DROUGHT-INDUCED ALTERATIONS IN GROWTH, OSMOTIC POTENTIAL AND *IN VITRO* REGENERATION OF SOYBEAN CULTIVARS

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Summary. Environmental stresses result in water deficiency for the plants, thus impairing its numerous biological roles. *In vitro* screening for stress tolerance will have its significance in identifying cultivars with optimal stress tolerance and productivity. In the present study, drought-induced alterations in growth, osmotic potential and *in vitro* shoot multiplication of soybean cultivars from India and Bulgaria were assessed with different concentrations of polyethylene glycol (PEG 6000 MW). *In vitro* callus cultures of both Indian (Hardee and JS 335) and soybean cultivars grown in Bulgarian (Collina and Korada) showed a reduction in callus growth during PEG treatment as compared with the control. The presence of PEG in the medium elevated dry matter content in all treatments compared with the control. Cotyledonary nodal explants subjected to 6 % PEG 6000 treatments resulted in 57.8 % and 68.5 % reduction in shoot induction in case of Hardee and JS 335, respectively, compared with the control. Our results can be used for *in vitro* screening and manipulations of soybean cultivars for improvement of drought tolerance.

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Key words: drought, *in vitro* screening, polyethylene glycol, soybean.

Abbreviations: PEG - polyethylene glycol, RGR - relative growth rate; DM - dry matter percentage; ψs – osmotic potential, MPa – mega pascal.

INTRODUCTION

Drought is one of the most common environmental stresses affecting plant growth and productivity (Boyer, 1982). Plant cell and tissue culture has been an useful tool to study stress tolerance mechanisms under *in vitro* conditions (Bajji et al., 2000). *In vitro* culture techniques minimize environmental variations due to defined nutrient media, controlled conditions and homogeneity of stress application. In addition, the simplicity of such manipulations enables studying large plant population and stress treatments in a limited space and short period of time. Polyethylene glycols (PEG) of high molecular weights have been long used to simulate drought stress in plants as non-penetrating osmotic agents lowering the water potential in a way similar to soil drying (Larher et al., 1993). Simulation of drought stress under *in vitro* conditions during the regeneration process constitutes a convenient way to study the effects of drought on the morphogenic responses.

Soybean (*Glycine max*) is an important legume crop, known for its high quality protein and oil content, and beneficiary secondary metabolites such as isoflavones, phenolic compounds and saponins (Sakthivelu et al., 2008). Soybean is highly amenable for tissue culture and *in vitro* techniques have been well established for this crop. There are, however, no reports on *in vitro* screening and assessment of drought tolerance in soybean, apart from a few reports at field level (Sinclair et al., 2006; Kosturkova et al., 2008). Hence, the aim of this study was to assess the changes in growth and osmotic potential of calli and *in vitro* shoot regeneration of two Indian and Bulgarian soybean cultivars as affected by polyethylene glycol (PEG)-induced water stress.

MATERIALS AND METHODS

Explant preparation

Soybean seeds of two Indian cultivars (cv. JS 335 and Hardee) and two cultivars introduced in Bulgaria (cv. Collina and Korada) were used in this study. Matured seeds of Indian cultivars were surface disinfected with 70 % ethanol for 2 min, followed by 10 min in 5 % sodium hypochlorite (v/v) and rinsed with sterile water for four to six times. Disinfected seeds were kept for germination in a ½ strength MS (Murashige and Skoog, 1962) basal medium (pH 5.7) with 30 gl^{-1} sucrose, 8 gl^{-1} agar. Cotyledonary node and hypocotyl (4 mm in length) from one-week-old seedlings were used as explants. Seeds from cvs. Collina and Korada were surface decontaminated in 70 % ethanol for 1 min, followed by 30 % v/v commercial bleach and rinsed three times in sterile distilled water. The basal medium of Murashige and Skoog (1962) was used for seed germination. Cotyledonary nodes excised from 10-14-day-old seedlings before the formation of the first leaf were used as explants.

In vitro culture conditions

For callus induction hypocotyl explants were placed in MS basal medium supplemented with 1 mgl^{-1} 2,4-D and 0.5 mgl^{-1} kinetin, and for *in vitro* organogenesis cotyledonary nodes were placed on shoot induction media (MS basal medium supplemented with 2 mgl^{-1} BAP for Indian cultivars and MS basal medium enriched with aminoacids, 0.4 mgl^{-1} benzylaminopurine and 0.1 mgl^{-1} 3-indolebutyric acid for Bulgarian cultivars) as per our previously reported protocol (Nedev et al., 2007). All the cultures were maintained at 23±1°C under 16 h illumination (40 μmol m^{-2} s^{-1}). Drought was simulated by the addition of polyethylene glycol (molecular weight 6000) at concentrations of 4 % and 6 % (w/v) to the media. The cultures were kept for six weeks to study their growth potential and regeneration capacity.

Relative growth rate and dry matter percentage

After six weeks the samples were analyzed for their relative growth rate (RGR), dry matter percentage (DM) and osmotic potential (ψs). Ten samples were analyzed for each treatment and the mean values were recorded. Callus RGR was calculated according to the following formula: RGR = $(FW_2 - FW_1)$/Number of days, where, FW_1 is the fresh weight of the callus at the beginning of the test period and FW_2 is the fresh weight of the callus at the end of the test period. The percentage of dry matter in callus tissues was estimated by the formula, DM = (DW_2/FW_2) X 100, where DW_2 is the dry weight of the callus at the end of test period.

Osmotic potential

Osmotic potential was determined with a Gonotec-Osmometer using sap extracts from fresh calli tissues. The tissue sap was extracted by centrifuging the calli at 5,000 rpm for 30 min. Osmolarity was expressed as MPa using the formula ψs = 0.00227 k, where k = osmolarity in mosmol kg^{-1}(Mohamed and Tawfik, 2006).

Statistical analysis

The experiments were set up in a completely randomized block design and each treatment was replicated three times, and the average values were estimated. An analysis of variance was performed and significant differences among treatment means were calculated by the least significant difference (LSD) test at a probability level of 0.05.

RESULTS

Relative growth rate and dry matter percentage

The relative growth rate, dry matter percentage and osmotic potential of stressed and non-stressed callus cultures of both Indian cultivars are summarized in Fig. 1. A significant decrease in the RGR of callus cultures

was observed for both Indian cultivars, with increasing PEG concentrations compared to the control (Fig. 1a). In both PEG treatments, cv. JS 335 showed significantly lower RGR than cv. Hardee. There was no significant difference in percentage of dry matter (DM) between the Indian genotypes under non-stressed (control) culture conditions (Fig. 1b) whereas, when subjected to PEG treatments, both cultivars showed increased DM percentage only at 6 % PEG evel compared to the control. Among the cultivars Hardee showed better DM content than JS 335 (Fig. 1b).

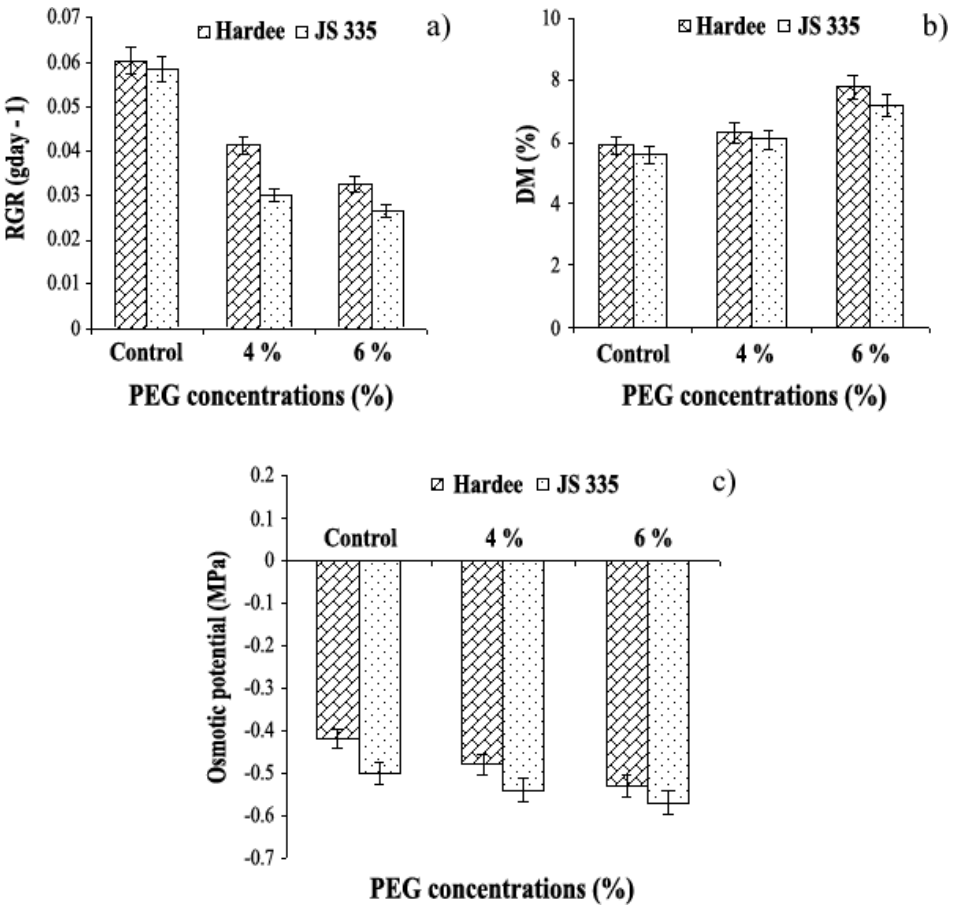

Fig. 1. (a) Relative growth rate, (b) Dry matter percentage and (c) Osmotic potential of soybean callus cultures of Indian cultivars on PEG 6000 media.

Table 1. Effect of PEG 6000 on shoot bud induction from cotyledonary nodes* of cv. Hardee and cv. JS 335.

Treatments	No. of explants inoculated	Hardee		JS 335	
		No. of explants responded	No. of shoots/ explant	No. of explants responded	No. of shoots/ explant
Control	50	38.7 ± 2.1	6.2 ± 1.1	34.3 ± 1.5	5.9 ± 0.3
PEG 4%	50	28.7 ± 3.5	4.3 ± 0.7	26.3 ± 2.5	3.7 ± 0.5
PEG 6%	50	16.0 ± 3.0	1.6 ± 0.3	14.3 ± 2.5	1.2 ± 0.4

* Values are means ± S.D. of three replicates.

Osmotic potential

The osmotic potential (ψs) of the medium without PEG was –0.30 MPa while it was -0.58 MPa and -0.74 MPa in the presence of PEG at 4 % and 6 %, respectively. The sap extract of non-stressed calli showed the lowest osmotic potential (ψs) values in both Indian cultivars compared to the stressed ones (Fig. 1c). In all treatments the osmotic potential was lower in cv. Hardee than cv. JS 335. The difference between the mean values of water deficit and control treatments showed that water stress induced a significant decrease in osmotic potential of callus tissues, irrespective of genotypes (Fig. 1c).

In vitro organogenesis

A sharp and significant decrease in the number of explants forming shoot buds was observed in the PEG-treated cotyledonary nodal explants of the Indian genotypes (Table 1). At 6 % PEG, the number of explants forming shoot buds was reduced by 57.8 % and 68.5% in cvs. Hardee and JS 335, respectively. The number of shoots per explant was also drastically reduced

Table 2. Effect of PEG on callus growth and adventitious bud formation of cv. Collina and cv. Korada.

Genotype	Collina				Korada			
Treatment	Callus		Organogenesis		Callus		Organogenesis	
	induction [%]	weight [g]	bud number	bud size [mm]	induction [%]	weight [g]	bud number	bud size [mm]
Control	100	400	2.2	1.2	100	620	2.6	2.0
PEG 6%	75	526	1.6	3.2	100	750	2.3	2.0

by 74.2 % and 79.6 % in cvs. Hardee and JS 335 at 6 % PEG compared to the control (Table 1).

As for soybean cultivars Collina and Korada, there was no significant difference in organogenesis. However, the mean number of buds per explant decreased by 28 % and 11.5 % in cvs. Collina and Korada, respectively (Table 2). PEG treatment increased the bud size in cv. Collina (3.2 mm) compared to the control (1.2 mm), while it was the same in cv. Korada (2 mm) upon both treatments. A significant difference in the percentage of callus formation compared to the control was also observed in cv. Korada.

DISCUSSION

The present study revealed the differences in callogenesis and regeneration capacity among the Indian and Bulgarian cultivars similar to our earlier studies (Nedev et al., 2007). The addition of PEG to the MS medium decreased the water potential of the media inducing water stress that adversely affected the callus growth and *in vitro* regeneration capacity of the soybean cultivars. Several authors reported the use of PEG for *in vitro* drought screening in crop plants (Dragiiska et al., 1996; Gopal and Iwama, 2007). PEG-induced water deficit produced substantial dehydration that led to elevated dry matter content and reduced RGR in callus tissues of all soybean cultivars. The decrease in osmotic potential is considered a potential cellular mechanism of drought resistance as it enables turgor

maintenance and growth continuation (Bajji et al., 2000; Munns, 1988). In the present study, cv. Hardee exhibited low osmotic potential in all treatments and thus it turned to be a better drought tolerant cultivar than JS 335. However, it also presents a metabolic cost due to synthesis and compartmentation of osmolytes (Bajji et al., 2000). The low initial ψs can act as a preexisting force to immediate dehydration buffering which was well documented in tepary beans (Mohamed and Tawfik, 2006).

The presence of PEG in the regeneration medium had a detrimental effect upon most parameters associated with plantlet regeneration. There was a gradual decrease in the total number of viable plantlets regenerated from cotyledonary nodes as the PEG concentration increased, in all soybean cultivars. A similar decrease in plantlet regeneration under *in vitro* stress conditions was reported in potato (Gopal and Iwama, 2007) and rice (Binh et al., 1992).

In conclusion, the soybean cv. Hardee showed better tolerance towards PEG-induced water stress compared to cv. JS 335. Similarly, genotype dependence was observed in the experiments with cvs. Korada and Collina, where the explants of cv. Collina were less affected by PEG. Thus, it is evident that *in vitro* screening can be used as an efficient tool to screen a large number of accessions or breeding lines for their drought tolerance. The results of the present study form an excellent guideline for *in vitro* screening of soybean cultivars for abiotic stresses like drought tolerance.

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