Response of Cotton to Irrigation, Fertilization and Plant Density in a Semi-Arid Region of Iran

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Summary

Fertilization and plant density are key factors in cotton yield, especially under conditions of water shortage. We conducted a two year- field experiment to investigate the nitrogen × PSB bacteria synergic effect on photosynthesis and water relation of cotton under water stress conditions and different plant densities. The experimental design was a 2^5 factorial with five factors I: Irrigation (moderate and restricted irrigation), N: Nitrogen (with and without nitrogen), D: Plant density (low and high plant density), B: phosphate solubilizing bacteria (PSB bacteria) (with and without incubation), P: Phosphorus (with and without phosphorus). Results revealed that the ratio (Fv/Fm) did not respond to restricted irrigation when N and B were consumed together, and one of these factors was also enough to prevent the decline in the relative water content (RWC) under I_R conditions. P had a reducing effect on RWC under I_R conditions, and its role in preventing LAI loss under restricted irrigation condition did not result in the improvement of yield (GLY). I_R reduced RWC and GLY in low density plots without nitrogen consumption but using N or higher plant density was enough to prevent this decline. The results conclude that P can be replaced with B under I_R conditions, and the synergic interaction of N×B can strongly reduce the effect of drought stress on cotton yield in high plant density.

Key words

irrigation, fertilization, plant density, chlorophyll fluorescence, relative water content, cotton

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Introduction

Bio-fertilizer is still an unclear technology in organic farming because of inadequate awareness about its use, benefits and disadvantages (Basu et al. 2017). Phosphorus precipitates with calcium and magnesium ions in alkaline and saline soils of the semi-arid areas and is not mostly absorbable for root (Silva et al. 2014; Shen et al. 2011). So, phosphate solubilizing bacteria (PSB bacteria) in sunflower, corn, cotton or other row crops are recommended to farmers as an organic way of introducing P into low-input cropping systems in Iran and some other Mediterranean regions (Latati et al. 2017). The other problem is the very poor yield of cotton due to interspecific competition for nitrogen, which shows the importance of some nitrogen use to decrease competition between plants for N and prevent cotton yield loss (Silva et al. 2014).

Row crops in higher plant density produce deeper roots than in low plant density, as Shao et al. (2018) reported that root growth in maize was enhanced under high plant density. Consequently, the competition between the plants in high plant density appears to be mainly for nutrients (Li et al. 2019), especially nitrogen and phosphorus which have an uniform distribution in the soil layers, rather than water, which is more available in the lower layers of the soil where cotton roots can access (Estrada et al. 2015). Li et al. (2017) and Shi et al., (2016) reported that an acceptable lint yield was achieved with nitrogen under high plant density, indicating the interaction of fertilizer × plant density on cotton and maize yield. High density can reduce flower and young bolls shedding in drought stress conditions, because at high densities, indicating significant interaction of irrigation × plant density (Shi et al. 2016).

So, the objective of this study is testing the idea of whether consumption of N and PSB-bacteria together and high plant density can alleviate the effect of water stress on cotton yield.

We hypothesize that PSB provides the phosphorus and if nitrogen is sufficiently available through consumption of some nitrogen in high plant density conditions, then P-related mechanisms such as better root system growth result in increased relative water content (RWC) of the leaves and reduced chlorophyll fluorescenc, which ultimately prevents a significant decrease in the yield of cotton under restricted irrigation.

Materials and Methods

Location and Plant Material

This experiment was carried out during the 2017 and 2018 growing seasons at Feizabad-Iran (latitude: 34°54'N, longitude: 58°70'E) with the cotton cultivar "Varamin". The growing season was about 100 days in both years, beginning from May 22 to August 31. In Feizabad, the temperature varied from 20 °C to 38 °C during the growing season and was rarely below 16 °C or above 41 °C. There was no rainfall during this period in both years. The soil used was a montmorillonite clay loam, low in total nitrogen (0.06%), low in soluble potassium (257 ppm) low in organic matter (0.6%), low in absorbable phosphate (12 ppm), with a pH of 8.0 and Ec of 0.89 dS m^{-1}.

Treatments and Experimental Design

The 2^5 factorial experiment consisted of five factors (I: Irrigation, N: Nitrogen, D: Plant density, B: PSB bacteria, P: Phosphorus), each with two levels with their combination. The levels of experimental factors included the following: I_R, I_O: Optimal and restricted irrigation, respectively; N_0, N_H: without and with nitrogen consumption, respectively (consumption of 133 kg ha^{-1} urea fertilizer contains 48% pure nitrogen at seven weeks after sowing); D_5, D_{10}: plant density of 5 and 10 plants per square meter respectively; B_0, B_H: without and with PSB bacteria consumption, respectively (Incubation with 100 g ha^{-1} PSB bacteria at two weeks after sowing); P_0, P_H: without and with phosphorus consumption, respectively (consumption of 133 kg ha^{-1} triple superphosphate fertilizer contains 46% pure phosphorus at sowing). A subplot size of 6 m × 3 m, having 6 rows of 6 m length was used.

Irrigation Scheduling

Furrow irrigation with siphons was applied. Up to seven weeks after sowing, all the experimental units were irrigated uniformly when the soil water content (SWC) reached $[\theta_{WP} + 75\% (\theta_{FC} - \theta_{WP})]$. After this stage, water content before irrigation in I_0 and I_R plots was $[\theta_{WP} + 75\% (\theta_{FC} - \theta_{WP})]$ and $[\theta_{WP} + 25\% (\theta_{FC} - \theta_{WP})]$, respectively. θ_{FC} and θ_{WP} were SWC at field capacity. SWC was measured daily by using granular matrix sensors according to the method that was described in our previous research (Madani et al. 2010). The amount of irrigation during the growing season was based on evapo-transpiration (ETP). Reference ETP (ETP0) was measured using class A evaporation pan. ETP_0 was then multiplied by the water stress coefficient (Ks) and the crop coefficient (Kc) to calculate the crop evapo-transpiration (ETPc). Ks values for different soil water contents and Kc values for cotton at different growth stages is reported by FAO irrigation and drainage-paper 56. (Allen et al. 1998). Net irrigation water requirement in moderate irrigation (I_0) plots was 1250 mm (2017) – 1500 mm (2018), and in restricted irrigation (I_R) plots was 970 mm (2017) – 1200 mm (2018).

Measurements

Chlorophyll content was measured with "SPAD 502 Plus" chlorophyll meter. The ratio Fv/Fm was measured with "Hansatech Pocket Pea" chlorophyll fluorimeter before and after irrigation. These two traits were measured at the start of R_2 and the end of R6 growth stages which were in accordance with the 56^{th} and 77^{th} days after sowing, respectively. The following formula was used to calculate the RWC (Barrs & Weatherley 1962):

$$RWC (\%) = [(W-DW) / (TW-DW)] \times 100,$$

where

W – Sample fresh weight of leaf disks (10 cm^2).

TW – Sample turgid weight (hydrated to full turgidity for 3-4h at 20 °C)

DW – Sample dry weight (dried at 80 °C for 24h).

An area of 2 m^2 was harvested to estimate the yield.

Data Analysis

Statistical analyses were performed using the GLM procedure with SAS 9.3. Duncan's multiple range test was applied for mean separations when F values were significant.

Results

Chlorophyll content (Chl) did not respond to the treatments. Light and dark-adapted Fv/Fm were measured before and after irrigation at different growth stages. Only dark-adapted Fv/Fm values measured before the irrigation and at the beginning of reproductive growth (R_2) were influenced by some experimental factors. The correlation of Fv/Fm with yield (GLY) was significant in both years only under restricted irrigation (Table 1).

Table 1. Separate ANOVA for 2017 and 2018 years, and Pearson coefficient correlations between Fv/Fm, Relative Water Content (RWC), and cotton yield (GLY) under moderate (I_0) and restricted irrigation (I_R)

	2017				2018		
			Pearson correlation				
			Optimal Irrigation (I_0)				
	Fv/Fm	RWC	GLY		Fv/Fm	RWC	GLY
Fv/Fm	-	NS	NS	Fv/Fm	-	NS	NS
RWC	NS	-	NS	RWC	NS	-	NS
			Restricted irrigation (I_R)				
Fv/Fm	-	NS	0.50 **	Fv/Fm	-	NS	0.57 **
RWC	NS	-	0.38 **	RWC	NS	-	0.42 **
			ANOVA				
I	**	**	**	I	**	**	**
N	ns	ns	**	N	ns	ns	**
I×N	ns	**	ns	I×N	ns	**	**
N×D	ns	ns	ns	N×D	ns	ns	**
I×B	ns	**	**	I×B	ns	**	**
I×P	ns	**	ns	I×P	ns	**	ns
I×N×D	ns	**	**	I×N×D	ns	**	**
I×N×B	**	ns	**	I×N×B	**	ns	**
I×N×P	**	ns	ns	I×N×P	**	ns	ns
	Leaf Area per Plant				Leaf Area per Plant		
I×P		**		I×P		NS	

Note: I: Irrigation; N: Nitrogen; D: Density; B: PSB bacteria; P: Phosphorus; **, * and ns: significant at $P < 0.01$, $P < 0.05$ and non-significant, respectively

Based on the average of years, restricted irrigation (I_R) caused a 14.9% (I_0=0.67, I_R=0.57, $LSD_{0.01}$=0.03) decrease in Fv/Fm. I×N×P and I×N×B interactions were significant on Fv/Fm in both years (Table 1). Based on the average of years, Fv/Fm did not respond to I_R when N and P were consumed together ($I_0N_HP_H$=0.62; $I_RN_HP_H$=0.60), while I_R resulted in a significant reduction in Fv/Fm when P ($I_0N_HP_0$=0.69; $I_RN_HP_0$=0.54) or N ($I_0N_0P_H$=0.69; $I_RN_0P_H$=0.56) or both ($I_0N_0P_0$=0.68; $I_RN_0P_0$=0.59) were not consumed (Fig. 1.A). A similar trend was observed when P was replaced with PSB bacteria (B). Based on the average of years, I_R resulted in decreased Fv/Fm when B ($I_0N_HB_0$=0.66; $I_RN_HB_0$=0.53) or N ($I_0N_0B_H$=0.69; $I_RN_0B_H$=0.59) or both ($I_0N_0B_0$=0.68; $I_RN_0B_0$=0.56) were not consumed (Fig. 1.B), while Fv/Fm did not respond to I_R when N and B were consumed together ($I_0N_HB_H$=0.65; $I_RN_HB_H$=0.62).

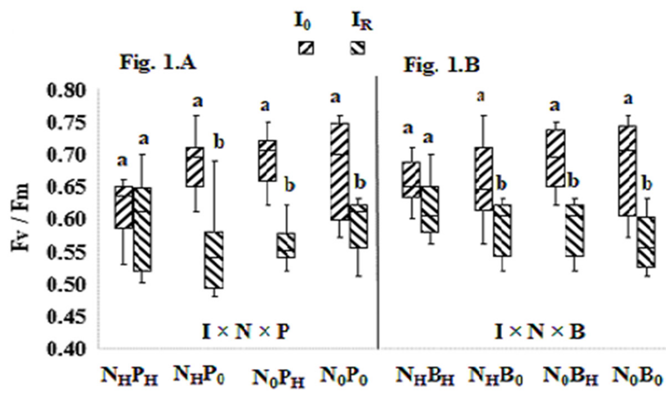

Figure 1. Interaction of some experimental factors on Fv/Fm based on the average of years. I_0 and I_R: moderate and restricted irrigation, respectively. N_0 and N_H: without and with nitrogen consumption, respectively. P_0 and P_H: without and with phosphorus consumption, respectively. B_0 and B_H: without and with PSB bacteria consumption, respectively

RWC was measured before (b) and after (a) irrigation. Based on the average of years, I_R significantly reduced RWC_b (I_0=70.6%; I_R=65.6%; $LSD_{0.01}$=3.8), while it did not affect RWC_a. The correlation of RWC with yield (GLY) was significant in both years only under restricted irrigation (Table 1).

Based on the average of the years, the response of RWC_b to I_R was not significant when N (I_0N_H=69.2%; I_RN_H: 66.3%) or B (I_0B_H=69.7%; I_RB_H=66.4%) was consumed (Fig. 2.A, Fig. 2.B), while I_R caused a significant decrease in RWC_b when N (I_0N_0=72.0%; I_RN_0=64.9%) or B (I_0B_0=71.5%; I_RB_0=64.9%) was not consumed (Fig. 2.A, Fig. 2.B), leading to a significant I×N and I×B interactions in both years (Table 1).

The role of P in the response of RWC_b to I_R was contrary to the role of N and B. Based on the average of years, I_R significantly reduced RWC_b when P was consumed (I_0P_H=72.2%; I_RP_H=64.3%), while RWC_b did not respond to I_R when P was not consumed (I_0P_0=69.1%; I_RP_0=67.0%), resulting in a significant I×P interaction on RWC_b in both years (Fig. 2.C and Table 1). I×P Interaction on leaf area per plant (LA) at R_6 was significant only in 2017 (Table 1). In 2017, when P was not consumed, I_R caused a 26.6% (I_0P_0=0.15 m^2 $plant^{-1}$; I_RP_0=0.11 m^2 $plant^{-1}$) significant decrease in LA at R_6, while LA did not respond to I_R when P was consumed (I_0P_0=0.14 m^2 $plant^{-1}$; I_RP_0=0.12 m^2 $plant^{-1}$).

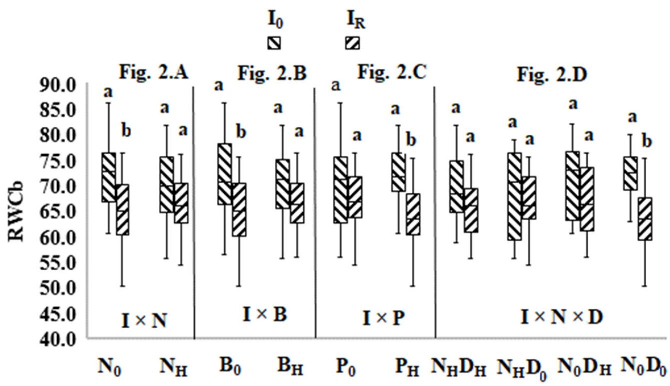

Figure 2. Interaction of some experimental factors on RWC based on the average of the years. I_0 and I_R: moderate and restricted irrigation, respectively. N_0 and N_H: without and with nitrogen consumption, respectively. P_0 and P_H: without and with phosphorus consumption, respectively. B_0 and B_H: without and with PSB bacteria consumption, respectively. D_0 and D_H: Low (5 plant m^{-2}) and high (10 plants m^{-2}) plant density, respectively

Among the N×D combinations, I_R reduced RWC_b only in N_0D_5 ($I_0N_0D_5$=72.8%, $I_RN_0D_5$=63.0), leading to a significant I×N×D interaction on RWC_b in both years (Table 1, Fig. 2.D).

Based on the average of years, I_R significantly decreased GLY (I_0=2641 kg ha^{-1}, I_R=1948 kg ha^{-1} $LSD_{0.01}$=3.6). I×N, I×B interactions on GLY was significant in both years (Table 1). Based on the average of years, when N or B was not consumed, I_R caused a 34.1% (I_0N_0=2437 kg ha^{-1}; I_RN_0=1604 kg ha^{-1}) and 33.8% (I_0B_0=2724 kg ha^{-1}; I_RB_0=1809 kg ha^{-1}) significant decrease in GLY, while GLY did not respond to I_R when N or B was consumed (Fig. 3.A, Fig. 3. B).

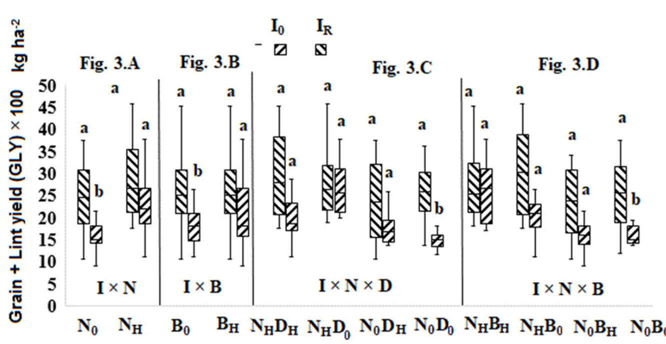

Figure 3. Interaction of some experimental factors on yield (GLY) based on the average of years. I_0 and I_R: moderate and restricted irrigation, respectively. N_0 and N_H: without and with nitrogen consumption, respectively. B_0 and B_H: without and with PSB bacteria consumption, respectively. D_0 and D_H: Low (5 plants m^{-2}) and high (10 plant m^{-2}) plant density, respectively

Based on the average of years, N consumption significantly increased GLY by 36.0% (N_0=2011 kg ha^{-1}, N_H=2 kg ha^{-1} $LSD_{0.01}$= 573). In 2018, GLY responded to N only in the low plant density (N_0D_5=1984 kg ha^{-1}, N_HD_5=2705 kg ha^{-1}; N_0D_H=2059 kg ha^{-1}, N_HD_H=2430 kg ha^{-1}), leading to a significant N×D interaction on GLY in 2018.

Among the N×D combinations, I_R reduced GLY only in N_0D_5 ($I_0N_0D_5$=2525 kg ha^{-1}, $I_RN_0D_5$=1453 kg ha^{-1}), leading to a significant I×N×D interaction on GLY in both years (Table 1, Fig. 3.C). Among the N×B combinations, I_R reduced GLY only in N_0B_0 ($I_0N_0B_0$=2503 kg ha^{-1}, $I_RN_0B_0$=1574 kg ha^{-1}), leading to a significant I×N×B interaction on GLY in both years (Table 1, Fig. 3.D).

Discussion

Reducing water consumption requires finding a way to prevent yield reduction under restricted irrigation (I_R). I_R led to less GLY due to reduced Fv/Fm and RWC. Consumption of N and P together prevented the photosynthetic efficiency (Fv/Fm) negative response to I_R (Fig. 1.A.). Shen and Li (2011) also reported that I_R impaired PS II function in wheat, and this effect was significantly ameliorated by NP fertilizer but not N alone. PSB bacteria (B) were able to play the role of P to reduce I_R effect on Fv/Fm, but not without N (Fig. 1.B.). N or B was able to reduce the effect of I_R on RWC (Fig. 2.A. and Fig. 2.B.). This trend was also observed for GLY. GLY did not respond to I_R when N and B were consumed together or alone (Fig. 3.A. and Fig. 3.B.). Researchers reported that increased resistance of photosynthesis to water stress at higher N conditions (Singh et al. 2016) or in PSB incubated plants (Shintu and Jayaram, 2015) resulted from improved RWC.

Jin et al (2015) reported that P supply under I_R conditions resulted in significantly greater LA in pea. P had a reducing effect on RWC in I_R conditions (Fig. 2.C.), and its role in preventing LA loss under I_R conditions in 2017 did not result in the improvement of GLY. The combination of results to this point clearly shows that P either alone or in combination with N is not suitable under I_R conditions, and its replacement with B is quite reasonable. The results indicate that P can be eliminated under I_R conditions when N and B are used together. Ding et al. (2014) reported that N application increased inorganic P uptake in soils with low P by improving the PSB performance.

N increased GLY without increasing Fv/Fm and RWC. However, I×N×D interaction on RWC and GLY in both years (Fig. 2.D. and Fig. 3.C.), and the N×D interaction on GLY in 2018 was significant. This result showed that I_R reduced RWC and GLY in low density plots without nitrogen consumption but using N or higher plant density was enough to prevent this decline (Fig. 2.D. and Fig. 3.C.) Researchers also found that plants treated with high N showed higher RWC (Chang et al. 2016) and Fv/Fm (Abid et al. 2016) than low N treatment under I_R conditions. Considering the necessity of simultaneous use of N and B to prevent Fv/Fm reduction under I_R conditions and also the results of I×N×B interaction on GLY (Fig. 3.D.), it does not seem that some N is not totally eliminated under I_R conditions.

The application of each fertilizer alone or just increased plant density did not change RWC and Fv/Fm. However, I×N×P and I×N×B interactions on Fv/Fm and also I×N, I×B and I×N×D interactions on RWC were significant. These results show that the alleviated negative effect of water shortage on GLY is attributed to fertilizer × plant density synergic effect. Singh et al. (2012) also reported that the potential effects of adopting high plant population with a minimum recommended dose of NPK fertilizer management offered an excellent opportunity to increase crop productivity in rainfed fields.

Conclusion

The study investigates the nitrogen × PSB bacteria synergic effect on photosynthesis and water relation of a crop under water stress conditions and different plant densities. I_R reduced GLY from 2437 kg ha^{-1} to 1604 kg ha^{-1} when N was reduced from 2724 kg ha^{-1}; to 1809 kg ha^{-1} when B was not consumed, while GLY did not respond to I_R when N or B was consumed together. In low plant density and without N consumption, I_R reduced GLY from 2525 kg ha^{-1} to 1453 kg ha^{-1}, while GLY did not respond to I_R in other nitrogen × plant density combinations. These results conclude that phosphorus can be replaced with PSB bacteria under restricted irrigation conditions, and the synergic interaction of nitrogen× PSB-bacteria can strongly reduce the effect of drought stress on cotton yield in high plant density.

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