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ORIGINAL ARTICLE

Physiological Performance of Soybean Cultivars under Drought Stress

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ABSTRACT
An experiment was conducted in Ilam, Iran, in order to study the effect of drought stress on yield and its associated traits of soybean cultivars, during 2010-2011 cropping season. The experiment was conducted as split plot arrangement based on Randomized Complete Block Design with three replications. Drought stresses during the 4 leaves stage (flowering, podding and seed filling stages) were assigned as main plots and three cultivars (M_7, M_9 and Hobit) were chosen as sub plots. The results showed that drought stress and cultivar were significant on plant height, fertile pod. Plant^{-1}, seeds.plant^{-1}, seed yield, biological yield, hundred kernels weight, seedspod^{-1}, harvest index, oil and protein percentage. M_9 cultivar had the best performance in seeds per pod, 100-seeds weight, seed yield and biological yield. The lowest seed yield was produced in the drought stress during the seed filling (2682 kg.ha^{-1}) and flowering stages (2918 kg.ha^{-1}), respectively. Under stress and non-stress conditions, the Hobit cultivar produced higher oil percentage. Protein percentage of M_7 cultivar was higher than other cultivars. It can be concluded that, M9 cultivar was better than the other cultivars and the grain filling stage was the most sensitive phonological one of soybean to drought stress in this region.
Keywords: *Drought stress, Phenological stages, Soybean, Yield, Yield components.*

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INTRODUCTION

Soybean is an important grain legume. The unique chemical composition of soybean has made it one of the most valuable agronomic crops [1]. Its protein has a great potential to be a major source of dietary protein. The oil produced from soybean grains is highly digestible and contains no cholesterol [2]. Soybean is one of the most important oil crops with 18–22 oil and 35 – 40 protein percent having the highest acreage of oil crop in the world which is known as the main source of plant oil and protein.

The crop growth and development are constantly influenced by environmental conditions such as stresses which are the most important yield reducing factors in the world [3]. Drought stress is considered as one of the crop performance limiting factors and a threat for successful crop production. Drought tolerance is important trait related to yield. To improve this trait, breeding requires fundamental changes in the set of relevant attributes, finally emerging as something named drought tolerance. Among the crops, soybean has the highest sensitivity to drought. This factor is the major limiting one in semi-arid regions. Therefore, soybean yield enhancement requires selection of tolerant and compatible cultivars in dry climate and low water [3].

Soybean has been reported to have a wide variation in drought tolerance. A cultivar is considered as a drought tolerant one when the yield is significantly higher than other cultivars in a drought condition but not in a non-drought condition and also when the yield difference among the cultivars is significant in both drought and non-drought conditions and the yield level is ranked higher under drought conditions [4, 5]. Brown *et al.,* [4] found a cultivar that exhibited less reduction in yield components, such as 100-seed weight and the number of seeds, under water stress conditions than other cultivars among the American cultivars. Along with water deficit which remains as a great concern, high temperature and low atmospheric humidity lead to drought [6, 7]. In such conditions, resistance to abiotic stress becomes a favorable trait of crops. However, due to the wide range of

plant stress responses with over lapping functions between their components, creating complex resistance mechanisms, selecting a new variety become a challenge [8, 9, 10].
Yield reduction due to water stress depends on genotype, developmental stage, severity and duration of water stress. But during the flowering, seed formation and seed filling stages, the negative effects of drought stress is more obvious [11]. In the early stage of soybean growth, from emergence to flowering, the effect of drought stress is less obvious than reproductive stage. Board [12] and Dennis and Bruening [8] suggested that under water stress, flowering and podding stages of soybean are the most sensitive stages and water stress during reproductive development decreases soybean yield through seed size reduction. Yordanov *et al.,* [10] claimed that water stress reduces the biomass, seed yield, number of pods in main stem, pod and seed number per plant. Rao *et al.,* [14] reported that in R5 stage, there is a strong positive relationship between yield and dry matter. They also found a linear relationship between biomass and seed yield.
Drought stress decreases the leaf area index in corn and those genotypes with greater leaf area showed a faster decline under stress [15]. Since the moisture requirements and sensitivity to drought stresses is varying in different crops, this experiment was conducted in order to study and determine the physiological performance of soybean cultivars under drought stresses, in Ilam's climatic condition.

MATERIALS AND METHODS

This experiment was carried out at the agricultural research station in Darreh Shahr, Ilam province, Iran, in 2010-2011 cropping season (46º 37′ E, 32º 9′ N and 650 m above sea level). This region has a moderate climate and average annual precipitation is 368 mm, most of which falls from late autumns to early spring. The soil type at the test site was a clay loam (sand: 36%, silt: 34%, and clay: 30%). Other soil properties are listed in Table 1. Monthly values of precipitation, temperature and relative humidity in the experimental field are shown in Table 2.This experiment was conducted as split plot design based on randomized complete block design with three replications. Drought stress treatments were arranged in main plots and soybean cultivars were allocated in sub plots. Drought stress treatments included: control treatment (D_1), drought stress at 4 leaves stage (D_2), drought stress at flowering stage (D_3), drought stress at podding stage (D_4) and drought stress at seed filling stage(D_5) that were arranged in main plots. Soybean cultivars including M_7, M_9 and Hobit were located in the sub plots.

Table 1. Soil properties of the experimental field in, 2010-2011 cropping season

EC	K (ppm)	P (ppm)	Total N (%)	O.C (%)	pH (Cº)
1.12	265	11.2	75	0.65	6.8

Table 2. Monthly value of precipitation, temperature and relative humidity in Ilam, Iran, 2010-2011 cropping season

Month	Evaporation (mm)	Ave.RH (%)	Precipitation (mm)	Min .temp (Cº)	Max .temp (Cº)
Jul.	511.9	21	0	26.5	43.4
Aug.	513.7	24	0	25	43.5
Sep.	425.8	32	0	21.9	39.5
Oct.	285.1	32	0	14.9	34.1

Each experimental plot consisted of 5 rows with intervals of 60 cm, row length of 5 m and plant distance of 10 cm. Irrigation control was implemented in the evaporation of 60 mm from the basin evaporation during the whole growing stage. Water stress and non -irrigation treatments were conducted in each stages in order to assess the responses of developmental stage to stress conditions. There was no rainfall during the study. Weather information for experimental field is presented in Table 1. Pre plant herbicide of allachlor was sprayed using a tractor to control the weeds of the field .
Before planting, soybean seeds were inoculated by *Brady rhizobium japonicum*. During growth stages, hand weeding was accomplished for 4 times. At physiological stage, harvesting was done from midline of the plot (after removal of half a meter of rows as edge effect).
Five plants were selected randomly and morphological traits and yield components were measured to evaluate the vegetative and reproductive traits in the experimental plot. Oil percentage of plots was measured after measuring the seed yield of each experimental plot using infra-metric system.

Traits studied were as follows: plant height, number of fertile pods per plant, number of seeds per pod and per plant, one hundred seeds weight, seed yield, biological yield, harvest index, seed oil percentage, and seed protein percentage. All statistical calculations were performed using SAS software and MSTATC and mean comparison of measured traits was performed using Duncan's multiple range test at 5% probability level.

RESULTS AND DISCUSSION

Plant height:

Analysis of variance indicated that the effect of drought stress and cultivars on plant height was significant at1% probability level but interactional effect between drought stress and cultivar on plant height was not significant (Table 3). Mean comparisons showed that drought stress in four-leaf stage reduced the plant height more than other treatments (Table 3). According to Austin [16] and Levitt [17], this reaction can be caused by mechanism of drought tolerance. When plants are exposed to drought stress, cell swelling, cell wall and synthesis enzymes are reduced and growth and plant height are decreased, consequently.

Plant height in both cultivars M7 and M9, did not significantly differ but Hobit cultivar showed significant difference as compared to other varieties (Table 3). In this experiment, Hobit and M7 cultivar has the lowest (23.07 cm) and highest (43.2 cm) plant height, respectively. Height increase is controlled by water and nutrients availability, which among the nutrients the nitrogen increases the plant nods and amount of available water increases internode distances respectively. Moammadi-Nasab *et al*., [18] reported that there was significant difference in terms of height among soybean cultivars.

Fertile pod numbers per plant:

Analysis of variances showed that the effect of drought stress and cultivars was significant on this trait at 1% and 5% probability level (Table 3). According to comparison results of mean values, drought stress in 4 leaf stage had the lowest effect on fertile pod numbers per plant and in seed filling stage it had highest effect on this trait (Table 4). Results showed that water deficiency in seed filling phase reduced the numbers of fertile pod per plant. Loss of flowers and pods during early reproductive phase is a possible reason for reduction of the number of pods per plant. Mean comparison at the level of 5% showed that, there was not a significant difference between M7 and M9 but Hobit had significant difference from the others.

M_7 and Hobit produced the highest (72) and lowest (50) fertile pod per plant, respectively (Table 3). Result of interaction effects between drought stresses and varieties on fertile pods per plant showed that M7 and M9 cultivars in different stresses have a higher number than Hobit cultivar (Table 5). As regards, M7 and M9 were indeterminate growth varieties and their flowering period was longer than Hobit cultivar, which means that these varieties had a better performance. This result is consistent with the results of Constable and Hearn [19] about the soybean responses to drought stress.

Seed numbers per pod:

Analysis of variance indicated that drought stress and cultivars effect on seed numbers per plant was significant at 1% probability level. Interaction between drought stress and cultivar on plant height was significant at 5% probability level (Table 3). Mean comparisons showed that the most decrease

Table 3. Analysis of variance for measured traits of soybean cultivars in drought stress treatments

		MS									
s.o.v	**df**	**plant height**	**Fertile pod . plant^{-1}**	**Seed.Pod^{-1}**	**Seed.plant^{-1}**	**100.seed.wt**	**Seed yield**	**Biological yield**	**Harvest index**	**Oil content**	**Protein**
Replication	2	6.26	21.99	0.02	59.1	0.007	358.2	3703.9	2.31	0.002	12.980
Stress (S)	4	25.11**	526.02**	1.11**	8374.2**	16.7**	63834.3**	98787.3**	229.4**	3.69**	28.414**
Error (a)	8	1.79	45.28	0.02	129.1	0.008	657.2	2929.31	3.7	0.0024	3.432
Cultivar	2	1925.1**	47.4**	0.45**	2124.1**	4.39**	107246.5**	406881.03**	98.8**	1.01**	25.880*
S×C	8	9.9	16.6*	0.06*	493.9	0.46**	651.4	3453.07	10.7	0.824**	13.259
Error	20	5.1	35.036	0.02	205.29	0.012	967.7	3790.3	6.5	0.0026	6.948
C.V (%)	-	6.26	9.22	6.2	8.71	0.085	8.67	8.66	6.45	0.073	7.41

*and **Significant at 5 %and 1 %probability levels, respectively in seed numbers per plant occurred in flowering stage (Table 4). The results showed that the flowering stage is the most vulnerable stage to stress that is consistent with the finding of Smiciklas *et al.,* [20].

Results showed that Hobit cultivar has the lowest seed numbers per plant and is significantly different from M7 and M9. Mean comparisons of the interaction effect of drought stress on cultivar indicated that M9 cultivar is the most tolerant one, because it has the minimum reduction of seeds per pod among all cultivars (Table 5).

Seed numbers per plant:

The results showed that this trait was affected by the impact of drought and cultivars but the interaction effect of these factors was not significant on this variable (Table 3). The greatest reduction in seed numbers per plant due to drought stress was observed at flowering stage. Stress at 4 leaf stage to flowering had a minimal impact on seed numbers per plant (Table 3). Seeds are considered the final sinks in plants and were affected by environmental conditions. Water deficiency is one of the most environmental factors that affect the seed numbers per plant. Bord and Hartville [21], under drought stress condition, seed number reduces in all plants, mainly because of the abscission of flowers and pods.

The effect of cultivar on seed numbers per plant was significant at 1%probability level (Table 3). Between M7 and M9 there was no significant difference, but the difference was significant (Table 4) as compared with the Hobit cultivar. Since the number of seeds per plant is multiplied by the pod numbers per plant and seeds per pods, it seems that there are significant differences between treatments due to the variation of the number of pods per plant.

100-seeds weight:

Analysis of variances for 100 seeds weight showed that the effect of drought stress, cultivar and drought stress was significant on the cultivar (Table 3) . Mean squares comparison showed that the drought treatments at grain filling stages have the greatest effect on reducing the yield (Table 4). It seems that the weight of grain is determined in the late reproductive stage therefore more

Table 4. Mean comparison of measured traits in drought stress treatments and soybean cultivars

	Plant height(cm)	Fertile pod . plant^{-1}	Seed. plant^{-1}	100-seed weight (g)	Seed .Pod^{-1}	Seed yield (kg.ha^{-1})	Biological yield (kg.ha^{-1})	Harv est Index (%)	Oil conten t (%)	Protein content (%)
Drought stress										
(D_1) Control	36.6 b	74.1 a	204.7 a	13.8 a	2.6 a	4653 a	10166 a	45.60 a	22.30 a	37.31 a
(D_2) 4-leaf	33.7 c	70.5 a	185.7 b	13.6 a	2.5 a	4228 b	9808 a	43.09 b	22.28 a	36.89 a
(D_3) Flowering	38.2 a	60.3 b	128.1 d	13.6 a	1.7 d	2918 d	7951 b	36.50 d	22.27 a	35.77 a
(D_4)Podding	35.3 b	58.4 b	150.6 c	13.4 a	2.1 c	3438 c	8485 b	40.08 c	22.25 b	34.81 ab
(D_5) Seed filling	36.7 b	57.3 b	152.8 c	10.5 b	2.3 b	2682 d	7953 b	32.90 e	20.80 c	32.87 b
Cultivars										
M7	43.2 a	71.9 a	185.3 a	12.8 b	2.3 a	3978 a	9736 a	40.4 a	21.82 c	36.87 a
M9	42.05 a	70.1 a	184.1 a	13.6 a	2.4 a	4168 a	9909 a	41.7 a	21.83 b	35.45 ab
HO	23.07 b	50.47 b	123.8 b	12.5 c	2.06b	2613 b	6973 b	36.7 b	22.29 a	34.25 b

Means in each column followed by similar letters are not significantly different at 5 %probability level, using Duncan's Multiple Rang Test

affected by drought stress at grain filling stage, this decrease can be due to loss of assimilate to seeds and then shrink them [10]. Other evidence indicates that the reduction in seed size is primarily due to a shortening seed filling period rather than an inhibition seed growth rate which leads to seed weight reduction [22]. In other drought stress treatments, no significant differences were observed in this trait. The mean square comparisons for 100-seed weight showed that the Hobit cultivar with 13.6 g produced the heavier seeds than M7 and M9 cultivars (Table 4). This heavier weight seems to be due to the better ability of Hobit for higher assimilating transfer to grains. in addition, the abundance of seed numbers per pod in M7 and M9 leads to the occurrence of high competition between the seeds to attract assimilates and finally leads to a reduction in dry matter in each grain. Interactions between drought stress and cultivars on this trait were significant at the level of 5 % of probability and Hobit had the highest seed weight at different drought stresses, while the control treatment had a less weight loss (Table 3&5).

Table 5. Interaction effect of drought stress treatments and soybean cultivar on fertile pod.plant^{-1}, 100.seed.wt, seed.Pod^{-1}and oil content

Drought stress	Fertile pod.plant^{-1}			100-seed weight (g)			Seed.Pod^{-1}			Oil content (%)		
	Cultivars			Cultivars			Cultivars			Cultivars		
	M7	M9	HO	M7	M9	HO	M7	M9	HO	M7	M9	HO
(D_1) Control	85.7 a	80.6 ab	56 ab	13.4 a	13.1 a	14.3 b	2.5 a	2.5 ab	2.6 a	22.2 ab	22.2 b	22.2 b
(D_2)4-leaf	78.2 a	72.5 bc	60.9 a	13.5 a	13 a	14.4 a	2.4 ab	2.75 a	2.3 b	22.25 a	22.36 a	22.26 b
(D_3) Flowering	62.06 b	67.6 cd	52.4 ab	13.4 a	12.9 a	14.5 a	1.8 c	1.65 c	1.8 d	22.27 a	22.27 b	22.33 a
(D_4) Podding	66.4 b	59.5 d	49.5 b	13.5 a	13 a	14.4 a	2.2 b	2.3 b	1.7 d	22.21 b	22.24 b	22.31 a
(D_5)Seed filling	67.2 b	70.2 bc	34.4 c	10.6 b	10.7 b	10.4 c	2.4 ab	2.5 ab	2.08 c	22.21 b	22.23 b	22.23 b

Means in each column followed by similar letters are not significantly different at 5 %probability level, using Duncan's Multiple Rang Test

Seed yield:
Analysis of variances showed that the effect of drought stress on soybean yield was significant at the level of 1% probability (Table 3). Results showed that the occurrence of drought stress at flowering and grain filling stages had the greatest effect on seed yield in this study (Table 4), which can be due to the reduction of fertile pod numbers per plant, seeds number per pod, and seeds weight. This conclusion is consistent with Samarah *et al.,* [23] and Demirtas *et al.,* [22].
As compared to the control, the lowest reduction in yield was observed in vegetative stage. It seems that drought stress in vegetative growth stage increases the root system and consequently the volume of soil that plant uses for water tension and thus reduces the damage. Average comparison of seeds yield indicated that among the cultivars, M9 had highest seed yield due to high seed weight and seeds number per pod. Smiciklas *et al.,* [20] stated that there was a difference between the yields of soybean cultivars.

Biological yield:
Analysis of variances showed that the effect of drought stress on biological yield was significant at the level of1% probability (Table 3). Results showed that there is a significant difference between reproductive stage (flowering, podding and pod filling stage) and drought stress occurrence in 4–leaf stage. According to the report of Georgiev [6], plants with stomatal closure have a less CO_2 absorption, assimilation and total dry matter production in severe drought stress at vegetative growth stage. Average comparison of biological yield indicated that the M9 produced higher dry matter than M7 and Hobit cultivar (Table 4).

Harvest Index
Analysis of variances showed that the effect of drought stress and cultivar on harvest index was significant at level of 1% probability (Table 3). Control treatment with 45.6% mean and drought occurrence at seed filling stage with 32.9% mean respectively, produced the highest and lowest harvest index (Table 4). Practically, harvest index is constant, because the drought stress does not change the yield total dry weight very much, unless severe drought stress reduces the grain yield in high level and consequently harvest index is reduced .
The loss of harvest index at grain filling stage due to reduced assimilate transfer, eventually leads to shrinkage and seed weight is reduced as a result of reducing the harvest index which is consistent with reported results of Ashley *et al.,* [24]. It seems that, the harvest index was reduced due to the loss of flowers and decrease in seed numbers per plant. According to mean of comparisons, M9 and

Hobit cultivar produced the highest (41.7%) and lowest (36.7%) harvest indices (Table 4). This result indicated that M9 has been more successful than Hobit cultivar because of the increasing carbohydrate transfer from photosynthetic organs to grains.

Oil content

Analysis of variances showed that the effect of drought stress, cultivars and interaction between drought stress and cultivars was significant on oil content at the level of 1% probability (Table 3). Mean comparisons showed that the lowest oil percentage (20.8%) was obtained in drought stress at grain filling stage which is consistent with the result of Smiciklas *et al.,* [20]. They suggested that drought stress occurrence during critical periods of seed formation and seed filling stages reduces the quantity and quality of seed oil. Mean comparisons indicated that Hobit cultivar has the highest (22.29%) oil percentage) (Table 4). Mohammadi-Nasab *et al*., [18] reported that, there was a significant difference among soybean cultivars in terms of oil percentage.

Protein content:

The results of this study showed that the effects of drought and cultivar had a significant effect on grain protein content (Table 3). Comparison showed that the lowest percentage of proteins in relation to stress in grain filling stage was observed in the treatment with 32.87 percent (Table 4). Franklin *et al.,* [3] also reported that increased stress decreases the grain protein. Comparison between the figures showed that theM7 cultivar with 36.87 percent protein, had the highest percentage of protein. Moammadi-Nasab *et al.,* [18] also reported that, there was significant difference among soybean cultivars in terms of protein content. It seems that the decrease was due to a severe decrease in photosynthesis. Photosynthesis is decreased in drought stress and materials for protein synthesis are not provided; therefore, protein synthesis is dramatically reduced or even stopped [25].

Simple correlation of traits:

Analysis of correlation between traits in drought stresses in soybean cultivars showed that the highest correlation was observed between seed and biological yield ($r=0.95^{**}$). The yield components including number of fertile pod per plant, seeds per pod and grain weight have a significantly positive correlation with yield and each other at the level of 1% probability. Seed yield with plant height, number of fertile pods per plant, and seed numbers per plant had a significantly positive correlation at the level of 1% probability (Table 6).

Georgiev [6] reported that seed weight, seed numbers per plant, number of pods per plant, and days to maturity had a significantly positive relationship with seed yield. A Positive correlation ($r=0.87^{**}$) was observed between seed yield and harvest index. Plant height has a significantly positive correlation with grain yield, number of fertile pods per plant, and seed numbers of plant at level of 1% probability .This relationship shows that plant height increases, plant level photosynthetic and total dry matter increase and ultimately grain yield also increases consistent to results of Smiciklas *et al.,* [20].The oil content had a significantly positive correlation with grain weight ($r=0.74^{**}$).

Table 6. Correlation between measured traits and seed yield in experimental treatments

Trait	plant height	Fertile Pod. plant^{-1}	Seed.plant^{-1}	Seed yield	Biological Yield	100.seed. wt	Seed.Pod^{-1}	Harvest Index	Oil content	protein
Fertile pod . plant^{-1}	0.67**									
Seed.plant^{-1}	0.61**	0.92**								
Seed yield	0.57**	0.89**	0.94**							
Biological Yield	0.70**	0.92**	0.94**	0.95**						
100-seed weight	0.21	0.34*	0.25	0.57**	0.43**					
Seed.Pod^{-1}	0.23	0.45**	0.75**	0.66**	0.61**	0.03				
Harvest Index	0.03	0.68**	0.75**	0.87**	0.68**	0.66**	0.62**			
Oil content	-0.32*	-0.13	-0.18	0.14	-0.05	0.74**	-0.19	0.3		
Protein	0.35*	0.45**	0.24	0.47**	0.34*	0.84**	0.23	0.48**	0.07	

*and **Significant at 5 %and 1 %probability levels, respectively

CONCLUSION

According to results of this study most of the traits including yield and yield components were affected by drought stresses. Among soybean cultivars, M9 cultivar showed a better performance than other cultivars. According to these results and results of water use comparison in different phenological stages, it seems that the drought stress occurrence in the 4-leaf stage (vegetative growth) has a less damage than other steps on final yield which is the best stage to save water and reduce its consumption.

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