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The effects of inoculation and N fertilization on soybean [*Glycine max* (L.) Merrill] seed yield and protein concentration under drought stress

O. Basal*, A. Szabó

Institute of Crop Sciences, Faculty of Agricultural and Food Sciences and Environmental management, Böszörményi út 138, Debrecen, Hungary

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Abstract. *An experiment was conducted during 2017 growing season in Debrecen, Hungary to investigate the effects of inoculation and N fertilization on the seed yield and protein concentration of soybean [Glycine max (L.) Merr.] cultivar (Boglár) under three irrigation regimes: severe drought (SD), moderate drought (MD) and control with no drought stress (ND). Three N fertilizer rates were applied: no N (0N), 35 kg/ha (35N) and 105 kg/ha of N fertilizer (105N). Half of the seeds in each treatment were inoculated with Bradyrhizobium japonicum inoculant. The results showed significant differences in the seed yield associated with both inoculation and irrigation, whereas there were no significant differences in the seed yield associated with fertilization. When seeds were inoculated, yield was increased when (35N) was applied compared to (0N); however, high rate (105N) reduced the yield to a level even less than (0N). When seeds were not inoculated, the highest rate of N increased the yield the most compared to the other two N fertilizer rates under drought. Under severe drought, inoculation was positively and significantly correlated with yield; however, adding N fertilizer increased the yield of non-inoculated plants compared to the inoculated ones, regardless of N-fertilizer rate. Protein concentration in the seeds was significantly affected by irrigation and by fertilization, but not by inoculation. Protein concentration increased as N fertilization rate increased, regardless of inoculation or irrigation; moreover, increasing N rate reduced the correlation coefficient of protein concentration with irrigation. It was concluded that adding N-fertilizer is not always recommended, especially when seeds are inoculated before being sown; however, it is very important under severe drought stress to sustain yield. Enhanced protein concentrations could be achieved by applying N fertilization, whether the seeds were pre-inoculated or not.*

Keywords: soybean, irrigation, N fertilization, seed quality

Introduction

Using mineral fertilization is increasing worldwide. It is well known that obtaining high yields of agricultural crops is due to this type of fertilization. However, negative impacts of mineral fertilization on soil and environment are becoming more obvious, with alternative methods being more necessary and applicable, especially with the current climatic changes, which has imposed serious abiotic stresses, such as drought (De Paola et al. 2014).

Soybean [*Glycine max* (L.) Merr.] is an important grain legume. It is known as the main source of plant oil and protein (Lei et al., 2006; Maleki et al., 2013). 60% of human vegetable protein comes from soybean (Rosenthal et al., 1998; Allen et al., 2009). The interaction (genotype×environment) determines the ratio of protein and oil in soybean seeds (Fehr et al., 2003; Wilson, 2004). Generally, high rate of protein in soybean seeds is negatively correlated with yield (Liang et al., 2010).

Changes in morphology and physiology are among many changes that occur within the soybean plant as a response to abiotic stresses such as drought stress (Bray, 1993; Cushman and Bohnert, 2000; Sekiet al., 2003; Yamaguchi-Shinozaki and Shinozaki, 2006); for example, changes include increased stomatal closure to reduce water loss, reduced leaf area and deeper and denser roots to enhance water absorption (Imsande, 1992); drought stress also decreases the number of soybean nodes by decreasing the rate of main stem nodes production (Frederick et al., 1989). Among crops, soybean is the highest drought stress sensitive (Maleki et al., 2013).

Nitrogen is one of the most important macronutrients for plant growth and yield; it is essential for total chlorophyll content and protein synthesis. N is essentially needed for the soybean vegetative growth in order to produce optimum biomass (Fabre and Plancho**n,** 2000; Fageria and Baligar, 2005). Soybean plants have a large N harvest index compared to other legumes (Lawn, 1989).

This research aims at investigating the effects of inoculation and N fertilization, solely and in interaction on the yield and the protein concentration of soybean [*Glycine max* (L.) *Merrill,* cultivar *Boglár*] under three different irrigation regimes.

Material and methods

Soybean cultivar *Boglár*, was grown on Debrecen University experimental site (Látókép) (N. latitude 47°33', E. longitude 21°27') in 2017. The soil type is calcareous chernozem. Half of the seeds were inoculated with *Bradyrhizobium japonicum*, and the other half was not inoculated. Three N fertilization rates (no N – 0N; 35 kg/ha N – 35N and 105 kg/ha N – 105N) under three different drought stress conditions; severe drought (no irrigation, just the precipitation amounts), moderate drought (precipitation + 2*25 mm irrigation at R2 and R4 stages) and no drought (precipitation + 2*50 mm irrigation at R2 and R4 stages) were applied in four replications. The experimental design was split-split plot design with the drought severity as the main plots, the inoculation as the sub-plots and the fertilization rates as the sub-sub-plots; each plot area was 67.5m²

* e-mail: oqbabasal@gmail.com

(2.7*25 m). The statistical analysis was made using SPSS ver.22 software, and two-way ANOVA was used to compare the means and significances.

Results

Yield

Significant differences in the seed yield associated with overall inoculation, irrigation and the interaction between them, whereas there were no significant differences in the seed yield associated with overall fertilization treatments alone or in interaction with overall inoculation or irrigation or both. When there was no drought, and considering the inoculation as the only source of N (without any N fertilizer engaged), the yield of inoculated treatment was increased to 5062.8 kg/ha from 4901.8 kg/ha of the non-inoculated counterpart (Table 1).

Table 1. The yield of the studied soybean cultivar under different irrigation regimes, and different N fertilizer rates

Irrigation regime	N Fertilizer rate (kg/ha)	Yield (kg/ha)	
		Inoculated	Non-inoculated
Severe drought (SD)	0 N	3854.0 [a]	4371.3 [a]
	35 N	3659.0 [a]	4351.3 [a]
	105 N	3753.3 [a]	4566.8 [a]
Moderate drought (MD)	0 N	4576.0 [a]	4712.8 [a]
	35 N	4716.8 [a]	4793.8 [a]
	105 N	4957.0 [a]	5067.0 [a]
No drought (ND)	0 N	5062.8 [a]	4901.8 [a]
	35 N	5378.8 [a]	5030.0 [a]
	105 N	4696.8 [a]	5047.5 [a]

* The same letter in each column within a particular irrigation regime indicates no significant difference at 0.05 level

When there was no drought, a slight addition of 35 kg/ha of N fertilizer increased the yield of the inoculated plants to 5378.8 kg/ha compared to 5062.8 kg/ha without any addition of N fertilizer; however, the high rate of N fertilizer (105 kg/ha) decreased the yield to 4696.8 kg/ha, which is even less than the control yield. On the other hand, the yield of non-inoculated plants increased as the N rate increased.

Under drought (whether severe or moderate) conditions, adding N fertilizer increased the yield of the non-inoculated plants compared to the inoculated ones, regardless of the amount of N. Moreover, adding relatively high amount of N to the non-inoculated plants resulted in the best yield (Table 1).

Regardless of inoculation, the mean yield of the three fertilization rates was better when the water amount increased. More specifically, severe drought stress reduced the seed yield of soybean more than moderate drought stress; the severe and the moderate drought decreased the yield by 25.6 and 5.9%, respectively, compared to the control. When not inoculated, the yield was the highest when 105 kg/ha of N was added, regardless of the irrigation scheme. This emphasizes the importance of N fertilizer to provide the N amounts needed by soybean with the absence of N_2 fixation process.

Protein concentration

Protein concentration in the seeds was significantly affected by overall irrigation and fertilization treatments, but not by overall inoculation treatments (Table 2). Under severe drought stress conditions, adding 35 kg/ha of N to the inoculated plants did not have an effect on the protein concentration (35.1% compared to 35.2% for the control – 0N), whereas the addition of relatively high amount of N (105 kg/ha) resulted in a noticeable increase (36.7%) in the protein concentration. On the other hand, the protein concentration gradually and noticeably increased with increasing the N rate addition to the non-inoculated plots (34.9, 35.4 and 36.4% for 0N, 35N and 105N treatments, respectively).

Under moderate drought stress, both the inoculated and the non-inoculated plots exhibited similar attitude; increasing the N rate turned out to increase protein concentration; the difference was significant between the (0N) and the (105N) treatments. Moreover, the effect was always higher on the non-inoculated plots compared to their counterpart inoculated plots. Similar results were observed when the drought was eliminated, however, there were no significant differences recorded among the three fertilization treatments (Table 2).

Table 2. The protein concentration of the studied soybean cultivar under different irrigation regimes, and different N fertilizer rates

Irrigation regime	N Fertilizer rate (kg/ha)	Protein concentration (%)	
		Inoculated	Non-inoculated
Severe drought (SD)	0 N	35.2 [a]	34.9 [a]
	35 N	35.1 [a]	35.4 [a]
	105 N	36.7 [a]	36.4 [a]
Moderate drought (MD)	0 N	34.9 [a]	35.3 [a]
	35 N	35.8 [ab]	36.3 [ab]
	105 N	36.9 [b]	37.5 [b]
No drought (ND)	0 N	36.1 [a]	36.2 [a]
	35 N	36.5 [a]	36.5 [a]
	105 N	37.0 [a]	37.1 [a]

* The same letter in each column within a particular irrigation regime indicates no significant difference at 0.05 level

Discussion

Yield

In our experiment, under no-drought conditions, with the inoculation being the only source of N, the yield of inoculated treatment was better than the non-inoculated counterpart (Table 1). Rurangwa et al. (2017) reported a yield increase by 19, 19 and 25% of inoculated soybean plots in three different sites compared to their non-inoculated counterparts. Albareda et al. (2009) established that inoculation with two different inoculants [*Sinorhizobium* (Ensifer) fredii SMH12 and *Bradyrhizobium japonicum* USDA110] resulted in significantly higher yields of 6071 and 6318 kg/ha, respectively, compared to 3715 kg/ha obtained from the non-inoculated control. Moreover, they reported that plants inoculated with one of the inoculants yielded higher than the non-inoculated plants which was fertilized by 200 kg N/ha, which is consistent with our findings as the yield of the inoculated plants without any N fertilizer addition was higher than all of the yields of the non-inoculated plants, regardless of the N rate provided (Table 1). Similar findings were published by Seneviratne et al. (2000), who reported the soybean cultivar Pb1 to yield 1458 kg/ha without inoculation and without N fertilization, whereas the sole N fertilization led to a yield of 2693 kg/ha, and the sole inoculation resulted in a yield of 2882 kg/ha. Zimmer et al. (2016) tested three early maturing soybean varieties in combination with four different *Bradyrhizobium* inoculants and compared with a non-inoculated control in two experimental sites: an organically managed site and a conventionally managed site; the average grain yield of the effectively inoculated soybeans increased by 57% and 16% in the two sites, respectively, compared to the non-inoculated control. Also, applying low rate (35 kg/ha) of N-fertilizer enhanced the yield of the inoculated plants compared to (0N) treatment, whereas the high rate (105 kg/ha) decreased the yield to a level even less than the control yield. On the other hand, increasing N rate resulted in increased yield of the non-inoculated plants (Table 1). Kaschuk et al. (2016) found that N fertilizer did not lead to enhanced yield quantity of two different soybean cultivar groups (determinate and indeterminate) which were inoculated. Harper (1974) and Imsande (1992) reported seed yield to be enhanced when N_2 fixation is associated with N fertilizer. Mac Kenzie and Kirby (1979) concluded that the yield was linearly correlated with N fertilizer amounts up to 90 kg/ha.

Under drought conditions, adding N fertilizer increased the yield of the non-inoculated plants compared to the inoculated ones; adding relatively high amount of N resulted in the best yield (Table 1), so, adding relatively high amounts of N fertilizer might be very important under severe drought stress. It was previously reported that N fertilizer is very important under drought stress conditions (Lyons and Earley, 1952; Obaton et al., 1982). Addition of N fertilizer to soybean increased drought tolerance as it enhanced the accumulation of both shoot nitrogen and shoot biomass under drought stress conditions, whereas under well-watered conditions, N decreased yield to 2597 kg/ha relative to 2728 kg/ha (Purcell and King, 1996).

The mean yield of the three fertilization rates was better when the water amount increased, regardless of inoculation. Many papers reported soybean seed yield to be decreased under drought stress conditions (Karam et al., 2005; Dogan et al., 2007; Bajaj et al., 2008; Gercek et al., 2009; Kokubun, 2011; Sadeghipour and Abbasi, 2012; Li et al., 2013). More specifically, severe drought stress reduced the seed yield of soybean more than moderate drought stress (Dornbos and Mullen, 1992), which is consistent with our results, as the severe and the moderate drought decreased the yield by 25.6 and 5.9%, respectively, compared to control (Table 1).

Applying high rate of N-fertilizer to the non-inoculated plants resulted in the highest yield, regardless of the irrigation regime (Table 1), which emphasizes the importance of N-fertilizer to provide the N amounts needed by soybean with the absence of N_2 fixation process. Salvagiotti et al. (2008) previously reported biologically fixed N_2 and mineral N to be the two main sources of N needed in soybean. If there is some deficiency in fixed-N_2 amounts, other sources (mainly through N fertilization) must be available (Yinbo et al., 1997; Fabre and Planchon, 2000), or else the N from leaves will be remobilized to the seeds, which in part will lead to decreased photosynthesis and eventually reduced yield (Salvagiotti et al., 2008).

Protein Concentration

Under severe drought stress conditions, the addition of high rate of N-fertilizer to the inoculated plants resulted in a noticeable increase in the protein concentration compared to the control, whereas the addition of low rate did not. On the other hand, the protein concentration was gradually and noticeably increased with increasing the N rate addition to the non-inoculated plots (Table 2). Previously, it was reported that protein content is increased when N is increased (Ham et al., 1975), whether the source of N is mineral fertilization or N_2 fixation. Harper (1974) and Imsande (1992) concluded that N_2 fixation, associated with N fertilizer, enhanced seed protein content. Moreover, Miransari (2016) concluded that N fertilizer rate has a significant effect on the seed protein content; the rate of 100 kg/ha increased seed protein just by 2%, whereas the rate of 200 kg/ha resulted in 14% increase in seed protein.

Conclusion

Although our experiment is a one-year experiment, we could come out with some initial conclusions concerning the combined effect of drought and N fertilization on yield and protein concentration; we could emphasize that adding N fertilizer is not always recommended, especially when seeds are inoculated before being sown as previously reported by some researchers; however, adding relatively small amounts of N fertilizer, along with inoculation may result in an increase in the yield, probably because the early vegetative stages need a starter amount of N as the symbiosis process wouldn't be initialized at those stages yet. On the other hand, adding N fertilizer is highly recommended when there is severe drought stress in order to maintain reasonable yield; this could be explained as an alleviation of drought negative effects by N. Moreover, the results suggest, under drought, not to inoculate soybean seeds; instead, to add relatively high rates of N fertilizer. Regardless of inoculation, enhanced protein concentrations could be achieved in our experiment by applying N fertilization, which emphasizes the important role of N in protein synthesis. Extending the experiments over the next years will lead to more precise results by involving the effect of different years by means of on-field weather conditions.

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Book chapter or conference proceedings: Author(s) surname and initials, year. Title. In: Title of the book or of the proceedings followed by the editor(s), volume, pages. Name of publisher, place of publication. Example:

Mauff G, Pulverer G, Operkuch W, Hummel K and Hidden C, 1995. C3-variants and diverse phenotypes of unconverted and converted C3. In: Provides of the Biological Fluids (ed. H. Peters), vol. 22, 143-165, Pergamon Press. Oxford, UK.

Todorov N and Mitev J, 1995. Effect of level of feeding during dry period, and body condition score on reproductive performance in dairy cows,IXth International Conference on Production Diseases in Farm Animals, September 11–14, Berlin, Germany.

Thesis:

Hristova D, 2013. Investigation on genetic diversity in local sheep breeds using DNA markers. Thesis for PhD, Trakia University, Stara Zagora, Bulgaria, (Bg).

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Animal welfare

Studies performed on experimental animals should be carried out according to internationally recognized guidelines for animal welfare. That should be clearly described in the respective section "Material and methods".

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