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Effects of osmotic stress on soybean varieties

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ABSTRACT: The drought tolerance of soybean cultivars viz., Shirale, Oscar, Con-II, Rainbow and 19H was investigated after exposure to drought stress at various growth stages in a pot experiment. Water stress was imposed at flowering and pod filling growth stages. Data of various physiological (leaf chlorophyll a & b, proline and protein contents) and agronomic attributes (number of pods/plant, seeds/pods, grain yield/plant) was recorded. The data revealed significant differences mong the various soybean genotypes for leaf chlorophyll a, b and proline accumulation. The chlorophyll a & b content of all the Napus genotypes declined due to drought stress at both the growth stages. Genotypes Rainbow showed least reduction (12%) in chlorophyll content during the flower initiation and pod filling stage. There were significant increases in osmosis-regulating substance proline under water stress. Rainbow accumulated highest proline. Drought treatment at different growth stages reduced grain yield significantly. Greater reduction in grain yield was observed when stress was imposed at flowering. Average yield was found greater in Rainbow and least in Oscar. The better osmoregulation ability under drought stress conditions proves Rainbow as drought tolerant cultivar. The findings of present research investigation suggested for growing of Rainbow in the areas of water scarcity to obtain economic yield.

***Keywords*:** Soybean, drought, growth stages, chlorophyll, proline.

INTRODUCTION

Soybean is a very important oil seed crop in the world. Its oil is of premium quality with low erusic acid and glucosinolates contents. It is a new crop in Pakistan, however, it achieved an important position due to fitting well in our cropping system. This crop is mainly grown in rainfed areas of Pakistan, where water availability is one of the most important limiting factors affecting plant growth and development. Soybean is generally considered to be more susceptible to drought. The yield is mainly affected by water shortages which occur during the stage from flowering to the end of the seed set. Plant growth is controlled by several factors, of which water plays a vital role. A small decrease in the availability of water to a growing plant immediately reduces its metabolic and physiological functions. Water stress induced a significant decrease in metabolic factors such as the decrease in chlorophyll contents and a increase accumulation of proline in soybean plants (Sakova *et al.* 1995; Gibon *et al.* 2000; Maharaj *et al.* 2003). Variations in the chlorophyll contents are often measured, because its loss is often assumed to be a symptom of stress injury (Majumdar *et al.* 1991). In Soybean cultivars, water stress reduced chlorophyll a + b content by 38% compared with the adequately watered plants (Paclik *et al*. 1996; Sharma *et al*. 1993). The extensive accumulation of active oxygen species and their contribution to cell damage induced by water deficit is well known. In order to deal with this effect, plants have evolved a number of protective defense mechanisms. Various metabolites accumulate in plant tissues upon reduction in leaf water status. Of these, free proline accumulation at high concentration has been shown to have adaptive mechanism of stress tolerance (Hare and Cress, 1997). Proline act as a osmoregulator, maintain membrane integrity and affect the solubility of various proteins due to its interaction with hydrophobic residues on the protein surface under the conditions of reduced water availability (Hare, 1995). The increase in the total hydrophilic area of the protein stabilizes it by increasing its solubility in an environment of low water availability. As the overall protein synthesis declines during drought stress, proline biosynthesis may substitute for protein synthesis (Hare, 1995). The yield and biochemical composition of a plant mainly depends on growth conditions, which is markedly affected by water availability (Sakova, *et al.* 1995. Paclik, *et at.* 1996). the most pronounced effects are observed when the water shortage occurs during the flowering period or pod-filling stages. At reproductive phase, water stress accelerates the process of flower and fruit drop and decreased seed yield (Gan, *et al.* 2004, Sinaki, *et al.* 2007). In Pakistan, soybean crop being grown in the semiarid regions is exposed to water deficiency which may limit seed yield. Therefore, the present research was conducted to study the behavior of soybean under drought stress conditions, with reference to the biochemical changes like chlorophyll, protein and proline cultivars. These findings could then be used to predict the susceptibility or tolerance of a cultivar as well as suitability of planting under drought stress conditions.

MATERIALS AND METHODS

Five soybean cultivars viz., Shirale, Oscar, Con-II, Rainbow and 19H were used in this study. These soybean varieties are mostly grown in the rainfed/arid areas of Pakistan. The plants were grown in pots in glasshouse in 12 L plastic containers, filled with a 10 kg sandy loam soil. Characteristics of the soil are given in Table 1. Each cultivar was replicated three times in separate pots having three plants per replicate. Total 45 pots were arranged in completely randomized design with a control in each cultivar. Basal dose of 100 mg nitrogen kg -1 as urea (2 splits), 50 mg kg -1 sulfur as potassium sulfate and 60 mg phosphorus as CaHPO4. 2H2O were applied before sowing. Sowing was done in the 2nd week of September 2009. Seedlings were thinned to 3 per pot at three leaf stage. The plants were watered to field capacity every two days until the treatments were imposed. The plants were exposed to drought stress at two growth stages i.e. flowering and pod filling stage. For imposing drought, water was withheld until the plants showed symptoms of wilting and leaf rolling (Sakova, *et al.* 1995, Siddique, *et al*. 2000). The control plants were irrigated continuously at the optimum moisture regime. Newly fully exposed leaves were collected from stressed and control plants for biochemical analysis at flowering after stress treatment. After sampling, the plants were re-watered. The leaves sampled were analyzed immediately in three replications for chlorophyll a+b concentration by Arnon, (1949), protein by Bradford, (1976) and proline by method of Bates *et al.* (1973). Data regarding yield and yield contributing parameters were recorded at maturity. The data were subjected to analysis of variance analysis (ANOVA) and least significant differences ($P<0.05$) were calculated using statistic software (MSTATC) using untransformed data.

RESULTS AND DISCUSSION

Chlorophyll

Drought stress reduced the chlorophyll a+b contents in all the soybean cultivars at flowering stages (Fig.1). The average reduction in chlorophyll a + b was 23.8 %. Drought caused highest relative reduction in chlorophyll a + b contents of cultivar; Oscar (45%) with respect to control whereas least reduction was observed in Rainbow and 19H (13.0 %). Drought not only causes dramatic loss of pigments but also leads to disorganization of thylakoid membranes, therefore reduction in chlorophyll contents is expected (Ladjal, et al. 2000). Decrease in chlorophyll content under water stress is a commonly observed phenomenon (Chaves, et al. 2003, Renolds, et al. 2005). Our results are in agreement with those of Kauser, et al. (2006). The decrease in chlorophyll under drought stress might be due to reduced synthesis of the main chlorophyll pigment complexes encoded by the cab gene family (Allakhverdiev, et al. 2002) or destruction of chiral macro-aggregates of light harvesting chlorophyll ‘a’ or ‘b’ pigment protein complexes (CHCIIs) which protect the photosynthetic apparatus (Sakovsky, et al. 2002) or due to oxidative damage of chloroplast lipids, pigments and proteins (Tambussi, et al. 2000). The variation in chlorophyll between the cultivars might be due to specific chlorophyll synthesizing enzymes such as chlorophyllase and peroxidase (Majumdar, et al. 1991). Singh, et al. (2003) found a significant reduction in chlorophyll contents in the leaves of mustard genotypes under drought stress. Kundu and Paul, (1997) observed decreased in chlorophyll a and b contents in rape under water stress at flowering but not at pod filling growth stage. Paclik, et al. (1996) observed that water stress reduced chlorophyll a and b contents by 38% compared with the adequately watered plants.

Proline

Proline contents in fresh leaves of different soybean cultivars are shown in Figure.2. Drought stress significantly enhanced accumulation of proline in the leaf of all the cultivars at flower initiation stage. This increase in proline was more in Rainbow (81%) and 19H (79%) and least in Oscar (58%) at flower initiation and 59% at pods filling stage). In all the soybean cultivars, the increase in proline contents was about same at both the reproductive stages. Deepak, et al. (1995) found that metabolic factors such as free proline contents in leaves increased significantly under severe drought stress. Kundu and Paul (1997) also observed higher proline accumulation in the

Brassica leaves at reproductive growth stages by water stress. Vartanian, et al. (1992) observed high proline accumulation through water shortage, reaching up to 4.6% of total dry matter. Proline accumulation during drought stress is an adaptive response that enhances survival and tissue water status (Chu et al. 1974).

Protein

Protein contents measured in the fresh leaves of soybean cultivars are shown in Figure.3. Drought stress did not affect the protein concentration in the leaves of soybean cultivars at both flower initiation and pod filling stages. There was a little increase or decrease in leaf protein but overall there were no significant differences. Sundarsan and Sudhakaran, (1995) observed no change in protein level in Cassava cultivars. Lazcano and Lowatt, (1999) found variable protein level in different cultivars of Phaseolus under water stress.

Yield

The seed yield per plant of soybean is a cumulative effect of various yield components like number of pods per plant, number of seeds per pods, and grain yield per plant. The data regarding seed yield of different soybean cultivars given in the Table. 2, 3, 4, revealed that grainyield and its components were significantly affected by drought stress applied at flower initiation and pods filling stages. The reduction was greater when drought stress was applied at flowering stage. Water stress during flowering and early pod development reduces yield of Soybean by reducing pods number and seed per pod. Early flowering of soybean ensure terminal drought escape as it allows the completion of seed development before onset of terminal\ drought (Debaeke and Aboudrare, 2004, Turner, 1997,
Hurling, 1991).

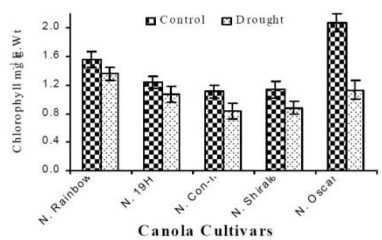

Figure 1. C a no la C ultiv a rs Fig.1. Effect of drought stress on leaf chlorophyll a + b content of soybean varieties at flowering stage (vertical bars represent standard error of mean)

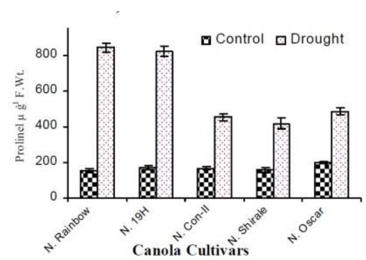

Figure 2. Effect of drought stress on leaf proline (μg g-1 f.wt) content of soybean cultivars at flowering stage (vertical bars represent standard error of mean)

The highest number of pods per plant (Table 2) and number of seeds per pod (Table 3) were recorded in Rainbow and lowest in Oscar. The difference in grain

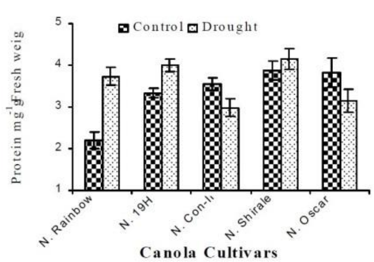

Figure 3. Effect of drought stress on leaf protein content (mg g-1 f.wt) of soybean cultivars at flowering stage (vertical bars represent standard error of mean)

Table 1. Physico-Chemical properties of the soil

Determinations	
pH (Sat. paste)	7.75
EC (dS m-1)	0.80
Organic matter	(%) 1.27
Kjeldah1 – N (%)	0.15
NO3 – N (mg kg-1)	3.46
P (mg kg-1)	5.2
K (mg kg-1)	112
Texture	Sandy Clay loam

Table 2. Effect of drought stress on pods plant-1 of soybean cultivars.

Treatments				
Varieties	Control	Flowering Stage	Pods filling Stage	Means
Rainbow	385.0c	227.7f	275.3e	296.0a
19H	410.0ab	211.3f	258.7e	293.3a
Con-II	424.7a	218.0f	261.0e	301.2a
Shirale	328.3d	165.0g	215.3f	236.2b
Oscar	397.7bc	148.3g	204.3f	250.1b
Means	389.1a	194.1c	242.9b	

LSD (0.01) for Varieties = 13.98, LSD (0.01) for Growth Stages = 9.985

Yield among varieties was non-significant. Nevertheless, the magnitude of reduction in grain yield was less in Rainbow and more in Oscar. Similar reduction in seed yield was also observed by Maharaj et al. (2003) in mustard genotypes. Richards and Thurling (1978) also showed that the drought markedly influenced seed yield in spring cultivars of oilseed rape species (Brassica compestris and Soybean L.). Deepak et al. (1995) suggested that the duration of post-flowering recovery phase may be a major determinant of yield under limited moisture conditions. Sinaki et al. (2007) found significant variation in seed yield and its components by drought stress applied at different developmental stages in various
Soybean cultivars.

Table 3. Effect of drought stress on seeds per pods of soybean cultivars

Treatments				
Varieties	Control	Flowering Stage	Pods filling Stage	Means
Rainbow	15.43	9.47	11.17	12.02a
19H	15.18	7.94	9.80	10.97a
Con-II	16.10	8.14	9.77	11.34a
Shirale	14.18	7.27	9.53	10.33ab
Oscar	13.50	5.44	7.20	8.71b
Means	14.88a	7.65c	9.49b	

LSD (0.01) for Varieties = 1.875, LSD (0.01) for Growth stages = 1.539

Table 4. Effect of drought stress on grain yield plant-1 (g) of soybean cultivars

Treatments				
Varieties	Control	Flowering Stage	Pods Filling Stage	Means
Rainbow	10.84	6.54	7.56	8.31a
19H	11.21	5.53	7.12	7.95a
Con-II	11.50	5.80	6.78	8.02a
Shirale	10.44	5.13	7.03	7.53ab
Oscar	10.87	4.33	5.63	6.95b
Means	10.97a	5.47c	6.82b	

CONCULSION

The data presented here indicated that the soybean cultivars accumulating greater proline produced higher grain yield under drought stress. The cultivars showing this potential may be used in breeding program to develop drought resistant cultivars for general cultivation under drought prone environment.

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