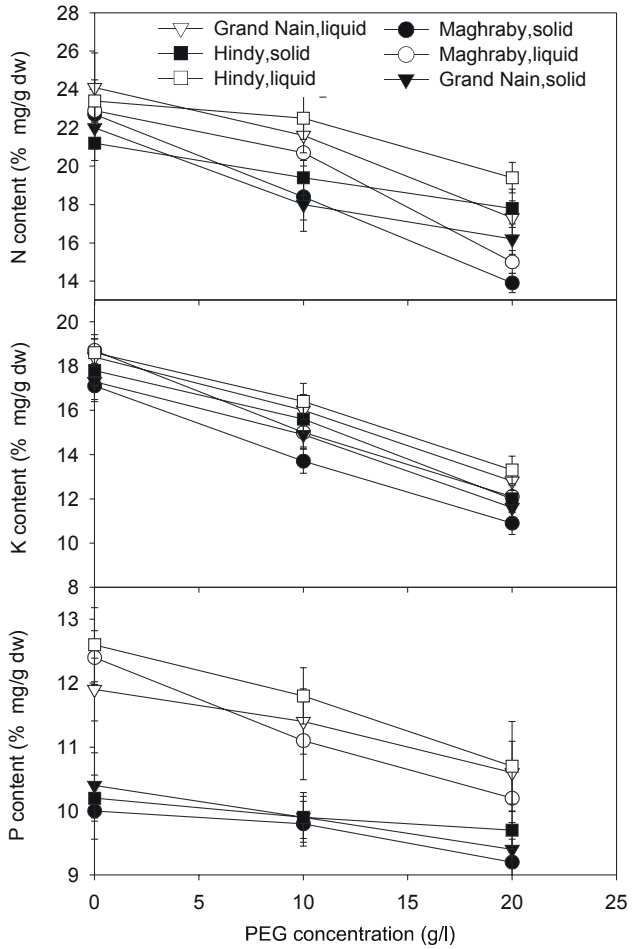

Fig. 3. N, P and K-content of shoots of *in vitro* cultured banana plantlets in presence of PEG on solid and liquid medium. Explants were cultured for 4 weeks on Murashige-Skoog basal medium containing

Discussion

Effects of PEG on osmotic performance had been reported for many plants (Bandurska, 2000; Pushpam and Rangasamy, 2000; Ronde *et al.,* 2000; Liu *et al.,* 2001). Predictably the growth of the small, micropropagated banana plants of cultivars Maghraby, Valery, Grand Nain and Hindy was influenced by application of PEG and, slightly, by the type of medium, but the magnitude of the response was consistently cultivar-dependent. The cultivar

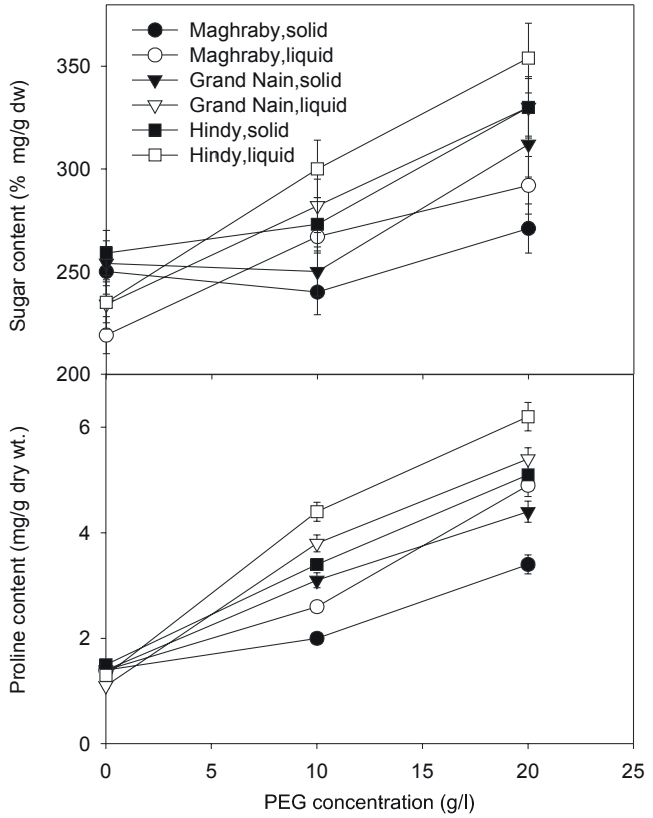

Fig. 4. Content of soluble sugars and proline in shoots of *in vitro* cultured banana plantlets in presence of PEG on solid and liquid medium. Explants were cultured for 4 weeks on Murashige-Skoog basal medium containing benzyladenine (5 mg/l, multiplication medium)

Hindy proved to be the most tolerant to PEG-induced water stress and cultivar Grand Nain was more tolerant than cultivars Valery and Maghraby. PEG in the medium led to less shoot growth and longer roots, thereby shifting the shoot/root ratio to lower values, an effect known for plant stress conditions (Brouwer, 1962). The cultivar Hindy, which turned out to be the most tolerant to PEG-treatment, was the one which had less shoot growth inhibition, but the strongest stimulation of root growth, *i.e.* it reacted strongly to the osmotic stress by favouring root growth and thereby minimized shoot inhibition. The cultivar Hindy was also the cultivar which exhibited the strongest stress response by increasing the content of proline 4-fold and of soluble sugar, mostly sucrose, 1.5-fold. The concentration of proline (4 mg/g d.wt.) however was much less than of sugars (200 mg/g d.wt.). Thus proline seems more indicative for the osmo-compatible solutes than really protective against osmotic stress. Nitrogen, potassium and phosphorus content decreased parallel with osmotic stress with only marginal cultivar differences. The absence of potassium increase was surprising because potassium does replace sugar as osmoticum in some sugar storing monocotyledons such as sugarcane (Glasziou and Gayler, 1972).

The comparison of solid and liquid medium appeared complex at first sight, but it may be explained on the basis of two simultaneous effects. Firstly agar exerts a small osmotic effect by itself, and secondly it slows down solute flux to the explants compared to flux in liquid medium. Therefore the banana plantlets in medium without PEG felt a small osmotic stress on agar and responded with higher values of sugar on solid medium compared to on liquid medium. In contrast, the addition of PEG to the medium had a larger effect on plantlets in liquid medium because they felt the osmotic stress immediately without noticeable diffusion barriers in their surroundings, whereas on agar a lower concentration of osmoticum may exist in the close environment of the roots due to localized nutrient uptake and extracellular metabolic degradation of solutes. In general it appears that plants on liquid medium give a better and clearer response to PEG treatments than plants on solid medium. Cultures in liquid media are also known to perform better in post-thaw regeneration (Khalil *et al.,* 2002).

Since it had been proven that banana plants from micropropagation show no differences in in-field behaviour compared to conventionally propagated plants (Cote *et al.,* 2000), it is suggested that the cultivar-specific osmotic response of the *in vitro* micropropagated banana plants is indicative of the drought tolerance of grown-up plants with the conclusion that cultivar Hindy may be the best for water-tight plantation conditions.

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