RESPONSE OF BULGARIAN AND INDIAN SOYBEAN GENOTYPES TO DROUGHT AND WATER DEFICIENCY IN FIELD AND LABORATORY CONDITIONS

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Summary. Drought is one of the limiting factors for better plant performance and higher yield. New variety selection is difficult due to the complex mechanism of drought resistance. Four Bulgarian soybean lines (selected after hybridization and mutagenesis), two Indian and one USA variety were used for field and laboratory experiments. Drought tolerance was estimated in irrigated and non-irrigated conditions. Bulgarian lines (compared to the world standard cv Hodson) had higher productivity by 17 - 44 kg/da in drought conditions. In laboratory experiments water deficiency was simulated by polyethyleneglycol (PEG) of MW 6000 in the following concentrations viz., 2, 4, 6, 8, 10 and 15 % (w/v) for seed germination. A linear and significant reduction in germination, shoot and root length, and their corresponding fresh and dry weight was observed for the genotypes as the concentration of PEG increased. The level of the negative effect of the osmoticum on developmental processes was genotype dependent with less damages for three Bulgarian lines, and especially for Line 5, which had highest seed yield under stress

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conditions. Among the Indian genotypes Hardee performed better than JS 335. However, reduction of seed germination of Indian varieties was stronger compared to the selected Bulgarian lines. A tendency of positive correlation between seed yield from one side and seed germination and seedling growth stages from another was observed. This can be used as criteria for predicting plant performance in field conditions under abiotic stress, evaluation of germplasm for higher drought tolerance and quick tests for screening of desired soybean genotypes.

***Key words*:** Drought, *Ex vitro*, *Glycine*, Osmotic stress, PEG, Soybean.
Abbreviations: PEG - polyethylene glycol; MW - molecular weight.

INTRODUCTION

Environmental stresses represent the most limiting factors for agricultural productivity. Nowadays a big concern is the water deficit, extremes in temperature and low atmospheric humidity leading to drought which is one of the most limiting factors for better plant performance and higher crop yield (Saxena et al., 1998; Szilgyi 2003; Hirt and Shinozaki, 2004; Georgiev, 2004). In such conditions resistance to abiotic stress is becoming one of the most desired trait of crops. However, new variety selection is difficult due to the wide range of plant stress responses with over lapping functions between their components creating complex mechanisms of resistance (Yordanov et al., 2003; Bartels and Sour, 2004; Feller, 2006). The latter necessitates interdisciplinary approach utilizing various methods of classical breeding, induced mutagenesis, genetic engineering, marker assisted selection (Cassells and Doyle, 2003; Quarrie et al., 2003; Wang, 2003). One of the prerequisites for successful breeding for drought tolerance is availability of reliable methods for screening of desirable genotypes. Classical breeding may be complemented with laboratory methods creating models for simulation of water deficiency and drought conditions. In this respect one of the most popular approach is to use high molecular weight osmotic substances, like polyethylene glycol (PEG), added to the medium for seed germination or plant/cell development (Griga et al., 2001; Kocheva and Georgiev, 2003; Turkan et al., 2005; Landjeva et al., 2008).

The aim of the presented work was to study the effect of water deficiency on seed development in *ex vitro* conditions (simulating osmotic stress) and on plant performance in the field searching for criteria for development of laboratory tests for screening of genotypes with higher drought tolerance.

MATERIALS AND METHODS

PLANT MATERIAL

Bulgarian and Indian soybean (*Glycine max* L.) genotypes were evaluated for drought and water deficiency conditions. A series of Bulgarian lines were selected using the methods of induced mutagenesis and hybridization, extensively used for soybean breeding in Bulgaria (Georgiev, 2005). For this study four perspective lines were chosen: Line 3 and Line 4 were obtained by γ-irradiation while, Line 5 and Line 6 - by hybridization using Bulgarian and foreign soybean varieties. The American variety Hodson was used as a reference standard.

Indian Soybean genotypes *viz.,* Hardee and JS 335 were obtained from Gandhi Krishi Vigyana Kendra, University of Agricultural Sciences, Bangalore, India. Both genotypes are high yielding, cultivated mainly in the central and northern parts of India.

FIELD TRIALS

Soybean was grown in the experimental field of Pavlikeni under irrigated and non-irrigated conditions. Field experiments were carried out in three years period (2005-2007) in four repetitions with 14 m^2 sowing and 5 m^2 yield plot size. Depending on the soil humidity during the critical phase of flowering - pod setting irrigation was applied once or twice in a norm of 70 - 90 m^3/da. Yield is presented in mean values for the three years.

LABORATORY EXPERIMENTS

Water stress was simulated by highly osmotic substance polyethelene glycol (PEG) of molecular weight (MW) 6000 (Duchefa Biochemie, The Netherlands). Seeds were washed with liquid soup and rinsed three times with distilled water and plated for germination on filter paper in Petri dishes containing 20 ml of water solutions of PEG in concentration 0 %, 2 %, 6 %, 10 % and 15 % w/v. Timol ($C_8H_9NO_2$), having bactericide and antiseptic properties, with no adverse effect on seed germination and development, was added in concentration of 1 g/l to the germination media for preventing pathogen development (Marinov-Serafimov et al., 2007; Moyer et al., 1997). Seeds were grown in thermostat chamber in dark at temperature of $22^o \pm 2^oC$. After a week seed germination percentage, seedling initial root length and hypocotyl were recorded. Forty seeds in two replicates were used for each variant.

The seeds of Indian soybean cultivars were subjected to germination in different concentrations of PEG 0, 2, 4, 6, 8, 10 and 15 % (w/v) by rolled

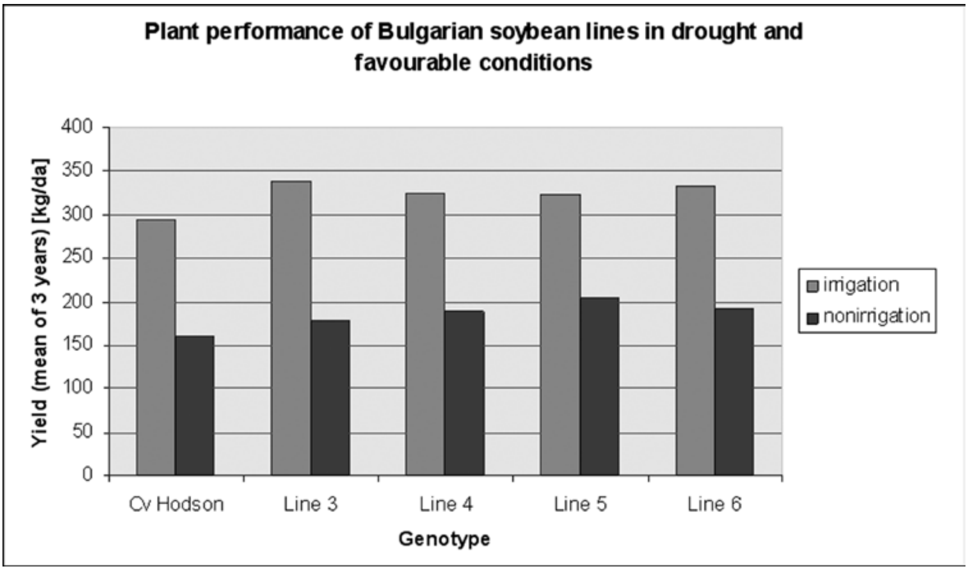

Fig. 1. Plant performance of Bulgarian soybean lines in stressed (without irrigation) and favourable (after irrigation) conditions.

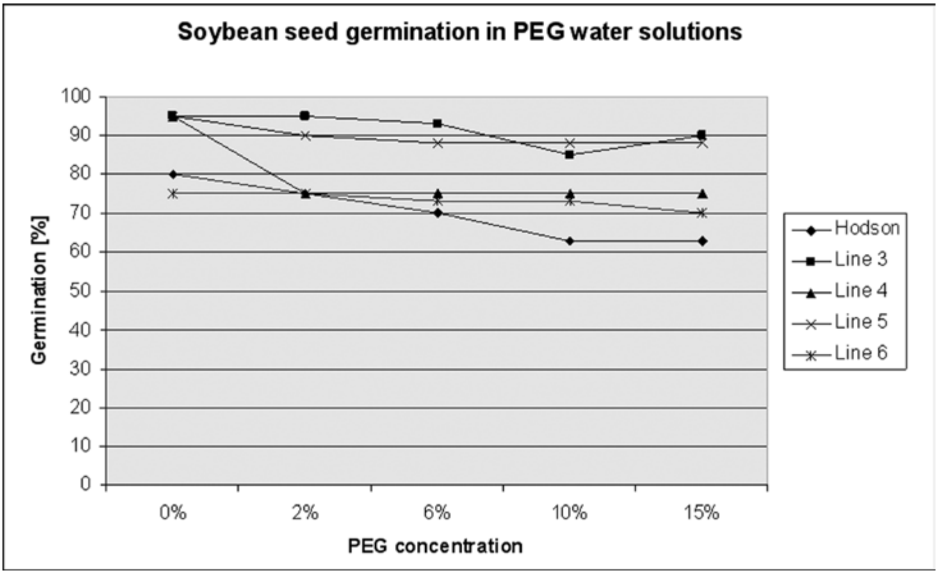

Fig. 2. Seed germination of Bulgarian soybean genotypes in PEG water solutions.

towel method. After one week, germination count and seedling growth parameters viz., shoot length, root length and their fresh and dry weight were recorded. Each treatment was replicated three times, and ten seedlings from each treatment were sampled randomly for measuring growth parameters.

RESULTS

In field experiments yield of the four selected Bulgarian lines varied in the range of 322 - 338 kg/da and 178 - 205 kg/da in irrigated and non-irrigated conditions, respectively (Fig 1). Compared to the control cv Hodson, all examined lines had higher productivity by 17 - 44 kg/da in drought conditions. Water deficiency reduced the yield by 45 % in cv Hodson, while yield reduction in stress conditions was lower for three of the lines – being 36 % (Line 5), 42 % (Lines 4 and 6) and 47 % (Line 3).

In the experiments *ex vitro* seed germination varied between 75 - 95 % in the control (water). and in the variant with the lowest concentration (2 %

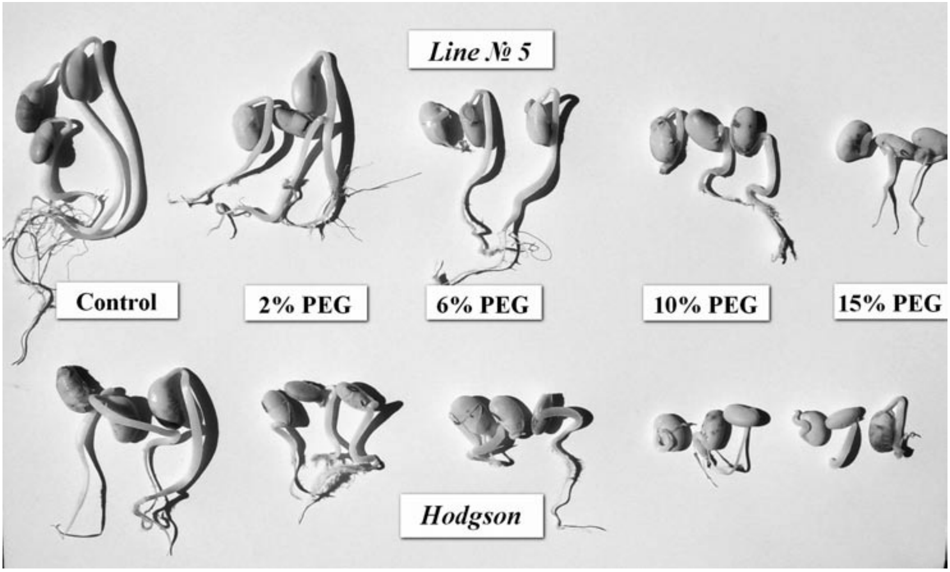

Fig. 3. Seed germination and root development of soybean seeds from Line 5 and Cv Hodson, grown in water solutions of different concentrations of PEG.

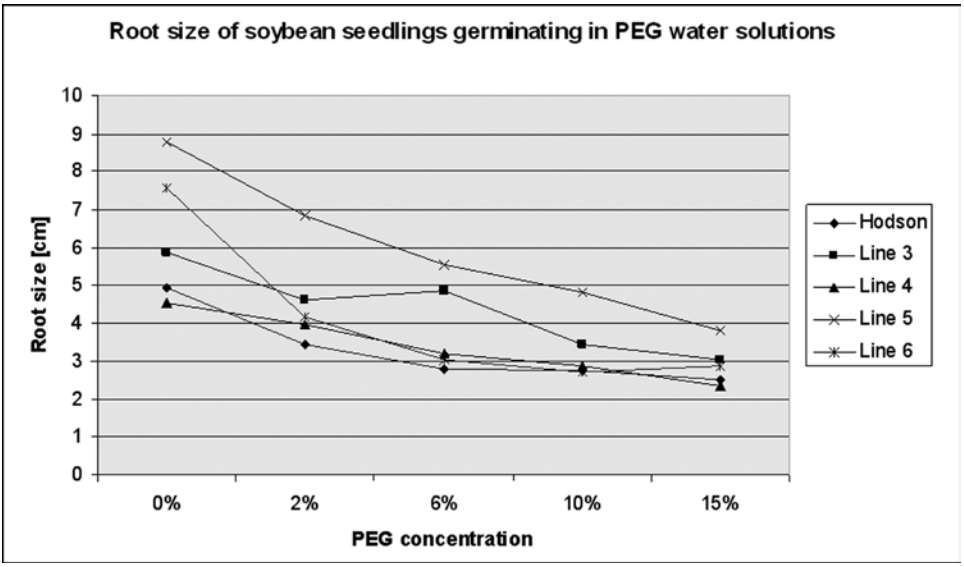

Fig. 4. Root size of Bulgarian soybean genotypes germinating in PEG water solutions.

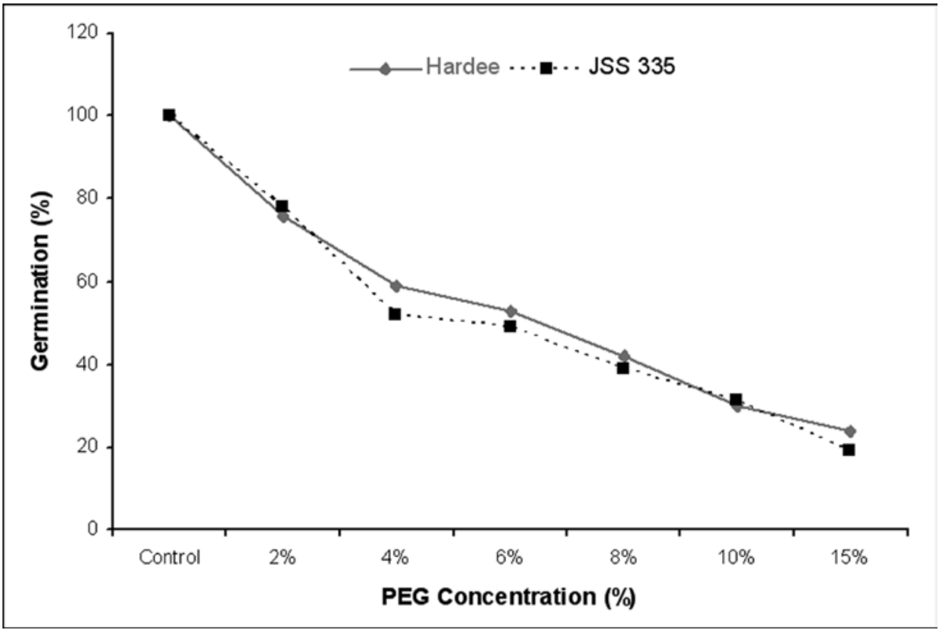

Fig. 5. Germination percentage of Indian soybean genotypes in PEG water solutions.

w/v) of polyethelene glycol. Addition of PEG in higher concentrations (6 %, 10 %, and 15 %) reduced seed germination progressively up to 30 % depending on the concentration. Slight differences, in the range of 12 - 20 % of germination suppression was observed between the Bulgarian genotypes. Compared to the control genotype (cv Hodson) suppression was less pronounced in Line 3 and Line 5 (Fig2). The negative effect of PEG was less stronger in Line 5 (Fig 3).

An increase in the PEG concentration reduced root growth by two to three times for the different genotypes. Suppression was less pronounced in all the examined Bulgarian lines, (particularly in Line 3 and Line 5) compared to the control cv Hodson (Fig 4).

A linear and significant reduction in the germination percentage was observed for both the Indian genotypes as the concentration of PEG increases (Fig. 5). Different responses were observed for shoot and root growth due to PEG treatment in both Indian genotypes. The shoot and root

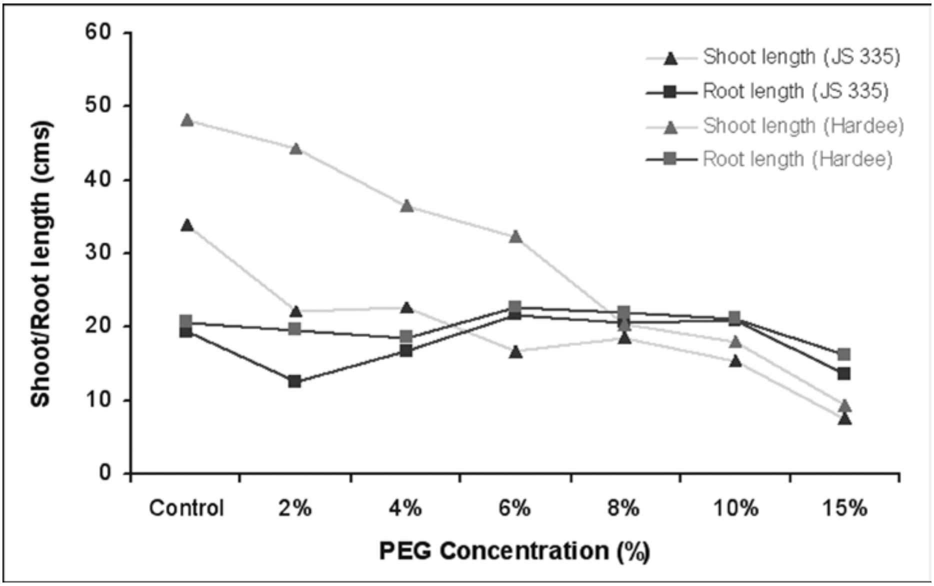

Fig. 6. Effect of PEG on root and shoot growth of Indian soybean genotypes.

length gradually decreased as the concentration of PEG increased, with root length showing a slight increase at 6 % PEG (Fig. 6). Shoot and root length was decreased by 77 % and 30 % for JS 335, while it was 80 % and 21 % for Hardee respectively at 15 % PEG. Similarly a corresponding decrease in shoot and root dry weight was recorded for both the genotypes (Table 1).

DISCUSSIONS

The present investigation demonstrated higher tolerance of the selected mutant and hybrid Bulgarian soybean lines to water deficiency in natural field and simulated laboratory conditions. Under drought conditions, seed yield losses of the Bulgarian genotypes (particularly Line 5) were up to 10 % less than that of the Hodson variety. In India soybean breeding is focused towards yield and quality improvement and not much of attention has been given towards breeding for abiotic stress tolerance. This may be due to the lack of screening methodologies for large collection of germplasms for

Table 1. Effect of PEG 6000 on seedling growth parameters* of cultivar Hardee and JS 335.

Concentration of PEG 6000	JS 335				Hardee			
	Shoot fresh weight [g]	Shoot dry weight [g]	Root fresh weight [g]	Root dry weight [g]	Shoot fresh weight [g]	Shoot dry weight [g]	Root fresh weight [g]	Root dry weight [g]
Control	1.110±0.26	0.215±0.034	1.100±0.18	0.231±0.002	2.210±0.58	0.365±0.021	1.542±0.15	0.356±0.002
2 %	1.169±0.29	0.228±0.035	0.579±0.25	0.122±0.002	2.652±0.62	0.448±0.026	1.251±0.18	0.281±0.005
4 %	0.865±0.35	0.148±0.065	0.160±0.22	0.036±0.003	1.586±0.35	0.264±0.034	0.264±0.22	0.056±0.004
6 %	0.727±0.54	0.128±0.054	0.138±0.35	0.029±0.004	1.248±0.58	0.120±0.058	0.251±0.35	0.052±0.002
8 %	0.600±0.64	0.108±0.065	0.100±0.48	0.022±0.005	1.024±0.84	0.175±0.054	0.234±0.38	0.049±0.005
10 %	0.649±0.46	0.110±0.058	0.115±0.54	0.025±0.004	0.964±0.78	0.160±0.032	0.364±0.48	0.071±0.003
15 %	0.537±0.32	0.092±0.045	0.225±0.34	0.048±0.003	0.615±0.54	0.118±0.041	0.308±0.24	0.064±0.003

* Values are Means ± S.D. of ten samples (n = 10)

their stress tolerance characters. Drought, due to its osmotic effect in natural and agricultural habitats can induce a wide number of responses ranging from growth inhibition, accumulation of osmolytes, enhancement of some antioxidant enzyme activities and reduction in yield. Thus prescreening of large number of accessions and breeding segregates for a few above mentioned characters can be a handy tool for breeding strategies.

In the present experiments suppressive effect of PEG on soybean seed development was recorded, as observed by other authors using this substance to create osmotic shock in various crops like common beans (Turkan et al., 2005), wheat, (Landjeva and Ganeva 2006; Landjeva et al., 2008) and barley (Kocheva and Georgiev, 2003). Similarly the level of the negative effect of the osmoticum on developmental processes was genotype dependent with less damages for three of the lines, and especially for Line 5, which has been the best performer under stress conditions. In both the Indian genotypes, PEG-induced water deficit resulted in decreased germination percentage and seedling growth parameters, with Hardee performing better than JS 335. However, reduction of seed germination of Indian varieties was stronger compared to the Bulgarian lines (Fig 5). Suppression in root and shoot growth is more pronounced for PEG concentrations higher than 8 %. Stem development is less effected by the stress.

Comparing parameters of seedling development *ex vitro* (germination, root length and stem size) and plant performance in the field, a tendency of positive correlation between seed yield from one hand and seed germination and seedling growth stages from the other was observed. The latter can be used as criteria for predicting plant performance in field conditions under abiotic stress, and evaluation of germplasm for higher drought tolerance as a quick test for screening of desired genotypes.

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References:

Bartels, D., E. Sour, 2004. Molecular responses of higher plants to dehydration. In: Plant Responses to Abiotic Stress. Eds. H. Hirt., K. Shinozaki, Springer, 13-37.

Cassells, A.C., B.M. Doyle, 2003. Genetic engineering and mutation breeding for tolerance to biotic and abiotic stresses: science, technology and safety.

Feller, U., 2006. Stomatal opening at elevated temperature: an underestimated regulatory mechanism?, Gen. Appl. Plant Physiology, Special Issue, 19-31.

Georgiev, G., 2004. Influence of moisture conditions on the yield of soybean variety Daniela 97, Plant Science (Sf), 5, 406-410.

Georgiev, G., 2005. State and prospects of soybean production in Bulgaria, Proceedings of the Jubilee Scientific Conference (with international participation) "Breeding and tehnological aspects of production and processing of soybean and other legume crops". 8th September 2005, Pavlikeni, 21-29.

Griga, M., G. Kosturkova, N. Kuchuk, M Ilieva-Stoilova, 2001. Biothechnology. In: Carbohydrates in Grain Legume Seeds: Improving Nutritional Quality and Agronomic Characteristics, Ed. C. Hedley, CABI Publishing, 145-207.

Hirt, H., K. Shinozaki, 2004. Plant Responses to Abiotic Stress. Springer, Berlin – Heidelberg - New York – Hong Kong – London – Milan – Paris – Tokyo.

Kocheva, K., G. Georgiev, 2003. Evaluation of the reaction of two contrasting barley (*Hordeun vulgare* L.) cultivars in response to osmotic stress with PEG 6000, Bulg. J. Plant Physiol, Special Issue, 290-294.

Landjeva, S., G. Ganeva, 2006. Changes in the seedling growth parameters in three common wheat (*Triticum aestivum* L) cultivars subjected to drought stress and subsequent re-hydration, Field Crop Studies, 2006, 3, 185-190.

Landjeva, S., K. Neumann, U. Lohwasser, A. Borner, 2008. Molecular mapping of genomic regions associated with wheat seedling growth under osmotic stress, Biologia Plantarum 52, 259-266.

Marinov-Serafimov, P., Ts. Dimitrova, I. Golubinova, A. Ilieva, 2007. Study of suitability of some solutions in allelopathic researches, Herbologia, 8, 1-10.

Moyer, J. R., and H. C. Huang, 1997. Effects of aqueous extracts of crop residues on germination and seedling growth of ten weed species, Bot. Bull. Acad. Sin., 38, 131 – 139.

Quarrie, S. A., D. Dodig, S. Pekic, J. Kirby, B. Kobiljski, 2003. Prospects for marker-assisted selection of improved drought responses in wheat. Bulg. J. Plant Physiol, Special Issue, 83-95.

Saxena, N.P., C. Johansen, M.C. Saxena, S.N. Slim, 1998. Selection for drought and salinity tolerance in cool-season food legumes. In " Breeding for Stress Tolerance in Cool-Season Food Legumes" ed. ICARDA, 245-259.

Szilagyi, L., 2003. Influence of drought on seed yield components in common bean, Bulg. J. Plant Physiol, Special Issue, 320-330.

Turkan, I., M. Bor, F. Zdemir, H. Koca, 2005. Differential responses of lipid peroxidation and antioxidants in the leaves of drought-tolerant *P. acutifolius* and drought-sensitive *P. vulgaris* subjected to polyethylene glycol mediated water stress, Plant Science, 168, 223–231.

Wang, W., B. Vinocur, A. Altman, 2003. Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta, 218, 1-14.

Yordanov, I., V. Velikova, T. Tsonev, 2003. Plant response to drought and stress tolerance, Bulg. J. Plant Physiol., Special Issue, 187-206.