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The effect of water stress on grain yield and protein of spotted bean (*Phaseolus vulgaris* L.), cultivar Talash

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ABSTRACT

In order to investigate drought stress and plant density the yield, yield components and protein of spotted bean grain (cultivar Talash), an experiment was carried out as split plots in randomized complete blocks with three replications in Yasouj, in 2012. Factors of the test including irrigation in three levels; without stress (control treatment), water stress in vegetative stage (interrupting the irrigation at the opening stage of the third to fourth true leaf), and water stress at reproductive stage (interrupting the irrigation at the stage of 50% flowering) as the main factors and four density level including 15, 25, 35 and 45 plants per m^2 were considered as sub-factors. The results showed that, the effect of water stress, plant density and interaction of water stress and plant density on grain yield, grains number in the pod, pods number in the plant and the weight of 100 grains was significant. The maximum yield was observed in the control plot and density of 45 plants per m^2, also the minimum yield was observed in the reproductive stress treatment and density of 25 plants per m^2 by 2398 and 1629 kg/ha respectively. Water stress treatment caused yield reduction at the stage of vegetative and reproductive growth by 10.4% and 32% respectively. The maximum amount of protein was obtained in the water stress treatment at the stage of reproductive growth. Considering the results of this experiment, the densities of 45 plants per m2 for normal irrigation conditions as well as 35 plants per m2 under water stress conditions are suggested.

Keywords: Spotted bean, Plant density, Water stress, Protein, Grain yield

INTRODUCTION

Drought is one of the most important environmental stressors which have detrimental effects on most of plant growth stages such as Germination, seedling growth, physiological activities and ultimately the plant yield (; Azami *et al*, 2013; Mohamadi and Rajaei, 2013; Saghafi *et al*, 2013; Dashti Marvili, 2013 and Azimi *et al*, 2013). With regard to the difference of bad effects severity of water stress in various

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growth stages, identification of critical or temporal stage in which the plant has the highest sensitivity to drought is very important. Appropriate irrigation regime is necessary for every region due to decreasing precipitations. Also due to the effect of plant density on plant establishment, weed control and product quality, evaluating of the most appropriate plant density is unavoidable to improve qualitatively and quantitatively the product (Askari Nejad, 2013; Ezzati kaklar and Moradi kor, 2013; Saeidian and rashidzadeh, 2013). Water stress during reproductive stage decreases the yield dramatically. Jalalian *et al*, (2005) announced that, effect of water stress in the yield reduction was more in reproductive growth stage than others. Mohammadzadeh *et al*. (2011) and Bayat *et al*. (2010) indicated that, water stress caused significantly to reduce pods number in plant, grains number in pod, weight of 100 grains and grain yield but, the amount of grain protein was increased. Nilson and Nelson (1998) stated that, stress in vegetative stage led to reduce the grain yield due to reduction of pods number in plant or grains number in pod. Stress at reproductive stage had the highest influence on the grain yield reduction. Among yield components, pods number in plant had the highest impact. Water stress in pod filling stage causes to make the highest percentage of hollow seeds as well as the lowest amount of grain protein. Generally, 20% to 30% of the beans grains consist of protein. Protein in the bean grain is considered as one of the secondary yields. The aim of this study is evaluation effect of water stress on grain yield and protein of spotted bean (*Phaseolus vulgaris* L.), cultivar Talash in Yasouj region (southwest of Iran).

MATERIALS AND METHODS

The experiment was done in Yasouj city which is located in longitude of western 55˚ 32′ and latitude of northern 32˚ 89′ and 1870 m elevation from sea level (figure 1).

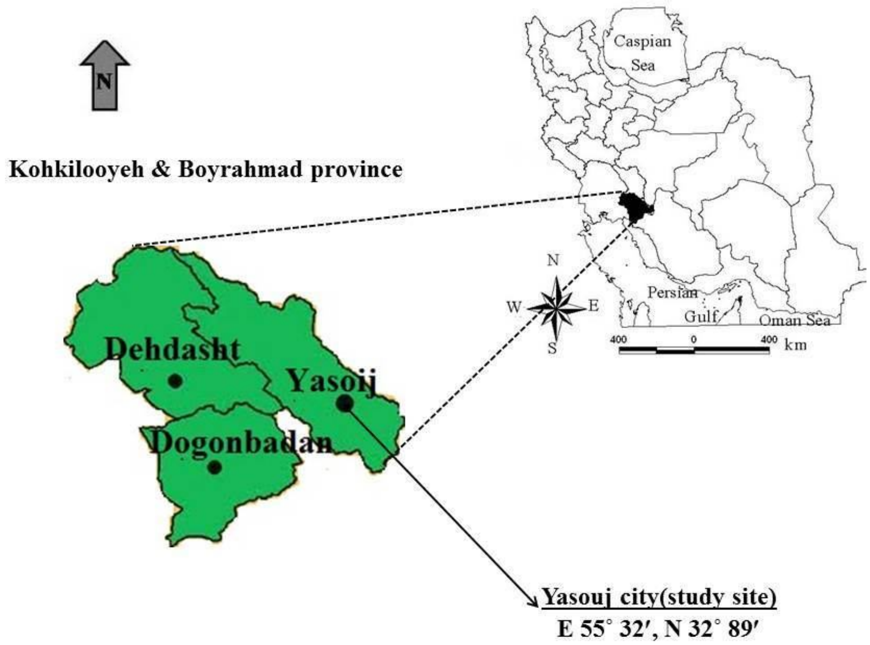

Figure 1: location of study area in the Kohkilooyeh & Boyrahmad province and Iran

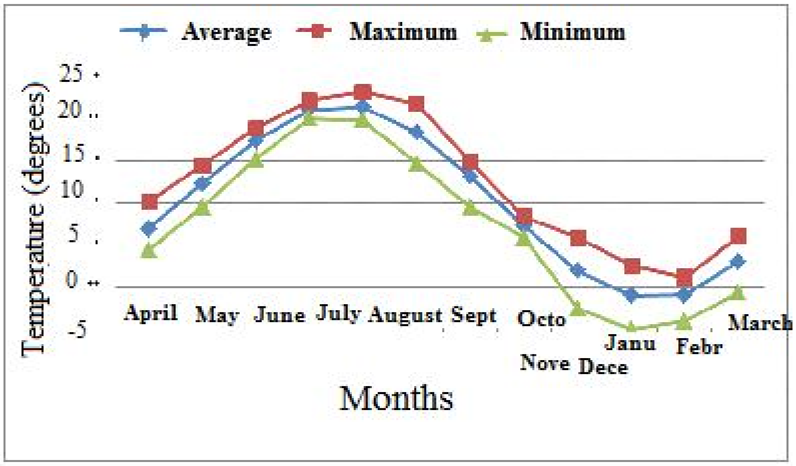

Figure 2: Changes in minimum, maximum and mean temperature during the growing season beans

According to the soil test conducted before planting, nitrogen fertilizer was given to the land from urea source by 50 kg/ha.

Table 1: Physical and chemical properties of soil test results

Absorbable Potassium	Manganese	Iron	Zinc	Absorbable Phosphorus	Percent of nitrogen	organic carbon %	eaction of saturated PH	trical conductivity$10^2\times$	Sampling depth(cm)
Mg/kg									
227	5.25	3.98	0.61	14.1	0.52	0.683	8.01	0.52	30

The experiment was performed as split plots in randomized complete blocks layout with three replications. Factors of the test including irrigation in three levels; without stress (control treatment), water stress in vegetative stage (interrupting the irrigation at the opening stage of the third to fourth true leaf), and water stress at reproductive stage (interrupting the irrigation at the stage of 50% flowering) as the main factors and four density level including 15, 25, 35 and 45 plants per m^2 were considered as sub-factors. Each sub-plot consisted of five planting lines with 5 m length also the distance between the rows was 50 cm. in order to eliminate the moisture effect resulted from irrigation and to prevent water leakage from the irrigated plots and furrows to the other plots, distance of the main plots from each side was considered 2 m, also distance of subplots from each other was considered by 1 m. planning operations was carried out after preparing the cultivation substrate and seed sterilization using Benomyl solution of 1% in the field. The studied field had kept as fallow in the year before performing the experiment was tilled 10 days before planting by moldboard plow. Then, aggregates were crushed by disc and complete leveling was done using trowel. Each experimental plot was formed by five planting lines with 5 m length. The distance of planting lines was considered by 50 cm. Primary irrigation was conducted each 3 to 4 days. After appearing the second main leaf and plant complete establishment, the desired densities were applied. By field investigations, an evaporation pan class A was installed close to the experiment location. In order to eliminate probable errors, the achieved results were compared with the results of meteorology ministry of Yasouj city. 60 mm evaporation from the evaporation pan was determined for normal irrigation as well as 90 mm evaporation to create water stress conditions. In order to distinguish irrigation time, the amount of evaporation was measured at the end of each day then, after reaching the considered amount, irrigation was carried out in the morning of the next day. Irrigation treatments were applied after thinning. In order to measure the grain yield, weight of 100 grains, pods number in plant and percentage of the grain protein, an area equal with 2 m^2 was cut from the center of plots using sampling frame. After counting the pods number in plants and grains of each pod, the grains were weighted (grain yield). The weight of 100 bean grains was calculated by separating 400 bean grains from each treatment. The grain protein was determined by measurement of nitrogen percentage existing in the bean grain. Variance analysis of the data was accomplished using statistical software SPSS and traits means were compared through Duncan test at 5% level.

RESULTS AND DISCUSSION

Pods number in plant

The results of variance analysis showed that, the effect of plant density and water stress on produced pods number became significant. Interaction of stress and density on the pods number in plant was not significant (Table 2).

Table2. Variance analysis of the traits related to the yield and some spotted beans traits

Protein percentage	Grain yield	Weight of 100 grains	Grain in pod	Pod in plant	df	Variations source
0/2382	0/0738	0/9297	0/0337	0/0172	2	Treat
0/4826 **	1/8357**	23/5577**	0/4247**	7/0978**	2	stress
2/5530 ns	0/0241 ns	0/3431 ns	0/0416 ns	0/0267 ns	4	Main error
0/2023 ns	1/0402**	87/8486**	1/8042**	17/1990**	3	density
0/1227 ns	0/1364**	11/0631**	0/327 ns	0/0737 ns	6	Density*stress
1/0654	0/0167	0/4451	0/0374	0/0397	18	Experiment error
7/2	3/7	3/0	3/2	7/9		Variations coefficient

** significant differences in 5% level, ** significant differences in 1% level ns non-significant differences*

Comparison of the means showed that the maximum number of pods in plant was in the density of 15 plants per m^2 by 12.04 pods, and the minimum number of pods in plant was found in the density of 25 plants per m^2 which did not have significant difference with the density of 45 plants per m^2 (Table 3).

Table3. Means comparison of the simple effects of various levels of water stress and plant density on the yield and some spotted beans trait

Grain yield (kg/ha)	Weight of 100 grains (gr)	Grain protein (%)	Grain in pod	Pod in plant	Levels	Treatments
2395 a	26/817 a	21/873 b	3/075 a	10/495 b	control	water stress
2146 b	24/950 b	21/816 b	2/914 b	10/728 a	vegetative stress	
1629 c	24/073 c	23/212 a	2/700 c	9/295 c	reproductive stress	
1868 b	29/578 a	21/766 a	3/484 a	12/043 a	15	density
1678 c	24/544 b	22/080 a	3/002 b	9/098 c	25	
2283 a	24/900 b	22/067 a	507 3/ a	10/392 b	35	
2398 a	22/098 c	22/079 a	2/592 c	9/158 c	45	

By increasing the density per area unit, the pods number in plant decreased. Because, by increasing the plant density, competition between the plants also increases to use available supplies but the supplies are limited for them (Koochaki & Sarmadnia, 2007). In the stress conditions, the maximum number of pods in plant was found in vegetative stress by 10.7 pods in plant. The lowest number of pods was obtained in reproductive stress treatment by 9.29 pods in plant which has been probably due to flower abortion, fertility decline, flowers falling and reduction of flowering period (Table 3). Therefore, flowering is the most sensitive growth stage of spotted bean to drought. These results are consistent with Vaezirad *et al.* (2008) and Nilson and Nelson (1998). Under such conditions, a reason of decreasing the pods number in plant can be the reduction of plant growth period which causes to reduce photosynthetic materials. This also has been emphasized by Wakerim *et al*. (2005). It seems that, decrease of photosynthetic material creation and increase of competition within the plant resulted from water stress along with flowers falling have led to decrease the pods number in plant (Santos *et al*. 2006). Although by increasing the density the pods number reduces due to less space and more competition, it is observed that, increase of grains number per area unit has made up the low number of pods and has caused to increase the pods number per m^2.

Grains number in pod

The results of variance analysis showed that, the effect of plant density and water stress on the grains number in plant became significant at level 1% (Table 3). Interaction of water stress and density on the grains number in pod was not significant. The highest and lowest numbers of grains in pod were related to the densities 15 and 45 plants per m^2 respectively (Table 4). Grains number in pod changes by changing the planting density and increase of density leads to reduce the grains number in each pod. Generally, the grains number decreases by increasing the plant density (Sadeghipoor *et al.*, 2004). Comparison of the means of applied levels of water stress on the grains number showed that, only in the treatment of water stress at flowering stage, the grains number in pod has been decreased dramatically and two others did not have any significant difference (Table 3). Therefore, application of water stress in vegetative stages does not cause a high effect in the grains number in pod which is much important in water efficiency management. Under water scarcity and limitation of production time conditions, photosynthetic materials decrease and consequently, the grains number in pod is reduced by aborted seeds. Bagheri *et al.*, (2001) reported that, bean has a high capacity to produce flower, fruit and seed. But, just a small part of these produced flowers becomes grain. One of the principal reasons of low yield of the bean under water stress conditions in reproductive stage is the lack of reservoir which mostly is related to flowers and fruits falling.

Table 4. Means comparison of interaction of water stress and plant density on the yield and some spotted beans traits.

Grain yield (kg/ha)	Weight of 100 grains (gr)	Grain protein (%)	Grain in pod	Pod in plant	Levels	Density
2104 ab	21/49a	31/00 a	3/67 a	12/32 a	control	15
1891 ac	21/62 a	29/43 f	3/38 ab	12/67e	vegetative stress	
1607 cf	22/21 a	28/30 df	3/40 ab	11/13 f	reproductive stress	
1809 c	22/27 a	23/96 bc	3/24 b	9/31 b	control	25
1812 c	21/70 a	24/40 be	3/06 b	9/68 d	vegetative stress	
1414 f	22/26 a	25/26 e	2/69 c	8/30 g	reproductive stress	
2737 de	21/80 a	27/43 d	2/69 c	10/61 c	control	35
2291 b	22/04 a	22/90 c	2/59 c	11/03 f	vegetative stress	
1821 c	22/36 a	24/36 be	2/24 d	9/53 bd	reproductive stress	
2931 d	21/92 a	24/86 be	2/69 c	9/73 d	control	45
2592 e	21/89 a	23/06 c	2/62 c	9/53 bd	vegetative stress	
1672 cf	22/41 a	18/36 g	2/46 cd	8/20 g	reproductive stress	

Weight of 100 grains

The effect of plant density, water stress and interaction of water stress and plant density on the weight of 100 beans grains became significant at level 1% (Table 2) so that, by decreasing the density, the weight of 100 grains increased. The maximum weight of 100 grains was observed in the density of 25 plants per m^2 by 29.57 gr and the minimum weight of 100 grains was observed in the density of 45 plants per m^2 by 22.9 gr (Table 3). The final grain size is affected by various environmental conditions (Masoodikia & Azizi, 2008). In high densities, the weight of 100 grains decreased due to increase of plant community respiration and shortened period of effective grain filling both of which limit the grain (Koochaki & Sarmadnia, 2007). Such a result has been reported about beans and soya by Sadeghipoor *et al.*, (2004) and Bord (2001). Significant effect of density on the weight of 100 grains is perhaps since, by increasing the density, competition between the plants decreases and consequently, use of foods by each pod increases, and since the grains number does not change, it shows its effect by increasing the weight.

Table 5. Correlation between the yield and some spotted beans traits

	Pod in plant	Grain in pod	Mean weight of 100 grains	Grain yield	Grain protein
Pod in plant					
Grain in pod	0/66 ns				
Mean weight of 100 grains	0/79*	0/73*			
Grain yield	0/2**	-0/21 ns	0/06 ns		
Grain protein	-0/79*	-0/68 ns	-0/72*	-0/33**	

** significant differences in 5% level, ** significant differences in 1% level ns non-significant differences*

Table of the means comparison shows that, under water stress conditions, the highest weight of 100 grains was observed in the control treatment by 40.33 gr and the lowest weight of 100 grains was observed in the treatment of stress in reproductive stage by 24.07 gr (Table 3). Reduction of the weight of 100 grains at the reproductive stage is due to the duration of the grain filling period and decrease of the photosynthetic material transmission to the grain. But, increase of the weight of 100 grains at the vegetative stress stage has been higher because of stimulating the vegetative growth and branching and consequently production of dry material and plant biomass (Vaezirad *et al*., 2008). These results are consistent with founding by Shekari (2001). In interaction of the effect of water stress and plant density, the maximum weight of 100 grains was obtained from the control treatment and the density of 15 plants per m^2 by 31 gr, also, the minimum weight of 100 grains was obtained from the reproductive treatment and the density of 45 plants per m^2 by 18.36 gr (Table 3). Mazhor *et al*., (1998) demonstrated that, irrigated canola postpones aging leaves and so, it cause increase and durability of the leaf area at the last growth stages which leads to increase the weight of 100 grains. Application of stress at reproductive stage with a high plant density causes to decrease the reservoir size and reduction of the weight of 100 grains. Although the mean weight of 100 grains was decreased by increasing the density and making water stress, the weight of 100 grains was not correlated with the grain yield (Table 4). The lack of correlation between the weight of 100 grains and the grain yield showed that, attempts to increase the yield through increasing the grain weight is not appropriate.

Grain yield

The effect of plant density, water stress and interaction of water stress and plant density on the grain yield became significant (Table 2). The maximum and minimum amounts of grain yield were respectively obtained from the densities of 45 and 25 plants per m^2. By increasing the density, the grains number in an area unit also increased (Table 3). It seems that by increasing the plant density, adequate leaf area index is provided during the grain filling stage and consequently, solar energy efficiency increases. This case leads to increase the grain yield per area unit in high densities. According to the table of means comparison, under water stress conditions, the maximum grain yield was achieved in the control treatment by 2395 kg/ha and the minimum grain yield was achieved in reproductive stress by 1629 kg/ha (Table 3). Basically, water stress in reproductive stages decreases the yield while, the conducted investigations before flowering are inconsistent. Schencut and Break (2003) and Emam *et al*., (2010) reported that, water stress causes to reduce the beans yield. But, the yield reduction is different considering the stress time and severity. The most sensitive stage of the beans life under stress conditions is in flowering stage so that, decrease of photosynthesis and photosynthetic materials lead to reduce the materials transmission to the grain and ultimately the grain yield reduction. Also, increase of flowers abortion and newly formed grains in pod is a factor to reduce the yield (Nilson & Nelson, 1998; Shekari, 2001). Comparison of the means of interaction of the effect of water stress and plant density on the grain yield shows that the highest grain yield was observed in the control treatment and the density of 45 plants per m^2 by 2931 kg/ha and the lowest grain yield was observed in reproductive stress treatment and the density of 25 plants per m^2 by 2931 kg/ha (Table 4). Engdy *et al*., (2003) stated that, the product yield is an accumulated response to the environment of growth season from planting to harvesting. So, determination of the effect of stress on the product depends on interaction of the factors associated with stress and products. According to the correlation table (Table 5), amount of the yield has the highest correlation with the pods number in plant. The grain yield is affected by the yield components. Therefore, the conditions which cause to change the yield components would change the grain yield.

The grain protein

Variance analysis showed that, the effect of water stress on the amount of grain protein was significant at level 1% (Table 1). The means comparison shows that, the highest amount of grain protein was observed in the treatment of water stress at reproductive stage by 23.21% and the lowest amount of grain protein was observed in the control treatment by 21.87% (Table 3). It seems that, the increase of grain protein under water stress conditions is associated with the increase of the ratio of protein to starch in the grain. Therefore, in water stress conditions, the amount of decline in starch synthesis is more dramatic which is consistent with Jalilian *et al*., (2005) and Mohammadzadeh *et al*., (2011. Also in water stress conditions, the amount of uptake and carbon fixation decrease by partial closure of stomata while, nitrogen remobilization from leaves to the grains does not decrease and it causes to increase percentage of the grain protein (Susa et al., 2004). The results of variance analysis showed that, the effect of various levels of density and interaction of water stress and plant density on the percentage of grain protein was not statistically significant and all of them were placed in a same group (Table 4). Therefore it seems that, the percentage of protein is one of the beans traits on which variations of plant density and interaction of water stress and plant density did not have a considerable effect, at least in the studied range in this research. Sadeghipoor *et al*., (2004) also reported the lack of effect of plant density on the percentage of the bean grain protein.

Conclusion

The overall results showed that, the effect of water stress, plant density and interaction of water stress and plant density on grain yield, grains number in the pod, pods number in the plant and the weight of 100 grains was significant. The maximum yield was observed in the control plot and density of 45 plants per m^2, also the minimum yield was observed in the reproductive stress treatment and density of 25 plants per m^2 by 2398 and 1629 kg/ha respectively. Water stress treatment caused yield reduction at the stage of vegetative and reproductive growth by 10.4% and 32% respectively. The maximum amount of protein was obtained in the water stress treatment at the stage of reproductive growth. Considering the results of this experiment, the densities of 45 plants per m2 for normal irrigation conditions as well as 35 plants per m2 under water stress conditions are suggested.

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