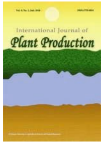

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Effect of water stress and plant density on canopy temperature, yield components and protein concentration of red bean (*Phaseolus vulgaris* L. cv. Akhtar)

M. Asemanrafat, T. Honar*

Irrigation Department, Shiraz University, Shiraz, I.R. of Iran.
*Corresponding author. E-mail: toorajhonar@yahoo.com

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Abstract

In order to study the effects of different irrigation regimes and plant density on yield component and protein concentration and crop water stress index (CWSI), under research field conditions a split plot arrangement was conducted in completely randomized block design during two years. The first factor of variables was the effects of 4 irrigation levels 120% (I_1), 100% (I_2), 80% (I_3) and 60% (I_4) of Standard evapotranspiration and the second factor was the spacing of 5 cm (D_1), 10 cm (D_2) and 15 cm (D_3) between plants within a row. Results indicate that the number of pods in each plant and the grains in each pod decreased when density increased, but the hundred-grains-weight and the height of bean plants increased. When irrigation increased, various increases were observed in the yield, the number of pods in each plant, the grains in each pod, the hundred-grains-weight and the height of bean plants, but the grain protein decreased. Protein concentration increased with more severe water deficit in the soil. The protein concentration directly correlated with total irrigation water during the growing season (TI) and the exponential equation $P=33^* \, e^{-4E\text{-}04TI}$ can be used for protein concentration prediction. The effect of the irrigation water was significant and CWSI increased with increased soil water deficit. The effect of the density for CWSI was not significant. Grain yield (GY) directly correlated with CWSI and the exponential equation, $GY=696.2(CWSI)^{-0.51}$ can be used for the prediction of grain yield. The CWSI value is useful for evaluating crop water stress in beans and thus it could be useful in timing the irrigation. Results of this research indicate that yield components such as height of bean plant, the number of pods in each plant and the number of grains in each pod were significantly different by applying the different irrigation strategies at the three different lengths of plant spacing within a row.

Keywords: Bean; Density; Standard evapotranspiration; Crop water stress index; Yield component; Protein content.

Introduction

Advancements in technology have led to wired and wireless thermal infrared instrumentation, which provides for direct and continuous recording of temperatures with various electronic data loggers and computer base stations, resulting in continuous crop surface temperature monitoring. Measuring multiple locations in the field can be done by a moving irrigation system such as a center pivot, which is regarded as a suitable platform for sensors (Phene et al., 1985; O'Shaughnessy et al., 2013).

Geo-referenced canopy temperature measurements made over a large area allow for spatiotemporal mapping of crop water stress (Sadler et al., 2002; Peters and Evett, 2008; O'Shaughnessy et al., 2011) and yield predictions (O'Shaughnessy et al., 2011).

Research has shown that plant temperature is strongly influenced by plant water status. Canopy temperature (T_c) has been suggested as an indirect method of quantifying crop water stress. Canopy temperature is used by detecting water stress based on the principle that the water which is lost through transpiration cools down the leaves below the temperature of the surrounding air when there is adequate irrigation applied. As the soil water becomes scarce, transpiration is reduced and leaf temperature increases. If transpiration is greatly reduced, leaf temperature will be greater than air temperature because of radiation absorbed by the leaf. The handheld infrared thermometer (IRT) is a remote sensory device which is used for rapid and convenient measurements of canopy temperatures across a large area, at all levels of water stress (Jackson, 1982). The standard of IRT measurements of canopy temperatures for crop water deficit assessment is recognized as an increasingly popular approach (Hatfield, 1990). It has been stated that the IRT has appeared as an important tool in irrigation scheduling and irrigation water management. Fuchs and Tanner (1966) proposed the first basic technique to employ IRT-determined crop temperatures in order to assess the severity of water deficits. They used the differences in (T_c) between various treatments, with a well-watered treatment usually being the reference (T_c).

Amini and Milani (2013) utilized the CWSI to evaluate crop water stress and also determined irrigation timing of lentil crops in research fields of Tabriz. Canopy temperature minus air temperature was discovered to be inversely and linearly correlated with the evapotranspiration in crops. Idsoet et al. (1977) found that the difference between the canopy temperature and the air temperature is related to the leaf water potential.

Researchers have established the water stress index for many commercial field crops. Studies have also been carried out on trees but, still, field crops have been studied more extensively (Sepaskhah and Kashefipour, 1994; Testi et al., 2008; Ben et al., 2009; Wang and Gartung, 2010; Paltineanu et al., 2013).

The red bean is being consumed as a prevalent source of protein, calories, fiber and minerals in many developing countries (Ramos et al., 1999; Singh et al., 1999). Plant distribution patterns and plant densities can practically affect the utilization of environmental resources to the extent that inter- and intra-plant competitions are influenced to a great degree. As a result, plant density is considered a vital factor when setting the aim to reach higher grain yield (Board and Harvile, 1996).

Proper selection of crop varieties are regional-specific and necessitate decisive managements since they have great effects on agricultural production projects. The pattern by which plants are spatially arranged within a given land area is a prime factor because an appropriate density of cultivated plants is a prelude to successful crop production systems. The geometric arrangement of plants (otherwise known as the planting pattern) can be modified by changing the width of rows and the spacing thereof. Hypothetically, the choice of narrow rows in plant spacing could be expected to increase the efficiency of resources and can also delay the onset of interplant competition. An optimum plant density for maximum economic yield would depend on the crop species, its variety and cultivation conditions. Accordingly, recent years of frequent research have partly focused on the regulation of plant populations based on the availability of production factors and thus have investigated how plant density can affect the quantity and quality of yield (Kochaki and Banyan Aval, 1993). By experience, the highest yields are achieved via optimum cultivation density which is in effect synonymous with the consistent distribution of plants. Furthermore, the structure of plant canopy isof great importance with regard to measurable factors of the yield (Mohamadzadeh et al., 2011). In a relevant research report, Nazaralizadeh et al. (2012) found that the row spacing and plant density can affect the growth of safflower in a manner that could improve yield when shorter row spacing was considered. This improvement in yield was claimed to have resulted from higher values of LAI and CGR with regard to the vegetative growth stage. A desirable plant density can be devised based on a variety of criteria including plant characteristics, growth period, the time and method of planting, soil fertility, plant size, available moisture, solar radiation, planting patterns and weeds status (Abbasi and Maleki, 2015).

Where annual precipitation is insufficient, drought stress is a common limiting factor in crop growth and yield. It can profoundly reduce dry matter production, yield and yield components due to the fact that leaf area decreases as a result of drought. Drought also accelerates the senescence of leaves (Emam and Seghatoleslami, 2005). The common bean could be grown as a seed legume where rain-fed crop-rotations are applied with winter wheat. Such crop-rotations can increase the production diversity and improve the yield at each rotation (Nielsen and Nelson, 1998; Emam et al., 2010).

There are hypothetical understandings that the common bean is susceptible to drought stress or water deficits. Nonetheless, the production of this crop on a global scale is often carried out where drought stress inevitably prevails, due to insufficient water supplies, sparse rainfall and/or inadequate irrigation (Machado and Durães, 2006). According to a report by FAO (2008), the global average yield of beans is 568 kg/ha. Total area under cultivation in Iran is 115833 ha and total production is 218858 tons, whereof 97.1% are cultivated under irrigated conditions and 2.9% are cultivated as dry farming or rain-fed. Due to limitations in the area of resourceful arable land and the common unfavorable climatic conditions, it would be imperative to increase the yield per area unit, should agricultural commodities succeed in being produced in amounts worthy of trade (Azami et al., 2013). The determination of plant density is one of the most important criteria for cultivation management whereby high yield comes in the frontline for farmers to pursue optimum harvest. An appropriate irrigation regime needs to be planned for every region where rainfall is scarce. Also, due to the effects of plant density on plant establishment, discernible improvements in the quality and quantity of productions would require management in weed control and overall product quality, besides outlining the most appropriate plant density. High harvesting index is a factor that considers the extent to which plant photosynthetic materials are dedicated to grain production and could accordingly guide towards higher economic yield. This index is an important criterion for the management of plant tolerance to water stress. Researchers have shown that water stress not only reduces grain yield, but also limits the total amount of biomass production and largely affects the harvesting index (Mohammadzadeh et al., 2011). This research studies the effects of water stress and different levels of cultivation densities of red bean (Akhtar cultivar) on canopy temperature, yield components and protein concentration of beans.

Materials and Methods

The predominant soil characteristics are known to be silty clay loam. Before sowing, 250 Kg/ha of urea fertilizer and 250 kg/ha of superphosphate was applied to the soil.

Beans (cv. Akhtar) were planted with different plant density of 66, 33 and 22 plant/ m^2 on May 26, 2013 and also on May 18, 2014 in the first and second years, respectively in six rows with 30 cm spacing between the rows and in a soil depth of 5 cm in each plot, by direct seeding in rows. A split plot arrangement was conducted in completely randomized block designs in both years. The primary variable was four levels of irrigation: 60 (I_4), 80 (I_3), 100 (I_2), 120% (I_1) of the crop standard evapotranspiration (ET_o) under surface irrigation. A second variable was the spacing of 5 cm (D_1), 10 cm (D_2) and 15 cm (D_3) between plants within each row, with 30 cm between the rows, in three replications. Soil water content at 0-0.2, 0.2-0.4 and 0.4-0.6 m depths was measured by the gravimetric method before each irrigation event. The irrigation interval was 7 days.

Reference evapotranspiration (ET_o) was calculated using modified Penman–Monteith equation for semi-arid environments in the study area (Razzaghi and Sepaskhah, 2012). Meteorological data were obtained from standard weather stations at the Agricultural College, located near the experimental field.

To ensure uniform germination and emergence, equal amounts of irrigation water were applied to all treatments in the initial stage. The experimental plots were irrigated by the furrow irrigation method by an applied efficiency of 90%. Before each irrigation event, the irrigation water requirements were estimated by multiplying the reference evapotranspiration values (ET_o) by k_c. In the first year, the k_c of the bean plant was obtained from the FAO-56 (Allen et al., 1998) but in the second year the k_c was derived from the soil water balance obtained in the first year. Irrigation water was applied every other week according to the four irrigation treatments: 1.1, 1, 0.8 and 0.6 multiplications of the plant's standard evapotranspiration of red bean. Due to the presence of a deep groundwater table enabled free drainage conditions. Each experimental plot was 6 meters long and 3 meters wide.

The canopy temperature (T_c) was determined using a hand-held infrared thermometer (Infrared thermometer model 5500) in the second year, 2014. The infrared thermometer (IRT) was operated with the emissivity adjustment set at 0.95. The IRT data collection was initiated on May 18 (Julian days, 172) and continued until September 1 (Julian days, 238) and data was read four days after irrigation. The canopy temperature was measured on 5 plants from 4 directions (east, west, north and south) when fully sunlit with oblique measurements at 20°-30° horizontally to minimize soil background temperature in the field of view. Measurements were then averaged.

The dry and wet bulb temperatures were measured with an aspirated psychrometer at a height of 2.0 m in the open area adjacent to the experimental plots. The mean T_a value was determined by the average of the dry bulb temperature readings during the measurement period. The mean value for vapor pressure deficit (VPD) was computed as the average of the calculated instantaneous VPD, using the corresponding instantaneous wet and dry bulb temperatures and the standard psychrometer equation (Allen et al., 1998).

The CWSI values were calculated using the procedures of Idso et al. (1981). In this approach, the measured crop canopy temperatures were scaled in relation to the minimum canopy temperature expected under non water-stress conditions and the maximum temperature under severe water stress. The non-water-stressed baseline for the canopy-air temperature difference (T_c-T_a) versus the vapor pressure deficit (VPD) relationship was determined by using data collected only from the control treatment (I-full). The upper (fully stressed) baseline was computed according to the procedures explained by Idso et al. (1981). To verify the upper baseline, the canopy temperatures of the plants under full stress (RDI treatment) were measured several times from May 18 (Julian days, 172) to September 1 (Julian days, 238). Using the upper and lower limit estimates, a CWSI can be defined as follows (Idso et al., 1981):

$$CWSI=\frac{BC}{AC}=\frac{(T_c-T_a)_m-(T_c-T_a)_{ll}}{(T_c-T_a)_{ul}-(T_c-T_a)_{ll}} \quad (1)$$

where T_c is the canopy temperature (°C), T_a the air temperature (°C), m is the measured point, ll is the non-water-stressed baseline (lower baseline) and ul is the non-transpiring upper base line.

The initial irrigation schedule here was to irrigate the land until the bean plants reached their four-leaf stage; thereafter, irrigation continued every other week according to the four irrigation treatments: 60, 80, 100 and 120% of standard evapotranspiration of red bean. Due to the presence of a deep groundwater table, suitable drainage conditions existed. Each experimental unit measured 6 meters long and 3 meters wide.

The canopy was sampled by the area of 2 m^2 from different experimental plots on harvest day in 2013 and 2014. The samples were dried at 80 °C in an oven and the grain yield (kg ha^{-1}) was measured. Also, the yield components of beans were measured in each treatment at harvest day (Moradi Kodoyi, 2013).

Protein concentration of seeds is determined after harvest by measuring its nitrogen concentration by the Kjeldahl method and by multiplying the nitrogen concentration by 6 so as to obtain the total protein concentration. The MSTAT-C software and Duncan test were used for the analysis of data variance.

Results and Discussions

Because the infrared ray gun requires sunny days to measure the water stress index, the measurement must be done at the same time (from 12 to 15 pm) when the crop water demand is high. Data were taken for all treatments based on Julian days: 172, 186, 201, 214, 221 and 238. Measurements were done four days after each irrigation.

Based on the method proposed by Idso et al. (1981), the parameters that define the lower and upper limits of the CWSI are presented in Figure 1. The equation that defines the lower CWSI baseline is:

$$T_c-T_a = 1.175-0.1019 \text{ VPD} \qquad r^2 =0.69, P<0.05, n=36 \quad (2)$$

where T_c-T_a is expressed in °C and VPD is in kPa. Idso (1981) reported the following relationship for the lower limit in tomato crops T_c-T_a = 2.86-1.96 VPD. For corn, Irmak et al. (2000) found the following relationship: T_c-T_a = 1.39-0.86 VPD. It can be observed that all relations are specifically different, a his fact which is in agreement with the results obtained by Bucks et al. (1985), who pointed out that the intercept and slope values vary depending on the climate, type of soil and the crop being cultivated.

The down-sloping line (Figure 1) represents the baseline without water stress, that is, the difference between the air temperature and the crop temperature during periods of adequate water supply at different VPD; in this case, stomata were supposedly open and the temperature difference was a function of VDP. An increase in VDP entails an increase in the drying power of the atmosphere and, consequently, in plant transpiration. The horizontal line (upper baseline, Figure 1) is the difference between the air temperature and the crop temperature associated with periods of greater stress (with water limitations), when there is no transpiration. The average value was 5 °C with n=36. For the bean crop, Irmak et al. (2000) determined an average value of 4.6 °C for corn, a value lower than that found in this study, which means that the bean plant is more susceptible to possible water stress than the corn crop.

A VPD equal to zero indicates that the air contains the maximum amount of water vapor possible (relative humidity = 100%). The lower limit of the CWSI changes as a function of vapor pressure due to the VPD. The CWSI varies between 0 and 1 when plants are subject to conditions ranging from appropriate irrigation to conditions of total water stress. The lower limit in this research was developed by VPD values ranging from 31 to 41 mbar. Gardner & Shock (1989) suggest that it is necessary for VPD values to range from 10 to 60 mbar in order to define a baseline which can be used in other locations too.

Calculation of CWSI can be done via a graphical approach starting from the following relation: CWSI = BC/AC, where point A is the difference between the temperatures of the leaf minus that of the air at the moment of measuring, point B is the difference in maximum temperature between the leaf and the air (superior limit) and point C the minimum difference (inferior limit) in the VPD conditions in which temperature measuring was carried out for the leaf and the air (A). Therefore, the CWSI is determined by the relative distance between the lower line that represents the conditions without stress and the u line where there is no transpiration.

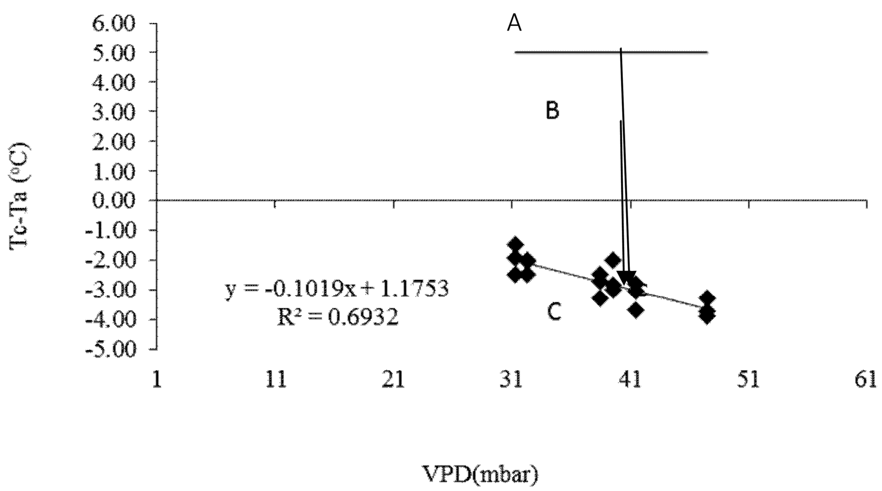

Figure 1. The lower and upper baselines for bean plant for determining the crop water stress index.

CWSI index four days after Irrigation

Figure 2 shows that the values of the CWSI index vary among the different treatments. The treatments of 120% ET_o and 100% ET_o rendered CWSI values that were nearly zero. By the treatment of 80% ET_o, the CWSI value ranged from 0.14 to 0.44 and by the treatment 60% ET_o, the CWSI was one. The value of the CWSI index by the 80% ET_o treatment indicates that the bean is very susceptible to water stress at that point (Figure 2).

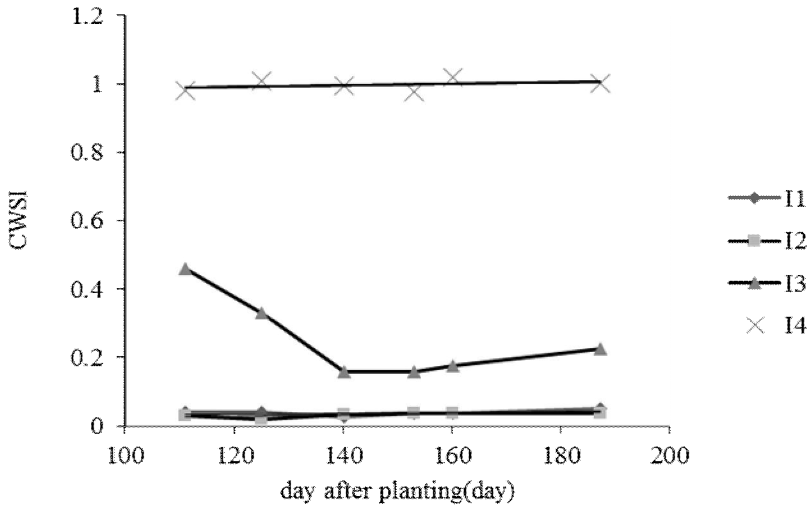

Figure 2. Variation in CWSI index for the I_1, I_2, I_3 and I_4, respectively indicating 120%, 100%, 80% and 60% of standard evaporation, during the growing season of 2014 for bean plant.

Effect of the total irrigation water (TI) on the water stress index

The analysis of variance showed that there are significant differences (P<0.01) between how various irrigation depths affect the CWSI during the different phenological stages of the crop. The effect of the density was not significant (P>0.05) while the effect of the interaction among irrigation, density and Julian days was significantly observed (P<0.01) among the differences (Table 1).

Table 1. Effect of the total irrigation depth on the water stress index during different Julian days in 2014.

Source of variation	Degree of freedom	CWSI
Replication	2	0.006^{ns}
Density	2	0.001^{ns}
Irrigation	3	11.59^{**}
Irrigation * Density	6	0.004^{**}
Julian days	5	0.036^{**}
Irrigation * Julian days	15	0.038^{**}
Density * Julian days	10	0.008^{**}
Irrigation *Density * Julian days	30	0.008^{**}
Error	136	00.002

Table 2 shows the relation between total irrigation depth and CWSI. In general, it can be noted that the treatment of 60% irrigation depth yields the highest CWSI values in the different stages of crop development, which is statistically significant compared to other levels. The lowest CWSI values were obtained by the irrigation depth of 100 and 120% of the ET_o treatments. This is because there was a normal water supply during the crop season. As water availability for the plant decreased, the CWSI value increased up to 1 by severe irrigation restrictions (60% ET_o).

Table 2. Effect of the total irrigation depth and plant spacing on the water stress index during different Julian days in 2014.

Treatment	Crop Water Stress Index					
	Vegetative		Reproductive		maturation	
Julian days	172	186	201	214	221	238
Within row spacing (cm)						
5	0.375	0.3792	0.315	0.298	0.3183	0.3233
10	0.405	0.3442	0.2967	0.2917	0.3242	0.3283
15	0.3625	0.3358	0.2992	0.3208	0.3092	0.4008
Irrigation regimes						
120% ET_s	0.041^f	0.042^f	0.026^f	0.037^f	0.038^f	0.05^f
100% ET_s	0.033^f	0.024^f	0.034^f	0.037^f	0.036^f	0.037^f
80% ET_s	0.46^b	0.33^c	0.159^e	0.158^e	0.178^e	0.228^d
60% ET_s	0.986^a	1.00^a	0.995^a	0.977^a	1.00^a	0.98^a

The relationship between the water stress index and the irrigation water is negative and exponential. As the irrigation increases, the CWSI decreases until it reaches 0 when the 100 or 120% of the ET_0 is applied. Simsek et al. (2005) observed that when the irrigation water decreases, the rate of transpiration by the crop also decreases, resulting in the increase in the crop's temperature and the CWSI. This reduces the crop yield (Figure 3). The exponential equation is as follows.

$$CWSI=624.55\ e^{-0.075\ T\ I} \qquad R=0.85,\ P < 0.01,\ n= 36 \qquad (3)$$

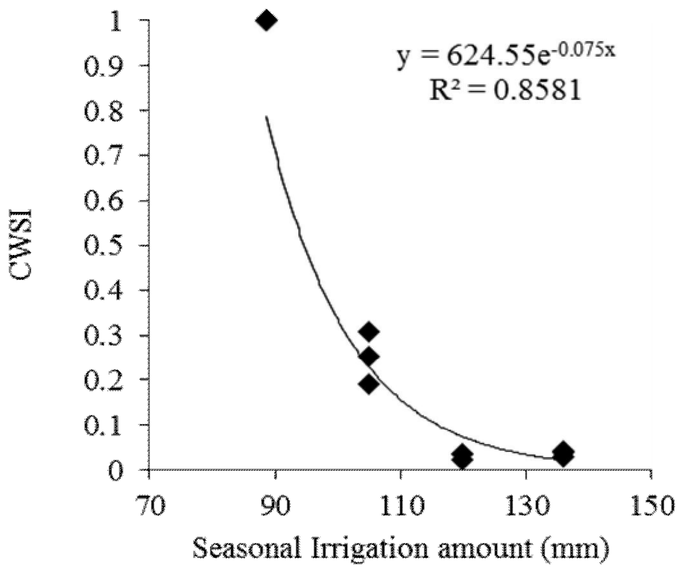

Figure 3. Crop Water stress index estimation in red bean, during the days after sowing in different treatment of irrigation in 2014.

Effect of the water stress index on the grain yield

The relationship between the water stress index and the grain yield (GY) is negative and exponential. The grain yield increases when the CWSI decreases (Figure 4). The exponential equation is as follows.

$$GY = 696.2(CWSI)^{-0.51} \qquad R=0.97,\ P < 0.01,\ n=36 \qquad (4)$$

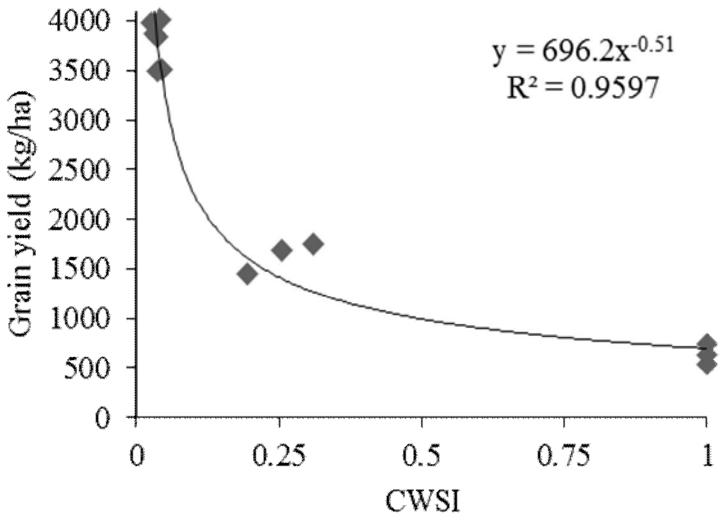

Figure 4. Grain yield as related to the mean value of CWSI.

The difference in our findings and those of others was at treatments for determined crop water stress index. The effect of the density for CWSI was not significant and for determined grain yield for own region can be used Equation 3 and 4 for water stress condition. The equation can be used to predict the yield of bean. Predicting yield response to crop water stress is important to farmers and researchers who aim at developing strategies and decisive plans concerning irrigation management under limited water conditions. The equation given above can predict the yield as a function of the CWSI. It proves to be a useful tool for such attempts. This result is in agreement with many other similar studies on different crops (Yazar et al., 1999).

Grain yield

Analysis of variance showed that the yield, grain yield, number of pods and the grains in pods, the pod length, plant height and protein were significantly affected by plant density, water stress and the interaction between water stress and plant density ($P<0.01$) (Tables 3, 6). The maximum yield was 3305.2 kg ha^{-1} in the state of 100% actual evapotranspiration and the row spacing of 5 cm. Minimum yield and water productivity was 1150.5 kg ha^{-1} when there was 60% actual evapotranspiration and the row spacing of 15 cm (Tables 4, 5, 7, 8). By increasing the plant density, it was observed that the grain yield increased. The reason lies in the fact that when plants are cultivated at higher densities, the sub branches are more likely to grow at the lower part of the plant which can be more productive (Gharib-Ardakani and Farajee, 2013). It has previously been reported that the grain yield of beans significantly decreases under water stress conditions (Emam et al., 2010). Similar reductions in the yield have frequently been reported, i.e. by Karam et al. (2005) in soybean, Cakir (2004) and Payero et al. (2006) in corn and Karam et al. (2007) in sunflower. Application of adequate water during flowering and pod development is the most significant factor in bean irrigation. Accordingly, similarities were observed in this study for the common bean that was cultivated in the semi-arid zone. Similar trends in the yield were seen for the yield components in all irrigation treatments of this study. Water stress combined with high temperature during flowering of the bean brought about a decrease in all yield components.

Number of plants per unit area (plant density)

Results show that plant density can substantially affect the number of plants per unit area that survive to the stage of maturity ($P<0.05$) (Tables 3, 6). By increasing the number of plants per unit area, grain yield increases. In 2013, the maximum yield was 3061.8 kg ha^{-1}, occurring at 120% of standard evapotranspiration and the 5 cm row spacing. Minimum yield was 834.2 kg ha^{-1} when there was 60% standard evapotranspiration and row spacing of 15 cm. In 2014, the maximum yield was 3305.2kg $ha^{-1}$when there was 100% standard evapotranspiration and row spacing of 5 cm. Minimum yield and water productivity was 1150.5 kg ha^{-1} when there was 60% standard evapotranspiration and row spacing of 15 cm (Tables 4, 5, 7, 8). By increasing the density of cultivating plants, the number of produced bean grains also increased per unit area (Tables 4, 5, 7, 8). Results obtained in a research by Ebrahimi (2011) concluded that despite the reduction of grain number per single plant, further modifications can be made to increase the plant density and thereby increase the number of produced grains per unit area. It seems that when the plant density increases, a more adequate leaf area index will appear to benefit the grain filling stage and, consequently, the use of solar energy increases in efficiency. This case thus culminates in an increased production of grain yield per unit area in high densities.

Number of seeds per pod

Plant density is shown to have a consistent influence on the number of seeds (grains) per pod in unit area ($P<0.01$) (Tables 3, 6). Yield is a complex outcome of multiple interacting components. Soper (1952) was among the first to explore the compensatory nature of yield components and how relevant responses by plants actualize as a result of changes in plant density. In beans, the most notable of components in yield are known to be seed weight, number of seeds per pod and number of pods per plant (Rowlands 1955). The number of pods per plant, more than other variables and factors, generally determines the amount of yield (Soper, 1952; Hodgson and Blackman, 1956). Korte et al. (1983) demonstrated that the yield and yield components such as number of pods per plant and number of grains in each pod of the beans significantly decreased under water stress conditions. Number of seeds per pod is a yield component that increases by higher plant density. A similar trend of increase in the number of seeds per pod has already been reported by Mcewen et al. (1988) and Wahab et al. (1986).

Plant Height

Plant density is shown by this research to affect plant height significantly (P<0.01) (Tables 3, 6). The interaction between soil moisture and density is also significant. In 2013, the maximum plant height reached 44 cm, occurring at 120% of standard evapotranspiration and the 5 cm row spacing. Minimum plant height reached 22 cm when there was 60% standard evapotranspiration and row spacing of 15 cm. In 2014, the maximum plant height reached 45 cm when there was 100% standard evapotranspiration and row spacing of 5 cm. However, minimum plant height reached 35 cm when there was 60% standard evapotranspiration and row spacing of 15 cm. Water stress suppressed the growth of plant height and the shortest plants were produced at higher water stress levels (Tables 4, 5, 7, 8). This finding is in agreement with the results of Nielsen and Nelson (1998) and Shenkut and Brick (2003) who reported that shorter plant heights were a result of severe environmental factors such as water stress. Morphologically, increasing the plants height can be an advantage, in terms of inter-specific and intra-specific competitions within the plant community. Furthermore, the increase in plant height could result in the formation of new and young leaves at the top of the canopy. This is where leaves are effectively located for the purpose of optimum photosynthesis (Martin and Downie, 2008). Results by this study show that the plant height increases significantly when plant population is increased per unit area. Similar findings were reached by Khalil et al. (2010) and Thalji (2010) who indicated that the denser the plant population, the taller the plant heights become due to the occurrence of competition among plants. The increase in plant height here happens because of intra-specific plant competition which results in taller plants being sparsely branched. When the plants are sown tightly close together, however, their stems are shaded from light when the plants enter their later stages of growth. This culminates in the accumulation of auxin, a major growth hormone that stimulates cell division and enlargement. On the other hand, a scattered population of plants would not provide shade and thus auxin destruction would become prevalent due to the presence of light. This then results in plants growing shorter (Mureithi et al., 2012).

Average weight of hundred seeds

This variable changed significantly against plant density (P<0.01) (Tables 3, 6). The interaction between soil moisture and density was also significant. In 2013, the maximum average weight of a hundred seeds was 44.2 grams, occurring at 120% of actual evapotranspiration and the 5 cm row spacing. Minimum average weight of a hundred seeds was 25.1 grams when there was 60% actual evapotranspiration and the row spacing of 15 cm. In 2014, the maximum average weight of a hundred seeds was 46 grams where conditions were of 100% actual evapotranspiration and the row spacing of 5 cm. Minimum average weight of a hundred seeds was 29.5 grams when there was 60% actual evapotranspiration and the row spacing of 15 cm (Tables 4, 5, 7, 8).

The highest average weight of a hundred seeds yield was obtained through a high planting density (Amany, 2014). Following water stress against the common bean, the accelerated maturity of crops is reported to have occurred concurrently with the reduction in grain yield and average weight of a hundred seeds (Molina et al., 2001). Water stress during the flowering and grain-filling periods reduced seed yield and seed weight but accelerated the maturity of bean plants (Zlatev and Stoyanov, 2005).

Grain protein

Variance analysis showed that the effects of water stress on the amount of grain protein was significant (P<0.01) but the effect of density on the amount of grain protein was not significant (Tables 3, 6). The interaction between soil moisture and density was also significant. In 2013, the minimum average grain protein was 22.5%, occurring at 120% of actual evapotranspiration. Maximum average grain protein was 24.97% when there was 60% actual evapotranspiration and the row spacing of 15 cm. In 2014, the minimum average grain protein was 22.6%, occurring at 120% of actual evapotranspiration. Maximum average grain protein was 24.7% when there was 60% actual evapotranspiration and the row spacing of 15 cm (Tables 4, 5, 7, 8). It seems that the increase of grain protein as a result of water stress is parallel to an increase in the grain's starch-protein ratio. Therefore, stressful drought circumstances can suppress starch synthesis to an extent that is more dramatic than any ordinary situation. This is consistent with Jalilian et al. (2005) and Mohammadzadeh et al. (2011). Furthermore, the occurrence of drought ostensibly puts limits on the amount of CO_2 uptake and carbon fixation which is commonly explained by the partial closure of stomata. Nonetheless, nitrogen remobilization from leaves to the grains does not decrease but causes a particular increase in the percentage of protein in the grain (Souza et al., 2004). Sadeghipoor et al. (2004) also reported that plant density does not affect the percentage of total protein in grains.

Table 3. Analysis of variance for bean yield and yield component (mean values for 2013).

Source of variation	Degree of freedom	Yield	Weight of hundred seeds	Protein	Length of pod	Number of seeds in pod	Number of pods in plant	Height of plant
Replication	2	10.24^{ns}	0.047^{ns}	0.0001^{ns}	0.023^{ns}	0.478^{ns}	1.99^{ns}	0.199^{ns}
Density	2	1846829.382^{**}	310.64^{**}	0.018^{ns}	3.72^{**}	2.288^{**}	185.3^{**}	57.43^{**}
Irrigation	3	3109500.734^{**}	94.11^{**}	10.42^{**}	8.54^{**}	2.295^{**}	109.93^{**}	87.91^{**}
Irrigation *Density	6	92910.147^{**}	12.935^{**}	0.028^{**}	0.431^{**}	0.149^{**}	25.61^{**}	1.903^{ns}
Error	18	2727.110	3.138	0.013	0.103	0.43	4.34	1.487

* Significant at P < 0.05; ** Significant at P < 0.01.

Table 4. Comparison of seed yield average and dry matter of bean affected by crucible density and irrigation (2013).

Treatment	Yield	Weight of hundred seeds	Protein	Length of pod	Number of seeds in pod	Number of pods in plant	Height of plant
5	2393^{a}	37^{a}	23.71^{a}	8.85^{a}	7.25^{c}	3.5^{b}	38.08^{a}
10	1991^{b}	31.72^{b}	24.02^{a}	8.33^{b}	10.00^{b}	4.0^{ab}	35.00^{b}
15	1611^{c}	29.42^{c}	23.88^{a}	7.7^{c}	12.10^{a}	4.5^{a}	33.17^{c}
Irrigation							
120% ET_o	2589^{a}	37.48^{a}	22.55^{c}	10.7^{a}	13.33^{a}	4.6^{a}	41.56^{a}
100% ET_o	2424^{b}	34.90^{b}	22.86^{c}	10.14^{b}	10.33^{b}	4.4^{a}	38.1^{b}
80% ET_o	1833^{c}	31.26^{c}	24.74^{b}	8.67^{c}	9.47^{b}	4.2^{a}	36.67^{c}
60% ET_o	1148^{d}	27.2^{d}	25.33^{a}	3.68^{d}	6.0^{c}	2.8^{b}	25.33^{d}

Each column in each treatment shows the difference between two mean values. Common letters denote insignificant differences between mean values by the Duncan test ($P < 0.05$).

Table 5. Comparison of average of mutual effects of crucible density and species on the yield and dry matter of bean (2013).

Treatment	Yield	Weight of hundred seeds	Protein	Length of pod	Number of seeds in pod	Number of pods in plant	Height of plant
$D_1 I_1$	3061.8^{a}	44.20^{a}	22.50^{d}	11.00^{a}	9.000^{ef}	4.000^{cd}	44.00^{a}
$D_1 I_2$	3001.2^{g}	41.20^{b}	22.40^{d}	10.81^{Ab}	8.000^{fg}	3.667^{de}	41.31^{b}
$D_1 I_3$	2023.3^{d}	33.29^{cd}	24.68^{b}	9.600^{c}	7.000^{gh}	4.043^{Bcd}	38.00^{c}
$D_1 I_4$	1485.2^{e}	29.30^{fg}	25.27^{ab}	4.000^{f}	5.000^{i}	2.333^{f}	29.00^{e}
$D_2 I_1$	2652.0^{b}	35.16^{c}	22.75^{cd}	10.80^{ab}	13.00^{b}	4.833^{abc}	40.00^{b}
$D_2 I_2$	2250.3^{c}	33.00^{cde}	22.75^{cd}	10.20^{b}	11.00^{cd}	4.333^{Abcd}	38.00^{c}
$D_2 I_3$	1938.1^{h}	31.50^{def}	24.83^{b}	8.700^{d}	10.00^{de}	4.233^{Abcd}	37.00^{c}
$D_2 I_4$	1125.4^{g}	27.20^{gh}	25.77^{a}	3.640^{f}	6.000^{hi}	2.667^{ef}	25.00^{f}
$D_3 I_1$	2052.1^{e}	33.07^{cde}	22.40^{d}	10.30^{b}	18.00^{a}	5.167^{ab}	40.67^{b}
$D_3 I_2$	2021.3^{d}	30.50^{ef}	23.43^{c}	9.400^{c}	12.00^{bc}	5.333^{a}	35.00^{d}
$D_3 I_3$	1537.0^{e}	29.00^{fg}	24.70^{b}	7.700^{e}	11.40^{bcd}	4.300^{abcd}	35.00^{d}
$D_3 I_4$	834.2^{i}	25.10^{h}	24.97^{b}	3.400^{f}	7.000^{gh}	3.333^{def}	22.00^{g}

Table 6. Analysis of variance for bean yield and yield component (mean values for 2014).

Source of variation	Degree of freedom	Yield	Weight of hundred seeds	Protein	Length of pod	Number of seeds in pod	Number of pods in plant	Height of plant
Replication	2	10.24^{ns}	0.047^{ns}	0.0001^{ns}	0.023^{ns}	0.478^{ns}	1.99^{ns}	0.199^{ns}
Density	2	1846829.382^{**}	310.64^{**}	0.018^{ns}	3.72^{**}	2.288^{**}	185.3^{**}	57.43^{**}
Irrigation	3	3109500.734^{**}	94.11^{**}	10.42^{**}	8.54^{**}	2.295^{**}	109.93^{**}	87.91^{**}
Irrigation *Density	6	92910.147^{**}	12.935^{**}	0.028^{**}	0.431^{**}	0.149^{**}	25.61^{**}	1.903^{ns}
Error	18	2727.110	3.138	0.013	0.103	0.43	4.34	1.487

* Significant at $P < 0.05$; ** Significant at $P < 0.01$.

Table 7. Comparison of seed yield average and dry matter of bean affected by crucible density and irrigation (2014).

Treatment	Yield	Weight of hundred seeds	Protein	Length of pod	Number of seeds in pod	Number of pods in plant	Height of plant
5	2682^{a}	40.88^{a}	23.23^{a}	10.66^{a}	8.58^{c}	4.16^{b}	42.25^{a}
10	2234^{b}	33.5^{b}	23.15^{a}	10.16^{b}	12.96^{b}	4.75^{Ab}	40.01^{b}
15	1900^{c}	31.13^{c}	23.20^{a}	9.55^{c}	16.42^{a}	5.0^{a}	37.88^{c}
Irrigation							
120% ET_o	2735^{a}	38.33^{a}	22.5^{c}	10.83^{a}	17.11^{a}	5.05^{a}	42.17^{a}
100% ET_o	2686^{b}	37.00^{a}	22.5^{c}	10.8^{a}	13.72^{b}	5.11^{a}	43.19^{a}
80% ET_o	2197^{c}	34.33^{b}	23.00^{b}	10.11^{b}	10.34^{c}	4.22^{b}	38.17^{b}
60% ET_o	1469^{d}	31.01^{c}	24.77^{a}	8.75^{c}	9.34^{c}	4.19^{b}	36.67^{c}

Each column in each treatment shows the difference between two mean values. Common letters denote insignificant differences between mean values by the Duncan test ($P < 0.05$).

Table 8. Comparison of average of mutual effects of crucible density and species on the yield and dry matter of bean (2014).

Treatment	Yield	Weight of hundred seeds	Protein	Length of pod	Number of seeds in pod	Number of pods in plant	Height of plant
D_1 I_1	3305^{a}	46.00^{a}	22.60^{c}	11.20^{a}	10.33^{ef}	4.367^{abcd}	45.00^{a}
D_1 I_2	3254^{a}	44.00^{a}	22.50^{c}	11.00^{ab}	9.167^{ef}	4.500^{abcd}	46.00^{a}
D_1 I_3	2361^{c}	40.00^{b}	23.00^{b}	10.70^{ab}	7.833^{ef}	3.750^{d}	40.00^{cde}
D_1 I_4	1807^{f}	33.53^{cd}	24.80^{a}	9.750^{d}	7.000^{f}	4.043^{cd}	38.00^{ef}
D_2 I_1	2636^{b}	36.00^{c}	22.50^{c}	10.80^{ab}	15.33^{bc}	5.167^{abc}	41.50^{bc}
D_2 I_2	2620^{b}	35.00^{cd}	22.40^{c}	10.90^{ab}	15.00^{bcd}	5.267^{abc}	42.56^{b}
D_2 I_3	2230^{d}	33.00^{cde}	22.90^{b}	10.04^{cd}	11.50^{cde}	4.367^{abcd}	39.00^{def}
D_2 I_4	1450^{g}	30.00^{ef}	24.80^{a}	8.900^{e}	10.00^{ef}	4.233^{cd}	37.00^{fg}
D_3 I_1	2265^{d}	33.00^{cde}	22.40^{c}	10.50^{bc}	25.67^{a}	5.633^{a}	40.00^{cde}
D_3 I_2	2185^{d}	32.00def	22.60^{c}	10.50^{bc}	17.00^{b}	5.567^{ab}	41.00^{bcd}
D_3 I_3	2000^{e}	30.00^{ef}	23.10^{b}	9.600^{d}	11.70^{cde}	4.567^{abcd}	35.50^{g}
D_3 I_4	1150^{h}	29.50^{f}	24.70^{a}	7.600^{f}	11.33^{de}	4.300^{bcd}	35.00^{g}

Relationship between protein and total irrigation

To study the correlation between protein (P) and total irrigation (I) in the years 2013-2014, all treatments were reviewed without considering the density of cultivation and the amount of irrigation water. Accordingly, the following equation is obtained from the regression between protein and total irrigation for all treatments at harvest day.

$$P=33.33 * e^{-4E-04\ I} \qquad R- 0.90,\ P < 0.01,\ n=72 \qquad (5)$$

The difference in the findings between ours and others was different at treatments for determined protein concentration. The effect of the density for protein concentration was not significant and for determined protein concentration for own field, Equation 5 can be used for water stress condition.

Conclusions

In this research, the mean CWSI value before applying irrigation was 0.07 under non-water-stress conditions. This CWSI value was consistent with the highest yield for bean. Grain yield (GY) also directly correlated with crop water stress index and the exponential equation of $GY=696.2(CWSI)^{-0.51}$. The effect of the irrigation water was significant and CWSI increased when there were more severe deficits of water in the soil. The effect of plant density for CWSI was not significant and this serves as a very important point in the timing of irrigation under field conditions. Investigations on yield and yield components of red bean show that appropriate irrigation treatments would cause a proper translocation of photosynthetic products to the grains, unless water stress is severe enough to cause a dramatic decrease of these products in the grains. When the plants were sown tightly close together, the effect of water stress on the amount of grain protein was significant, but density alone did not significantly affect the amount of grain protein. The increase in grain protein as a result of water stress is parallel to an increase in the grain's starch-protein ratio. The protein concentration directly correlated with total irrigation water during the growing season (TI) and the exponential equation $P= 33* e^{-4E-04TI}$ can be used for the prediction of protein concentration.

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