Scientific Bulletin. Series F. Biotechnologies, Vol. XVIII, 2014
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

ESTABLISHMENT OF TESTS FOR FACILITATING SCREENING OF DROUGHT TOLERANCE IN SOYBEAN

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Abstract

Abiotic stress due to environmental changes causing water deficit, extreme temperatures and low atmospheric humidity decreases plant productivity. One of the elements of overcoming the stress is to develop new varieties. However, drought tolerance has a complex nature which makes this problem difficult to solve and involves different approaches and methods. The process of creating new varieties could be facilitated by screening of genotypes with higher resistance. In this respect laboratory tests in in vitro and ex vitro conditions could complement in vivo experiments. To make stress models availability of a factor causing or imitating the stress is required. In addition, choosing appropriate criteria of correspondence between responses in field and in laboratory conditions is crucial.
The high molecular weight substance polyethylene glycol (PEG) causes osmotic shock which is one of the components of the drought. Different concentrations (2-10 %) of PEG were used in our experiments. PEG was added into the media for germination of seeds and its effect on water absorption and seed development was followed. Soybean lines obtained previously and varieties (used as standard) were studied. Positive correlation between the level of drought tolerance of the genotypes in the field and in laboratory experiments was identified. The results are interesting for establishment of quick screening tests for identification of lines appropriate for breeding programs of soybean, commonly used grain legume with multiple product application.

***Keywords**: drought tolerance, Glycine max, PEG, soybean, screening.*

INTRODUCTION

Soybean (Glycine max (L.) Merrill.), an oil and protein rich plant is the largest cultivated pulse crop in the world, providing more than half of the world's phytoprotein. Soybean oil is the basic one for cocking in the countries of Asia and is the preferred one in other countries like USA where its consumption has the biggest share of 61 % of the market of edible vegetable oil [US DAERS 2011]. Soybean is attracting greater interest with its nutraceutical value and as a component of wholesome food and health based nutrition programs. Soya foods reduce the risk of heart disease, osteoporosis and certain forms of cancer. [Messina and Messina 2010; Vij et al. 2011]. Compared to other grain legumes soybeans have higher fat and protein content, and lower carbohydrate content. The grain differs not only in macronutrients but in micronutrients, too, being rich in mineral elements, vitamins and specific constituents promoting health benefits. It contains phytochemicals such as isoflavones which have been a subject of intensive research during the last years due to their phytoestrogen activity [Kosturkova and Mehandjiev, 2002; Sakthivelu et al., 2008a]. Soybean global importance is growing up, too, due to its multipurpose applications not only as a food for humans and feed for animals but as a source of more than 200 industrial products like environmentally friendly solvents, lubricants, cleaners and paints.
Despite great success in soybean breeding and improvement of cultivation this crop still faces the *problems* caused by biotic and abiotic stress which reduces production and grain quality. [Szilagyi, 2003; Hirt and Shinozaki, 2004; Todorova and Kosturkova 2010]. In soybeans, drought causes 50% yield losses. Plants have developed a number of molecular, cellular, and

physiological mechanisms to cope with environmental stress. Due to the complex nature of the tolerance improvement of plant performance include both traditional and modern breeding studies. Success in creating better adapted varieties depends upon the efforts by various research domains [Landjeva et al., 2008; Amudha and Balasubramani, 2011, Ji Huang et al., 2013]. Classical breeding have been complemented with new methods of plant biotechnology and molecular biology allowing simulation of the desired stress *in vitro*, selection on cell level and exploitation of genes for resistance [Raia et al, 2011, Arumingtyas et al., 2012].

The objectives of the present study were to find out appropriate criteria for simple and quick screening of soybean genotypes with higher tolerance to drought using different approaches. Response of plants to drought conditions in the field and seed germination *in vitro* under osmotic stress were studied to find out a parallel between stress response at different stages of development.

MATERIALS AND METHODS

Plant material. Bulgarian soybean (*Glycine max* L.) lines, obtained by induced mutagenesis and/or hybridization were object of the present investigations. The American variety Hodson and the Bulgarian Variety Daniela were used as a world and a national standards.

Field trials. Soybean was grown in the experimental field of Pavlikeni under irrigated and non-irrigated conditions. Field experiments were carried out in four repeats with 14 m^2 sowing and 5 m^2 yield plot size. Seeds from each genotype are sown in four rows at 70 cm distance. 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.

Laboratory experiments. Water stress was simulated by polyethelene glycol (PEG) with molecular weight (MW) 6000 (Duchefa Biochemie, The Netherlands). Seeds were plated for 24 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 measured by the difference of initial seed weight and imbibed seed.

In vitro 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. Seeds were plated *in vitro* on solidified basal Murashige and Skoog (MS) medium [Murashige and Skoog, 1962] Osmotic stress *in vitro* was simulated by adding PEG in two concentrations 5 % w/v and 8 % w/v to MS medium before autoclaving. Seeds were grown in culture rooms at temperature of $24^o \pm 2^o$ C. After 10 days seed germination percentage, seedling initial root length and shoot size were recorded. Twenty seeds in two replicates were used for each variant.

RESULTS AND DISCUSSIONS

The present study revealed differences in plant performance between the physiological and agronomical characteristics of the tested selected soybean lines. Vegetation period of plants in drought conditions is with two days shorter (102-117 days) than that of plants being irrigated (104-119 days). Four of the tested lines (L1, L2, L3, L5) did not show great differences between the vegetation period under irrigation and non-irrigation conditions like the Bulgarian standard variety Daniela. This may suppose higher tolerance to drought. Comparing the lines with the international standard Hodson, six of the lines (L1, L4, L5, L7, L8, L10) had longer vegetation period in simulated drought conditions which supposes that these lines have higher tolerance than cv Hodson. All the lines under water deficiency were more productive than the international standard Hodson (60 kg/da) but less yielded (with exception of Line 1) compared to the Bulgarian standard Daniela (155 kg/da). Comparing yield of tested lines under favourable and stress conditions less reduction of yield (by 59 kg/da, 80 kg/da and 80 kg/da) was recorded for Lines 1, 6 and 7, respectively (Figure 2). Reduction of yield for the both standards and the other lines was 110-120

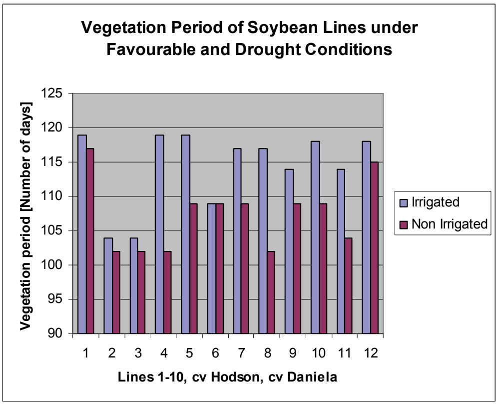

Figure 1. Vegetation period of soybean lines under favourable and drought conditions reps. with irrigation and without irrigation.

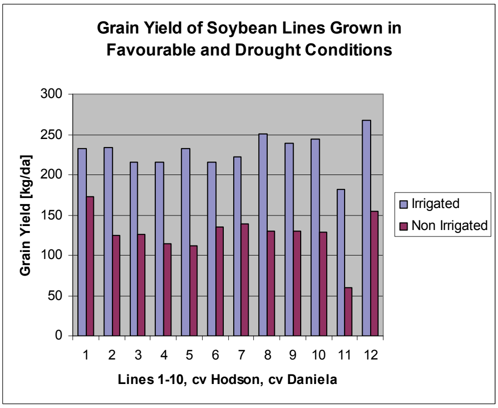

Figure 2. Yield of soybean lines grown in favourable and drought conditions resp. with irrigation and without irrigation.

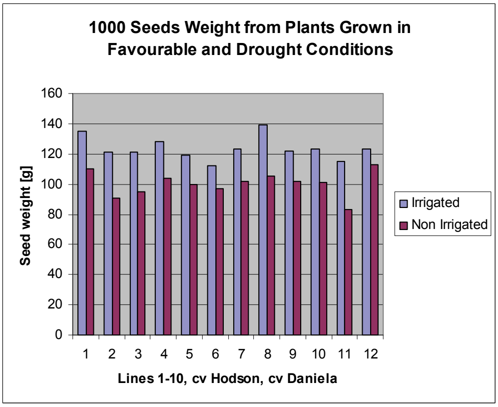

Figure 3. Seed yield of soybean lines grown in favourable and drought conditions resp. with irrigation and without irrigation.

kg/da. Concerning seed weight – the largest seeds were observed in line L8, and the smallest – in lines L2 and L3. The weight of 1000 seeds is around 20 g less in plants under drought stress (Figure 3).

Rate of water absorption by seeds during the first day differs among the genotypes in the range of 25 % (Figure 4). There is a tendency of slower absorption under osmotic stress caused by PEG presence in the soaking solution. Suppression of imbibing under stress is less profound in lines L1, L6, L7, L8, L9, and L10 which are ranked as more productive in the field. These lines had relatively bigger grains, too.

Seed germination under control conditions in the field varied among the lines in the range of 23 % marked by the lowest and the highest values of the both varieties, Daniela (59 %) and Hodson (82 %) (Figure 5). These margins were wider for germination *in vitro* on MS medium for the varieties Daniela (50 %) and Hodson (90 %) and even bigger for two lines with lowest values for L8 (30 %) and L1 (40 %). A tendency for positive correlation in two thirds of the cases between *in vivo* and *in vitro* germination was observed. Addition of PEG (both concentrations) to the MS medium inhibited germination of the both varieties, particularly Hodson. Lower number of germinated seeds was observed in half of the selected lines. Suppression caused by PEG (both concentrations) was not profound for most of the lines and even stimulation of germination was observed for some of them (L1, L5, L8, L9) but strict parallel with their yield in the field was not drawn.

In control conditions on MS medium root length of seedlings of the both varieties Daniela and Hodson was similar being 25 mm and 28 mm, respectively (Figure 6). Most of the lines had longer roots than the national and international standards. On MS medium containing 5 % PEG roots were longer in half of the lines (L2, L3, L7, L9, L10), shorter in two lines and without significant difference in the rest ones. Stimulation of root growth under the mild osmotic stress was observed for lines which had better performance in the field under drought conditions (L1, L6, L7). All seedlings developing on medium with the higher concentration of PEG 8 % had shorter roots. However, suppression of root elongation was stronger in lines with lower tolerance to water deficiency in the filed experiments.

Shoot development was more diverse in both varieties and the lines as well. In the control size of Daniela shoots (17 mm) was nearly twice less than that of Hodson (40 mm) (Figure 7). Most of the tested lines had bigger initial stem ranging from 60 mm to 90 mm. Addition of PEG 5 % in the medium caused slight decrease of the growth in the both standards and different levels of depression in the lines.

Stronger osmotic shock caused greater suppression but stimulation in two lines as well (L2 and L10). It was difficult to draw any parallel between the shoot size and the productivity of lines in the field. However, concerning weight of 1000 seeds, some observations were made that growth of seedlings on media containing PEG was suppressed more in lines with smaller seeds (L7, L10, L3, L5).

Several authors reported the use of PEG for *in vitro* drought screening in crop plants [Maan and Punia 2006, Gopal et al, 2007; Kosturkova et al, 2008, Sakthivelu et al 2008b, Govindaraj et al. 2010]. Seed germination and seedling growth characters under different water stress regimes was studies for screening the drought tolerant genotypes under *in vitro* conditions.

Only 11 % seed germination with no seedling and shoot development was observed on MS liquid medium supplemented with 20 % PEG [Maan and Punia 2006]. Significant genotype

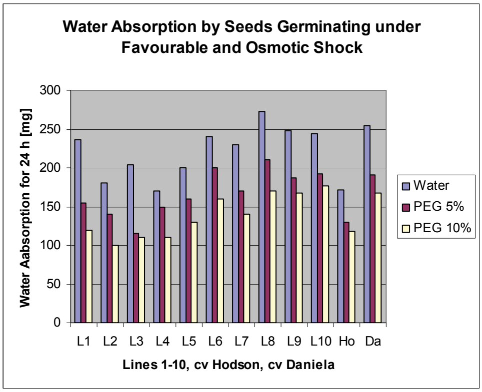

Figure 4. Water absorption by soybean seeds soaked for 24h in water (favourable conditions) and water solution of PEG (MW 6000) causing osmotic stress

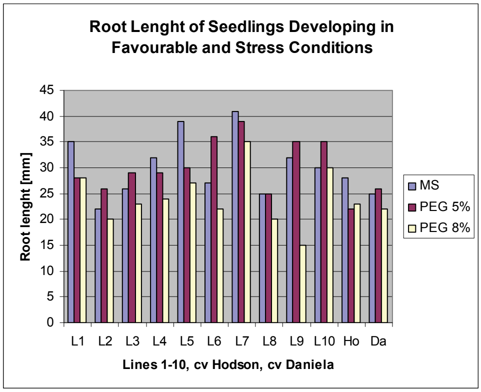

Figure 6. Root length of seedlings after 10 days of *in vitro* cultivation on MS medium (control) and under osmotic stress caused by PEG added to the MS medium

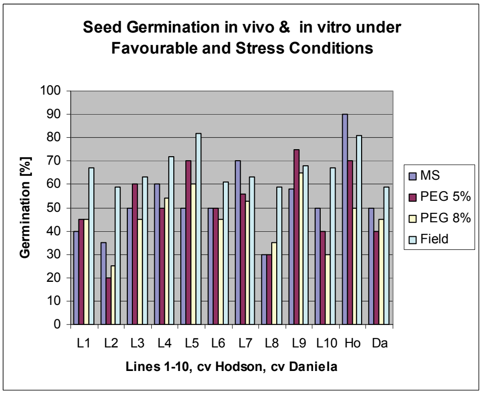

Figure 5. Seed germination *in vivo* in the field and *in vitro* under favourable conditions on MS medium and under osmotic stress on MS medium supplemented with PEG.

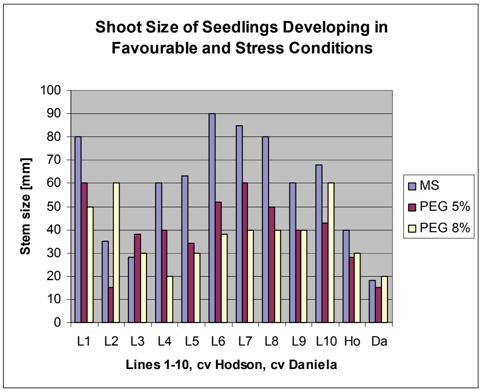

Figure 7. Shoot size of seedlings after 10 days of *in vitro* cultivation on MS medium (control) and under osmotic stress caused by PEG added to the MS medium

differences were recorded studying response of various traits (germination percentage, root length, shoot length, root / shoot ratio) to five different moisture stress levels by using PEG-6000 [Govindaraj et al. 2010].

This study is an extension to our previous experiments [Kosturkova et al, 2008] for comparing parameters of seedling development and plant performance in the field. Different criteria like seed germination, root length and stem size in water solution of PEG in different concentrations (2-15 %) were followed. Clear tendency of positive correlation between seed yield from one hand and seed germination and seedling growth stages from the other was observed allowing predicting of 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.

CONCLUSIONS

The present study revealed that the selected lines obtained after induced mutation and hybridization were less effected by osmotic shock in field conditions. Valuable information was obtained about their yield. It was observed in the laboratory experiments that more productive lines absorb bigger amount of water during the first 24 h of germination under osmotic stress caused by different concentrations of PEG added to the water. This could be a criterion for preliminary quick screening for drought tolerance. Development of the selected lines in *in vitro* conditions gave valuable information about the effect of osmotic shock caused by presence of PEG in the media. Suppression and stimulation of seed germination *in vitro* caused by PEG was observed but strict parallel with the yield in the field could not be drawn and could not be used as a criterion. Similar is the situation concerning the diverse results concerning shoot size. However, seedling growth on media containing PEG was less suppressed in lines with larger seeds. Suppression of root elongation was stronger in lines with lower tolerance to water deficiency in the filed experiments. These two characteristics of *in vitro* development are possible candidates for criteria in developing models and quick tests for preliminary screening of drought tolerance. This study examines not only the effects of the drought conditions and the water access deficiency *in vitro* simulated by the high osmotic substance PEG on the development and productivity of new soybean genotypes. Here an attempt is made to find correlation between field performance and *in vitro* growth parameters what could not been seen in many of the publications examining possibilities for establishment of tests for quick screening.

ACKNOWLEDGEMENTS

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

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