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Effects of Nitrogen and Water Deficiency on Agronomic Properties, Root Characteristics and Expression of Related Genes in Soybean

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Abstract: Drought and insufficient nutrient supply are the main limiting factors for field crop production; therefore, the present study aimed to investigate the responses of four registered soybean varieties to limited nitrogen (N) supply in combination with drought stress. Plants were grown in tubes filled with silica sand, under open-air conditions. Water shortage was initiated at the three-leaf stage by providing 50% of water, and N deficiency was obtained by reducing the N content of the half-strength Hoagland solution by 50%. N deficiency did not influence the grain yield, while it significantly increased the root dry biomass in Boglár and Pannónia Kincse. Compared with nitrogen supply, the effects of the water shortage were dominant in root development. The expression levels of the investigated genes also showed genotypic variations. The expression of the Inducible nitrate reductase1 gene increased under N-deficient conditions in Boglár and decreased in Pannónia Kincse under drought conditions. The expression level of the aldehyde dehydrogenase gene and abscisic acid 8'-hydroxylase 3 increased under combined stress conditions. Summarising the agronomic and physiological characteristics, Boglár and Sinara were sensitive to drought, Bagera was sensitive to N deficiency but produced the highest yield under limited watering in each nitrogen treatment, while Pannónia Kincse was tolerant to nitrogen deficiency under well-watered conditions.

Keywords: root structure; legume crop; SPAD; chlorophyll content; gene expression; grain yield

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1. Introduction

Soybean (*Glycine max* [L] Merr.) is the most important oilseed crop in the world [1]; it is used mainly for human nutrition and animal feed. In 2019, soybean was harvested on more than 120 million hectares worldwide, and the average yield was 2.77 t/ha [2]. The contribution of the “EU-28” countries to the total soybean production of the world in 2019 was only 0.84% [2], but there is a relatively high demand for genetically non-modified (non-GM) soybean in the EU in comparison with other parts of the world [3]. Cultivation technology for non-GM soybean varieties is more complex than that of the herbicide-tolerant transgenic hybrids; therefore, the selection and breeding of stress-tolerant genotypes could be the driver of achieving effective and competitive soybean production in countries where GM crops are not allowed to be grown [4,5].

Ongoing climate change has brought severe challenges to agriculture; among these is the intensifying of drought events, which reduce yield stability [6]. Based on future climate projections, dependence on precipitation will continue to threaten soybean yield variability

among years and growing areas, even in Hungary [7]. Previous studies have reported a great yield loss when the water availability was limited in the vegetative stages of development [8,9]; therefore, soil water deficit is the most important limiting environmental variable contributing to yield reductions in soybean in the Carpathian Basin [10]. Soybean is relatively resistant to low and very high temperatures, but growth rates decrease above 35 °C and below 18 °C. The minimum temperature for growth is about 10 °C. Extreme heat stress, when the average daily temperature exceeds 26–27 °C, occurring at the generative developmental stage, could retard the plant growth and yield formation in soybean [11]. Besides drought, nitrogen (N) shortage is the other important yield-limiting environmental factor in soybean production [12]. However, nitrogen use efficiency is influenced by many factors, such as the form of nitrogen fertiliser, soil properties, agronomic practices and crop rotations, and there are also significant differences between the cultivated species and genotypes. Plants usually absorb less than 50% of the applied N fertiliser [13], and more than half of the applied nitrogen is not used by the crops, as it dissipates into the environment and contaminates water, or passes from the soil into the atmosphere [14].

The nitrogen utilisation of crops needs to be improved to reduce the costs of production and mitigate environmental damage. However, this necessity rarely emerges in connection with soybean since—as a legume—soybean can live in a symbiotic relationship with N-fixing bacteria (BNF). In soybean, N derived from the atmosphere via BNF can range from 0 to 98% of the total N uptake, representing 0 to 337 kg N ha⁻¹ [15], but the yield response of soybean to N fertiliser has been found to be inconsistent [16]. However, legume nodulation is suppressed by high levels of soil N [17]. This is a homeostatic mechanism that presumably allows the plant to balance out the high cost of N fixation with its N requirements [18]. At the molecular level, plants can adjust their capacity to acquire different forms of N in a range of concentrations by modulating the expression and function of genes in different N uptake systems. Modulation of plant growth and development, most notably changes in the root system architecture, can also greatly impact plant N acquisition in the soil [19]. Abiotic stresses, such as drought, may negatively affect plant N availability. Plants can adapt to a water-limited environment and low nitrogen supply by adjusting their root architecture [20], which can be an important ability for surviving limited rainfall periods or unfavourable nutrient availability [21–23]. The root system has primary importance in improving crop productivity and stress tolerance, as well as increasing the utilisation of resources, such as water and nutrients [23,24]. Although root traits are crucial in maintaining crop yields in soils characterised by insufficient water and nutrients [25,26], due to the complexity of root development and its interactions with external factors, selecting for genotypes with improved abiotic plasticity is difficult under field conditions. Selecting and breeding genotypes with improved water and nitrogen uptake capabilities may improve productivity and could also have environmental benefits. The main aim of this study was to examine the effects of limited water and nitrogen availability and their combinations on four commonly grown soybean genotypes. We were particularly interested in investigating the following problems: (1) How does N supply affect the main agronomic parameters of soybean plants? (2) How does water limitation affect the responses of the plants to different N levels? (3) How do various soybean varieties differ in their abiotic stress tolerance?

2. Materials and Methods

2.1. Plant Material and Growth Conditions

Four soybean varieties that are widely grown in the Carpathian Basin (Boglár, Pannónia Kincse, Bagera, Sinara) were tested in a model experiment at the Georgikon Faculty, University of Pannonia, between 21 April and 15 August 2017 (Table 1).

Table 1. Origin and main agronomic properties of the studied genotypes.

Genotype	Ripening	Special Property	Growth Type	Origin
Bagera	extra early	drought-tolerant	semi-determined	Switzerland
Boglár	early	high stem stability	semi-determined	Hungary
Pannónia Kincse	mid	high-yielding capacity	non-determined	Hungary
Sinara	extra early	high-yielding capacity	semi-determined	Austria

Plants were grown in sand-filled tube brewing vessels [27] in a randomised complete block design with 4 replicates under open-air conditions, covered with a glass roof to exclude rainwater. The vessels consisted of PVC tubes—75 cm in height and 10 cm in diameter—and polythene tubing bags sleeved into the PVC tubes filled with 9.5 kg dry silica sand #30 (particle size: 600 µm). Nutrients were supplied with a half-strength Hoagland solution [28]. Pre-germinated soybean seeds were planted on 21 April 2017. In the control treatment, the plants were irrigated during the vegetation with half-strength Hoagland solution according to the plants' needs (N100_C). When the plants reached the 3-node stage (BBCH 13) [29], nitrogen deficiency was obtained by reducing the N content of the nutrient solution by 50% (N50 treatment), and the drought was initiated by providing 50% of water to the formerly well-watered plants (DS treatment) (Table 2). The concentration of the other compounds in the solution was not modified.

Table 2. Summary of the applied treatments.

Treatment	Abbreviation
100% water and 100% nitrogen	N100_C
100% nitrogen and 50% water	N100_DS
50% nitrogen and 100% water	N50_C
50% nitrogen and 50% water	N50_DS

2.2. Data Collection

The chlorophyll content of the leaves was recorded by SPAD-502 (Konica-Minolta, Inc., Tokyo, Japan) twice a week, and plant height was recorded once a week in the vegetative phases. The measurements were taken on the youngest fully developed leaves and the mean of 10 readings was presented. A comprehensive plant analysis was carried out when the plants reached the final maturity stage (BBCH 99) [29]. The analyses involved measurement of plant height, pod number, grains per pod, grain weight and the total aboveground biomass. The harvest index was calculated as the ratio of grain yield to aboveground biomass. To retrieve intact root systems, each polythene bag was pulled out of the PVC tube, laid on a screen frame in a tube and cut lengthwise. The intact root system was brought to the water surface and washed carefully. Besides measurement of the total root dry biomass, the root system was divided into shallow (0–30 cm) and deep (30–75 cm) sections.

2.3. Chlorophyll-a Fluorescence Induction Measurements

Chlorophyll-a fluorescence induction was measured using a PAM 2100 portable fluorometer (Walz Ltd., Effeltrich, Germany). Measurements were carried out at BBCH 75 stage under natural light condition, between 11:00 a.m. and 2:00 p.m. The quantum yield of the Photosystem II fluorescence induction parameter (defined as $(F_m' - F_s)/F_m'$, where F_m' and F_s represent the maximum and steady-state fluorescence levels in the light-adapted state, respectively) was calculated according to Genty et al. [30].

2.4. Gene expression Studies

For gene expression analysis, the middle leaflets of the second trifoliolate leaves were collected at BBCH 78 stage from each biological replication. Total RNA was extracted from the samples using TRI Reagent (Zymo Research Ltd., Irvine, CA, USA) according to the

manufacturer's instructions, and the samples were treated with DNase I enzyme (Promega Ltd., Madison, WI, USA).

The purity and concentration of RNA were assessed by using Nanodrop 1000 (Thermo Scientific Ltd., Wilmington, DE, USA), and the samples were also run on agarose gel stained with ethidium bromide to check the integrity of total RNA. cDNA synthesis was made from 1 µg of total RNA using M-MLV Reverse Transcriptase (Promega Ltd., Madison, WI, USA). The expression levels of the target genes were determined by real-time RT-qPCR, using a CFX96 thermocycler (Bio-Rad Ltd., Hercules, CA, USA), with the primers detailed in Table 3 [31,32]. The relative gene expression values were determined via the $\Delta\Delta C_t$ method [33]. Threshold cycle (C_t) values were normalised using the C_t values of the housekeeping gene encoding Gm β -actin (GMU60500).

Table 3. Primers used for RT-qPCR studies.

Gene Name		Primer Sequences (5' → 3')	Reference
<i>Gmβ-actin</i>	Forward	GAGCTATGAATTGCCTGATGG	[31]
	Reverse	CGTTCATGAATTCAGTAGC	
<i>GmDREB1B</i>	Forward	GTAAAGATTGTTTCGTATGGGACAAG	[32]
	Reverse	ACACCTAAAATGAGCAACCGTACTA	
<i>GmCYP707A3b</i>	Forward	GGCTAACCTTCTGACTTTCC	[32]
	Reverse	CAAGTGTCTGTTCTGAGGT	
<i>GmTP55</i>	Forward	CGAAAAGGGAGAGGAGGACTTC	
	Reverse	TCTGGGTCACCGAAAGGCAA	
<i>GmINR1</i>	Forward	TCAATACGGCACCCACATAA	NCBI Reference Sequence: NM_001251161.1
	Reverse	GTCGTCGTTTTGGTCTTCGT	

2.5. Statistical Analysis

Multivariate analysis was performed to test for significant main effects of the genotype, nitrogen supply and watering levels and their interactions using R, version 3.4.1 [34]. Means were compared by Tukey's honest significance test with the support of the agricolae package [35]. Plots were generated by ggplot2 [36] and Microsoft Excel.

3. Results

3.1. Phenological and Yield Parameters

Based on the ANOVA, all factors (genotype, water supply and nitrogen fertilisation rate) and their interactions had significant effects on the phenological parameters (pod number, grain number, biomass and harvest index).

Water shortage significantly decreased the plant height in each variety, except for Bagera under normal nitrogen supply (−18.25% in average compared with the well-watered control); however, limited water supply significantly reduced the plant height (−24.25%) in each variety under limited nitrogen fertilisation (Table 4). Boglár and Sinara gave the most intensive responses to drought stress (−31.8% and 42.3% in N50_DS compared with N50_C, respectively). Generally, limited nitrogen availability induced plant growth in all the genotypes, which was found to be more intensive under well-watered conditions (32.4%) than under drought stress (7.1%) (Table 4).

All the stress treatments resulted in a decrease in pod number per plant, but the effects of water shortage were more intensive than those of limited nitrogen supply (Table 4). Water withdrawal decreased the number of pods by 50.6% in the N100_DS treatment compared with N100_C, and a similar trend was observed under limited nitrogen supply (−48.45% reduction in the number of pods in the N50_DS treatment compared with N50_C). The decrease was significant in each genotype except for Sinara. The nitrogen supply reduced the number of pods in each genotype (−35.1%, −37.2% and −52.4% in Boglár, Pannónia Kincse and Sinara, respectively) except for Bagera under well-watered conditions. However, as a result of the reduced nitrogen supply, a significantly lower number of

pods was found under limited water availability in Bagera (−53.1%) and Pannónia Kincse (−55.1%). The lowest values were recorded under the combined stress treatment (Table 4).

Table 4. Influence of the nitrogen and water treatments on plants' phenological parameters. Different lowercase letters within a column indicate significant differences based on Tukey's HSD test ($p < 0.05$).

Genotypes	Treatments	Plant Height (cm)	Pod Number per Plant	Grain Number per Pod	Aboveground Dry Biomass	Harvest Index
Bagera	N100_C	64.2 ^c	9.5 ^{de}	2.38 ^{de}	10.04 ^c	0.42 ^{bcd}
	N100_DS	82.4 ^b	8.0 ^e	2.07 ^{hi}	6.24 ^e	0.37 ^{cde}
	N50_C	99.0 ^a	8.75 ^{de}	2.61 ^{abc}	11.90 ^b	0.35 ^{def}
	N50_DS	85.6 ^b	3.75 ^{fg}	2.66 ^a	5.75 ^e	0.31 ^{efg}
Boglár	N100_C	26.5 ^{gih}	12.32 ^{bc}	2.26 ^{efg}	10.13 ^c	0.41 ^{bcd}
	N100_DS	23.2 ⁱ	4.25 ^{fg}	2.59 ^{abc}	3.34 ^g	0.27 ^g
	N50_C	37.0 ^{ef}	8.0 ^e	2.71 ^a	8.50 ^d	0.44 ^{abc}
	N50_DS	25.3 ^{hi}	2.25 ^g	2.08 ^{ghi}	3.51 ^g	0.37 ^{cde}
Pannónia Kincse	N100_C	46.3 ^d	21.5 ^a	2.37 ^{de}	13.48 ^a	0.28 ^{fg}
	N100_DS	33.2 ^{fg}	10.75 ^{cd}	1.93 ⁱ	6.63 ^e	0.29 ^{efg}
	N50_C	44.7 ^d	13.5 ^d	2.47 ^{bcd}	14.09 ^a	0.35 ^{def}
	N50_DS	40.5 ^{de}	4.75 ^{fg}	2.43 ^{cde}	4.60 ^f	0.31 ^{efg}
Sinara	N100_C	31.5 ^{fgh}	10.5 ^{cde}	2.28 ^{ef}	7.77 ^d	0.45 ^{ab}
	N100_DS	27.3 ^{ghi}	4.75 ^{fg}	2.09 ^{ghi}	3.73 ^{fg}	0.34 ^{defg}
	N50_C	44.6 ^d	5.0 ^f	2.65 ^{ab}	6.51 ^e	0.51 ^a
	N50_DS	25.4 ^{hi}	3.75 ^{fg}	2.13 ^{fgh}	2.69 ^g	0.46 ^{ab}

The reactions of the genotypes to water shortage varied under optimum nitrogen fertilisation: significantly decreasing trends were detected for Bagera, Pannónia Kincse and Sinara. The number of grains per pod was 14.7% higher in Boglár under the N100_DS treatment than under N100_C. Under reduced nitrogen supply, the additional drought stress induced a significant decrease in grains per pod in Boglár and Sinara (−23.2% and −19.6%, respectively). The nitrogen shortage level significantly increased the grains per pod under dry conditions in Bagera (23.8%) and Pannónia Kincse (25.6%), while the opposite tendency was observed for Boglár (19.7%).

The water shortage consequently decreased the aboveground biomass significantly under either 100% or 50% nitrogen supply in each genotype. The negative impact was the most intense when the water shortage was combined with a reduced nitrogen supply. However, the genotypes showed different reactions to the reduced nitrogen supply because a significant decrease was observed for Boglár and Sinara (−16.2% and −16.2%, respectively), and biomass production was improved in Bagera (18.5%) under well-watered conditions. When the nitrogen supply was reduced under drought conditions, a significant reduction in biomass could be observed only for Pannónia Kincse. Under 100% nitrogen fertilisation, the water shortage had significant effects only on the harvest indices of Boglár (−33.5%) and Sinara (−24.7%). The opposite tendencies were observed for Boglár and Sinara under drought conditions, where nitrogen deficiency induced a 35.0% and 34.1% increase in the harvest index, respectively. Significant differences were observed among the genotypes and between the two watering levels, and the interaction of these factors was also significant. The nitrogen application rate did not influence the harvested yield. Under 100% N fertilisation, the yield capacity of Bagera and Boglár was significantly higher than that of Sinara under optimum watering, but water shortage significantly reduced the harvested yield in each genotype.

Under limited nitrogen supply (Figure 1b), the yield of all genotypes varied significantly (PK > Bagera > Boglár > Sinara) under normal watering, while the water shortage induced a significant reduction in this parameter, which was more severe than that under

100% nitrogen fertilisation (Figure 1a). Bagera produced the highest yield under limited watering in each nitrogen treatment.

Figure 1. Grain yield of the soybean varieties under 100% (a) and 50% (b) nitrogen supply. C: optimum water supply; DS: drought stress treatment. Different lowercase letters indicate significant differences based on Tukey's HSD test ($p < 0.05$).

3.2. Root Parameters

Based on the multivariate ANOVA, the effects of all three factors and their interactions on root biomass were statistically significant. Under control conditions (N100_C), the root mass of Sinara was significantly lower than that of Bagera and Pannónia Kincse. The water shortage under 100% nitrogen availability induced a significant decrease in root mass, except in Pannónia Kincse. Under optimum watering, reduced nitrogen fertilisation significantly increased root development in Boglár and Pannónia Kincse by 37.0% and 42.4%, respectively, while no changes were observed for Bagera and Sinara (Figure 2).

Figure 2. Total root biomass of the soybean varieties under optimum and limited water and nitrogen supply. Abbreviations are explained in Table 2. Different lowercase letters indicate significant differences based on Tukey's HSD test ($p < 0.05$).

Water shortage combined with 50% nitrogen supply (N50_DS) significantly reduced the root mass of the examined genotypes compared with the well-watered plants (N50_C). However, under the combined stress, the root weight of Pannónia Kincse was significantly higher than that of the other genotypes. Regarding the root mass, no significant differences were observed between the N100_DS and N50_DS treatments, which indicates that the main stress factor in the experiment was watering (Figure 2).

Under control conditions (N100_C), the root mass of Bagera and Pannónia Kincse was significantly greater in the upper 30 cm than that of Boglár and Sinara. The water shortage did not influence the root mass of Boglár, Pannónia Kincse and Sinara, but a significant decrease was observed for Bagera in the N100_DS treatment compared with N100_C (Figure 3). The limited nitrogen supply resulted in a significant increase of 86.0% in the root mass of Boglár in the upper 30 cm under optimum watering. Compared with the N50_C treatment, the combined stress (N50_DS) significantly reduced the root mass in each genotype, but the difference between N50_DS and N100_C was not statistically significant in Boglár. This may indicate that the nitrogen limitation could enhance root development in this genotype, which could be achieved otherwise only under well-watered conditions (Figure 3).

Figure 3. Root biomass of the soybean varieties under optimum and limited water and nitrogen supply in the upper 30 cm. Abbreviations are explained in Table 2. Different lowercase letters indicate significant differences based on Tukey's HSD test ($p < 0.05$).

Under optimum watering and 100% N supply, the root mass between 30 and 75 cm significantly differed between Boglár and Sinara (Figure 4). In this layer, water withdrawal (N100_DS) significantly reduced the root mass only in Boglár by 80.2% compared with the control (N100_C), but the responses of the other genotypes to water shortage were not significant. Limited nitrogen fertilisation under well-watered conditions (N50_C) increased the root dry weight (107.5%) in the 30–75 cm layer in Pannónia Kincse compared with the control (N100_C), but the shortage in nitrogen supply combined with drought (N50_DS) significantly reduced the root mass in Boglár and Sinara by 81.2% and 57.3%, respectively, compared with the N50_C treatment. Regarding the root biomass of the examined genotypes, no significant differences were found between the drought stress treatments in the deeper layer (30–75 cm), irrespective of the nitrogen supply (Figure 4).

Figure 4. Root biomass of the soybean varieties under optimum and limited water and nitrogen supply in the 30–75 cm layer. Abbreviations are explained in Table 2. Different lowercase letters indicate significant differences based on Tukey's HSD test ($p < 0.05$).

3.3. Chlorophyll Content of Leaves

A decrease in the chlorophyll content of the leaves contributes to the reduced photosynthetic capacity of the canopy. No differences were found between the varieties and treatments until the beginning of seed set. The highest SPAD values were generally measured in the control treatment, and the highest was in Boglár (Figure 5b). The reduction in nitrogen supply combined with water withdrawal resulted in a more intensive drop in the SPAD values in Bagera and Boglár (Figure 5a,b), while no significant differences were observed for Pannónia Kincse (Figure 5c). In Sinara, the responses to drought were more intensive than the reactions to the limited nitrogen supply, but the most intensive decrease in the chlorophyll content of each variety was induced by the combined stress (Figure 5d).

Figure 5. Cont.

Figure 5. Changes in the chlorophyll content of the examined soybean genotypes under different watering regimes and nitrogen supply levels: (a): Bagera; (b): Boglár; (c): Pannónia Kincse; (d): Sinara.

3.4. Activity of Photosystem 2

The actual quantum efficiency of Photosystem 2 was measured in the R5 growth period. The reduction in nitrogen supply induced a significant decrease in the activity of Photosystem 2 in Bagera and Sinara, while the effects of limited water availability were significant in Boglár and Sinara (Figure 6). The combined stress treatment resulted in a significant reduction in the quantum efficiency of Photosystem 2 in each genotype except for Pannónia Kincse. The SPAD values and quantum yield parameters reflected the favourable stress tolerance and adaptation capacity of Pannónia Kincse.

Figure 6. Changes in the quantum yield of Photosystem 2 (PS2) measured in the second phase of the growth period in four soybean varieties. Abbreviations are explained in Table 2. Mean + SD, $n = 18$. *, ** and *** represent significant differences at $p < 0.05$, 0.01 and 0.001, respectively, compared with the control plants.

3.5. Gene Expression Studies

To better understand the molecular background of the responses of soybean genotypes to nitrogen and/or water deficiency, the expression levels of the selected genes related to nitrogen metabolism or stress responses were investigated in two selected genotypes, Boglár and Pannónia Kincse (Figure 7a), which showed the most different sensitivity to N and water supply. The expression of the *INR1* (Inducible nitrate reductase (NADH) 1) gene substantially increased under reduced N supply in Boglár under both well-watered and drought-stressed conditions.

Figure 7. The relative expression levels of (a) inducible nitrate reductase (*INR1*), (b) aldehyde dehydrogenase (*TP55*), (c) abscisic acid 8'-hydroxylase 3 (*CYP707A3b*) and (d) the abscisic acid-dependent transcription factor (*DREB1*) genes measured in Boglár and Pannónia Kincse. Abbreviations are explained in Table 2. Mean + SD, $n = 3$. *, ** and *** represent significant differences at $p < 0.05$, 0.01 and 0.001, respectively, compared with the control plants.

In contrast to this, the expression of *INR1* decreased slightly but statistically significantly in Pannónia Kincse under drought conditions, but the N level did not affect the expression of *INR1*. The behaviour of the *TP55* gene, which encodes an aldehyde dehydrogenase, was similar in both genotypes: a significant increase could only be detected under combined stress conditions (Figure 7b). Similar results were observed for *CYP707A3b* but the increase was more pronounced in Boglár than in Pannónia Kincse (Figure 7c). The expression of the *DREB1* gene increased significantly only under the combined stress condition in Boglár, however, it also significantly increased under reduced N availability, especially when it was combined with drought stress in Pannónia Kincse (Figure 7d).

4. Discussion

In the present experiment, soybean varieties grown under different water and nitrogen supplies were examined with particular attention to the properties involved in stress tolerance. We aimed to identify those genotypes that used the available water and/or nitrogen sources the most efficiently. The experimental design allowed us to regulate the water and nutrient supplies precisely and to investigate the root mass accurately, even in a solid growth medium. In our experiment, the effects of water shortage and genotype on grain yield were statistically significant, but the nitrogen dosage did not significantly influence yield production. However, Onor et al. [37] confirmed that supplementing soybean with a low concentration of nitrogen may improve growth and production. The highest aboveground biomass and grain yield production under limited nitrogen supply was found for Pannónia Kincse, while these showed the greatest decrease in the case of Boglár, under all stress treatments. The depth of intensive root development under dry conditions was in line with the drought tolerance, since root growth at deeper soil layers improves the resilience of soybean to induced water shortage [38]. In order to take up water and nutrients, shallow rooting systems and more branching can occupy a greater topsoil volume than taproot systems. Shallow systems benefit from climates with regular rainfall

during the growing season. Taproots play a crucial role in water uptake from deeper soil layers [39]. The results of Ye et al. [40] indicated that drought-tolerant lines tend to elongate their rooting depth significantly more than sensitive lines under drought stress. Our results confirmed that there is phenotypic plasticity for rooting characteristics, and root traits contribute to sustaining crop performance under drought conditions [20]. The root dry weight of Pannónia Kincse increased significantly as a result of the reduced nitrogen fertilisation rate under optimum watering, and an increase in root weight occurred in the deeper layer, while that of Boglár took place in the upper layer. The biomass and grain yield of the other examined varieties decreased because of drought and combined stress but not because of nitrogen deficiency. Our results support that a low concentration of nitrogen supply may improve the growth of soybeans [37]. Lynch and Wojciechowski [41] found that the genotypes with shallower rooting and higher HI might produce higher yields if the soil resources are abundant. In our experiment, the smallest HI was calculated for Pannónia Kincse in all treatments, while Sinara and Bagera reached medium values.

A decrease in the chlorophyll content of leaves weakens the photosynthetic capacity of the canopy and thus it restricts the yield potential. The positive effect of enhanced root growth in Pannónia Kincse under the stress treatments was manifested in the chlorophyll content of the leaves and the quantum efficiency of Photosystem 2, while Boglár proved to be the most sensitive to the applied stresses, especially when low N and low water were combined.

Acclimation to a changing environment also involves adjustment of the expression levels of various regulating genes. Hao et al. [42] also found that several soybean genes were differentially expressed among the N-tolerant and N-sensitive varieties under N-limited conditions. The expression levels of the stress- or nitrogen-related genes were also determined in two selected genotypes: in Boglár and Pannónia Kincse.

Nitrate reductase (NR) is a key enzyme involved in the first step of nitrate assimilation in plants. Soybean has been characterised as having two constitutive nitrate reductase isoforms and one substrate-inducible nitrate reductase isoform in the leaves [43]. Expression of the *INR1* gene substantially increased under reduced N conditions in Boglár regardless of the water supply. In contrast to this, the N level did not affect the expression of *INR1* in Pannónia Kincse, but it decreased significantly under drought conditions. These results indicate that better adaptability cannot be related to higher expression levels of the *INR1* gene. The higher expression of *INR1* in Boglár could indicate that this genotype was susceptible to nitrogen supply, which could be confirmed by the intensive decrease in grain yield production, especially under optimum irrigation. The reaction observed for Pannónia Kincse is consistent with earlier findings, where repression of nitrate and nitrite reductase activity under abiotic stress conditions, including osmotic stress, has also been reported [44,45].

GmTP55 is an aldehyde dehydrogenase gene which encodes an antiquitin-like soybean protein involved in drought tolerance [26]. Earlier, it was assumed that the stress-induced *GmTP55* might be involved in the acclimation processes by reducing lipid peroxidation-derived reactive aldehydes under oxidative stress [46]. In our study, the expression of the *GmTP55* gene was not influenced by the water shortage or the nitrogen supply treatment, but a significant increase was observed in the expression level of the aldehyde dehydrogenase gene in both genotypes under the combined stress (reduced N and water availability) conditions. The expression level of *GmTP55* was not correlated with the measured phenological and yield parameters. The *Cyp707* gene encodes an ABA 8'-hydroxylase-induced protein, which plays a central role in regulating ABA levels during rehydration [47]. As was expected, based on Shukla et al. [32], the expression level of the *Cyp707* gene was not affected by water limitation in our experiment. However, interestingly, when water limitation was combined with limited N supply, a significant increase could be detected in its expression level, especially in the sensitive genotype Boglár. *DREB* genes encode an abscisic acid-dependent transcription factor, which regulates drought responses in plants [32]. Expression of the *DREB1* gene increased only under the combined stress

condition in Boglár, but it also increased under the reduced N treatment and especially under the combined stress in Pannónia Kincse. These results suggest that its expression level was more upregulated by N reduction than by water limitation.

5. Conclusions

The present results show that N deficiency contributed to the effects of drought in soybean; however, the plant responses were genotype dependent. Boglár and Sinara were relatively sensitive to drought, and Bagera was sensitive to N deficiency but produced the highest yield under limited watering under each nitrogen treatment, while Pannónia Kincse was tolerant to nitrogen deficiency under well-watered conditions. The most relevant changes were observed in the root system: N deficiency forced the root growth of the upper part in Boglár and Bagera, while intense root growth was observed in the deep layer in the case of Pannónia Kincse and Sinara. Selection for these traits may be important in breeding programmes. However, based on our results, the role of water supply overtook the effects of nitrogen supply; fertilisation intensity could have relevance, especially when the water availability is limited. Fertilisation management should be designed by considering the nitrogen reactions of the harvested genotypes as well as the capacities of the fields. The expression levels of the investigated genes also showed genotypic variations and changes under the different treatments. Since no direct correlation was found between the expression levels of each gene and the yield parameters, further studies are needed to better understand the detailed stress-regulating mechanisms.

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