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INOCULATION ENHANCES SOYBEAN PHYSIOLOGY AND YIELD UNDER MODERATE DROUGHT

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Abstract

With a high seed content of protein and oil, soybean is one of the most widely-grown legumes worldwide. Inoculation process enables soybean to achieve most, and sometimes all, of his nitrogen requirements through N₂-fixation process, however, this process, like soybean plant itself, is drought-sensitive. Drought is globally-increasingly imposed as a result of climatic changes, negatively affecting soybean production. An experiment was conducted in Debrecen, Hungary in 2017 and 2018 to evaluate the influence of moderate drought stress on some physiological parameters of both inoculated and non-inoculated soybean plants. Results showed that drought negatively affected soybean's physiology and yield, regardless of inoculation; however, inoculated plants could maintain better values of studied parameters relative to their non-inoculated counterparts. On the other hand, drought occurring during R4 stage had more noticeable effects on soybean plants as compared to drought occurring earlier (at V2 stage) during vegetative

period. It was concluded that inoculation could be a beneficial strategy in order for soybean to reveal better physiology and, consequently, better yield under moderate drought conditions, and that the timing of drought stress occurrence is crucial regarding soybean's vigor and final seed yield.

Keywords

Inoculation, Moderate Drought, Physiology, Soybean, Yield

1. Introduction

Soybean (*Glycine max* (L.) Merrill), a very important legume, is reported to be one of the main sources of plant oil and protein (Lei et al., 2006; Maleki et al., 2013) as it counts for 60% of human vegetable protein (Allen et al., 2009). Abiotic stresses are considered to be major limiting factors of plant growth inhibition, resulting in a considerable yield loss (Mahajan and Tuteja, 2005). Soybean is the highest in terms of sensitivity to drought stress compared to the other legume crops, (Maleki et al., 2013). As a response to drought stress conditions, changes in both morphology and physiology are to take place in soybean plants (Seki et al., 2003; Yamaguchi-Shinozaki and Shinozaki, 2006).

Biologically-fixed N₂ is one of the main sources of nitrogen needed by soybean plants (Salvagiotti et al., 2008). Well-nodulated soybean does not need N fertilizer as shown by many field experiments, as the sole inoculation with *Bradyrhizobium* is enough as N source (Hungria and Mendes, 2015; Kinugasa et al., 2012). Drought stress can widely affect the establishment and the activity of the legume-*Rhizobium* symbiosis (Abaidoo et al., 2007). As such, even moderate levels of drought can decrease legume productivity (Saxena et al., 1993; Subbarao et al., 1995). Herrmann et al. (2014) reported drought stress to cause low soybean nodulation in a two-year experiment.

In soybean, nitrogen fixation's highest rate takes place between flowering and pod filling stages (Harper, 1974; Obaton et al., 1982), consequently, drought stress occurring during this period is expected to have major influence on this process. Moreover, pods-per-plant trait is determined at the beginning of pod filling stage as well (Dybing et al., 1986); during which active cell division in the young ovules and rapid pod expansion are executed; both processes are highly sensitive to water-deficiency stress (Westgate and Peterson, 1993).

In this study, we aimed at determining the influence of inoculation on some physiological traits of soybean (*cv. Boglár*) when exposing to moderate drought stress during two different vegetative stages; V2 or R4 stages.

2. Materials and Methods

Soybean (*cv. Boglár*) was sown in Debrecen University's experimental site (Látókép) (N. latitude 47° 33', E. longitude 21° 27') in 2017 and 2018. The soil type is calcareous chernozem. Half of the seeds were inoculated with *Bradyrhizobium japonicum*, and the other half were not inoculated. In both years, irrigation was applied whenever needed in order to match the average annual precipitation during the period between 2001 and 2010 (Hungarian Meteorological Service, 2018) with two exceptions; in 2017, moderate drought stress was managed to occur at V2 stage (during May), whereas it was managed to occur at R4 stage (during July) in 2018 (Fig. 1). Each of the two treatments (inoculated and non-inoculated) were applied in four replications. Each plot's area was 67.5m² (2.7*25 m). The statistical analysis was made using SPSS ver.25 software, and two-way ANOVA was used to compare the means and significances.

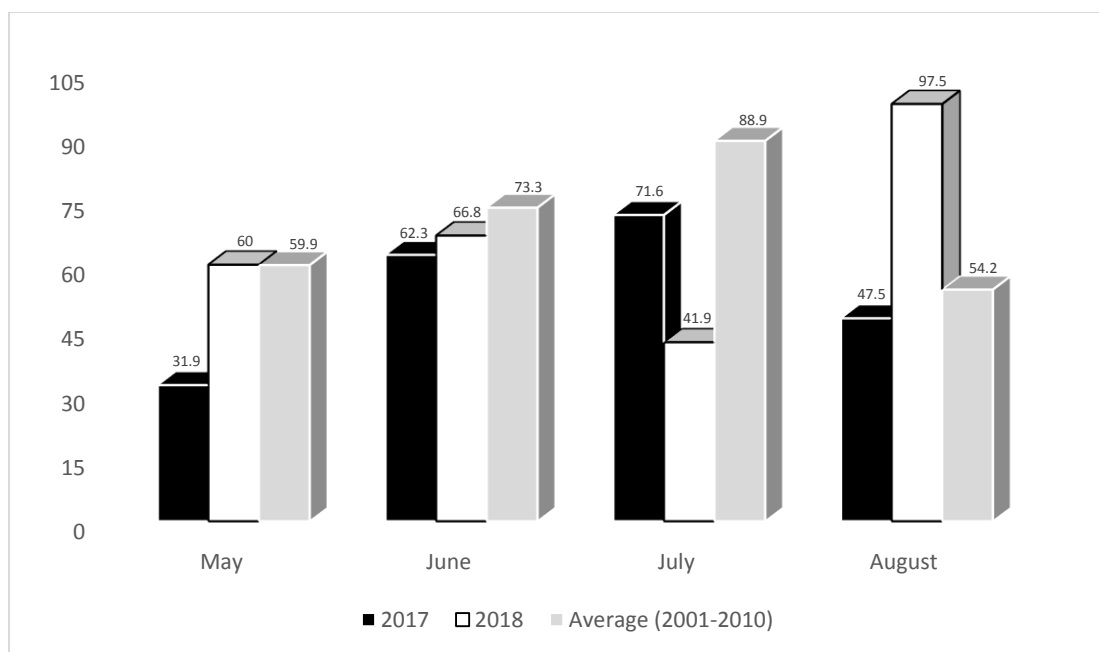


Figure 1: Water amounts (mm) provided to plants in 2017 and 2018 as compared to 2001-2010 average annual precipitation in Debrecen, Hungary. Adopted from the Hungarian Meteorological Service's official website (https://www.met.hu/eghajlat/magyarorszag_eghajlata/eghajlati_adatsorok/Debrecen/adatok/havi_adatok/)

3. Results and Discussion

3.1 Plant Height (cm)

On average, the inoculated plants tended to be taller (84.1 cm) than the non-inoculated counterparts (81.8 cm) (Table 1); however, the stage at which drought stress occurred had different effects; when drought occurred early during V2 stage in 2017, non-inoculated treatment resulted in shorter plants (92.1 cm) as compared to inoculated counterpart (97.2 cm), whereas drought at R4 stage had an opposite influence; it resulted in slightly shorter plants in the inoculated treatment (70.9 cm relative to 71.5 cm for non-inoculated treatment) (Table 2). Previously, Dadson (1984) reported that soybean plants inoculated with *R. japonicum* were taller than non-inoculated plants. A reduction by 4.3% in seedling height when drought stress was applied on soybean was reported (Navari-Izzo et al., 1990), similar results were reported at other different stages of plant's vegetative period (Atti et al., 2004; Demirtas et al., 2010; Hao et al., 2013; Mak et al., 2014). This reduction might be the result of drought causing cell swelling and synthesis enzymes decrease, and consequently, reducing growth and plant height (Austin, 1989; Levitt, 1980).

For both treatments, drought during R4 stage resulted in shorter plants than did drought during V2 stage (Table 2), which was reflected on other traits in this experiment as demonstrated later; taller plants had more flowers and pods, and consequently more final yield was achieved. It was previously reported that seed yield has a significantly positive correlation with plant height (Georgiev, 2004; Maleki et al., 2013).

Table 1: Yield (kg ha^{-1}), pod number (pod plant^{-1}), flower number (flower plant^{-1}) and plant height (cm) of inoculated and non-inoculated soybean (cv. Boglár) under moderate drought stress conditions averaged between 2017 and 2018 in Debrecen, Hungary

Yield	Inoculated	4918 ^a
	Non-inoculated	4740 ^a
Pod number	Inoculated	55.0 ^a
	Non-inoculated	44.4 ^b
Flower number	Inoculated	60.9 ^a
	Non-inoculated	48.5 ^b
Plant height	Inoculated	84.1 ^a
	Non-inoculated	81.8 ^a

- Same letter indicates no significant differences at .05 level between the two inoculation treatments within certain trait.

3.2 Flower Number (flower plant⁻¹)

Significantly higher flower number per plant was produced from inoculated plants in both years as compared to non-inoculated plants (Table 1). In 2017, inoculated treatment had a flower number of 64.5 flower per plant, whereas the flower number per plant in non-inoculated treatment was 50.3 flower per plant. In 2018, inoculated treatment produced 57.3 flower per plant compared to 46.8 flower per plant for the non-inoculated counterpart. The flower number per plant, for both treatments, was better in 2017 than in 2018, and the reduction percentage for inoculated treatment (11.2%) was higher compared to the reduction in the non-inoculated treatment (7.0%), indicating more negative effect of drought stress on inoculated soybean as compared to non-inoculated counterpart (Table 2). It was previously concluded that decreases in producing flowers and pods and also abortions of both flowers and pods are major factors influencing the final seed yield, and drought stress at pod production stage negatively affected the final seed yield (Fang et al., 2010; Leport et al., 2006).

Table 2: Yield (kg ha⁻¹), pod number (pod plant⁻¹), flower number (flower plant⁻¹) and plant height (cm) of inoculated and non-inoculated soybean (cv. Boglár) under moderate drought stress conditions in 2017 and 2018 in Debrecen, Hungary

Trait	Year	inoculated	non inoculated
Yield	2017	5379 ^{a1}	5030 ^{a1}
	2018	4456 ^{a1}	4450 ^{a1}
Pod number	2017	57.8 ^{a1}	47.3 ^{a2}
	2018	52.3 ^{a1}	41.5 ^{a2}
Flower number	2017	64.5 ^{a1}	50.3 ^{a2}
	2018	57.3 ^{b1}	46.8 ^{a2}
Plant height	2017	97.2 ^{a1}	92.1 ^{a1}
	2018	70.9 ^{b1}	71.5 ^{b1}

- Same letter indicates no significant differences at .05 level between the two years within certain inoculation treatment.
- Same number indicates no significant differences at .05 level between inoculation treatments within certain year.

3.3 Pod Number (pod plant⁻¹)

The number of pods per plant was significantly higher in inoculated treatment in both years of experiment and also in the two years' average (Tables 1 and 2). Drought during R4 stage resulted in less pod per plant, regardless of inoculation; however, the reduction was less for inoculated treatment (by 9.5%) compared to non-inoculated treatment (by 12.3%) (Table 2), whereas it was more effective on the flower number per inoculated plant, as discussed previously, indicating that drought has more negative effect on podding process (flowers turning into pods) of non-inoculated plants compared to inoculated ones; in other words, inoculation has a positive effect on podding process under drought stress occurring during R4 stage. Miao et al. (2012) reported that exposure of soybean to drought decreases pod number and grain yield, moreover, Liu et al. (2003) concluded that drought stress at podding stage significantly increases pod abortion rate which, in part, reduces the final seed yield. Later, Atti et al. (2004) suggested that yield is decreased under drought stress conditions as a result to the decrease in pod number per plant.

3.4 Yield (kg ha⁻¹)

In both years, inoculated treatment resulted in better yield compared to the non-inoculated counterpart, however, the difference was insignificant. In 2017, yield of inoculated treatment (5379 kg ha⁻¹) was noticeably higher than non-inoculated treatment (5030 kg ha⁻¹), whereas the difference was very slight in 2018 (Table 2). It was previously concluded that under drought conditions, inoculation caused a decrease in water consumption, an increase in growth rate, biomass and final yield. This indicates that symbiosis might take part in ameliorating the effects of climatic changes and further expanding marginal lands' agricultural production (Redman et al., 2011). Similar to our results, many previous papers reported that symbiosis enhances soybean yield (Couto et al., 2011; Egamberdiyeva et al., 2004; El-Shaarawi et al., 2011; Silva et al., 2013).

As drought occurred during V2 stage in 2017, whereas it occurred during R4 stage in 2018, it can be concluded that inoculated plants were less negatively-affected when drought occurred early in the vegetative period, however, when occurring during reproductive stages (particularly R4), both inoculated and non-inoculated plots were negatively-influenced by drought to the same extent. Many papers concluded that drought stress reduces soybean seed yield (Bajaj et al., 2008; Dogan et al., 2007; Gercek et al., 2009; Karam et al., 2005; Li et al., 2013; Sadeghipour and Abbasi, 2012; Sincik et al., 2008).

From another point of view, drought occurring at R4 stage reduced soybean yield (regardless of inoculation) to measurable extent as compared to yield loss resulting from drought occurring early during vegetative stages (Table 2). Many studies concluded that drought stress during the vegetative stages does not affect the yield much (Ashley and Ethridge, 1978; Elmore et al., 1988; Specht et al., 1989) whereas during the reproductive stages it could lead to significant yield loss. More particularly, It was previously found that drought stress during beginning of pod formation (R3) and full pod formation (R4) stages caused higher yield loss rate compared to drought occurring during beginning of flowering (R1) and full flowering (R2) stages (Doss et al., 1974; Sionet and Kramer, 1977). Song (1986) reported pod setting and filling to be the most susceptible stages to drought stress; he associated that with the reductions in seed size and number; this conclusion was demonstrated later (Jin et al., 2005; Xie et al., 1994).

4. Conclusions

Inoculation has positive effects on soybean's physiology and yield under moderate drought conditions; it resulted in better flower and pod number per plant and, consequently, better seed yield. However, inoculation is affected by moderate drought stress during R4 more than during V2 stage. Regardless of inoculation, moderate drought at both V2 and R4 stages negatively affected soybean plants, however, exposing soybean to drought during R4 stage had more negative influence as compared to drought occurring during V2 stage.

As inoculation's area of establishment is the roots, our future research will focus on studying the influence of inoculation on this part of soybean plants under different drought severities occurring at different stages of lifecycle.

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