Turkish Journal of Agricultural and Natural Sciences
Special Issue: 1, 2014

TÜRK
TARIM ve DOĞA BİLİMLERİ
DERGİSİ

TURKISH
JOURNAL of AGRICULTURAL
and NATURAL SCIENCES

www.turkjans.com

Screening of soybean against water stress mediated through polyethylene glycol

Georgina KOSTURKOVA[a], Rositsa TODOROVA[b], Krasimira TASHEVA[a], Margarita DIMITROVA[a]

[a]In vitro Development and Genomic Regulation Group, Regulation of Plant Growth and Development
[b]Department, Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria
Soybean Experimental Station AD -Pavlikeni, 5200 Pavlikeni, Bulgaria
*Corresponding author: georgina_kosturkova@abv.bg

Abstract

Glycine max (L.) Merrill is the world largest cultivated pulse crop, rich in protein and oil. Soybean provides phytoprotein to more than half of the world's population and is desired wholesome and healthful food in many diet programs. Despite its global importance soybean production and grain quality suffer the problems of biotic/abiotic stress causing damages at all levels of cell and plant development. Hence, success in creating better adapted varieties depends upon the efforts of various scientific domains. Classical breeding have been enriched with up-to-date approaches and techniques. The objectives of the present study were to screen *in vivo* soybean genotypes and to find out relevant criteria for simple and quick identification of lines with higher drought tolerance. Different varieties and lines created by the authors were used. Various approaches for field and laboratory screening were applied creating water deficit in field and laboratory conditions. Data about the response of tested genotypes were obtained. Productivity of three (Srebrina, Richy and Daniela) out of four tested Bulgarian varieties was higher than the world standard Hodgson under non irrigated conditions indicating their higher drought tolerance. Genotypic differences in the response to osmotic stress evaluated by water absorption by seeds soaked in PEG solution were more indicative in the period between 6th and 20th hour at 5% PEG. A tendency for correlation between drought tolerance in the field and root growth depression of seedlings in PEG solutions was observed. These data could be a basis for rapid preliminary tests for evaluation of drought tolerance of a great number of soybean genotypes.

Keywords: *Glycine max*, soyben, drought, *in vivo, in vitro*

Introduction

Soybean (Glycine max) is an annual plant and is the most important grain legume in the world with multiple uses as food and feed, as natural fertilizer, as raw material for industry. The species is widely cultivated as a valuable oilseed crop (20 % fat) and rich in protein (40 %), too. It is a substantial source for human food (1/3 of the world human consumption of oils) and farm animals feed (2/3 of the world consumption of protein meals for animals) (Reda Helmy Sammour, 2011). Soybean is used for improving nutritional balance and value of the food and feed products due to its high content of essential amino acids, vitamins (A, B, C and D), mineral elements (calcium, iron, magnesium, phosphorus, potassium, etc) and various phytochemicals. Interest to its nutraceutical properties is increasing constantly. Soybeans contain isoflavones most of them being natural estrogens (phytoestrogens) controlling the actions of hormones and protecting against cancer (Sakthivelu et al. 2008a). Genistein is especially beneficial for prostate cancer preventing synthesis of the enzymes responsible for the formation of tumors. Soybeans are rich in lecithin (key player in the metabolism of fat in the body), phytic acid (antioxidant which slows down the progression of tumors), saponins (chemical substances which destroy cancer cells). However, nutrition value and plant performance are influenced by environmental stresses.

Stress tolerance is a complex of genetic, physiological, biochemical and other mechanisms requiring complex and multidisciplinary approach to study it and resolution of susceptibility to negative factors. The studies are carried out on several levels –organismic, cellular and molecular (Sanchez et al 2001, Emery et al. 2001).

Despite the great soybean importance the cultivated areas in Bulgaria are decreasing.

Drought occurs more often causing demand for varieties with higher tolerance. Bulgarian major soybean research is concentrated in the Experimental station of soybean in Pavlikeni where intensive investigations on soybean germplasm and its improvement have been carried for more than 50 years. Application of the methods for induced mutagenesis and hybridization and their combined use brought to development of new varieties with valuable traits like higher yield, higher tolerance to biotic and abiotic stress, better nutrition value (Alexieva and Todorova 2000, Todorova et al., 2001, Todorova 2005, Todorova and Goranova, 2009, Todorova and Kosturkova, 2010)

Evaluation of physiological and biochemical parameters of the sustainability of soybeans at different stages of growth and development to water and heat stress is not sufficiently studied. The effect of stress on growth and yield of soybeans depends on the level of stress, variety and stage of growth in which it occurs. The drought in later phases of the development R4-R6 (end of flowering and maturation of grain) has a greater effect on the reduction in yield compared to the other phenophases (Wang et al 2003).

Among the abiotic factors affecting yield and quality of production water insufficiency is the most frequent cause of damage. Due to the complex nature of drought tolerance, the problems related to plants ability to withstand water deficiency require investigations at various levels (Saxena et al. 1993). Study of physiological processes in plants under suboptimal or stress conditions could contribute to our knowledge of metabolic plasticity and could reveal some of the mechanisms involved in overcoming the effects of unfavorable environmental conditions. Induction of stress in laboratory has certain advantages over field experiments as controlled growth conditions offer better reproducibility of results and the possibility to separate evaluation of a particular limiting factor thus avoiding the interference of stressors. The use of polyethylene glycol (PEG) solutions for the simulation of osmotic stress provides an option for precise control of the degree of dehydration within a wide range of osmotic potentials (Filek et al. 2012). PEG is suitable for *in vitro* studies of the osmotic stress on cell development in controlled conditions, creating *in vitro* models and tests for faster screening for higher drought tolerance (Saktivelu et al. 2008b, Kosturkova et al. 2008).

The aim of this study is to find out appropriate criteria for simple and quick screening of Bulgarian soybean varieties with higher tolerance to drought, to create a parallel between stress responses at different stages of development.

Materials and Methods

Plant material. Bulgarian soybean (Glycine max L.) varieties were developed in the Experimental Station of Soybean, Pavlikeni. Object of the present investigations cv Rosa, cv Daniela, cv Richy and cv Srebrina were obtained by induced mutagenesis and/or hybridization (Todorova and Goranova 2009). The American variety Hodgson and the Bulgarian variety Daniela were used as world and national standards.

Laboratory experiments. Seeds were washed with liquid soup surface disinfected by dipping in 70% v/v ethanol for 1 min, followed by 30% v/v commercial bleach and rinsed three times in autoclaved distilled water.

Water stress was simulated by polyethelene glycol (PEG) with molecular weight (MW) 6000 (Duchefa Biochemie, The Netherlands). Seeds were plated for 30 hours on filter paper in Petri dishes containing distilled water as control and water solution of PEG in two concentrations (5% w/v and 10% w/v) for evaluation the very first response to the osmotic stress. Water absorption was evaluated by the difference of initial seed weight and the weight of imbibed seeds at different intervals of soaking time period. Water absorption coefficient was defined as the weigh difference divided by initial weigh.

The cultures were maintained in cultivation room at temperature of 23-24^{o} C and light (40 $\mu Mm^{-2}s^{-1}$) and photoperiod 16/8 h. Response of 30 to 40 seeds was examined for each variant and Sigma Plot 11.1 applied for statistics.

Results

Investigations on physiological adaptation of plants to stress are in the focus of soybean research for improvement of drought tolerance which is the main limiting factor for the production. Differences in the yield values under irrigation and non-irrigation conditions are indicator for the level of drought tolerance. The present study revealed differences in plant performance between the physiological and agronomical characteristics of the tested selected soybean lines. Productivity of the investigated varieties is in the range of 174 – 186 kg/da and 289 – 343 kg/da in the field without and with irrigation (Table 1). Bulgarian varieties had higher drought tolerance in the field under non irrigated conditions. Cv. Srebrina is the most productive without irrigation while cv. Rosa has highest yield under irrigation conditions.

Table 1. Productivity and vegetation period of soybean varieties under irrigated and non irrigated conditions

Varieties	Yield [kg/da]		Vegetation period [days]	
	irrigated	Nonirrigated	irrigated	nonirrigated
Hodgson	312	174	116	106
Daniela	289	180	116	106
Srebrina	303	186	120	112
Richy	329	180	116	106
Rosa	343	174	120	112

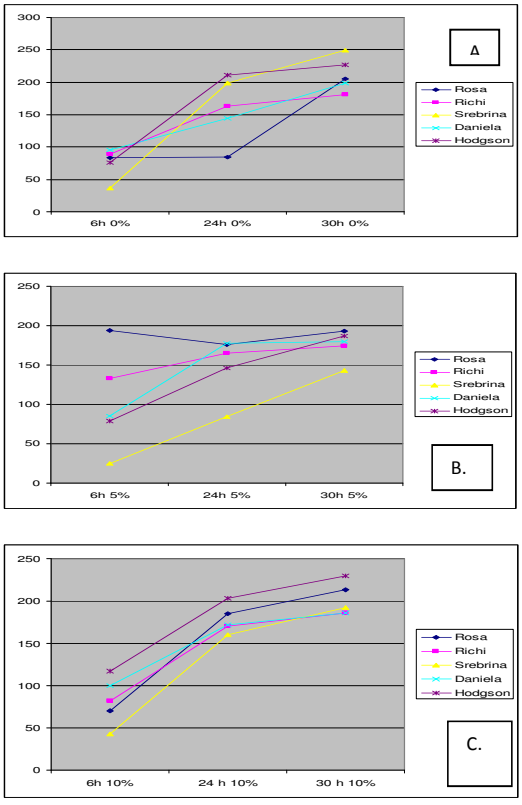

Figure 1. Water absorption (mg) of seeds after different soaking time (6h, 24h and 30h) on different PEG concentrations: A – 0 % PEG (Control); B – 5 % PEG; C – 10 % PEG.

Vegetation period is longer with 8 – 10 days under favourable conditions, though the differences are not so profound compared to the yield.

One of the most important prerequisite for the success of breeding programs is the availability of quick and reliable tests for screening and selection of the tolerant genotypes. Genotypic differences could be recognized by the depression of root or stem growth of juvenile plants. Levels of depression correlate often with the levels of tolerance of the mature plant.

Seed water absorption.

Seeds of various varieties absorbed water with different rate which was best demonstrated on the 24th hour for the control and on the 6th hour under mild osmotic stress (5% PEG) (Figure 1). The genotypic differences decrease on the 24th hour, as well as when higher osmotic stress was applied (10% PEG). The period between 6th and 20th hour at 5% PEG could be appropriate for discremination of the genotypes. Cv Srebrina which has the highest yield under drought conditions absorbs slower water while Cv Rosa which has the highest yield has higher absorption under osmotic stress at 5% and 10% PEG. These correlations could be indicative for fast preliminary screening tests.

Figure 2. Seed germination of different varieties (A - cv. Srebrina; B - cv. Rosa) on different PEG concentrations: 1 - 0% PEG, Control; 2 - 5% PEG; 3 - 10 % PEG on 7th day

Seed germination.

Seed germination of the varieties was in the range of 40-70% in control conditions and differed slightly in PEG solutions (Table 2). Germination rate, both, of Srebrina and Rosa was 70% and was not influenced by the effect of PEG. However, root growth was suppressed for Rosa but not for Srebrina and Richy (Figure 2, Table 2). Similar were results for the other Bulgarian varieties. The standard Hodgson which had lowest yield differed in its response compared to the Bulgarian genotypes. Growth depression observed for less tolerant genotypes could be a creterion for screening.

Figure 2. Seed germination of different varieties (A - cv. Srebrina; B - cv. Rosa) on different PEG concentrations: 1 - 0% PEG, Control; 2 - 5% PEG; 3 - 10 % PEG on 7th day

Table 2. Root development of seedlings germinating in water (control) and under osmotic stress in PEG solution of 5% and 10%

Varieties	Seed germination [%]			Root length [cm]		
	0 % PEG	5 % PEG	10 % PEG	0 % PEG	5 % PEG	10 % PEG
Hodgson	40	60	60	1.7	4.0	3.2
Daniela	40	40	30	1.5	2.7	2.7
Srebrina	70	70	70	5.4	3.8	5.4
Richy	50	70	70	3.7	4.1	3.8
Rosa	70	70	70	3.8	1.7	2.0

Conclusions

Obtained results demonstrate differences between the investigated Bulgarian genotypes under favorable conditions and under water deficiency. Productivity of three (Srebrina, Richy and Daniela) out of four tested Bulgarian varieties was higher than the world standard Hodgson under non irrigated conditions indicating their higher drought tolerance. Genotypic differences in the response to osmotic stress evaluated by water absorption by seeds soaked in PEG solution were more indicative in the period between 6th and 20th hour at 5% PEG. A tendency for negative correlation between drought tolerance in the field and root growth depression of seedling development in PEG solutions was observed. These data could be a basis for development of rapid preliminary tests for evaluation of drought tolerance of a great number of soybean genotypes under laboratory conditions using *in vivo* and *ex vitro* models for simulating the stress by polyethylene glycol (PEG).

Aknowledgement

The research was funded by the Bulgarian NSF under the Intergovernmental Program for S&T between Bulgaria and India (projects DNTS-01/2 2013-2016)

References

Alexieva, A., Todorova, R., 2000, Study on the biological effect of gamma rays and EMS applied separately or in combination on four soybean varieties. Plant science, 37, 571 - 575.

Emery, R. J. N. and Salon, C., 2001 Apparent aquaporin mediated water entry into pressurised detached root systems of pea,

soybean and tomato follows saturating facilitated diffusion kinetics. Towards the Sustainable Production of Healthy Food, Feed and Novel Products, ed. AEP, 308-309.

Filek, M., Walas, S.W., Mrowiec, H., Rudolphy-Skórska, E., Sieprawska, A., Biesaga-Kościelniak, J., 2012. Membrane permeability and micro- and macroelement accumulation in spring wheat cultivars during the short-term effect of salinity- and PEG-induced water stress. Acta Physiologia Plantarum, 34, 985–995.

Kosturkova G., Todorova, R., Sakthivelu, G., Akitha Devi, M. K., Giridhar, P., Rajasekaran, T., Ravishankar, G. A., 2008. Response of Bulgarian and Indian soybean genotypes to drought and water deficiency in field and laboratory conditions. Journal of General and Applied Plant Physiology, 34(3-4), 239-250.

Reda Helmy Sammour, 2011. Genetic Diversity and Allele Mining in Soybean Germplasm, Soybean - Genetics and Novel Techniques for Yield Enhancement, Prof. Dora Krezhova (Ed.), ISBN: 978-953-307-721-5, InTech, 3 – 22. DOI: 10.5772/18682

Sakthivelu G., Akita Devi M.K., Giridhar P., Rajasekaran T., Ravishankar G., Nikolova. M.T., Angelov G., Todorova R., Kosturkova G., 2008a. Isoflavone composition, phenol content, and antioxidant activity of soybean seeds from India and Bulgaria. Journal of Agricultural and Food Chemistry, 56(6), 2090-2095.

Sakthivelu G., Akitha Devi, M. K., Giridhar, P., Rajasekaran, T., Ravishankar, G. A., Nedev, T., Kosturkova, G., 2008b. Drought induced alterations in growth, osmotic potential and in vitro regeneration of soybean cultivars. Journal of General and Applied Plant Physiology, 34 (1-2), 103-112.

Sanchez, F. J., Manzanares, M., De Andres, E. F., Tenorio L. and Ayerbe, L., 2001. Variability of some physiological traits associated with drought tolerance in pea plants subjected to water stress. "Towards the Sustainable Production of Healthy Food, Feed and Novel Products", ed. AEP, 304-305.

Todorova, R., Georgiev , I., Vladova, R., Mehandjiev, A., 2001. Some possibilities of using irradiation mutagenesis for obtaining soybean lines resistant to stem cancer, Comptes rendus de L'Academie des Sciences (Biologie genetique), 54(1), 89-92.

Todorova, R. 2005. Study of biological effect of application of physical and chemical mutagens in two soybean varieties. "Breeding and technological aspects in production and processing of soybean and other legume crops" 8 September 2005, Pavlikeni, 86 - 93.

Todorova, R., Goranova, K., 2009 Richy-new Bulgarian soybean varieties, Field Crops Studies, Dobroudja Agricultural Institute, 5(II), 157-162.

Todorova, R., Kosturkova, G., 2010. Achievements, problems and perspectives in soybean breeding. "Breeding & Technological Aspects in Production of Soybean" 2010, Pavlikeni, 27-36.

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