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Soybean Response to Drought and Seed Inoculation

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Abstract: Drought is one of the most important factors that limits N_2 fixation, growth and yield of soybean. The aim of this study was to determine the effects of water stress, starter N application and seed inoculation on yield and yield components of soybean (cv. Williams). The experiment was conducted in 2010 at the Islamshahr town that located in 12 km of South-west of Tehran, the capital of Iran. The experiment was laid out in a split plot on the basis of randomized complete block design with three replications that placed different irrigation levels (irrigation after, E0= 40, E1= 80 and E2= 120 mm evaporation from the class "A pan" evaporation) in the main plots and seed inoculation treatments (I0= 0 kg N ha^{-1} + un-inoculation, I1= 20 kg N ha^{-1} + inoculation with *Rhizobium japonicum*, I2= 20 kg N ha^{-1} + inoculation with *Azotobacter chroococcum*, I3= 20 kg N ha^{-1} + inoculation with *Azospirillium brasilense*, I4= 20 kg N ha^{-1} + inoculation with mix of bacteria, I5= 20 kg N ha^{-1} + un-inoculation and I6= 100 kg N ha^{-1} + un-inoculation) in the sub plots. Results showed that water stress decreased bacteria activity, number of pods per plant, number of seeds per pod, seed weight and seed yield. Under optimum and water stress conditions, application of 20 kg N ha^{-1} as starter and seed inoculation especially with mix of bacteria, produced maximum yield.

Key words: Water stress · Seed inoculation · Yield · Soybean

INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) is one of the most important world crops. It is mainly cultivated for oil and protein and is a major food and feed source [1]. Legumes are agronomically and economically important in many cropping systems because of their ability to assimilate atmospheric N_2 and this importance is anticipated to increase with the need to develop sustainable agricultural practices. Indeed, biological N_2 fixation is the most significant natural pathway for the introduction of N into the biosphere [2]. Rhizobia are very minor components of the soil microflora and reach their maximum numbers in association with plant roots. They are stimulated by carbon compounds either leaked by or sloughed-off plant roots and are controlled by microbial competition, antagonism, lysis and predation as well as by their physical and chemical environment. Their ability to infect legume roots and multiply within the resulting root nodule, protected from the soil environment, provides a special advantage over their competitors [3]. One of the important challenges facing crop physiologists and agronomists is understanding and overcoming the major abiotic stresses in agriculture which reduces crop productivity and yield [4]. Interest in crop response to environmental stresses has increased greatly in recent years because of severe losses that result from drought, salinity, heat and cold stress [5]. Drought is one of the major causes of reduced growth, development and yield in field crops [6-10]. In leguminous plants, drought also reduced nitrogen fixation and its related traits [11]. As with other grain legumes, soybean is very sensitive to drought stress which leads to reduced yield and seed quality. Drought stress is a major factor affecting symbiosis and leads to decreased nodule formation, reduced nodule size and N_2 fixation [1]. Leguminous plants in association with *Rhizobium* species have the potential to fix large amounts of atmospheric N_2 which contributes to the soil N pool provided that the N_2 fixation is not restricted by other environmental or microbial factors [12]. Seed inoculation with specific *Rhizobium* sp. increased nodulation by absorbing atmospheric nitrogen symbiotically. Rainfall, drought, salinity, acidity, low P and the presence of toxic ions hinders the establishment of symbiotic N_2 fixation [13]. Seed inoculation increases yield and yield component of soybean [13, 14].

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Investigations have been shown that, N- fertilizer reduces N_2 fixation through a reduction in the number, weight and activity of nodules [15, 16]. However, this effect depended on a variety of factors such as amount of N applied and type of soil, climate and farming system [17]. Studies revealed that small amounts of starter-N applied at sowing could improve root growth and N uptake by soybean prior to full nodulation [18, 19]. Coating soybean seeds with rhizobial inoculants has been suggested as a way to improve N_2 fixation [20, 21], but the success of inoculation was found to be highly variable [22]. Coutinho *et al*. [23] found that inoculation, increased soybean grain yields in the Cerrado region of Brazil by up to 750 kg ha^{-1}. This positive effect has been confirmed in other studies and seed coating with inoculants is now being recommended as a standard practice for soybean growers in Brazil [24]. The objective of this study was to determine the effect of water stress, nitrogen application and seed inoculation on yield and yield components of soybean at the Islamshahr town in Iran.

MATERIALS AND METHODS

The experiment was conducted in 2010 at the Islamshahr town that located in 12 km of South-west of Tehran, the capital of Iran. The research field is located in an arid climate where the summer is hot and dry and the winter is cool and dry. Longitude, latitude and altitude of region are 51°10′ E, 34°42′ N and 1165 m, respectively. The mean annual precipitation and temperature are 231.7 mm and 17.1°C respectively. The experiment was laid out in a split plot on the basis of randomized complete block design with three replications that placed different irrigation levels (irrigation after, E0= 40, E1= 80 and E2= 120 mm evaporation from the class "A pan" evaporation) in the main plots and seed inoculation treatments (I0= 0 kg N ha^{-1} + un-inoculation, I1= 20 kg N ha^{-1} + inoculation with *Rhizobium japonicum*, I2= 20 kg N ha^{-1} + inoculation with *Azotobacter chroococcum*, I3= 20 kg N ha^{-1} + inoculation with *Azospirillium brasilense*, I4= 20 kg N ha^{-1} + inoculation with mix of bacteria, I5= 20 kg N ha^{-1} + un-inoculation and I6= 100 kg N ha^{-1} + un-inoculation) in the sub plots. The soil of experimental field was clay loam with pH 7.8, organic carbon 2.1%, total nitrogen 0.19%, available phosphorus 32 ppm, exchangeable potassium 264 ppm and EC 2.96 mmohs cm^{-1}. N fertilizer was applied in the form of urea at the sowing time by side dressing with the help of hand drill. In I6 treatment, half dose of nitrogen fertilizer was applied as basal at the sowing time by side dressing with the help of hand drill and remaining half nitrogen was top dressed 40 days after sowing. Experimental field was uniformly fertilized with 40-20 kg PK ha^{-1} in the form of triple superphosphate and muriate of potash at the time of final land preparation. For seed inoculation, sugar solution was prepared and bacteria were mixed uniformly as the inoculums could stick the seeds. The seeds were put on paper under shade for drying and then immediately were sown in the morning. The sowing of soybean (cv. Williams) was done with the help of a single row hand drill in rows on 2nd June, 2010. Seeds were treated with Bavistin before sowing to control the seed borne disease. Row to row and plant to plant distance was 50 cm and 10 cm respectively. Each sub plot had 4 planting rows with length of 6 m. Size of each plot was 12 m^2. Crop management practices such as weeding, thinning and plant protection measures were done as per requirement. Irrigation was done as per treatments. At physiological maturity ten plants $plot^{-1}$ were selected randomly, sun dried and were recorded number of pods $plant^{-1}$, number of seeds pod^{-1} and 100-seeds weight. To determine seed yield, plants in 3 m^2 were harvested by hand of each plot that were sun dried properly. Collected data were analyzed statistically using MSTAT-C program and the means were compared by Duncan's Multiple Range Test at 5% probability level [25].

RESULTS

Number of Pods $Plant^{-1}$: Data showed that irrigation levels, seed inoculation and their interactions had significant effect on number of pods per plant (Table 1). The number of pods per plant was decreased significantly at the mild and severe water stress conditions (Table 2). At the all irrigation levels, the highest number of pods per plant was observed in treatment I4 (20 kg N ha^{-1} + inoculation with mix of bacteria), while the lowest number of pods per plant was recorded in treatment I0 (0 kg N ha^{-1} + un-inoculation) (Table 3).

Number of Seeds Pod^{-1}: The analysis of variance indicated significant differences among irrigation levels, seed inoculation and their interactions (Table 1). Mean comparisons showed that number of seeds per pod was decreased from optimum irrigation level to mild and severe water stress conditions in all treatments of seed inoculation (Table 2). The maximum number of seeds per pod in optimum irrigation, mild and severe water stress

Table 1: The mean squares of ANOVA for effect of water stress and seed inoculation on yield and yield components of soybean

S.O.V	df	Pods plant^{-1}	Seeds pod^{-1}	100-Seeds weight	Seed yield
Replication	2	22.35ns	0.000007ns	54.69**	51.85**
Water stress	2	396.08**	0.48181**	72.32**	84.07**
Error (a)	4	13.09ns	0.00064ns	24.17**	3.86ns
Seed inoculation	6	4783.62**	0.21843**	10.74*	49.91**
Water stress×Seed inoculation	12	410.37**	0.22921**	8.16ns	17.86*
Error (b)	36	15.76	0.02137	4.45	6.77
C.V (%)	---	7.19	7.69	16.96	14.55

ns, * and ** : Non significant, significant at the probability levels of 5% and 1% respectively

Table 2: Mean comparison of yield and yield components of soybean as affected by water stress and seed inoculation

Treatments	Pods plant^{-1}	Seeds pod^{-1}	100-seeds weight (g)	Seed yield (g m^{-2})
Water stress				
E0	65.00a	2.04a	14.36a	380.83a
E1	54.45b	1.89b	12.72b	261.80b
E2	45.99c	1.72c	10.25c	162.16c
Seed inoculation				
I0	21.07e	1.66d	11.42c	79.89d
I1	40.41c	1.87bc	11.55c	174.56c
I2	69.03b	1.89bc	13.28b	346.52b
I3	66.33b	1.96b	11.83c	307.60b
I4	82.00a	1.79c	14.11a	414.21a
I5	33.60d	2.17a	11.57c	168.72c
I6	73.56ab	1.84bc	13.35b	361.38b

Means with the same letter in each column and treatment are not significantly different at the probability level of 5% using DMRT.

E0= Irrigation after 40 mm evaporation from the class “A pan” evaporation

E1= Irrigation after 80 mm evaporation from the class “A pan” evaporation

E2= Irrigation after 120 mm evaporation from the class “A pan” evaporation

I0= 0 kg N ha^{-1} + un-inoculation

I1= 20 kg N ha^{-1} + inoculation with *Rhizobium japonicum*

I2= 20 kg N ha^{-1} + inoculation with *Azotobacter chroococcum*

I3= 20 kg N ha^{-1} + inoculation with *Azospirillium brasilense*

I4= 20 kg N ha^{-1} + inoculation with mix of bacteria

I5= 20 kg N ha^{-1} + un-inoculation

I6= 100 kg N ha^{-1} + un-inoculation

conditions was obtained in treatments I3 (20 kg N ha^{-1} + inoculation with *Azospirillium brasilense*), I5 (20 kg N ha^{-1} + un-inoculation) and I5, respectively. The minimum number of seeds per pod in optimum irrigation, mild and severe water stress conditions was produced in treatments I4 (20 kg N ha^{-1} + inoculation with mix of bacteria), I4 and I0 (0 kg N ha^{-1} + un-inoculation), respectively (Table 3).

100-Seeds Weight: There were significant differences between irrigation levels and seed inoculation treatments, but interaction effect of their, was not significant (Table 1). 100-Seeds weight was decreased with increase in intensity of water stress (Table 2). At the all irrigation levels, the highest 100-seeds weight was observed in treatment I4 (20 kg N ha^{-1} + inoculation with mix of bacteria), while the lowest 100-seeds weight in optimum irrigation, mild and severe water stress conditions was recorded in treatments I0 (0 kg N ha^{-1} + un-inoculation), I3 (20 kg N ha^{-1} + inoculation with *Azospirillium brasilense*) and I5 (20 kg N ha^{-1} + un-inoculation), respectively (Table 3).

Seed Yield: Seed yield was affected by water stress and seed inoculation as well as their interactions (Table 1). Mean comparisons indicated that seed yield was decreased significantly at the mild and severe water stress conditions (Table 2). At the all irrigation levels, the maximum seed yield was obtained in treatment I4 (20 kg N ha^{-1} + inoculation with mix of bacteria), while the minimum seed yield was produced in treatment I0 (0 kg N ha^{-1} + un-inoculation) (Table 3).

Table 3: Interaction effects of water stress and seed inoculation on yield and yield components of soybean

Treatments combination	Pods plant^{-1}	Seeds pod^{-1}	100-seeds weight (g)	Seed yield (g m^{-2})
E0 × I0	28.73l	2.02cd	12.26abcd	142.30gh
E0 × I1	43.70i	2.04bcd	13.33abc	237.67ef
E0 × I2	74.40cd	2.05bcd	14.73a	449.32b
E0 × I3	71.20de	2.16bc	15.18a	466.91b
E0 × I4	103.50a	1.97cd	15.46a	630.44a
E0 × I5	42.00ij	2.03cd	14.52a	247.59ef
E0 × I6	91.50b	2.04bcd	15.08a	562.97a
E1 × I0	25.30l	1.97cd	12.16abcd	121.21gh
E1 × I1	41.23ij	1.84de	12.63abcd	191.63efg
E1 × I2	69.50defg	1.88de	13.41abc	350.43c
E1 × I3	65.10fgh	1.88de	10.16bcde	248.69ef
E1 × I4	78.80c	1.83de	14.12ab	407.23bc
E1 × I5	31.00kl	1.99cd	13.31abc	164.22fg
E1 × I6	70.20def	1.83de	13.28abc	341.20cd
E2 × I0	9.20m	1.00g	9.85cde	18.12i
E2 × I1	36.30jk	1.73ef	8.70de	109.27gh
E2 × I2	63.20gh	1.74ef	11.72abcd	257.76de
E2 × I3	62.70h	1.86de	10.14bcde	236.51ef
E2 × I4	63.73fgh	1.59f	12.77abcd	258.80de
E2 × I5	27.80l	2.49a	6.90e	95.53h
E2 × I6	59.00h	1.67ef	11.69abcd	230.36ef

Means with the same letter in each column are not significantly different at the probability level of 5% using DMRT.

E0= Irrigation after 40 mm evaporation from the class "A pan" evaporation

E1= Irrigation after 80 mm evaporation from the class "A pan" evaporation

E2= Irrigation after 120 mm evaporation from the class "A pan" evaporation

I0= 0 kg N ha^{-1} + un-inoculation

I1= 20 kg N ha^{-1} + inoculation with *Rhizobium japonicum*

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I3= 20 kg N ha^{-1} + inoculation with *Azospirillium brasilense*

I4= 20 kg N ha^{-1} + inoculation with mix of bacteria

I5= 20 kg N ha^{-1} + un-inoculation

I6= 100 kg N ha^{-1} + un-inoculation

DISCUSSION

In this study, water stress decreased number of pods per plant, number of seeds per pod, 100-seeds weight and seed yield of soybean (cv. Williams). Water stress increases the abortion of flowers and pods also decreases fertilization values, photosynthates mobilization to seeds and seed filling period. The decrease in yield and yield components of soybean, due to water stress, has also been reported by other researchers [26-28]. Drought is the major limitation of crop yields worldwide. In soybean, drought not only results in losses in CO_2 accumulation and leaf area development but also its symbiotic N_2 fixation is especially vulnerable to drought. With declining soil water content, soybean has decreased N_2 fixation rates in advance of declines of other physiological processes. This means a decrease in N availability to support cell and tissue development throughout the plant [29]. In present research, in both, optimum and water stress conditions, application of 20 kg N ha^{-1} as starter and seed inoculation especially with mix of bacteria, produced maximum yield (treatment I4). In severe water stress as compared with optimum conditions, seed yield was decreased (87%) and (43%) in treatment I0 (0 kg N ha^{-1} + un-inoculation) and treatment I2 (20 kg N ha^{-1} + inoculation with *Azotobacter chroococcum*) respectively. This results showed that starter N and seed inoculation improved yield of soybean in optimum and water stress conditions. Drought is by far the most important environmental factor contributing to crop yield loss, especially in soybean, where symbiotic fixation of atmospheric nitrogen (N_2) is sensitive to even modest soil water deficits. Decline of N_2 fixation with soil drying causes yield reductions due to inadequate N for protein production, which is the critical seed product [29]. Nitrogen fertilization is sometimes needed to achieve a substantial yield of legumes (e.g., soybean) when the symbiotic N_2 fixation is unable to provide enough nitrogen. However, fertilizer rates exceeding those exerting a "starter nitrogen" effect generally reduce nodulation and N_2 fixation. The response of the *Rhizobium*-legume symbiosis to added nitrogen fertilizer is definitely determined by time of application (growth stage), level and form of N and the legume species [30]. Siddiqui *et al.,*[3] recommended that biofertilizer should be inoculated which fix atmospheric nitrogen in the root nodules with the inorganic nitrogen at the level of 50 kg ha^{-1} where maximum output could be achieved. The occurrence of rhizobial populations in desert soils and the effective nodulation of legumes growing therein emphasize this fact that rhizobia can exist in soils with limiting moisture levels, however, population densities tend to be lowest under the most desiccated conditions and to increase as the moisture stress is relieved [30]. According to our research, drought, decreased bacteria activity, however, response of bacteria species was different. Both nodulation and N_2 fixation in soybean are sensitive to drying soil and this can have a negative effect on yield [31]. Serraj *et al*., [2] established that drought stress leads to decreased N_2 fixation, mainly as a result ureide accumulation in shoots and asparagine in nodules. The highest soil moisture level guaranteed better nodulation and a higher efficiency of this process were modulated by the inducer. It is important to produce inoculants which have been obtained from induced media, because they will not only increase nodulation and N_2 fixation, but can also help under adverse conditions of water stress [1]. Benefits of inoculation and N application are generally thought to be higher if N is a limiting factor, either because of low N availability in the soil [22] or because of large N demand of high yielding cultivars [32]. Purcell and King [33] observed that N fertilizer application had no effect on irrigated plots but that drought-stressed soybean benefited from fertilizer N due to lower flower and pod abortion rates. They concluded that application of N fertilizer increased drought tolerance of soybean because of the extreme sensitivity of N_2 fixation to drought. Based on study of Kubota *et al.,* [17] starter N application (50 kg ha^{-1}) and seed inoculation appears to offer some protection against intermittent drought in the shortened growth period after delayed sowing of soybean. They also revealed that N fertilization alone did not increase yield. A positive effect of N application was only detected when seeds had been coated with rhizobial inoculants. This raises the questions about the nature of additional benefits incurred by inoculation.

In conclusion, results of present study showed that, drought decreased bacteria activity, yield and yield components of soybean. Effect of bacteria species on yield of soybean was different. In optimum and drought conditions, starter N (20 kg ha^{-1}) + seed inoculation especially with mix of bacteria, improved seed yield.

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