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The Effects of Drought and Nitrogen on Soybean (*Glycine max* (L.) Merrill) Physiology and Yield

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Abstract**—**Legume crops are able to fix atmospheric nitrogen by the symbiotic relation with specific bacteria, which allows the use of the mineral nitrogen-fertilizer to be reduced, or even excluded, resulting in more profit for the farmers and less pollution for the environment. Soybean (*Glycine max* (L.) Merrill) is one of the most important legumes with its high content of both protein and oil. However, it is recommended to combine the two nitrogen sources under stress conditions in order to overcome its negative effects. Drought stress is one of the most important abiotic stresses that increasingly limits soybean yields. A precise rate of mineral nitrogen under drought conditions is not confirmed, as it depends on many factors; soybean yield-potential and soil-nitrogen content to name a few. An experiment was conducted during 2017 growing season in Debrecen, Hungary to investigate the effects of nitrogen source on the physiology and the yield of the soybean cultivar '*Boglár*'. Three N-fertilizer rates including no N-fertilizer (0 N), 35 kg ha^{-1} of N-fertilizer (35 N) and 105 kg ha^{-1} of N-fertilizer (105 N) were applied under three different irrigation regimes; severe drought stress (SD), moderate drought stress (MD) and control with no drought stress (ND). Half of the seeds in each treatment were pre-inoculated with *Bradyrhizobium japonicum* inoculant. The overall results showed significant differences associated with fertilization and irrigation, but not with inoculation. Increasing N rate was mostly accompanied with increased chlorophyll content and leaf area index, whereas it positively affected the plant height only when the drought was waived off. Plant height was the lowest under severe drought, regardless of inoculation and N-fertilizer application and rate. Inoculation increased the yield when there was no drought, and a low rate of N-fertilizer increased the yield furthermore; however, the high rate of N-fertilizer decreased the yield to a level even less than the inoculated control. On the other hand, the yield of non-inoculated plants increased as the N-fertilizer rate increased. Under drought conditions, adding N-fertilizer increased the yield of the non-inoculated plants compared to their inoculated counterparts; moreover, the high rate of N-fertilizer resulted in the best yield. Regardless of inoculation, the mean yield of the three fertilization rates was better when the water amount increased. It was concluded that applying N-fertilizer to provide the nitrogen needed by soybean plants, with the absence of N_2-fixation process, is very important. Moreover, adding relatively high rate of N-fertilizer is very important under severe drought stress to alleviate the drought negative effects. Further research to recommend the best N-fertilizer rate to inoculated soybean under drought stress conditions should be executed.

Keywords**—**Drought stress, inoculation, N-fertilizer, soybean physiology, yield.

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I. Introduction

SOYBEAN (*Glycine max* (L.) Merrill) is among the 10 world widely most grown crops [1], fourth most important food crop [2], most widely grown seed legume, providing an inexpensive source of protein [3] and most widely grown oilseed crop [4]; moreover, it is an important crop regarding biodiesel production [5]. Soybean is mostly sown under rain fed conditions, which has put this crop, with the current global climatic changes, under drought stress in many regions as soybean is reported to be drought-susceptible, especially at certain growth stages [6]; the annual soybean yield reductions caused by drought are enormous [7], [8], reaching up to 40% [9]. As a response to drought stress, many morphological and physiological changes are revealed by soybean plants, which in part, lead to growing and development fluctuations [10], e.g. alleviated stomatal closure to reduce water loss, decreased leaf area and deeper and denser roots to improve water uptake [11]; drought stress also decreases the number of soybean nodes [12] which lead to reduced plant height. In addition, light absorption is affected by drought through changes in the leaf chlorophyll content [13].

Plant height reveals soybean ability to produce more nodes on the main stem, and consequently more flowers, pods and seeds. It has been previously reported that plant height is decreased under water deficiency [14], [15], and different decrease curves been reported at different growth stages of soybean when water deficiency occurred [16], [17]. Decreases in plant height under water deficiency conditions might decrease both leaf area and yield [18], [19].

The leaf area index (LAI) is the canopy density of a crop population and has an important effect on the final yield [20]. Normally, shading happens to the lower leaf levels and consequently reduces the LAI, but drought stress decreases the LAI more than mutual shading does [20], resulting in less LAI values under drought conditions.

Plant height shows the ability of the soybean plants to produce more nodes, and consequently more flowers, pods and seeds. Many papers reported plant height to decrease when drought stress is imposed at different stages of soybean lifecycle [16], [17].

Water deficiency negatively influences soybean growth resulting in yield loss [7], [21]-[23]; moreover, the timing of water deficiency during soybean lifecycle (e.g. at pod formation [24], at seed filling [25]) results in different amounts of yield reduction. Reference [26], [27] concluded that water deficiency has the most negative effect on soybean if occurred at flowering stage (R_2). However, soybean genotype also plays a role [1].

Chlorophyll content is one of the most important physiological traits, as it reflects the plant photosynthesis, and consequently, yield's potentials. Drought stress influences the chlorophyll content and reduces its value as reported by many researchers [3]. Total chlorophyll content and protein synthesis essentially need nitrogen (N), which is one of the most important macronutrients for plant growth and yield. Moreover, N is also essentially needed for the soybean vegetative growth in order to produce the optimum biomass [28], [29].

Biologically-fixed N_2 and mineral N are the two main sources of N needed in soybean [30]. If there is some deficiency in fixed N_2 amounts, other sources (mainly through N fertilization as a quick and partially-convenient method of providing N to plants) must be available [28], [31], or else N from leaves will be remobilized to the seeds, which in part, will lead to decreased photosynthesis and eventually reduced yield [30]. Although applying N fertilizer at appropriate rates can enhance seedling growth by becoming established at the beginning of the season until the initiation of biological N_2-fixation by rhizobia [32], [33], higher amounts of N fertilizer can negatively affect *B. japonicum* activity and, hence, N_2-fixation [34], [35], yet it is still a better solution than exposing the plants to N-deficiency which can result in growth delay, especially if it happens during the vegetative stages [30]. Therefore, the determination of N-fertilization influence on the growth and the yield of soybean crop is very important in order to maximize yield and economic profitability in a particular environment [11]. Reference [36], [37] reported seed yield and seed protein content to be enhanced when N_2-fixation is associated with N fertilizer, particularly during pod filling [30].

N-fertilizer is very important under abiotic stresses [36] like drought stress [38]. The 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 [39].

The aim of this paper is to study the combined effects of drought stress and N (fixed through symbiotic N-fixation and achieved from mineral N-fertilizer) on the morphology, physiology and yield of soybean cv. **'***Boglár***'**.

II. Materials and Methods

Soybean cv. *Boglár* was sown in Debrecen University's experimental site (Látókép) (N. latitude 47° 33', E. longitude 21° 27') on April 26th, whereas the harvest was on September 1st, 2017. The soil type is calcareous chernozem, the average annual precipitation is 565.3 mm, whereas the precipitation between sowing and harvesting dates was 213.3 mm (Fig. 1).

Half of the seeds were inoculated with *Bradyrhizobium japonicum*, and the other half was not inoculated. Three N fertilizer rates; 0, 35 and 105 kg ha^{-1} of ammonium nitrate (NH4NO3) (0 N, 35 N and 105 N, respectively) were applied under three irrigation regimes; severe drought (SD), moderate drought (MD) and no drought (ND).

LAI values were recorded using SS1 – SunScan canopy analysis system (Delta- T Devices, UK) at three growing stages [40]; fourth node V4 (LAI 1), full bloom R2 (LAI 2) and full pod R4 (LAI 3). The chlorophyll content was measured using SPAD-502Plus (Konica Minolta, Japan) at V4, R2 and R4 growing stages. Plant height was measured manually using a ruler at R2 stage. In every measurement, 10 plants were randomly chosen from each plot, and the average was calculated.

The statistical analysis (2-way ANOVA) was made using SPSS (ver.22) software.

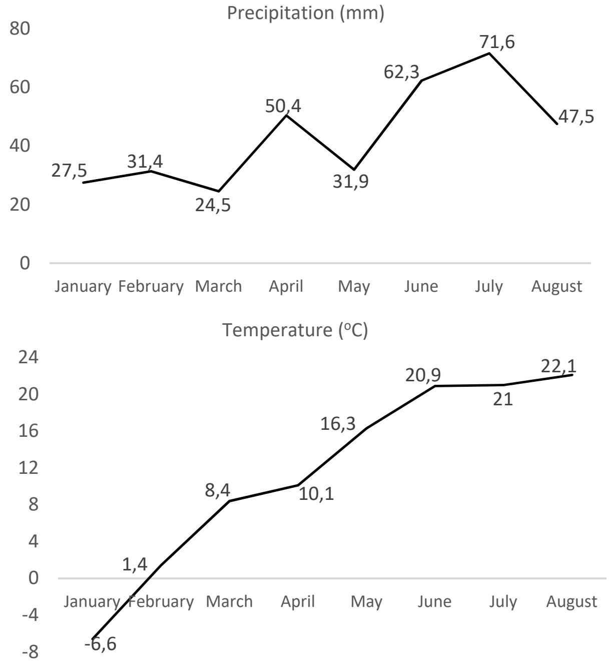

Fig. 1 The precipitation (mm) and the temperature (°C) from the beginning of the year of experiment till the harvest date

III. Results and Discussion

A. Chlorophyll Content

Both irrigation and inoculation did not affect the general trend of N-fertilizer's influence on chlorophyll content; increasing N rate was accompanied with increased chlorophyll content. However, under severe drought conditions, adding N-fertilizer (whether at high or low rates) resulted in better chlorophyll content of the non-inoculated plots compared to their inoculated counterparts at V4 stage, whereas, in the latter stages, it was the contrary. High N rate resulted in significantly higher chlorophyll content, compared to non-fertilized counterpart, at R4 for the inoculated plots, and at both V4 and R4 for the non-inoculated plots (Table I). Reference [4] reported chlorophyll content to be better when N-fertilizer (at a rate of 200 kg ha^{-1}) (20.04 and 20.04 µg cm^{-2} in 2013 and 2014, respectively) was applied to non-inoculated soybean plants compared to the non-fertilized counterparts (19.68 and 19.46 µg cm^{-2} in 2013 and 2014, respectively) in their two-year experiment; however, the difference was insignificant in both years. Under moderate drought conditions, the non-inoculated plots were always better compared to their inoculated counterparts, except for 35 N treatment at R4 stage. Again, 105 N treatment resulted in significantly higher chlorophyll content than did (0 N) treatment in both inoculated and non-inoculated plots at R4 stage. Similar results were obtained when the drought was waived (Table I).

At the vegetative stage (V4), adding N fertilizer, regardless of rate, enhanced the chlorophyll content of the inoculated plots under fully irrigated compared to severe drought, whereas it enhanced chlorophyll content of the non-inoculated plots under drought compared to non-drought conditions (Table I).

At early reproductive stage (R2) and under no-drought conditions, inoculated plots resulted in better chlorophyll content in 0 N and 35 N treatments compared to non-inoculated counterparts, whereas the high rate of N (105 N) resulted in less chlorophyll content compared to non-inoculated counterpart. On the other hand, the non-inoculated plots resulted in less chlorophyll content under severe drought stress than under moderate or no-drought stress, regardless of N application and rate (Table I).

At R4 stage, adding N enhanced chlorophyll content, regardless of inoculation and irrigation regime; moreover, the high rate of N fertilizer (105) enhanced chlorophyll content more than did the low rate (35) (Table I).

B. Leaf Area Index (LAI)

Similarly to chlorophyll content, the irrigation regime did not play a role in LAI trait; under severe drought conditions, LAI tended to increase with increased N rate, except for a non-significant decrease for inoculated 35 N compared to 0 N treatment at V4 stage (Table I). Reference [36] reported LAI values to be increased with increasing N rates. The high N rate resulted in significantly higher LAI compared to non-fertilized counterpart at R2 stage in the inoculated plots, and at both V4 and R2 stages in the non-inoculated plots (Table I). Previously, [41], [42] reported that adding N fertilizer before reproductive stages enhances growth and LAI. Under moderate drought conditions, similar results were obtained except for a non-significant decrease in 105 N treatment compared to the other two treatments at R4 stage in the non-inoculated plot; however, LAI values of the high N fertilizer rate plots were significantly higher than those of non-fertilized plots at R2 stage, regardless of inoculation. When drought was waived off, LAI increased as N-fertilizer rate increased (Table I).

At V4 stage, both inoculated and non-inoculated plots resulted in better LAI under drought compared to non-droughted counterparts when N-fertilizer was not applied (0 N); moreover, severe drought resulted in better LAI than did moderate drought. When N-fertilizer was applied at low rate (35 N), the inoculated plots tended to slightly increase LAI with increasing available water, whereas the non-inoculated plots followed same trend of non-fertilized plots. When high rate of N fertilizer (105 N) was applied, the fully irrigated plots resulted in the best LAI values, regardless of inoculation (Table I). Reference [43] reported drought stress to cause a reduction in the leaf area, and thus reduced protein synthesis and led to yield reduction [39]. Drought stress reduces the active photosynthetic leaf area and the interception of radiation by the crop canopy, which decreases seed yield [18], [19].

At R2 stage under severe stress, LAI values of the inoculated plots were the lowest when no N-fertilizer was applied (0 N), whereas they were better when N-fertilizer was applied, regardless of application rate (Table I), indicating that N-fertilizer could alleviate the negative effects of severe drought at this stage of plant development.

At R4 stage, inoculated plots resulted in better LAI values as available water increased, regardless of N application and rate. Non-inoculated plots followed different trend; non-stress-droughted treatment always resulted in the highest LAI, whereas moderate stress always resulted in the lowest LAI values (Table I).

C. Plant Height (cm)

Under severe drought conditions, adding low rate of N-fertilizer (35 N) resulted in shorter plants compared to both (0 N) and (105 N) treatments, regardless of inoculation; no significant differences were recorded. However, under moderate drought, the low rate of N-fertilizer was accompanied with better plant height compared to non-fertilized counterparts, regardless of inoculation. Interestingly, high rate of N-fertilizer (105 N) resulted in the longest plants in the inoculated plots, and the shortest plants in the non-inoculated plots. When the drought was waived off, the plant height tended to increase as N-fertilizer rate increased, regardless of inoculation (Table I).

Regardless of inoculation and N-fertilizer application and rate, plant height was the lowest under severe drought (Table I), which is consistent with many previous papers [3], [14], [16], [17]. This reduction might be caused as cell swelling, cell wall and synthesis enzymes are reduced, consequently, growth and plant height are decreased [44], [45].

For inoculated plots, when drought was waived off, plant height was better when N-fertilizer was not applied (0 N); however, the addition of N-fertilizer, regardless of rate, resulted in longer plants under moderate drought. For non-inoculated plots, under moderate drought, plant height was better when 0 N and 35 N were applied, however, the addition of high rate of N-fertilizer (105 N), though it enhanced plant height compared to severe drought, resulted in shorter plants compared to the non-drought-stressed (ND) counterparts (Table I).

D. Yield (kg ha^{-1})

Significant differences in the seed yield were associated with overall inoculation, irrigation and their interaction, whereas there were no significant differences in the seed yield associated with fertilization alone or in interaction with inoculation or irrigation or both.

When drought was waived off and considering inoculation as the only source of N (0 N), yield of inoculated treatment was increased to 5062.8 kg ha^{-1} relative to 4901.8 kg ha^{-1} of non-inoculated counterpart (Table I). Reference [46] reported a yield increase (by 19, 19 and 25%) of inoculated soybean plots in three different sites compared to their non-inoculated counterparts. Reference [47] reported 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^{-1}, respectively), compared to 3715 kg ha^{-1} 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 were fertilized by 200 kg N ha^{-1}, 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 N-rate provided (Table I). Similar findings were reported [33], who reported the soybean cultivar *Pb1* to yield (1458 kg ha^{-1}) without inoculation and without N-fertilization, whereas the sole N-fertilization leads to a yield of (2693 kg ha^{-1}), and the sole inoculation resulted in a yield of (2882 kg ha^{-1}). Reference [48] tested three early maturing soybean varieties in combination with four different *Bradyrhizobium* inoculants and compared to 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.

When drought was waived off, a slight addition (35 N) of N-fertilizer increased the yield of the inoculated plants to 5379 kg ha^{-1} compared to 5063 kg ha^{-1} without any addition (0 N) of N-fertilizer; however, the high rate of N-fertilizer (105 kg ha^{-1}) decreased the yield (to 4697 kg ha^{-1}), which is even less than the control's yield. On the other hand, the yield of non-inoculated plants increased as N-rate increased (Table I). Reference [49] reported that N-fertilizer did not lead to enhanced yield quantity of two different soybean cultivar groups (determinate and indeterminate) which were inoculated. References [11], [37] reported seed yield to be enhanced when N_2 fixation is associated with N-fertilizer. Reference [50] concluded that yield was linearly correlated with N-fertilizer amounts up to (90 kg ha^{-1}).

Under drought (whether severe or moderate), adding N-fertilizer increased the yield of the non-inoculated plants compared to the inoculated counterparts, regardless of N amount. Moreover, adding relatively high amount of N to the non-inoculated plants resulted in the best yield (Table I), 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 [38]. Adding 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^{-1} relative to 2728 kg ha^{-1}) [39].

TABLE I
CHLOROPHYLL CONTENT, LAI, PLANT HEIGHT (CM) AND YIELD (KG HA^{-1}) OF INOCULATED AND NON-INOCULATED SOYBEAN CV. '*BOGLÁR*' UNDER DIFFERENT N-FERTILIZER RATES AND DIFFERENT IRRIGATION REGIMES

Trait	N-fertilizer rate (kg ha^{-1})	Inoculated			Non-inoculated		
		SD	MD	ND	SD	MD	ND
SPAD1	0 N	37.5 [a]	37.0 [a]	37.1 [a]	36.6 [b]	38.2 [a]	37.5 [a]
	35 N	38.0 [a]	36.9 [a]	38.7 [a]	38.8 [ab]	40.0 [a]	38.1 [a]
	105 N	38.1 [a]	42.2 [a]	40.9 [a]	43.5 [a]	42.3 [a]	40.7 [a]
SPAD2	0 N	35.0 [a]	34.4 [a]	37.9 [a]	35.8 [a]	37.0 [a]	37.2 [a]
	35 N	35.8 [a]	38.3 [a]	39.2 [a]	37.7 [a]	38.8 [a]	38.0 [a]
	105 N	38.9 [a]	36.8 [a]	38.0 [a]	38.5 [a]	40.3 [a]	39.0 [a]
SPAD3	0 N	40.2 [b]	36.8 [b]	36.5 [a]	39.0 [b]	37.4 [b]	35.0 [b]
	35 N	41.5 [ab]	42.1 [ab]	39.2 [a]	40.6 [ab]	40.8 [ab]	38.9 [ab]
	105 N	44.5 [a]	43.5 [a]	40.1 [a]	44.4 [a]	44.8 [a]	42.6 [a]
LAI1	0 N	2.8 [a]	2.0 [b]	1.8 [b]	2.4 [b]	2.0 [a]	1.9 [b]
	35 N	2.1 [a]	2.2 [ab]	2.3 [ab]	2.5 [b]	2.1 [a]	2.1 [b]
	105 N	3.1 [a]	2.8 [a]	3.3 [a]	3.2 [a]	2.7 [a]	3.3 [a]
LAI2	0 N	4.5 [b]	5.0 [b]	5.1 [b]	5.6 [b]	5.2 [b]	5.4 [a]
	35 N	6.2 [ab]	6.0 [ab]	5.9 [ab]	6.5 [ab]	6.9 [a]	6.9 [a]
	105 N	7.9 [a]	7.0 [a]	7.3 [a]	8.2 [a]	8.1 [a]	7.1 [a]
LAI3	0 N	7.6 [a]	8.9 [a]	9.5 [a]	9.1 [a]	8.2 [a]	9.3 [a]
	35 N	8.1 [a]	9.0 [a]	9.6 [a]	9.2 [a]	8.4 [a]	9.9 [a]
	105 N	9.0 [a]	9.4 [a]	10.6 [a]	9.3 [a]	7.9 [a]	10.0 [a]
Height	0 N	67.0 [a]	67.5 [a]	68.3 [a]	69.5 [a]	72.0 [a]	69.5 [a]
	35 N	66.0 [a]	71.0 [a]	70.0 [a]	66.0 [a]	72.8 [a]	71.8 [a]
	105 N	68.8 [a]	72.8 [a]	72.5 [a]	68.0 [a]	71.5 [a]	73.3 [a]
Yield	0 N	3854 [a]	4576 [a]	5063 [a]	4371 [a]	4713 [a]	4902 [a]
	35 N	3659 [a]	4717 [a]	5379 [a]	4351 [a]	4794 [a]	5030 [a]
	105 N	3753 [a]	4957 [a]	4697 [a]	4567 [a]	5067 [a]	5048 [a]

Same letter in the same column within certain trait indicates no significant difference at .05 level.

Regardless of inoculation, the mean yield of the three fertilization rates was better when the water amount increased

(Table I). Many papers reported soybean seed yield to be decreased under drought stress conditions [2], [21]-[23]. More specifically, severe drought stress reduced the seed yield of soybean more than moderate drought stress [51], 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 I).

When not inoculated, the yield was the highest when relatively high rate (105 kg ha^{-1}) of N was added, regardless of the irrigation regime (Table I); this emphasizes the importance of N-fertilizer to provide N amounts needed by soybean with the absence of N_2 fixation process. Reference [30] previously reported biologically-fixed N_2 and mineral N to be the two main sources of N needed in soybean, and so if there is some deficiency in fixed-N_2 amounts, it is necessary to be provided from other sources [28], [31], or else leaves' nitrogen will be remobilized to the seeds, resulting in decreased photosynthesis and yield [30].

IV. Conclusions

Based on our results, it could be concluded that N fertilization has more influence on the chlorophyll content than does the inoculation or the irrigation regime. At V4 stage, the addition of N-fertilizer is recommended to the inoculated plants if there is no drought, whereas it is recommended to the non-inoculated plants under drought conditions. At R2 stage, it is not recommended to apply high N rate to the inoculated plants as it reduced the chlorophyll content; however, N fertilization could alleviate the negative effects of drought stress.

Adding high rate of N fertilizer is not always recommended, especially if there is no drought stress and the seeds are inoculated before being sown; however, adding relatively small rate of N-fertilizer, along with inoculation, can increase the yield, regardless of water availability.

Our results suggest, under drought, to not inoculate soybean seeds, but to add relatively high rate of N-fertilizer instead in order to achieve high yields. More intensive research should be conducted to investigate the exact rate of N-fertilizer under drought which leads to the best yield. Moreover, it would be of much importance to investigate the growth stage of soybean in which the N-fixation process is mostly affected by drought stress, in order to supply the plants with N-fertilizer to overcome N deficiency.

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