The effects of N fertilization on soybean (*Glycine max* L. Merrill) yield and quality under different drought stress levels

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Abstract: As a result to continuous exploitation in agriculture, soil nutrients decrease, and one way of re-fertilizing is by mineral fertilization. However, applying mineral fertilizers should be controlled and pre-evaluated in terms of quantity to be added, as the excessive amounts could negatively affect both plants and soil. Fertilization is very important under abiotic stress conditions, like drought stress which has negative effects on both quantity (yield) and quality (seed content) of crops, especially drought-sensitive crops such as soybean; it is a very important legume with high content of both protein and oil.

In order to study the influence of both nitrogen fertilization and drought stress on the yield and the seed quality of two soybean cultivars, an experiment was conducted in Debrecen, Hungary in 2017. Three N fertilization rates; 0, 35 and 105 kg ha^{-1} were applied under three irrigation regimes; severe drought (SD), moderate drought (MD) and no drought (ND). The results showed drought stress to negatively affect the yield of both cultivars by different extents; it also manipulated both protein and oil concentrations. (N) fertilization could enhance the yield of (MD) and (ND), but not (SD) treatment when applied in a relatively-low rate, whereas it negatively affected the yield when high rate was applied to (ND) treatment. The protein concentration increased as the (N) fertilization rate increased, whereas the oil concentration was not affected by (N) fertilization, but rather by drought.

It was concluded that the high-rate application of nitrogen is not always recommended for soybean, especially when water is available for plants. (N) fertilization has a noticeable effect on the protein but not on the oil concentration. Further studies on the best N rate when drought stress is applied at certain growth-stage will help to better understand the combined effects of both traits on soybean yield and quality.

Keywords: Soybean, drought stress, (N) fertilization, seed quality.

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Introduction

Soybean (*Glycine max* (L.) Merrill) has the greatest global area-harvested among seed legumes; it is the main source of relatively-cheap protein and vegetable oil (Maleki et al., 2013; Mutava et al., 2015; Wang et al., 2006). 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).

Soybean yield is greatly affected by several abiotic stresses, with drought stress being one of the major ones (Fan et al., 2013); drought intensively increased over the past decades affecting the world's food security (Vurukonda et al., 2016), which makes it very important to improve the knowledge of plant response to abiotic stresses (Morison et al., 2008). Drought negatively affects quantity (yield) and quality (seed content) of soybean (Vurukonda et al., 2016) as soybean is highly-sensitive to drought stress compared to other crops (Maleki et al., 2013) especially during certain periods of plant lifecycle (Liu et al., 2004). Many studies reported soybean seed yield, when exposed to drought stress, to be reduced (Kokubun, 2011; Li et al., 2013; Rose, 1988; Sadeghipour & Abbasi, 2012); yield reduction was found to be genotype-dependent (Bellaloui & Mengistu, 2008; He et al., 2017).

Protein and oil concentrations in soybean seeds are the most important parameters determining nutritional value (Chung et al., 2003). Under drought stress conditions, there is no effect on protein concentration (Sionit & Kramer, 1977), or less protein concentration (Boydak et al., 2002; Carrera et al., 2009; Rose, 1988; Specht et al., 2001) depending on the timing (stage) and the severity of applied drought stress (Carrera et al., 2009).

In general, protein concentration in soybean seeds is negatively correlated with oil concentration (Chung et al., 2003). Few papers reported oil concentration to be increased under drought stress (e.g. Boydak et al., 2002; Specht et al., 2001).

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Nitrogen (N) 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 & Planchon, 2000; Fageria & Baligar, 2005). Biologically-fixed N_2 and mineral (N) are the two main sources of (N) needed by soybean plants (Salvagiotti et al., 2008). 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 (Fabre & Planchon, 2000; Miransari, 2016; Yinbo et al., 1997), or else (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). 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 (Ferguson et al., 2010; Seneviratne et al., 2000). 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 (Caliskan et al., 2008).

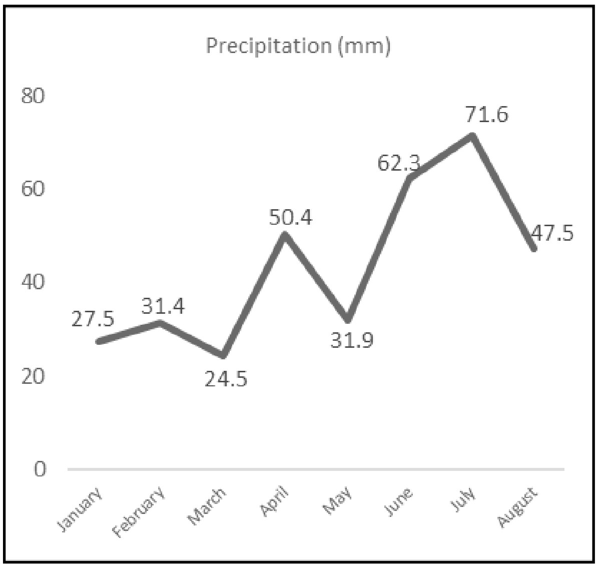
(N) fertilization is particularly very important under abiotic stress conditions (Caliskan et al., 2008) like drought stress (Obaton et al., 1982); adding (N) fertilizer to soybean increases drought tolerance as it enhances the accumulation of both shoot nitrogen and shoot biomass under drought stress (Purcell & King, 1996).

Our experiment aimed at revealing the effects of different (N) fertilization rates on both yield and seed quality of two soybean cultivars under drought stress conditions.

Materials and Methods

Two soybean cultivars; *'Boglár'* (00 maturity group) and *'Pannonia kincse'* (I maturity group) (Bonefarm, Hungary) were sown in Debrecen University's experimental site (Látókép) (N. latitude 47° 33', E. longitude 21° 27') on April 26th and harvested 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.

Three (N) fertilizer rates; 0, 35 and 105 kg ha^{-1} of ammonium nitrate (NH_4NO_3) (0 N, 35 N and 105 N, respectively) were applied under three irrigation regimes; severe drought (SD) (where the precipitation amount of 213.3 mm was the only source of irrigation water), moderate drought (MD) (where an additional 50 mm of irrigation water was supplied) and no drought (ND) (where an additional 100 mm of irrigation water was supplied). The experimental design was split-split-plot design, with the cultivars being the main plots, the irrigation treatments being the sub-plots and the fertilization treatments being the sub-sub plots. The final plot number was 18 (2 cultivars



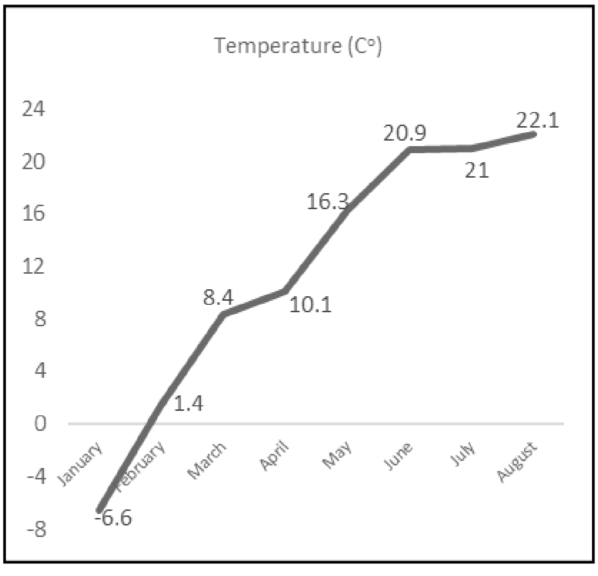


Figure 1: The precipitation (mm) and the temperature (C°) from the beginning of the year of experiment till the harvest date.

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* 3 fertilization rates * 3 irrigation regimes) * 4 replications = 72 plots. The dimensions of each plot were 9.2 * 5.4 = 49.68 m² with 12 rows in each plot. Both the protein and oil concentrations were measured using NIR analyser Granolyser (Pfeuffer, Germany).

The analysis of variance (ANOVA) was conducted to compare the means of each treatment, and then tukey post-hoc test was conducted to indicate the statistically-different means using SPSS (ver.25) software.

Results and Discussion

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

For cultivar *'Boglár'*, the fertilization rate did not play a noticeable role in the yield under severe drought stress conditions, moreover, applying (N) fertilizer insignificantly reduced the yield (to 3659 and 3753 kg ha^{-1} for 35 N and 105 N treatments, respectively) compared to the non-fertilized control (3854 kg ha^{-1}) (table 1). Previously, Kaschuk et al. (2016) concluded that (N) fertilizer did not lead to more yield of two different soybean cultivar groups (determinate and indeterminate) whether (N) application was done at sowing time, during reproductive stages or both; same conclusion was previously reported (Hungria et al., 2006; Mendes et al., 2008). However, the fertilization did play a role in the resulted yield under moderate drought stress conditions; the yield increased as the fertilization rate increased (4576, 4717 and 4957 kg ha^{-1} for 0 N, 35 N and 105 N, respectively) (table 1). Some researchers concluded that (N) fertilizer addition increases yield (Ham et al., 1975; Gault et al., 1984; Kuwahara et al., 1986; Nakano et al., 1987; Norhayati et al., 1988; Takahashi et al., 1991; Watanabe et al., 1986) by reducing abortions of flowers and pods (Brevedan et al., 1978). When drought was waived off, the low rate of (N) fertilizer (35 N) enhanced yield (to 5379 kg ha^{-1}), whereas, interestingly, the high rate (105 N) decreased it (to 4697 kg ha^{-1}) to a level even less than the control (0 N) (5063 kg ha^{-1}) (table 1), which implies that when plants does not suffer from stress, high rates of (N) negatively affect the yield. Fabre & Planchon (2000) reported a significant correlation between yield and (N) fertilizer during flowering stage. MacKenzie and Kirby (1979) concluded that yield was linearly correlated with (N) fertilizer amounts up to 90 kg ha^{-1}, whereas Salvagiotti et al. (2008) concluded that less than 50 kg ha^{-1} of (N) fertilizer has lead to the largest agronomic efficiency.

The reasons for alteration in the response to (N) are not accurately specified; however, initial soil fertility, nodulation capacity, inoculant presence in soil and pre-sowing inoculation and the timing of (N) application all have a role (Gault et al., 1984; Peoples et al., 1995).

Regardless of fertilization application and rate, (SD) significantly resulted in the least yield compared to the other two irrigation regimes (table 1). It was reported that soybean seed yield decreases under drought stress conditions (Ashley & Ethridge, 1978; Bajaj et al., 2008; Dogan et al., 2007; Doss et al., 1974; Gercek et al., 2009; Heatherly & Elmore, 1986; Karam et al., 2005; Kokubun, 2011; Li et al., 2013; Rose, 1988; Sadeghipour & Abbasi, 2012; Sincik et al., 2008). The yield increased in (MD) compared to (SD), regardless of (N) fertilizer rate; this result is consistent with Dornbos & Mullen (1992) conclusion that severe drought stress reduced the seed yield of soybean more than did moderate drought stress. Moreover, the yield further increased when the drought was waived off for both (0 N) and (35 N) treatments, but decreased for (105 N) (table 1), which emphasizes the harmful effect of high (N) fertilizer rate on the expected yield.

The effect of irrigation (calculated as Eta Square) on the yield was noticeable (60.5%), which means that over 60% of the yield differences were resulted by the different irrigation regimes.

For cultivar *'Pannonia kincse'*, applying high rate of (N) fertilizer under severe drought stress resulted in a better yield (4276 kg ha^{-1}) compared to the low rate application (3960 kg ha^{-1}); however, the difference was not significant (table 1). This result gives an impression that (N) fertilizer could alleviate the negative effect of severe drought for this cultivar. Previous papers reported (N) fertilizer to be very important under abiotic stresses (Caliskan et al., 2008; Salvagiotti et al., 2008) such as drought stress (Lyons & Earley, 1952; Obaton et al., 1982). It was reported by Purcell & King (1996) that (N) fertilizer significantly increased the yield (to 2798 kg

Table 1: Yield (kg ha^{-1}), protein concentration (%) and oil concentration (%) of soybean cultivars *'Boglár'* and *'Pannonia kincse'* under different N-fertilizer rates {0 kg ha^{-1} (0 N), 35 kg ha^{-1} (35 N) and 105 kg ha^{-1} (105 N)} and different irrigation regimes {severe drought (SD), moderate drought (MD) and no drought (ND)}.

	Boglár			*Pannonia Kincse*		
	SD	MD	ND	SD	MD	ND
Yield						
0 N	3854^{a2}	4576^{a12}	5063^{a1}	4335^{a1}	4220^{a1}	4746^{a1}
35 N	3659^{a2}	4717^{a1}	5379^{a1}	3960^{a1}	4325^{a1}	4526^{a1}
105 N	3753^{a2}	4957^{a1}	4697^{a12}	4276^{a1}	4185^{a1}	4470^{a1}
Protein Concentration						
0 N	35.2^{a1}	34.9^{b1}	36.1^{a1}	36.1^{b1}	36.1^{a1}	37.8^{a1}
35 N	35.1^{a1}	35.8^{ab1}	36.5^{a1}	36.9^{b1}	37.8^{a1}	38.1^{a1}
105 N	36.7^{a1}	36.9^{a1}	37.0^{a1}	39.6^{a1}	39.2^{a1}	39.2^{a1}
Oil Concentration						
No N	23.5^{a1}	22.8^{a1}	22.7^{a2}	22.7^{a1}	22.3^{a12}	21.4^{a2}
35 N	23.4^{a1}	22.6^{a1}	22.7^{a1}	22.8^{a1}	21.8^{a12}	21.3^{a2}
105 N	23.0^{a1}	22.6^{a1}	22.3^{a1}	22.4^{a1}	22.1^{a1}	22.2^{a1}

Same number indicates no significant differences at .05 level between irrigation regimes of certain cultivar and within certain N-Fertilizer rate.

Same letter indicates no significant differences at .05 level between N-Fertilizer rates of certain cultivar and within certain irrigation regime.

ha^{-1}) compared to (2373 kg ha^{-1}) without (N) fertilizer; they related this increase to increased seed number because of decreased flower and pod abortion. Moreover, they concluded that 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. However, under well-watered conditions, (N) decreased yield (to 2597 kg ha^{-1}) relative to (2728 kg ha^{-1}) (Purcell and King, 1996). Chen et al. (1992) reported that under severe drought stress, every (1 kg ha^{-1}) of (N) fertilizer resulted in extra (1.2 kg ha^{-1}) seeds.

When stress was relatively moderate, the low rate of (N) Fertilizer resulted in a higher yield (4325 kg ha^{-1}) than did the high rate (4185 kg ha^{-1}) (table 1) which, similarly to *'Boglár'*, was the lowest; this result was also similar when the drought stress was waived off, which, once more, reflects the negative effect of high (N) fertilizer rate on the yield.

Unlike *'Boglár'*, the irrigation did not noticeably affect the yield of this cultivar (11.8%); Garcia et al. (2010) reported that genotypes significantly differ in yield production under drought stress conditions and also within the interaction between drought stress and genotype; similar conclusions were reported (Bellaloui & Mengistu, 2008; Brown et al., 1985; He et al., 2017; Maleki et al., 2013). Also, the fertilization's effect on the yield of this cultivar was very low (2.2%).

2. Protein Concentration (%)

For cultivar *'Boglár'* under severe drought (SD), both (0 N) and (35 N) treatments resulted in very similar protein concentrations (35.2 and 35.1%, respectively), however, reducing the severity of drought (to MD) enhanced the protein concentration for (35 N) treatment (to 35.8%), whereas decreased it for (0 N) treatment (to 34.9%). Moreover, eliminating drought stress (ND) resulted in the best protein concentration for both fertilization treatments (36.1 and 36.5% for 0 N and 35 N, respectively) (table 1). On the other hand, the high rate (105 N) resulted in the best protein concentration compared to the other (N) rates, regardless of water availability, reflecting the importance of (N) in protein

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synthesis. It was previously concluded that protein content increased when (N) was increased (Ham et al., 1975); (N) fertilizer dose had a significant effect on seed protein content, as the dose of (100 kg ha^{-1}) increased seed protein just by (2%), whereas the dose of (200 kg ha^{-1}) resulted in (14%) increase in seed protein (Miransari, 2016).

In (0 N) treatment, protein concentration increased under (SD) compared to (MD), which is consistent with many papers that reported increased protein content under drought stress (Bellaloui & Mengistu, 2008; Dornbos & Mullen, 1992; Kumar et al., 2006; Rotundo & Westgate, 2009; Wang & Frei, 2011); this might be explained as a result to a reduction in seed number associated with an increase in seed size (Borras et al., 2004), or caused by remobilizing nitrogen from leaves to seeds rapidly as a result of drought stress (Brevedan & Egli, 2003; DeSouza et al., 1997) which leads to increased protein concentration.

In our experiment, protein concentration increased under (ND) treatment compared to both (SD) and (MD) treatments; few studies showed no effect (Sionit & Kramer, 1977) or lower protein concentration (Boydak et al., 2002; Carrera et al., 2009; Rose, 1988; Specht et al., 2001; Turner et al., 2005) under drought stress conditions; the relationship between drought stress and soybean seed composition remains controversial (Medic et al., 2014), and differences among the reported conclusions were suggested to be due to timing and intensity of drought stress during the different stages (Carrera et al., 2009).

The effect of (N) fertilization on protein concentration was noticeable (32.1%), whereas the irrigation effect was not (12.5%). For *'Pannonia kincse'*, regardless of irrigation regime, protein concentration increased as the (N) fertilizer rate increased (table 1). Rotundo & Westgate (2009) reported, in their meta-analysis study, that adding (N) fertilizer increased protein content about (27%) in all study environments; particularly, the increase was about (8%) in field studies. Increasing water availability resulted in increased protein concentration for both (0 N) and (35 N) treatments, whereas it slightly decreased it for (105 N) treatment (table 1); this tendency was different compared to *'Boglár'*; Bellaloui & Mengistu (2008) suggested that the plant's response to drought stress, in terms of seed composition, might be cultivar-dependent.

Though the irrigation did not relatively affect protein concentration (3.6%), yet the fertilization noticeably did (31.8%).

3. Oil Concentration (%)

For *'Boglár'*, except for a slight increase in (35 N) treatment under (ND) (22.7%) compared to (MD) (22.6%), oil concentration decreased as the drought stress decreased, regardless of (N) fertilizer application and rate (table 1). Few reports showed increased oil content with water deficiency conditions (e.g. Boydak et al., 2002), whereas others indicated that water deficiency reduced oil content in the seed (Bellaloui & Mengistu, 2008; Rose, 1988; Rotundo and Westgate, 2009). The timing of drought stress was reported to have an important effect on oil content; the early-stage drought did not affect the oil content, whereas drought stress during seed filling stage resulted in a reduction of oil content by 35%. The effect of Irrigation on oil concentration was noticeable (31.6%).

Under drought stress (both SD and MD), applying (N) fertilizer decreased oil concentration; high (N) rate decreased oil concentration more than did low (N) rate, whereas when drought stress was waived off (ND), the application of low (N) rate (35 N) resulted in the same oil concentration (22.7%) as did the control (0 N); however, the high (N) rate decreased the oil concentration (to 22.3%) (table 1). The effect of fertilization was not noticeable on oil concentration (6.3%).

The correlation between oil and protein concentrations was slightly negative ($r = -0.16$). Chung et al. (2003) reported soybean seed protein content to negatively correlate with the amount of seed oil.

For *'Pannonia kincse'*, similarly to *'Boglár'*, decreasing drought decreased oil concentration, regardless of (N) application and rate. Under drought (whether severe or moderate), control (0 N) treatment resulted in better oil concentration compared to (105 N) treatment, whereas it was the opposite when drought was waived off (table 1). For this cultivar, the correlation between oil concentration and yield was negatively significantly-high ($r = -0.44^{**}$). Same to

'Boglár', the fertilization did not relatively affect the oil concentration (1.5%), whereas the irrigation effect was noticeable (34.0%).

Conclusions

Our work was a single-year experiment only, yet some preliminary conclusions could be interpreted; it was concluded that drought stress decreases soybean yield of both studied cultivars; it also affects protein and oil concentrations to some extent. Depending on the cultivar, (N) fertilization is not always recommended for soybean, especially high rate, as it has a negative influence on the yield; however, it is important under drought stress conditions as it could alleviate the negative effect on the yield. Also, it plays an important role in increasing protein concentration in soybean seeds, whereas it has a very little effect on the oil concentration.

More intensive research should be conducted to investigate the exact rate of (N) fertilizer under drought which leads to the best yield with maintaining relatively high protein concentration in the produced seeds. Moreover, it would be of much importance to investigate the growth stage of soybean in which nitrogen availability is mostly affected by drought stress (majorly because of N_2-fixation malfunction caused by drought), in order to apply (N) fertilizer to overcome N-deficiency negative effects.

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