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ARTICLE

Morphological and physiological behavior in soybean (*Glycine max*) genotypes to drought stress implemented at pre- and post-anthesis stages

Nesa Sepanlo, Reza Talebi*, Asad Rokhzadi, Hayedeh Mohammadi

Department of Agronomy and Plant Breeding, College of Agriculture, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran

ABSTRACT In order to evaluate the morphological and physiological responses of soybean genotypes to water deficit, a field experiment with 3 different soybean genotypes at three different irrigation regimes was carried out. Plants were grown either under optimum condition (irrigated), drought stress implemented before the flowering (pre-anthesis) and pod-filling stage (post-anthesis). Seed yield and measured morphological characters, except for number of seeds per plant and seed protein content, decreased from normal irrigation regime to water deficit stress in both flowering and pod filling growing stages. Leaf relative water content (RWC) was significantly decreased in all genotypes by water deficit at both growing stages, as well as both stressed environments had progressive fall in chemical osmolytes and chlorophyll content. With the present results, it can be concluded that drought stress retards the growth and metabolic activity of soybean genotypes. These parameters showed considerable variability under drought stress at different growth stages in soybean. **Acta Biol Szeged 58(2):109-113 (2014)**

KEY WORDS

Soybean
drought stress
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Plants are subjected to several harsh environmental stresses that adversely affect their growth, metabolism, and yield. Drought is a meteorological term and defined as a period without sufficient rainfall for crop growth and productivity. This limitation for water supply in agriculture is likely to increase in the future due to growth of population and economical sectors other than agriculture (Araus 2004). Soybean is considered a species sensitive to several abiotic stresses (Van Heerden and Krüger 2000), when compared with other tropical legumes, such as *Vigna unguiculata* and *Phaseolus vulgaris* (Roy-Macauley et al. 1992; Silveira et al. 2003), as well as others species as *Gossypium hirsutum*, *Sorghum bicolor* (Younis et al. 2000) and chickpea (Talebi et al. 2013). Drought stress is the most important limiting factor at the initial phase of plant growth and establishment (Jaleel et al. 2009). Soybean is particularly sensitive to the lack of moisture during the blooming process (growth stages R1 and R2) and during the legume and seed growing processes (growth stages R3 – R6) (Doss et al. 1974; Sionit et al. 1987). Mederski et al. (1973) claim that water stress during the blooming process (growth stages R1 and R2) and legume growing process (growth stages R3 and R4) was noticed as a factor responsible for a flower and legume abortion, however, the seed size was reduced by the stress during the seed growing process (growth stages R5 and R6) (Krivosudská and Filová 2013). The responses of different crops to the decrease of water potential caused by drought may vary considerably among species (Save et al. 1995). In most of the crops, yield losses might be the result of decreasing in water supply during the vegetative phase, due to drought during reproductive development or due to terminal drought at the end of the crop cycle (Serraj et al. 2004). In term of morphological responses to drought stress, the remarkable reduction in growth, dry mater and harvestable yield in a number of plant species were recorded, but the tolerance of species to this menace varies remarkably (Serraj et al. 2004; Talebi et al. 2013). Various physiological responses of plants to drought with their tolerance mechanisms were reported in different crops (Dhanda et al. 2004; Serraj et al. 2004; Benjamin and Nielsen 2006; Kalefetoğlu and Ekmekci 2009; Praba et al. 2009; Talebi et al. 2013). Soybean is planted over a wide range of conditions, but information related to morphological and physiological changes in the plant organs under drought stress is lacking. The aim of the present work was to examine the effects of drought on several morphological and physiological parameters of soybean under different water regimes.

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*Corresponding author. E-mail: srtalebi@yahoo.com

Materials and Methods

Three soybean (*Glycine max* cv. Sambaiba) accessions (Habit, L17 and M7) were chosen for the study based on their reputed differences in growth habit and yield performance. Experiments were conducted at the experimental field of Islamic

Azad University of Sanandaj (35°10'N, 46°59E; 1393 m above sea level), in Kurdistan province (northwest of Iran) in 2012. Some of the soil physicochemical characteristics were: sand 25.4%, silt 32.6%, clay 42%, pH 7.6, organic carbon 0.62%, electrical conductivity 0.50 dS m–1, and available P and K 9.3 and 340 mg L^{-1}, respectively. The experiment was laid out in a split-plot arrangement with randomized complete block design and three replications. Three different irrigation regimes including irrigation every 4th day (a1), irrigation every 4th day with withholding irrigation at flowering stage for 15 days (a2) and irrigation every 4th day with withholding irrigation at pod filling stage for 15 days (a3) were compared in main plots. In well-watered control experiment, water level at field capacity (between 80 to 90%) was maintained throughout the experiment. For drought treatments, water was withheld for 15 days until the water level at field capacity decreased to 50%. Three different soybean genotypes were assigned in sub-plots. Each sub-plot contained three sowing rows 3 m in length. Inter- and intra-row spacing was 50 and 15 cm, respectively.

Six plants were randomly chosen from each plot to measure the number of seeds per plant, number of seeds per pod, plant height, 100-seeds weight, biological yield and grain yield (g m^{-2}) was measured by harvesting each plot at crop maturity. Leaf relative water content (RWC) was determined according to Turner (1981), based on the following equation:

$$RWC = (FM - DM) / (SM - DM) \times 100$$

where FM is leaf fresh mass, DM is dry mass of leaves after drying at 85 °C for 3 days, and SM is the turgid mass of leaves after soaking in water for 4 h at room temperature (approximately 20 °C). Half of the third (from the top) fully expanded leaf was used. Samples for chlorophyll and carotenoid determination were taken from soybean leaves using a 0.8 cm diameter cork borer, weighted quickly in pre-weighted clean glass vials and 5 ml of 80% acetone was added to these samples. The leaf material was bleached and decanted off. The optical density was read at λ = 663, 646 and 470 nm using 80% acetone as a blank by a spectrophotometer (Spectronic Genesys-5, Milton Roy). Content of chlorophyll a, chlorophyll b and carotenoids (μg g^{-1}) was calculated according to Lichtenthaler and Wellburn (1983) using the following formulae:

$$\text{Chlorophyll } a = 12.21\ OD_{663} - 2.81\ OD_{646};$$

$$\text{Chlorophyll } b = 20.13\ OD_{646} - 5.03\ OD_{663};$$

$$\text{Carotenoids} = (1000\ OD_{470} - 3.27\ \text{Chlorophyll } a - 104\ \text{Chlorophyll } b) / 229$$

Soluble sugars were determined based on the method of phenol-sulfuric acid (Dubois et al. 1956). 0.5 g fresh weight of soybean leaves was homogenized with deionized water, extract was filtered and treated with 5% phenol and 98% sulfuric acid, mixture remained for 1 h and then absorbance at 485 nm was determined by spectrophotometer. Contents of soluble sugar were expressed as mg g^{-1} FW.

Leaf soluble proteins were extracted from 2 g leaf dry weight of each sample into 5 ml Tris-HCl buffer (pH=8.0) containing 26.8 ml 0.2N HCl, 17.2% sucrose, 1% ascorbic acid and was then centrifuged. 1 ml of reagent D was added into 0.05 ml of the resulting solution and kept at room temperature. Then, 3 ml of reagent E was added and the sample was kept in Bain-marie at 50 °C. The absorbance was measured spectrophotometrically at 625 nm. Protein was calculated based on μM g^{-1} FW. For seed oil content, crude oils were extracted with n-hexane in a Soxhiet apparatus for 12 hs. After drying the solution with anhydrous sodium sulphate, solvent was removed by vacuum distillation at 30 °C. Oil percentages were determined by weight difference. Seed protein content was determined by the Kjeldahl method and it was converted to protein content by using the conversion factor 6.25 (AOAC 1980).

All collected data were subjected to analysis of variance operations and means of treatments were compared with the least significant difference (LSD) test at $P \leq 0.05$. The statistical calculations were performed with MSTAT-C software version 2.10.

Results

Seed yield and yield components

Drought stress had significant effects on seed yield and morphological traits. Seed yield and measured morphological characters, except for number of seeds per plant and seed protein content, decreased from normal irrigation regime to water deficit stress in both flowering and pod filling growing stages (Table 1). Interestingly, seed oil content increased significantly by water deficit stress. Between the genotypes, Habit showed higher seed yield and oil content (Table 1). The interactive effects of irrigation regimes and genotypes for seed yield and morphological characters are presented in Table 2. Seed yield and most of the measured traits were significantly decreased in stress treatments compared to normal irrigation environment (Table 2). Habit showed higher seed yield and 100-seed weight in stressed environments compared to other genotypes, while seed oil content in L17 and M7 was dramatically higher than in Habit (Table 2).

RWC and leaf biochemical characters

Leaf relative water content (RWC) was significantly decreased in all genotypes by water deficit at both growing stages as well as both stressed environments (a1 and a2) had progressive fall in chemical osmolytes and chlorophyll content (Table 3). Interactive effects of genotypes and irrigation regimes for RWC and leaf biochemical attributes showed less decrease in early flowering stress (a2), while when plants subjected to water stress at pod filling stage (a3) these characters significantly decreased and genotypes differed in response

Table 1. Main effects of irrigation regimes and genotypes on seed yield and yield components traits. Data are means from three replications. Means followed by same letters in a group of a column are not significantly different at $P \leq 0.05$ according to LSD test.

Treatment	NPP	NSP	TSW	Y (t/ha)	BY (t/ha)	PH	Seed oil (%)	Seed protein (%)
Normal irrigation (a1)	127.7±11.22 a	2.4±0.41 a	13.1±1.12 a	6.23±2.14 a	15.05±3.78 a	90.2±7.49 a	15.9±0.49 b	34.2±0.77 a
Water stress at flowering (a2)	73.3±8.63 b	2.1±0.28 a	11.7±0.58 b	2.26±0.98 b	6.42±2.18 b	63.5±7.91 b	16.8±0.78 b	34.2±0.33 a
Water stress at pod filling (a3)	80.9±9.11 b	2.5±0.29 a	11.3±0.47 b	2.67±1.08 b	7.46±2.58 b	91.7±8.17 a	19.04±1.14 a	28.9±0.79 a
Habit	110.9±9.01 a	2.2±0.11 a	13.4±0.98 a	5.38±0.34 a	11.43±1.07 a	60.6±4.78 b	15.7±0.30 b	33.03±0.77 a
L17	87.2±7.14 b	2.3±0.09 a	10.3±0.68 b	2.93±0.45 b	8.73±0.91 b	94.7±7.11 a	17.4±0.24 a	31.70±0.46 a
M7	83.8±6.89 b	2.4±0.07 a	12.5±0.49 a	2.86±0.51 b	8.81±1.08 b	90.1±8.79 a	18.7±0.41a	32.80±0.24 a

NPP=Number of pods/plant; NSP=Number of seeds/plant; TSW=100-seed weight; Y=seed yield (t/ha); BY=Biological yield (t/ha); PH=Plant height (cm)

Table 2. Interaction effect of irrigation regimes × genotype on seed yield and morphological characteristics. Data are means from three replications. Means in each column followed by same letters are not significantly different at $P \leq 0.05$ according to LSD test.

Treatment	Genotype	NPP	NSP	TSW	Y
	Habit	129.5±10.11 a	2.35±0.19 a	13.96±0.97 a	10330±478.9 a
Normal irrigation (a1)	L17	149.6±9.77 a	2.47±0.17 a	11.27±1.08 ab	5036.6±325.8 b
	M7	103.7±11.07 ab	2.44±0.10 a	14.01±1.45 a	3340±279.58 c
	Habit	94.5±9.14 b	2.06±0.08 a	13.62±1.11 a	2793.4±215.21 c
Water stress at flowering (a2)	L17	51.4±8.11 c	2.02±0.09 a	9.74±0.54 b	1346.7±425.14 c
	M7	74.2±7.19 c	2.19±0.11 a	11.77±1.57 a	2636.6±478.14 c
	Habit	108.56±10.11 ab	2.29±0.12 a	12.54±1.14 a	3010±521.14 c
Water stress at pod filling (a3)	L17	60.56±4.01 c	2.53±0.19 a	9.75±0.78 b	2400±615.25 c
	M7	73.5±5.14 c	2.55±0.21 a	11.6±1.86 ab	2603±397.78 c

Treatment	Genotype	BY	PH	Seed oil (%)	Seed protein (%)
	Habit	19330±2478.15 a	46.5±28.37 d	13.86±2.47 b	36±1.28 a
Normal irrigation (a1)	L17	13476.6±1987.45 b	115.66±29.14 a	16.43±0.98 ab	34.24±2.12 a
	M7	12363.3±2135.17 b	108.99±17.15 a	17.56±1.65 a	32.35±1.48 a
	Habit	7000±2745.25 c	56.44±8.14 cd	16.10±1.73 ab	33.87±1.38 a
Water stress at flowering (a2)	L17	5316.6±1798.54 c	69.88±5.18 c	15.90±1.98 ab	33.24±1.47 a
	M7	6953.3±1796.35 c	64.22±6.16 c	18.50±1.11 a	35.52±1.65 a
	Habit	7880±2014.34 c	79.44±8.19 bc	17.21±1.36 a	25.24±1.29 b
Water stress at pod filling (a3)	L17	7400±1978.24 c	98.66 ±10.14ab	20±0.98 a	26.36±1.37 b
	M7	7101.3±2078.39 c	97±14.77 ab	19.91±0.79 a	30.38±2.79 ab

NPP=Number of pods/plant; NSP=Number of seeds/plant; TSW=100-seed weight; Y=seed yield (t/ha); BY=Biological yield (t/ha); PH=Plant height (cm)

to drought stress (Table 4). It seems that, M7 is able to keep or accumulate more RWC and other biochemical osmolytes when compared to Habit and L17, while Habit showed higher seed yield and other yield components than the other two genotypes. The yield reductions in M7 and L17 under water deficit stress are less than that in Habit. The role of RWC and osmolytes accumulation in tolerance to water deficit in L17 and M7 is noticeable.

Discussion

Irrigation regimes and genotypes both differed significantly for all morphological characters, except for number of seeds per plant and seed protein content, that can be considered useful for screening soybean germplasm under water stress. Compared with control conditions (a1), progressive water stress decreased number of pods per plant, seed yield, biological yield and plant height. The reduction in the number of pods and seed size in the stress treatments appears through the reduction of dry matter production (Oya et al. 2004). The reduction in relative water content (RWC) in both stress environments (a1 and a2) was provoked by the water losses in soil, because during the photosynthesis water loss occurs through the stomatal mechanism and the water assimilation rate is negatively affected during drought stress (Verslues et al. 2006; Lobato et al. 2008). The reduction in the total soluble proteins detected in the plants under water stress is

Table 3. Main effects of irrigation regimes and genotypes on relative water content (RWC) and some leaf biochemical characteristics. Data are means from three replications. Means followed by same letters in a group of a column are not significantly different at $P \leq 0.05$ according to LSD test.

Treatment	RWC	Soluble sugar	Soluble protein	*Chl a*	*Chl b*	Carotenoid
Normal irrigation (a1)	0.68±0.6 a	0.072±0.07 ab	0.061±0.04 a	13.2±1.12 a	16±1.37 a	1.6±0.14 a
Water stress at flowering (a2)	0.64±0.5 a	0.080±0.06 a	0.053±0.02 b	9.47±0.79 b	7.31±2.79 b	1.6±0.27 a
Water stress at pod filling (a3)	0.55±0.3 b	0.062±0.05 b	0.041±0.04 c	7.81±1.09 b	16.81±3.97 a	0.6±0.71 b
Habit	0.64±0.7 a	0.061±0.05 b	0.059±0.07 a	9.3±0.63 a	12.3± 0.77 b	1.2±0.01 a
L17	0.62±0.7 a	0.072±0.04 a	0.062±0.08 a	10.8±0.99 a	12.6 ±0.71 b	1.2±0.01 a
M7	0.62±0.6 a	0.063±0.04 a	0.042±0.09 b	10.3±0.83 a	15.2±1.01 a	1.3 ±0.01a

RWC=Relative water content; *Chla*=Chlorophyll a; *Chlb*=Chlorophyll b

Table 4. Interaction effect of irrigation regimes × genotype on physiological parameters. Data are means from three replications. Means in each column followed by same letters are not significantly different at $P \leq 0.05$ according to LSD test.

Treatment	Genotype	RWC (%)	Soluble sugar	Soluble protein	*Chl a*	*Chl b*	*Carotenoid*
	Habit	0.63±0.11 a	0.07±0.01 a	0.063±0.009 a	10.71±1.13 b	13.65±0.54 b	1.47±0.39 bc
Normal irrigation (a1)	L17	0.43±0.08 b	0.07±0.02 a	0.063±0.007 a	13.35±0.45 a	15.24±1.21 a	1.38±0.27 bc
	M7	0.51±0.05 a	0.06±0.02 a	0.057±0.005 a	15.50±1.88a	19.35±2.89 a	1.57±0.49 bc
	Habit	0.50±0.03 a	0.07±0.01 a	0.047 ±0.006ab	10.66 ±1.15b	7.62±1.28 c	0.73±0.47 c
Water stress at flowering (a2)	L17	0.43±0.04 b	0.08±0.03 a	0.053±0.005 a	9.77±1.34 b	7.80±1.24 c	0.65±0.42 c
	M7	0.41±0.04 b	0.08±0.02 a	0.060 a	7.77±1.01 b	6.48±2.64 c	0.77±0.38 c
	Habit	0.47±0.04 ab	0.06±0.02 a	0.040±0.003 b	6.51±1.12 c	16.04 ±1.25a	2.24±0.66 b
Water stress at pod filling (a3)	L17	0.47±0.03 ab	0.06 ±0.01a	0.037±0.006 b	9.28±0.77 b	14.69±0.57 a	3.78±0.59 a
	M7	0.51±0.01 a	0.07±0.01 a	0.047±0.005ab	7.56±0.88 bc	19.72±3.24a	1.39±0.77 bc

RWC=Relative water content; *Chla*=Chlorophyll a; *Chlb*=Chlorophyll b

due to probable increase of the protease enzyme activities, in which these protease enzymes promote the breakdown of the proteins and consequently decrease the protein amount present in the plant under abiotic stress conditions (Debouba et al. 2006; Lobato et al. 2008; Jaleel et al. 2009). In inadequate conditions to the plant the pathway of protein breakdown is active, because the plant uses the proteins for the synthesis of nitrogen compounds as amino acids that might be auxiliary for the plant osmotic adjustment (Sankar et al. 2007). In conclusion, alternative and significant variation was found for morphological and physiological characteristics in tested soybeans under water deficit environments, which underlines the susceptibility of this crop to drought stress. Based on the present results it can be concluded that drought stress retards the growth and metabolic activity of soybean genotypes. These parameters showed considerable variability under drought stress at different growth stages in soybean. This study may help to understand some adaptive mechanisms developed by soybean genotypes and contribute to identify useful traits for soybean breeding programmes.

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