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The effect of water stress and plant density on yield and some physiologic traits of spotted bean (*Phaseolus vulgaris* L.), cultivar Talash in Yasouj region

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Abstract

In order to investigate the effect of water stress and plant density on yield and some physiologic traits of spotted bean (*Phaseolus vulgaris* L.), cultivar Talash, an experiment was carried out as split plots layout 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 biologic yield, grain yield, harvesting index and qualitative traits was significant. The maximum grain yield was observed in the control plot and density of 45 plants per m^2 by 2398 kg/ha, also the minimum yield was observed in the reproductive stress treatment and density of 25 plants per m^2 by 1629 kg/ha.

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Introduction

Although, beans has been introduced as a sensitive plant to drought and dehydration, its production is carried out in most parts of the world which are exposed to drought due to lack of water. According to FAO (2008) report, global average of beans yield is 568 kg/ha. Total area under cultivation in Iran is 115833 ha and total production is 218858 Tons of which 97.1% is cultivated as irrigated and 2.9% as dry farming. Due to limited arable land and inappropriate climatic conditions, so, increasing the yield per area unit should be considered to increase agricultural products (Azami *et al*, 2013; Mohamadi and Rajaei, 2013; Saghafi *et al*, 2013; Dashti Marvili, 2013 and Azimi *et al*, 2013). Determination of plant density is one of the most important methods for cultivation management to achieve high yield. 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. High harvesting index is due to more dedication of plant photosynthetic materials to the grain production and consequently higher economic yield and is an important criterion for the plant tolerance to the water stress. Researches have shown that, water stress significantly decreases the grain yield, biologic yield and harvesting index (Mohammadzadeh *et al.*, 2011; Bayat *et al.*, 2010). Decrease of cellular inflammation is the first effect of drought which causes to reduce final size and growth rate of the product. Then, the rate of stem and leaf growth decreases since decrease of the photosynthetic units number, production of photosynthetic materials and its transmission to various sections and finally, the yield decreases. In photosynthetic organs, carotenoids play an essential role in photosynthetic activity and have a high physiologic activity and protect automatic oxidation in the center. In non-photosynthetic organs, carotenoids have relation with oxidation mechanism stop. Chlorophyll stability has been suggested as a criterion of drought resistance to choose resistant varieties. Researches have shown that, by increasing water stress, the chlorophylls number also increases so, chlorophyll in these conditions decreases but, chlorophyll ratio a/b increases (Salehi *et al.*, 2005). With regard to the complexity of the process of resistance to drought, some researchers believe that, evaluation of plants only based on the yield is not an appropriate approach to investigate the amount of resistance to drought. Therefore it seems that, the yield study under stress conditions along with the selection of traits which are correlated with a mechanism of drought resistance such as avoidance and tolerance can make more rapid progress. Accordingly in this research, the effects of water stress and plant density on the grain yield and biologic yield and harvesting index were evaluated and compared. Also, physiological index of proline, and ability of chlorophyll a, b preservation and carotenoid in stress and density were investigated.

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).

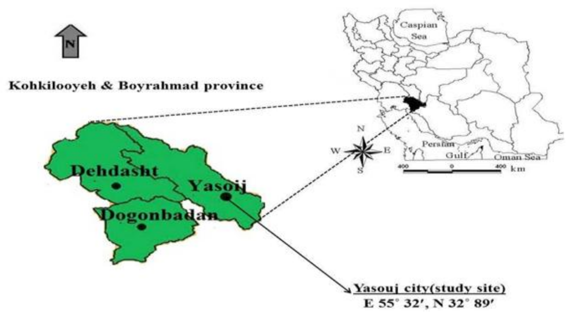

Fig. 1. location of study area in the Kohkilooyeh & Boyrahmad province and Iran.

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

Methods

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 m² 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 sub-plots 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.

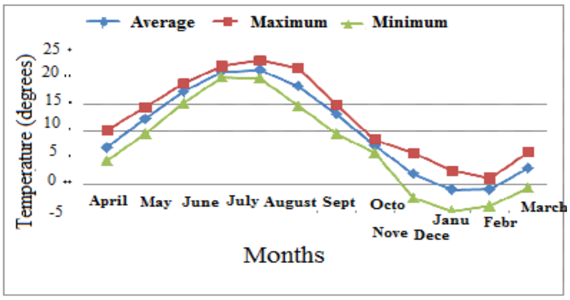

Fig. 2. Changes in minimum, maximum and mean temperature during the growing season beans.

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 the stage of physiological handling, an area equal with 2 m² was cut manually from the center of plots by eliminating the margins using sampling frame. After the plants weighting in all treatments (biomass yield), the grains were separated and were weighted separately (grain yield). Harvesting index was obtained through dividing the grain yield by biological yield. Also, the amount of proline, chlorophyll (Arnon, 1949) and carotenoid (Lichtentaller, 1968) of the plant leaf were measured. Variance analysis of the data was accomplished using statistical software SPSS and the traits means were compared through Duncan test at 5% level.

Results and discussion

Grain yield

Variance analysis showed that, the effect of plant density, water stress and interaction of water stress and plant density on the grain yield became significant (Table 2).

Table 1. Physical and chemical properties of soil test results.

Absorbable Potassium	Manganese	Iron	Zinc	Absorbable Phosphorus	Percent of nitrogen	organic carbon %	Reaction of saturated PH	electrical conductivity10²×EC	Sampling depth(cm)
Mg/kg									
227	5.25	3.98	0.61	14.1	0.52	0.683	8.01	0.52	30

The maximum and minimum amounts of grain yield were respectively obtained from the densities of 45 and 25 plants per m². By increasing the density, the grains number in an area unit also increased (Table 3). The results of Ebrahimi's (2010) research showed that, despite the reduction of grains number in single plant, by increasing the plant density of bean, the grains number in an area unit has increased. 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 2).

Table 2. Variance analysis of the traits f yield and some qualitative traits of spotted bean.

Mean squares								
Carotenoid	Chlorophyll a	Chlorophyll b	Proline	Grain yield	Biological yield	Harvesting index	df	Variation sources
/0001	0/0002	0/0003	0/0002	0/0738	0/9342	1/6944	2	Variations source
0/0244**	0/0272**	0/0119**	0/0965**	1/8357**	3/6573**	174/5278**	2	Treat
0/0005**	0/0004^{ns}	0/0002^{ns}	0/0002^{ns}	0/0241^{ns}	0/1714^{ns}	1/9444^{ns}	2	stress
0/0020**	0/0015*	0/0028**	0/0011^{ns}	1/0402**	10/6837**	22/0000**	3	Main error
0/0029**	0/0021**	0/0013**	0/0001^{ns}	0/1364**	1/5751**	21/0833**	6	density
0/0001	0/0004	0/0001	0/0004	0/0167	0/1305	0/8611	18	Density*stress
3/5	3/5	2/5	3/2	3/7	4/4	3/2		Experiment error

** Significant differences in 5% level, ** significant differences in 1% level ^{ns} non- significant differences.*

Basically, water stress in reproductive stages decreases the yield while, the conducted investigations before flowering are inconsistent. 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).

Table 3. Comparison of the means of simple effects of various water stress levels and plant density on spotted bean qualitative traits.

Carotenoid	Chlorophyll a	Chlorophyll b	Proline	Grain yield	Biological yield	Harvesting index (%)	Levels	Treatments
(micrograms per gram of the leaf wet weight)				(kg/ha)				
0/348 c	0/278 b	0/167 a	0/893 c	2395 a	6071 a	40/333 a	control	water stress
0/410 b	0/272 b	0/159 b	0/929 b	2146 b	5757 b	37/250 b	vegetative stress	
0/435 a	0/192 a	0/109 c	1/060 a	1629 c	4997 b	32/750 c	reproductive stress	
0/381 c	0/238 b	0/133 a	0/947 b	1868 a	5101 a	36/559 b	15	density
0/417 a	0/266 a	0/159 b	0/960 ab	1678 c	4344 c	38/889 a	25	
0/393 bc	0/238 b	0/148 c	0/957 b	2283 b	6232 b	36/556 b	35	
0/399 b	0/246 b	0/142 d	0/980 a	2398 b	6756 d	35/111 c	45	

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 m² by 2931 kg/ha and the lowest grain yield was observed in reproductive stress treatment and the density of 25 plants per m² (Table 4). According to the correlation table, among the investigated traits in this research, the amount of chlorophyll and harvesting index have the highest positive correlation with the yield. Therefore by increasing the chlorophyll a and harvesting index, the amount of yield increases (Table 5).

Table 4. Correlation between the yield, yield components and some qualitative traits of spotted beans.

Grain yield (kg/ha)	Carotenoid	Chlorophyll a	Chlorophyll b	Proline	Protein (%)	Water stress	density
	(micrograms per gram of the leaf wet weight)						
2104 ab	0/350 a	0/272 ab	0/144 a	0/873 a	21/493 a	control	15
1891 ac	0/383 b	0/267 ab	0/154 c	0/917 bc	21/623 a	vegetative stress	
1607 cf	0/410 de	0/176 e	0/101 f	1/050 e	22/210 a	reproductive stress	
1809 c	0/380 b	0/312 c	0/183 b	0/890 ab	22/277 a	control	25
1812 c	0/420 de	0/262 ab	0/151 c	0/930 cd	21/703 a	vegetative stress	
1414 f	0/450 fg	0/223 df	0/135 g	1/060 e	22/260 a	reproductive stress	
2737 de	0/290 c	0/273 ab	0/185 b	0/880 ab	21/800 a	control	35
2291 b	0/430 df	0/289 bc	0/162 d	0/930 cd	22/040 a	vegetative stress	
1821 c	0/460 g	0/153 e	0/097 f	1/060 e	22/360 a	reproductive stress	
2931 d	0/370 ab	0/253 ad	0/155 c	0/930 c	21/923 a	control	45
2592 e	0/407 e	0/269 ab	0/169 e	0/940 d	21/897 a	vegetative stress	
1672 cf	0/420 de	0/217 f	0/101 f	1/070 e	22/417 a	reproductive stress	

Biological yield

The results of variance analysis showed that, the effect of plant density and water stress and interaction of stress and density on biological yield became significant at the level 1% (Table 2). By increasing the plant density, biological yield increases. Because in higher densities, sub branches which are placed at the lower part of the plant, do not have a significant participation in the grain yield and mostly increase biological yield. Comparison the means of various density levels on biological yield shows that, the maximum biological yield was obtained from the density of 45 plants per m² by 4344 kg/ha (table 3). By increasing the plant density, the single plant weight decreases. Also under water stress conditions, the minimum amount of biological yield was observed in treatment of stress at reproductive stage by 4997 kg/ha and the maximum biological yield was achieved in the control treatment by 6071 kg/ha (Table 4). The dry mass yield of the beans significantly decreased under water stress conditions (Emam *et al.,* 2010). By applying the water stress in reproductive stage, stimulation of plant growth and increase of the yield seem to be rational.

Comparison of the means of interaction of water stress and plant density on biological yield shows that, the maximum biological yield was achieved in the control treatment and density of 45 plants per m² by 7931 kg/ha and the minimum biological yield under water stress conditions was obtained at reproductive stage and density of 25 plants per m² by 3969 kg/ha which did not have significant difference with the control treatment (Table 4).

Harvesting index

The results of variance analysis showed that, the effect of plant density and water stress and interaction of stress and density on harvesting index became significant at the level 1% (Table 1). Comparison of harvesting index means in various densities shows that by increasing the plant density, harvesting index has decreased significantly. The maximum harvesting index was found in density of 25 plants per m² by 38.19 and the minimum amount of harvesting index was in density of 45 plants per m² by 35.11 (Table 3). The reason of this happening can be the increase of biological yield by increasing the density. It seems that, most of sub branches have role in the grain production in low densities but, in higher densities, sub branches which are placed at the lower part of the plant, do not have a significant participation in the grain yield and mostly increase biological yield (Sekeston *et al.*, 1994). In water stress conditions, the highest harvesting index was found by 40.33% in the control treatment and the minimum harvesting index was obtained in the treatment of stress in reproductive stage by 32.75% (Table 4). Probable reason of this happening is that, mobility of the materials to the grain has increased at the end of growth period due to lack of available water which causes the grain yield decline. Also, pods number in plant which has an important role in the production yield, is an important reason of harvesting index reduction in the stress treatments which is consistent with Bayat *et al.*, (2010). In the interaction of water stress and plant density, the maximum harvesting index was observed in the control treatment and density of 25 plants per m² by 45.66% and the minimum harvesting index was found in the control treatment and density of 45 plants per m² by 31.33% which did not have a significant difference with density of 25 plants per m² under the same conditions (Table 4). Sooriano *et al.*, (2004) believe that, harvesting index under water scarcity conditions is a function of the ratio of used water after pollination which if increases, harvesting index would increase.

Table 5. Correlation between the yield, yield components and some qualitative traits of spotted beans.

	Harvesting index	Biological yield	Grain yield	Proline	Carotenoid	Chlorophyll a	Chlorophyll a
Harvesting index							
Biological yield	-0/1 ns						
Grain yield	0/3**	0/91*					
Proline	0/21 ns	-0/29**	-0/18 ns				
Carotenoid	-0/14 ns	-0/06 ns	-0/12 ns	-0/14 ns			
Chlorophyll a	0/82 ns	0/11 ns	0/45**	0/03 ns	0/14 ns		
Chlorophyll a	0/1 ns	-0/12 ns	-0/06 ns	-0/17 ns	-0/06 ns	-0/15 ns	

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

Amount of proline

The results of variance analysis showed that, the effect of various levels of water stress was significant on the amount of beans leaf proline (Table 2). Water stress causes to increase the amount of proline. Comparison of the means showed that, the maximum amount of proline was observed in the treatment of stress at reproductive stage and the minimum proline was obtained in the control treatment by 1.060 and 0.893 respectively (Table 3). Increase of proline concentration as a reaction to environmental stresses has been reported by Ghorbanali and Niakan (2005), Delakrda *et al.*, (2003) in sorghum, also by Sou *et al.*, (2003) in rice. Activity reduction of proline enzyme of oxidase is one of the most important factors of increasing proline concentration under stress conditions (Pearson & Haw, 1984). Among the studied densities, the maximum amount of proline concentration was related to the density of 45 plants per m² and the minimum proline was in the density of 15 plants per m² (Table 3). Investigating the interaction of water stress and plant density demonstrated that, the highest amount of proline was achieved in reproductive stress treatment and density of 45 plants per m² which did not show any significant difference with other applied levels in the same conditions. The minimum proline concentration also was observed in the control treatment and density of 15 plants per m² (Table 3).

Amount of chlorophyll a

The results of variance analysis showed that, the effect of water stress and interaction of water stress and plant density was significant at the level 1%, also the impact of density on the amount of chlorophyll in the beans leaf at the level 1% became significant (Table 2). Comparison of the means indicated that, the maximum chlorophyll was observed in the control treatment and density of 25 plants per m^2 and the its minimum was from the density of 35 plants and reproductive stress treatment by 0.312 and 0.153 micro grams per gram of the leaf weight respectively (Table 3).

Water stress also led to reduce the amount of chlorophyll a. The maximum amount of chlorophyll a was observed in the control treatment and the minimum was in reproductive stress treatment by 0.278 and 0.192 micro grams per gram of the leaf wet weight (Table 3). The amount of chlorophyll (a) decreases due to increase of density and stress conditions which causes light absorption, decrease of photosynthesis and decrease of the grain yield. Chlorophyll stability has been suggested as a criterion of resistance to drought. Under stress conditions, the amount of the leaf chlorophyll decreases but the ratio of chlorophyll a to b increases (Salehi *et al.*, 2005).

Amount of chlorophyll b

The results of variance analysis showed that, the impact of water stress treatment, plant density and interaction of water stress and plant density on the amount of chlorophyll b was significant at the level 1% (Table 2). Comparison of the means showed that, the maximum amount of chlorophyll b was observed in the control treatment and density of 35 plants per m^2 and the minimum amount of chlorophyll b was in treatment of water stress at reproductive stage treatment and density of 35 plants per m^2 by 0.185 and 0.097 micro grams per gram of the beans dry weight respectively. Also under water stress conditions, the highest amount of chlorophyll in the stress treatment was obtained in the control treatment and the lowest was related to reproductive stress treatment by 0.167 and 0.109 micro grams per gram of the beans leaf wet weight. In density treatment also, the maximum amount of chlorophyll b was in density of 25 plants per m^2 and its minimum was in density of 15 plants per m^2 (Table 3). There are different reports about the effect of water stress on the amount of chlorophyll. Movahedi Dehnavi *et al.*, (2004) reported that, amount of chlorophyll b increases because of water stress which is inconsistent with Yadavi *et al.*, (2004).

Amount of carotenoid

The results of variance analysis indicated that, the impact of water stress and plant density and interaction of water stress and density on the amount of beans leaf carotenoid was significant at the level 1% (Table 2). Comparison of the means showed that, in water stress treatment, the maximum amount of carotenoid belonged to the treatment of stress at reproductive stage and the minimum amount of carotenoid was observed in the control treatment by 0.435 and 0.348 micro grams carotenoid per gram of the beans wet weight. Also in the density treatment, the maximum amount of carotenoid belonged to the density of 25 plants per m^2 and the minimum carotenoid belonged to the control treatment by 0.417 and 0.381 micro grams carotenoid per gram of the beans wet weight (Table 3). Investigating the interaction of the impact of water stress and plant density on the amount of carotenoid showed that, the maximum amount of carotenoid belonged to the density of 35 plants per m^2 and treatment of water stress at reproductive stage and the minimum amount of carotenoid belonged to the control treatment in the same density by 0.46 and 0.29 micro grams carotenoid per gram of the beans wet weight respectively (Table 4).

Various researches have shown that, there is a strong correlation between oxidative stresses which is caused by environmental stresses, and increase in concentration of antioxidant enzymes in photosynthetic plants. Lascano *et al.*, (2005) showed that, the concentration of antioxidant enzymes would increase as two times under stress conditions, therefore, it causes to increase resistance to oxidative stresses. Increase of the amount of carotenoid under environmental stresses is because of its protective role which protects the green pigment of chlorophyll in stress and competition conditions (Tise and Zyeger, 2006). Also, it transmits the absorbed light to the chlorophylls and causes to increase the chlorophyll efficiency.

Conclusion

Overall investigation of qualitative traits shows that, there is a proper path transmission from photosynthetic products to the grains in appropriate irrigation situation but, by occurrence of the water stress, there would be a dramatic decrease of these products in the grains. Considering parallel decrease of other yield components, it can be mentioned that, the reason of decreasing the weight of 100 grains is the amount of photosynthesis and not the significant variation in sharing the materials with grains; and the most important reasons of this happening include the decrease of grains filling, decrease of pigment and photosynthetic enzymes which is consistent with Vaezirad (2008). Destruction of chloroplasts and chlorophyll degradation resulted from chlorophylase and peroxidase enzymes activity are considered as effective factors on the reduction of this pigment concentration in water scarcity conditions (Sundersun & Sudacaren, 1995). According to Psarkly (1999), preservation of the leaf chlorophyll and photosynthesis durability under water stress can be effective in the plants resistance for water stress conditions.

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