Variations in Polyamines and Growth Regulators under Different Conditions of Water Stress in Cell Suspension Cultures of Two *Acacia* Species

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Abstract: Changes in polyamines and growth regulator contents were investigated as associated with water stress exerted on cells of two *Acacia* species namely *A. farnesiana* and *A. nilotica*. This has been conducted in cell suspension cultures established from the callus cultures of both species as mentioned in the methods. Cells of both species were subjected to different levels of water stress induced by PEG as well as control cells grown in PEG-free medium were transferred to different levels of water stress to evaluate tolerance potential of the selected cells upon the non-selected cells. Some of the cells were transferred to PEG-free medium for recovery (recovered cells) to asses physiological activity on relief of the stress. On the other hand, cells of both species were exposed directly (shocked cells) or gradually (adapted cells) to high levels of water stress. The most interesting results were the accumulation of putrescine, spermidine, and spermine in the selected and non-selected cell lines of both *Acacia* species exposed to low and moderate levels of water stress. Stressed cells of both *Acacia* species contained higher concentrations of all polyamines than the recovered cells. Shocked cells of *Acacia farnesiana* accumulated high levels of putrescine, spermine and total polyamines, while in the adapted cells, a slight decrease in spermine and total polyamines and an increase in putrescine were observed. The most interesting,. In general, a reduction in the concentrations of IAA and gibberellic acid (GA_3), and enhancement of IBA levels were observed, particularly in the stressed and shocked cells and to a lower extent in the non-selected and selected recovered, and adapted cells of both species. Meanwhile, a general increase in total cytokinins was obtained in the non-selected and selected cell lines of both species, with a drop after stress relief. Furthermore, total cytokinins of the stressed, shocked and adapted cells of *A. nilotica* were higher than those of *A. farnesiana*. Abscisic acid (ABA) levels showed a positive correlation with the extent of water stress tolerance where higher levels were recorded in the more tolerant *A. farnesiana* than the less tolerant *A. nilotica*, under different conditions.

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1. Introduction

Acacia is an important genus of family *Leguminosae;* subfamily *Mimosoideae* (Boulos, 1999). This genus is widely distributed throughout the arid and semiarid regions of the world. *Acacia farnesiana* (sweet *Acacia* or sponge tree) is naturalized and cultivated in Egypt (Duke, 1981), whereas *Acacia nilotica* (Egyptian *Acacia*, Nile *Acacia*, Gum Arabic tree or Sant) is an endemic species commonly seen on the banks of the River Nile and along the water channels (Boulos, 1999). This plants, either acclimated or originally growing in their natural habitat, represent a valuable resource for elucidating mechanisms of acclimation to environmental constraints (Brosche *et al.*, 2005). In this respect, plant cell and tissue cultures provide a unique system for studying the problem at the cellular level, especially when the stress agent is directly applied to plant cells in suspension cultures (Collin and Edwards, 1998). Polyamines and plant growth regulators are among the controlling factors that regulate and integrate the different physiological and biochemical processes affected by water stress (Wittenmayer and Merbash, 2005 and Christmann *et al.*, 2006).

The diamine putrescine, and to a lesser extent the polyamine spermidine, accumulate in response to various environmental stresses (Crozier *et al.*, 2000; and Jian Chang *et al.* 2004). MingGong *et al.* (2004) reported that the contents of putrescine, spermidine and spermine were increased under water stress in seedlings of drought-resistant wheat strains. They added that exogenous application of spermidine obviously increased the resistance of drought-sensitive wheat to water stress. Scaramagli *et al.* (2000) stated that acclimation to low water potential in potato cell suspension cultures led to an increase in soluble putrescine over the unacclimated cells. Capell *et al.* (2004) found that transgenic plants produced much higher levels of putrescine, spermidine and spermine under stress, thus ultimately protecting the plants from drought. On the other hand, as compared with the wild-type, transgenic *Arabidopsis thaliana* plants exhibited a significant increase in spermidine synthase activity and spermidine content in leaves together with enhanced tolerance to various stresses including

drought (Kasukabe *et al.*, 2004).

Stresses generally alter the level of specific hormones and the sensitivity of plants to them (Munns, 2002). Under drought stress, indoleacetic acid (IAA) content generally shows a significant decrease (Xiao *et al.*, 2005 and Li *et al.*, 2005). On the other hand, Sofo *et al.* (2005) found that the patterns of IAA oxidase activity in olive trees (2-year old) ran in alliance and showed an increase in relation to the degree of drought. Contradictory the results reported by Ghasempour *et al*. (2001) showed that IAA had no effect on the protoplasmic drought tolerance (PDT) of cell suspensions of the resurrection grass *Sporobolus stapfianus*. Ludwig- Muller *et al.* (1995) reported that maize seedlings growing *in vitro* under drought conditions or osmotic stress showed a dramatic increase in the activity of indolebutyric acid (IBA) synthetase. This increase involved IAA as a direct precursor and appeared likely as an escort to enhanced abscisic acid (ABA) (Ludwig-Muller, 2000).

Xiao *et al.* (2005) recorded a reduction in the level of GA_3 in the roots of rice under drought stress. Exogenous application of gibberellic acid (GA_3) in cotton plants (60-day- old) one day prior drought stress treatment alleviated the effect of drought on the relative water content (Renu *et al.,* 2004). Ghasempour *et al.* (2001) reported that exogenous application of GA_3 improved protoplasmic drought tolerance (PDT) of cell suspensions of the resurrection grass *Sporobolus stapfianus*.

ABA plays an important role in drought-stress adaptation of plants (Xiong and Zhu, 2003; Wittenmayer and Merbash, 2005; Christmann *et al.,* 2006). ABA increased significantly under water deficit in *Phaseolus vulgaris* (Wakrim *et al.,* 2005), in *Populus Kangdingensis* (Yin *et al.,* 2005) and in two cultivars of Japanese mint (Priti *et al.*, 2005). Zhang *et al.* (2005) found that the dry climate ecotype of *Populus davidiana* exhibited higher ABA as affected by low soil water contents than the wet climate ecotype. Higher ABA contents were also recorded in drought tolerant genotypes (Aruna and Sairam, 2001) and selected calli of rice under water stress (Perales *et al.*, 2005). ABA might influence the accumulation or operation of other hormones such as IBA (Ludwig-Muller, 2000), gibberellins (Benson *et al*., 1990), and ethylene or growth regulators as polyamines (Upreti and Murti, 2005). Moreover, Sugiharto *et al.* (2002) reported the presence of a drought-inducible gene (*SoDip22*) in sugarcane leaves which functions for adaption to drought stress in the bundle sheath cell, where the signaling pathway for the induction is, at least in a part, mediated by ABA.

Under stress, cytokinin may be involved indirectly in the control of cell division and turnover of development in the growing regions (Munns and Cramer 1996). Lower concentrations of zeatin and zeatin riboside (Zr) were found in stressed plants (Nikolaou *et al.* 2003 and Li *et al.* 2005). Water stress led to a decline in Zr in leaves of four onion cultivars (Upreti and Murti, 2004), in roots of a rice cultivar (Xiao *et al.*, 2005) and in sunflower plants after a transient rise (Hansen and Dorffling 2003). On the other hand, Li *et al.* (2003) did not find obvious differences in the concentrations of Zr between drought stressed and control apple trees in both leafless and leafy types. Foliar application of benzyl adenine (BA) exhibited alleviatory effect on drought in cotton plant (Renu *et al.,* 2004). Pospisilova *et al.* (2005) added that pretreatment with ABA further increased cytokinin content in water stressed bean and tobacco, while BA pretreatment increased that of cytokinin in sugar beet and tobacco after rehydration.

Thus, the present work intended to investigate the impact of drought stress on the metabolism of two species of *Acacia* namely *Acacia farnesiana and Acacia nilotica* growing in Egypt. Cell suspension cultures were obtained from callus tissues of these species, then a comparison was carried out between the controls, stressed, shocked, recovered, and adapted cells. This aimed to determine which of the two species under study could gain more tolerance to drought and the possible role of polyamines and phytohormones in this respect.

2. Materials and Methods

Materials

Legumes of *Acacia farnesiana* were obtained from trees grown along the motorway between Cairo and Ismaelia. Legumes of *Acacia nilotica* were obtained from EL-Orman Garden, Cairo, Egypt. Murashige and Skoog medium (MS) and all the chemicals used in the present work were obtained from Duchefa Company, Netherlands and Sigma Aldrich Chemical Company. The agar used in this study was a purified bacteriological agar obtained from Marine Chemicals, India.

Methods

Preparation of explants, callus induction and cell suspension

All the procedures of tissue culture techniques were performed under aseptic conditions using Laminar Air-flow Cabinet. Uniform seeds of *Acacia farnesiana* of similar size, shape, and colour were sterilized and germinated as described in Ismail (2000). The hypocotyls were separated from the produced seedlings, cut into small sections (1cm each), and used as explants for callus induction. Green legumes of *Acacia nilotica* were rinsed thoroughly with water, surface sterilized and dissected aseptically and the immature green seeds including embryos were taken off and used as explants for callus induction. Explants of *Acacia farnesiana* hypocotyls or *Acacia nilotica* immature green seeds were placed on the surface of previously cooked MS culture media supplemented with 1.5 mg/L 2,4-D and 0.5 mg/L benzyl amino purine (BAP) for *A. farnesiana* and 3 mg/L 2,4-D and 5 mg/L BAP for *A. nilotica*. The media were also supplied with 3% sucrose and the pH was adjusted to 5.8 ± 0.1 prior to the addition of agar (0.8 % w/v). Explants

were incubated at 25˚C ± 2 and continuous light (intensity of 35 µmol m^{-2} s^{-1}).

The Calli were firstly subcultured regularly every four weeks until a callus line was achieved. After four passages, the calli were divided aseptically into small pieces and transferred to quick-fit jars containing MS liquid medium with the same growth regulators mentioned before. Different concentrations of polyethylene glycol (PEG) were added to the liquid medium according to the type of the respective experiment. Cell suspension cultures were incubated at the same conditions mentioned above.

1. Effect of different levels of PEG- induced water stress on selected and non-selected cell lines

Cell suspension culture of each species was divided into two groups; the first was grown and subcultured twice in liquid MS medium with the same hormone composition and 15% PEG for *A. farnesiana* and 10% PEG for *A. nilotica* (these concentrations of PEG were the moderate according to the tolerance of the two species, Binggeli, 1997). Cells, after these two passages, were harvested (necrotic cells were discarded visually) and termed "selected cells". The second group of suspension cultures (control) were continuously grown and subcultured twice in PEG-free MS liquid medium with the same hormone composition. These cells were then harvested and represented "non-selected cells". In the third subculture, the selected and non-selected cells of both species were transferred to MS medium supplemented with the same hormone composition in addition to different concentrations of PEG (0, 10, 15, 20 or 25% corresponding to – 0.34, – 0.55, – 0.83, – 1.33 and – 1.67 MPa, respectively). Each experiment was represented by three replicates. Three weeks later, cells of both groups were collected by filtration.

2. Effect of exposure to and recovery from PEG-induced water stress

In this experiment, established cell suspension cultures of both *Acacia* species were sub cultured in liquid MS medium as mentioned before in exp. 1 (first group). After three weeks stress period, half of the materials were preserved frozen and termed "stressed cells". The remaining materials were transferred to PEG-free liquid MS medium for another three weeks as a recovery period. These cells were termed "recovered cells".

3. Effect of sudden or gradual exposure to high levels of PEG- induced water stress

Cell suspension cultures of both species were divided into two groups. Cells of group (1) were transferred directly to liquid MS medium with the same growth regulators and 25% PEG. After three weeks, cells were then collected and represented "shocked cells". Cells of group (2) were transferred to MS medium with the same growth regulators in addition to 10% PEG. The same cells were then transferred every one week to fresh medium with stepwise increase of PEG by 5% each time until it reached 25% PEG (i.e. 10, 15, 20 and 25% PEG). Cells were then collected and represented "adapted cells".

Fresh materials of different cell lines of both *Acacia* species were collected for the analysis of polyamines and growth hormones.

Extraction and determination of polyamines

The extraction procedure was similar to that followed by Flores and Galston (1982), as adopted by Deabes (2000). The dansylated samples were stored at -20 ºC. Under these conditions, samples were found to still stable for several months. Aliquots of 5-10 µl were taken for injection in the HPLC. Standards were treated in a similar way, with up to 50 µl of each polyamine in the reaction mixture. For HPLC analysis of different PAs (Put, Spd & Spm), the parameters described by Smith *et al.* (1985) were applied with some modifications as described by Ismail (2000).

Extraction and determination of phytohormones

The extraction procedure of acidic hormones (IAA, GA_3, and ABA) and cytokinins was done according to Shindy and Smith (1975).To estimate the amounts of acidic hormones, the plant hormone fractions and standard ones were methylated according to Vogel (1975) to be ready for gas chromatography (GC) analysis. For identification and detection of cytokinin fractions, the procedure of Müller and Hilgenberg (1986) was used.

3. Results and Discussion

Changes in polyamine contents

The data presented in Table (1) show a progressive depression of all polyamine fractions in both cell lines of the two *Acacia* species under investigation with increasing the level of PEG over 15%. It is also worth mentioned that putrescine (put) was dominant in the selected cells of *A. farnesiana* and both the selected and non-selected cells of *A. nilotica*, whereas spermine (spm) was dominant in the non-selected cells of *A. farnesiana*. Moreover, those of the selected line of *A. farnesiana* contained higher putrescine and spermidine (spd) and lower spermine than the non-selected cells under the influence of all the used concentrations of PEG. Such high levels of put, followed by spd in the selected cells of the more tolerant species *A. farnesiana* seems to be meaningful since put, as a precursor for polyamines, might be responsible of enhancing the potentiality of the selected cells to synthesize more polyamines for improving cell acclimation to the

applied stress (Kasukab *et al*., 2004). Recent studies also proved that several genes which control polyamine biosynthesis appeared to be controlled stringently by stress signaling (Panicot *et al.*, 2002).

In the second experiment, it could be generally outlined that the stressed cells of *A. farnesiana* (grown at 15% PEG) and those of *A. nilotica* (grown at 10% PEG) showed markedly high levels of total polyamine contents (TPAs), as compared with corresponding controls (Table 2). On recovery of the stressed cells in PEG-free media, the polyamine fractions became more or less comparable with the control levels in *A. farnesiana*, whereas a noticeable reduction in put, spd and TPAs concentrations was shown in *A. nilotica*. Moreover, the modulation of polyamine levels in the stressed and the recovered cells of both *Acacia* species is in agreement with the results of many other workers in this respect (e.g. Capell *et al*., 2004; JianChang *et al*., 2004).

In the third experiment, when cells of *A. farnesiana* were suddenly exposed to a high level of water stress (shocked cells), they accumulated high concentrations of put, spm, and TPAs (Table 2). The same polyamine fractions showed a reversed pattern in the shocked cells of *A. nilotica*, where their concentrations were highly depressed. Gradual exposure of the cells to PEG (adapted cells) resulted in a slight decrease in spm and TPAs, a sharp decrease in spd, and increased put in *A. farnesiana*. In the adapted cells of *A. nilotica*, put, spd and consequently total polyamines (since spm level was not changed) were markedly decreased. According to Nayyar *et al*. (2005), such a decrease in the adapted cells of *A. nilotica* might indicate higher stress injury in these cells.

In this connection, polyamines, chiefly spd, were found to modulate cell activities to cope with stress (Liu *et al.*, 2000b). In the polyamine pool, spm usually represents a stored form, which can compensate shortage in minor forms under cell requirements (Shoji *et al*., 2000). Thus, on the bases of the results obtained in the present work, it was assumed that the introversions between put (the precursor) and higher polyamines (the tri-amine spd and tetra-amine spm) might be genetically determined according to the magnitude of cell resistance to a certain stress factor. Consequently, the higher tolerance of *A. farnesiana* to water stress would then be mainly channeled via spm so that, on need, it would easily furnish the cell with lower polyamines. This assumption might be supported by MingGong *et al.* (2004) who revealed that PEG-induced osmotic stress was underlined with increased contents of put, spd and spm in the seedlings of a drought resistant strain of wheat, whereas in those of the drought sensitive strain, only put was enhanced.

Table (1): Concentrations (mg/100g d.wt. equivalent) of putrescine (Put) , spermidine (Spd), spermine (Spm), and total polyamines (TPAs) of the non-selected and selected cell lines of *Acacia farnesiana* and *Acacia nilotica* exposed to different concentrations of PEG (0, 10, 15, 20 or 25%).

PEG CONC. (%)	Concentrations (mg/100 g d.wt equivalent)															
	Acacia farnesiana								*Acacia nilotica*							
	Non- selected cell line				Selected cell line				Non- selected cell line				Selected cell line			
	Put	Spd	Spm	TPAs	Put	Spd	Spm	TPAs	Put	Spd	Spm	TPAs	Put	Spd	Spm	TPAs
0	2.29	1.99	9.10	13.38	5.78	2.25	4.53	12.56	24.95	7.16	2.58	34.69	6.06	4.57	2.63	13.26
10	8.14	1.81	21.66	31.61	13.75	8.53	5.77	28.05	33.91	8.16	6.63	48.70	8.98	4.61	7.01	20.60
15	10.43	0.75	23.10	34.28	19.45	3.01	7.24	29.70	22.96	5.64	4.37	32.97	19.67	6.25	3.22	29.14
20	7.11	0.62	17.10	24.83	6.71	2.02	6.40	15.13	6.94	3.90	4.19	15.03	18.10	4.63	2.40	25.13
25	7.87	0.546	11.15	19.56	7.47	1.94	5.57	14.98	2.23	0.23	2.05	4.51	6.19	1.53	0.797	8.52

Table (2): Concentrations (mg/100g d.wt. equivalent) of putrescine (Put), spermidine (Spm), spermine (Spm), and total polyamines (TPAs) of the control (0% PEG), stressed cells (exposed to 15% and 10% PEG for *Acacia farnesiana* and *A. nilotica*, respectively), recovered cells (stressed cells transferred to 0% PEG)), shocked cells (exposed directly to 25% PEG) and adapted cells (exposed gradually to 25% PEG) of *Acacia farnesiana* and *A. nilotica*.

Treatment	Concentrations (mg/100 g d.wt equivalent)							
	Acacia farnesiana				*Acacia nilotica*			
	Put	Spd	Spm	TPAs	Put	Spd	Spm	TPAs
Control	2.29	1.99	9.10	13.38	24.95	7.16	2.58	34.69
Stressed cells	10.43	0.75	23.10	34.28	33.91	8.16	6.63	48.70
Recovered cells	5.78	2.25	4.53	12.56	6.06	4.57	2.63	13.26
Shocked cells	7.87	0.546	11.15	19.56	2.23	0.23	2.05	4.51
Adapted cells	3.20	0.397	8.36	11.96	3.01	0.50	2.5	6.01

Growth regulating substances

The results obtained in the present work generally showed progressive decrease in the concentration of indoleacetic acid (IAA) with the increase of PEG- induced water stress in either the selected or the non-selected cells of both *Acacia* species (Figure 1). A reverse pattern was evident with indolebutyric acid (IBA), which showed maximum concentration at the highest level of PEG (25%). A reduction in IAA concentration was also observed in the stressed and recovered cells (stressed transferred to PEG-free medium) of both species (Figure 2). On the other hand, IBA showed accumulation in the stressed and recovered cells of *A. farnesiana* and the stressed cells of *A. nilotica*, but was decreased in its recovered cells. In the shocked and adapted cells of *A. farnesiana* and *A. nilotica* (Figure 3), IAA concentration was lower than the corresponding control level, whereas a reverse pattern was recorded with IBA (Figure 3). All these results agreed to a wide extent with those of other authors concluding auxin depression under the influence of drought stress (Xiao *et al*, 2005), where such a change was concomitant with the reduced growth rates (Munns, 2002). The decreased levels in IAA could be mainly correlated with enhanced IAA oxidase activities (Sofo *et al*., 2005). In this instance, Pustovoitova *et al*. (2000) indicated involvement of IAA in the adaptation of tobacco plants to drought, and showed that tolerant transgenic plants were carrying the recessive *iaaM* and *iaaH* genes of auxin biosynthesis. The transduction pathways of the stress signals *via* auxins might be conceivable through regulation of a calmodulin gene (*MBCaM-1*; Botella and Arteca, 1994), a calcium-dependent protein kinase (*VrCDPK-1*; Botella *et al*., 1996), and a 1-aminocyclopropane-1-carboxylic acid (ACC) synthase gene (*AIM-1*; Botella *et al*., 1995). The enhancement of IBA levels in cells of both *A. farnesiana* and *A. nilotica* could also be supported by the results of Ludwig-Muller (2000), which showed increased IBA accumulation under osmotic and drought stresses; a process which involved IAA as a direct precursor and appeared likely as an escort to elevated abscisic acid (ABA) levels.

The trends of changes in GA_3 in cell suspension cultures of *A. farnesiana* and *A. nilotica* were approximately similar to those of IAA in being depressed with the increase of applied stress. In this respect, *A. nilotica* was retaining relatively higher levels of this hormone than *A. farnesiana* (Figure 1). A similar conclusion was also generally shown, with regard to the changes in GA_3 concentrations in the stressed and recovered cells, as well as the shocked and adapted cells of both species (Figure 2). Such a trend was also observed by Itai (1999). Herein, it might be speculated that the decrease in GA_3 levels under stress might represent a consequence of retarded cell activity, as an adaptive step (Munns, 2002). However, recent studies on the gene level for regulation of hormone biosynthesis and deactivation by other hormones indicated that GA biosynthesis depends on auxin and at the same time gibberellins can be viewed as component of the auxin signal transduction pathway (Reid *et al*., 2004). It should also be added that ABA is the hormone mostly associated with stress (Itai, 1999). Inverse relations for the levels of ABA, gibberellins, and cytokinins would be expected on the basis of sharing a general precursor (isopentenyl diphosphate; C_5), whether from the chloroplast-derived isoprenoids (forming methylerythritol phosphate) or the cytosol-derived isoprenoids (forming mevalonate) (Schwartz and Zeevaart, 2004). This could be observed from the present results with ABA (Figure 1), which showed elevated levels of ABA in response to drought; a typical response of drought-induced water deficit (Hansen and Dorffling, 2003). This is assumed to play a role in osmotic adjustment and plant adaptation (Wittenmayer and Merbach, 2005). In addition, the decreased ABA contents in the recovered cells of *A. farnesiana* and *A. nilotica* (Figure 2) could be supported by the conclusion of Zhang *et al*. (2006) that ABA accumulation in the alarm phase of stress is met by an equally rapid drop in the regeneration (recovery) phase when such stresses are relieved.

The changes in cytokinins (zeatin or zeatin riboside) showed enhanced levels at 15% and 10% PEG in *A. farnesiana* and *A. nilotica*, (Figure 4). This result could be further supported by those of Thomas *et al*. (1995), Pospisilova *et al*. (2005), who reported enhanced cytokinin levels as signs of plant responses to adverse conditions, including water stress. In this connection, there is evidence that some of the genes expressed under stress are likewise known to be transcriptionally regulated by ABA and cytokinins (Itai, 1999). The most interesting point in this respect might be the interaction of ABA with cytokinins for triggering the synthesis of specific protein kinases included within the signal transduction cascades, converting water stress cues into outputs such as control of gene expression and metabolic regulations (Bano *et al*., 1993 & 1994, Davies, 2004). On the other hand, the decreased total cytokinin contents in the non-selected and selected cell lines of *A. farnesiana* and *A. nilotica*, at higher levels of drought stress, as well as after stress relief in *A. farnesiana* (Figure 4), might be supported by the results of some other workers who revealed low cytokinin levels

associated with stress (Goicoechea *et al*., 1995; Badenoch *et al*., 1996). However, Vaseva-Gemisheva *et al*. (2005) reported that complicated regulating mechanisms of the enzyme cytokinin oxidase/dehydrogenase (CKX), responsible of modulating the cytokinin pool during stress, take place throughout the duration of environmental adverse conditions. Furthermore, the lower total cytokinins in the selected, shocked, and adapted cells of the less tolerant *A. nilotica* than the more tolerant *A. farnesiana* (Figure 5), in the present work, might be reinforced by the conclusion of Upreti and Marti (2004), revealing that cultivars with higher ABA concentrations and lesser decline in cytokinin would be relatively with higher drought tolerance. Thus, on the bases of the results obtained, herein, it may be assumed that conflicting changes in cytokinin levels are shown throughout the duration of water stress prevalence, where an enhancement is achieved at the starting stage of subjection to water stress. It should also be added that difference in the physiological modes of the two species under study was accompanied by only quantitative changes in cytokinin levels, while their trends under different conditions were approximately similar.

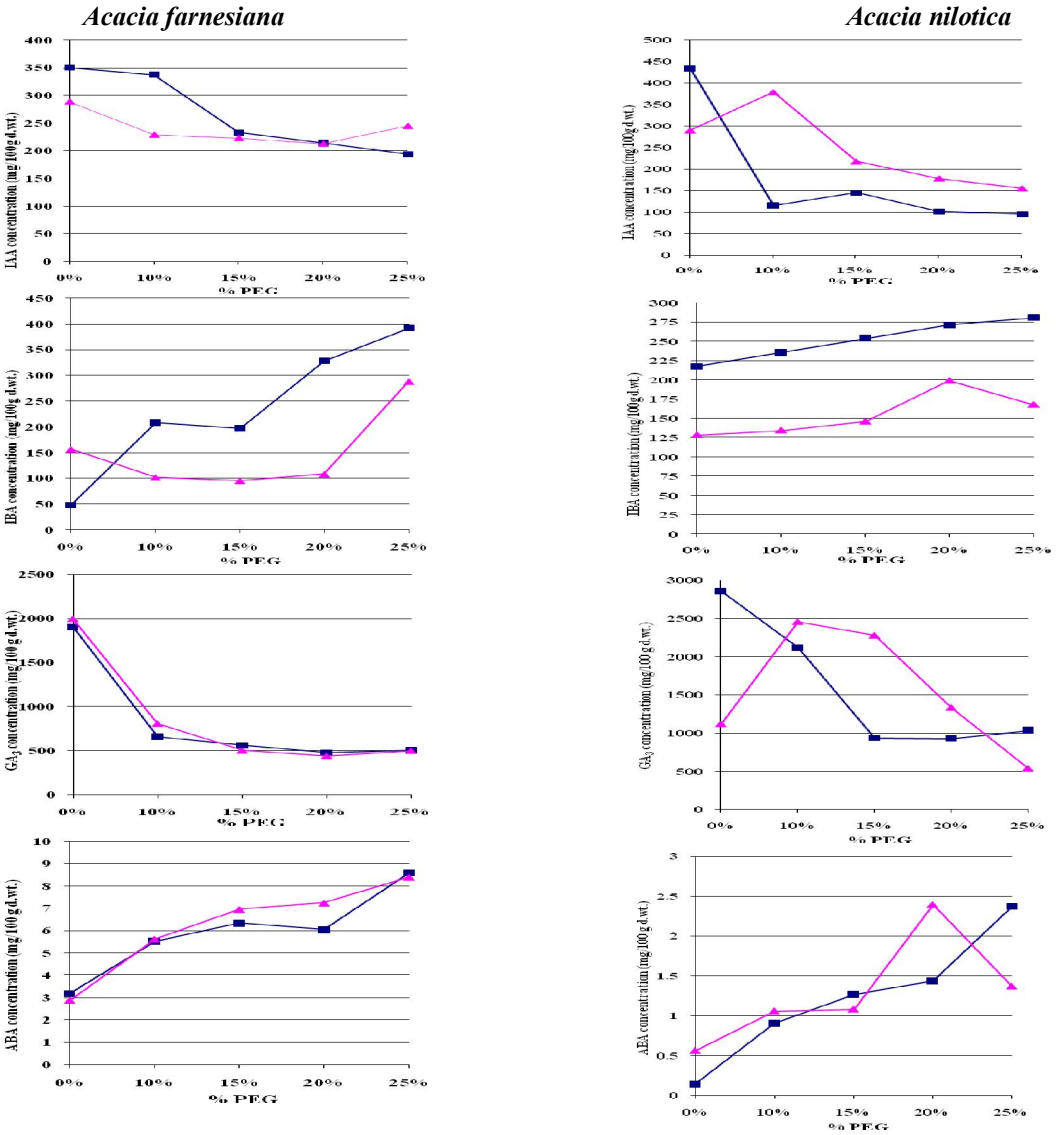

Figure (1): Concentrations (mg/100g d.wt. equivalent) of indole acetic acid (IAA), indole butyric acid (IBA), gibberellic acid (GA_3) and abscisic acid (ABA)of the non-selected and selected cell lines of *Acacia farnesiana* and *A. nilotica* exposed to different concentrations of PEG (0, 10, 15, 20 or 25%).

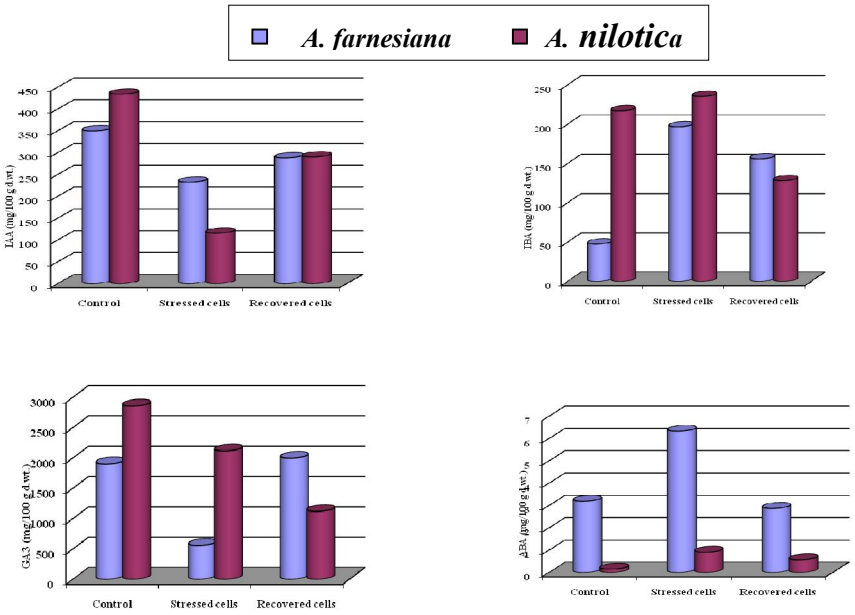

Figure (2): Concentrations (mg/100g d.wt. equivalent) of indoleacetic acid (IAA), indolebutyric acid (IBA), gibberellic acid (GA_3) and abscisic acid (ABA) of the control (0% PEG), stressed cells (exposed to 15% and 10% PEG for *Acacia farnesiana* and *A. nilotica*, respectively) and recovered cells (stressed cells transferred to 0% PEG) of *Acacia farnesiana* and *A. nilotica*.

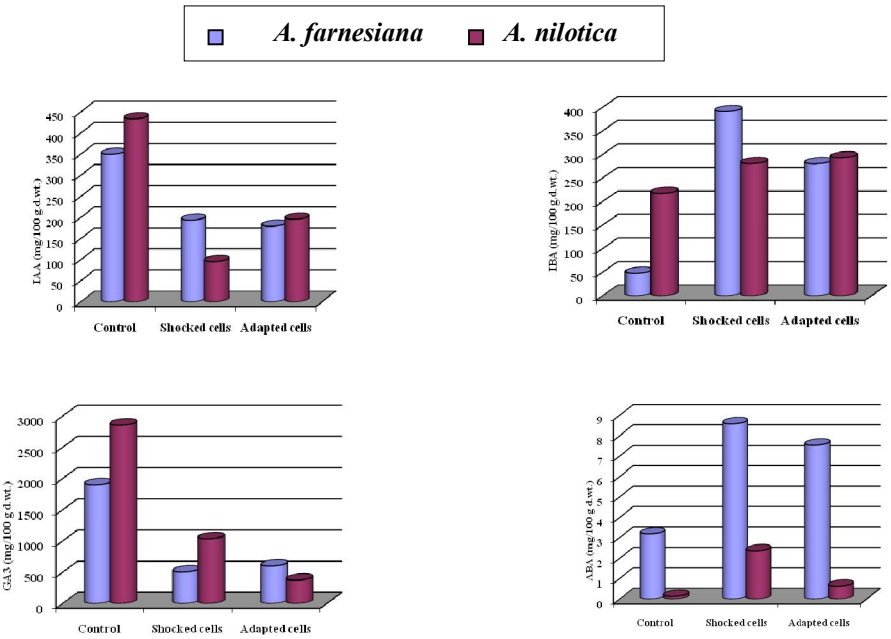

Figure (3): Concentrations (mg/100g d.wt. equivalent) of indole acetic acid (IAA), indole butyric acid (IBA), gibberellic acid (GA_3) and abscisic acid (ABA) of the control (0% PEG), shocked cells (exposed directly to 25% PEG) and adapted cells (exposed gradually to 25% PEG) of *Acacia farnesiana* and *A. nilotica*.

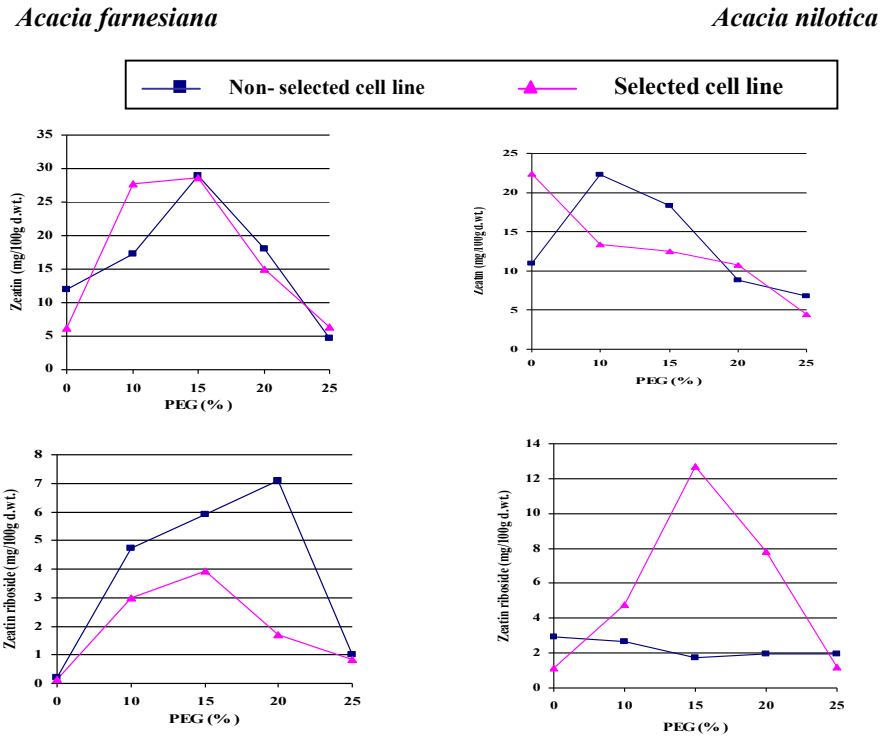

Figure (4): Concentrations (mg/100g d.wt. equivalent) of zeatin and zeatin riboside of the non-selected and selected cell lines of *Acacia farnesiana* and *A. nilotica* exposed to different concentrations of PEG (0, 10, 15, 20 or 25%).

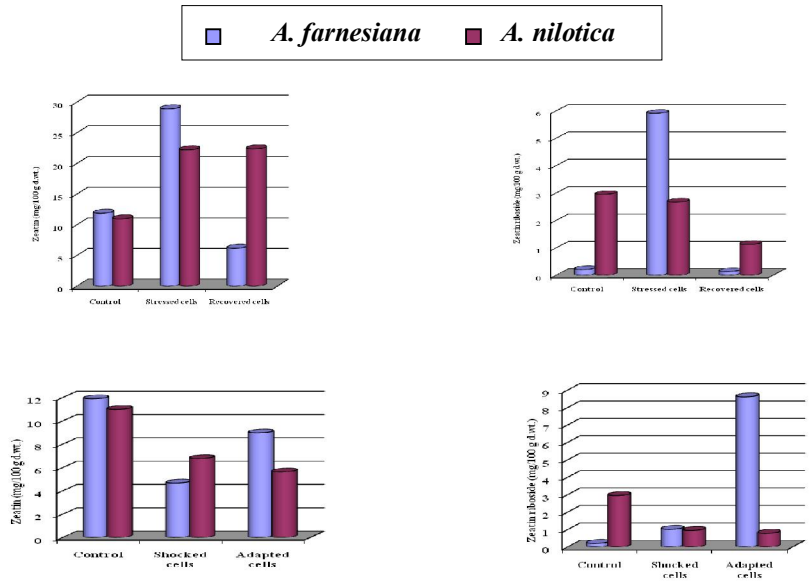

Figure (5): Concentrations (mg/100g d.wt. equivalent) of zeatin and zeatin riboside of the control (0% PEG), stressed cells (exposed to 15% and 10% PEG for *Acacia farnesiana* and *A. nilotica*, respectively), recovered cells (stressed cells transferred to 0% PEG) and the control (0% PEG), shocked cells (exposed directly to 25% PEG) and adapted cells (exposed gradually to 25% PEG) of *Acacia farnesiana* and *A. nilotica*.

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