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**THE EFFECTS OF DROUGHT STRESS ON SOYBEAN (*GLYCINE MAX* (L.)
MERRILL) GROWTH, PHYSIOLOGY AND QUALITY**

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**THE EFFECTS OF DROUGHT STRESS ON SOYBEAN (*GLYCINE MAX* (L.)
MERRILL) GROWTH, PHYSIOLOGY AND QUALITY**

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1. INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is one of the most important food legumes because of its high protein (about 40%) and oil (about 20%) concentrations (Wang et al., 2006; Maleki et al., 2013), in addition to carbohydrates and minerals (Miransari, 2016). It is a cheap source of protein (Hao et al., 2013), and 60% of human vegetable protein is provided by soybean (Allen et al., 2009; Hao et al., 2013). According to FAO statistics, soybean has the highest average harvested area among all legumes, and it has the highest harvested area of all oilseed crops as well (Mutava et al., 2015; FAO, 2019).

The top 5 countries in soybean production are USA, Brazil, Argentina, India and China; these countries represent 86.4% of area harvested and 89.1% of soybean production (Table 1). In Europe, Russia and Ukraine represent the top soybean production areas, followed by Italy and Serbia. Hungary comes eighth in the area harvested and ninth in the production of soybean in Europe (Table 2), however, major advancements in both harvested area and production are recently recorded (Fig. 1).

Table 1. Area harvested (1000 ha) and production (1000 ton) of soybean in certain regions averaged among 2009 – 2018.

Region	Area harvested/ 1000 ha	Production/ 1000 ton
World	113 142	293 609
Europe	4 066	7 681
EU	633	1 783
Hungary	51	121
USA	32 535	101 510
Brazil	28 630	86 173
Argentina	18 243	48 837
India	10 783	11 826
China	7 529	13 358

Source: FAO, 2019

The current global climatic changes have put this crop under certain periods of drought stress during different stages of its vegetative growth, and soybean is reported to be sensitive to several abiotic stresses as compared to other legumes and crops (Silveira et al., 2003; Fan et al., 2013; Talebi et al., 2013). Moreover, soybean is currently sown as a rainfed crop in many regions. Hence, drought is continuously affecting soybean

production and quality (Liu et al., 2004; Manavalan et al., 2009), especially with the fact that drought intensively increased over the past decades, altering precipitation amounts and distribution (De Paola et al., 2014), and is predicted to further increase in frequencies and intensities (Zhao and Running, 2010; Turner et al., 2011). As such, soybean production, along with other sensitive crops, is put under serious challenges and raising the concern about the food security of the world (Oh and Komatsu 2015; Vurukonda et al., 2016), especially with the fact that global population is continuously increasing and expected to reach 9.1 billion in 2050 (Sto, 2011).

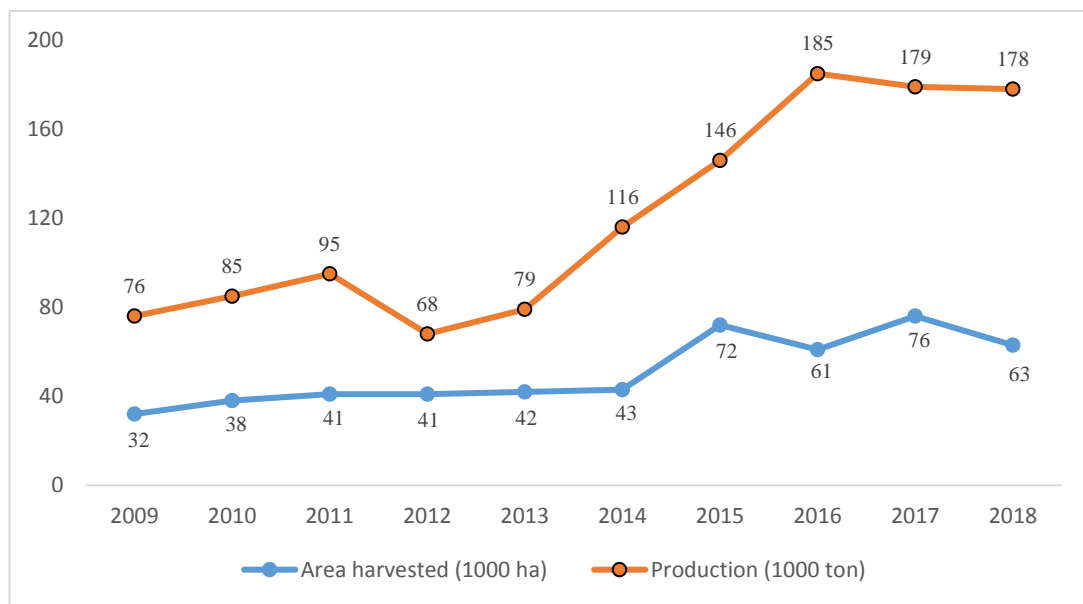


Fig. 1. Area harvested (1000 ha) and production (1000 ton) of soybean in Hungary between 2009 and 2018.

Based on these issues, understanding the influence of drought stress on crops becomes vital, as such understanding can be exploited in irrigation-scheduling practices which, in part, reduces drought-related fluctuations in food production (Wei et al., 2018). However, the response to drought stress is a very complex process that involves multiple mechanisms on different morphological, physiological and metabolic levels (Seki et al., 2003; Mattana et al., 2005; Yamaguchi-Shinozaki and Shinozaki, 2006; Reynolds and Tuberosa, 2008; Rahdari and Hoseini, 2012). For example, restrained germination is likely to happen when exposing germinating seeds to severe stress (Swigonska and Weidner 2013). Generally in plants, roots sense drought stress before the other organs (Davies et al., 2002; Wilkinson and Davies 2002; Oh and Komatsu 2015), consequently, plants can form fine roots which can penetrate smaller soil pores and increase, to some

extent, water uptake (Komatsu and Hossain 2013), or they can develop a deep rooting system to maximize water uptake and/or intensely control the stomata in order to minimize water loss (Levitt, 1980; Martinez-Ferri et al., 2004).

Table 2. Area harvested (1000 ha) and production (1000 ton) of soybean in the top European countries averaged among 2009 – 2018.

Country	Area harvested (1000 ha)	Production (1000 ton)
Russia	1 703	2 287
Ukraine	1 503	3 062
Italy	228	792
Serbia	172	468
Romania	99	222
France	85	230
Croatia	64	165
Austria	46	125
Hungary	51	121

Source: FAO, 2019

Another mechanism that plants modify under drought stress conditions is light absorption through changes in leaf's chlorophyll content (Sandoval-Villa et al., 2002; Dong et al., 2015). Chlorophyll has a major role in light quantum's absorption and transmission, and chlorophyll content represents light use ability by plant (Bornman et al., 1991). Under drought conditions, chlorophyll pigments and photosynthetic electron transport system could be damaged, leading to reactive oxygen species (ROS) production (Zgallai et al., 2005) in higher concentrations (Shigeoka et al., 2002), resulting in cellular damage as a result of gene alteration, protein degradation and enzyme inactivation (Mahajan and Tuteja, 2005) and, eventually, cell death (Upadhyaya et al., 2007). However, despite the fact that high concentrations of ROS cause damages to the cells, yet low concentrations play the role of signaling molecules that can ease several processes like germination and growth (Dowling and Simmons, 2009). For example, it was reported that ROS play noticeable role in regulating stomatal closure in order to optimize water use efficiency (Huang et al., 2009). Hydrogen peroxide (H₂O₂) is a compound that belongs to non-radical ROS (Matilla-Vázquez and Matilla, 2012); it regulates many physiological mechanisms such as growth and development under both normal and stressed conditions, playing a major role in activating various signal molecules in plants leading to inducing

different mechanisms of tolerance (Wendehenne et al., 2004; Bright et al., 2006; Foyer and Noctor, 2009). Many reports have demonstrated that treating plants with suitable concentrations of H_2O_2 increases tolerance to abiotic stresses (Chao et al., 2009; Xu et al., 2011; Gondim et al., 2013; Hossain and Fujita, 2013). Ishibashi et al. (2011) concluded that spraying soybean plants with H_2O_2 resulted in better net photosynthesis (P_n) and that this application made the plants more tolerant to drought stress; similar conclusion was also reported on melon plants (Ozaki et al., 2009) and on cucumber seedlings (Sun et al., 2016). Liu et al. (2010) reported improved osmotic stress resistance when two cucumber varieties were pre-treated with H_2O_2 as a result of the activation of antioxidant system. It was suggested that the mechanism by which plants exogenously-sprayed with H_2O_2 better tolerate stress could be by ROS-detoxification modification or by the regulation of multi-pathways that respond to stress (Hossain et al., 2015); other papers reported that H_2O_2 alleviated the negative stress effects by either a regulated stomatal closure (Kolla et al., 2007; Quan et al., 2008; Wang and Song, 2008) or by promoting the biosynthesis of the oligosaccharides and, accordingly, maintaining the leaf water content (Ishibashi et al., 2011). Many efforts were made to exploit this characteristic in enhancing stress tolerance in plants (Jubany-Marí et al., 2009; Liu et al., 2010).

Nitrogen (N) is one of the most important macronutrients for plant vegetative growth and development, affecting several functions and components such as enzymes, proteins and cell walls to name a few (Fageria and Baligar, 2005). In addition, N represents a major component of the chlorophyll (Blackmer and Schepers, 1995); as such, it affects chlorophyll formation and, consequently, photosynthesis (Jongschaap and Booij, 2004; Mauromicale et al., 2006). Moreover, N is essentially needed for soybean in order to produce optimum biomass (Fabre and Planchon, 2000; Fageria and Baligar, 2005). Soybean plants have a large N harvest index compared to other legumes (Fageria and Baligar, 2005), and N deficiency causes N from leaves to be remobilized to the seeds, which in part will lead to decreased photosynthesis and eventually reduced yield (Salvagiotti et al., 2008; Lindström et al., 2010). Because of its high protein concentration in the seeds, soybean plants have high N requirements (Bellaloui et al., 2015). Kaschuk et al. (2016) reported that to yield 1000 kg of soybean seeds, about 80 kg of nitrogen are needed. N demand is at its maximum during pod filling (Kaschuk et al., 2010; Hungria and Mendes, 2015), and nitrogen fixation was reported to be in its maximum rate during

this stage (Zapata et al., 1987), so, the timing of N application has an important role in yield (Yinbo et al., 1997). The two main sources of nitrogen for soybean plants are biologically-fixed N₂ and mineral N fertilizer (Salvagiotti et al., 2008). One benefit of fixed N₂ is that plants immediately use it, with no potential losses due to any environmental factors. Another point is that commercial inocula are much cheaper than chemical N- fertilizer (Miransari, 2016). Moreover, inoculation process can enhance plant's resistance to abiotic stresses (Gurska et al., 2009); for example, Redman et al. (2011) reported that under drought stress conditions, inoculation decreased water consumption, whereas enhanced yield and biomass.

Some experiments reported that well-nodulated soybean does not need N-fertilizer application, as the sole *Bradyrhizobium* inoculation is enough as N source (Sogut, 2006; Kinugasa et al., 2012; Hungria and Mendes, 2015), which was demonstrated by Kaschuk et al. (2016) who concluded that N fertilizer did not lead to more yield of two different soybean cultivar groups (determinate and indeterminate) whether the N application was at sowing time, during reproductive stages or both; it even resulted in a slight yield loss when it was applied at full flowering stage, which was previously reported (Hungria et al., 2006; Mendes et al., 2008). Moreover, many researches reported N fertilizer application to reduce the soybean yield (e.g. Deibert et al., 1979; Hardarson et al., 1984; Herridge and Brockwell, 1988; Jefing et al., 1992). However, other researchers reported otherwise (e.g. Ray et al., 2006; Caliskan et al., 2008; Lindström et al., 2010) as fixed N₂ was reported to provide soybean plants, on average, with 50–60% of required N (Salvagiotti et al., 2008). If there is some deficiency in fixed-N₂ amounts, other sources (mainly through N fertilization) must be available (Fabre and Planchon, 2000). Although adding N fertilizer can temporarily prohibit nodulation (Welch et al., 1973; Hardarson et al., 1984; Jefing et al., 1992; Hungria et al., 2005), 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 (Salvagiotti et al., 2008). MacKenzie and Kirby (1979) concluded that yield was linearly correlated with N fertilizer amounts up to 90 kg ha⁻¹, and Fabre and Planchon (2000) reported a significant correlation between yield and N fertilizer during flowering stage. Salvagiotti et al. (2008) concluded that less than 50 kg ha⁻¹ of N fertilizer has led to the largest agronomic efficiency. Some other researchers concluded also that N fertilizer addition increases yield (Kuwahara et al., 1986; Nakano et al., 1987; Norhayati et al., 1988; Takahashi et al., 1991) by reducing abortions of

flowers and pods (Brevedan et al., 1978). Moreover, Harper (1974) and Imsande (1992) reported seed yield and seed protein content to be enhanced when N₂ fixation is associated with N fertilizer, particularly during pod filling (Imsande, 1998; Salvagiotti et al., 2008). The reasons for alteration in the response to N fertilization are not accurately specified; however, environment and stresses, initial soil fertility, nodulation capacity, inoculant presence in soil and pre-sowing inoculation and the timing of N application all play a role (Gault et al., 1984; Peoples et al., 1995).

N is particularly important under drought stress conditions (Caliskan et al., 2008; Salvagiotti et al., 2008) for improving shoot nitrogen and shoot biomass accumulation (Purcell and King, 1996). The same authors reported that under well-watered conditions, N decreased yield to 2597 kg ha⁻¹ relative to 2728 kg ha⁻¹. Chen et al. (1992) concluded that under severe drought stress, every 1 kg ha⁻¹ of N fertilizer resulted in extra 1.2 kg ha⁻¹ seeds.

Accordingly, N fertilization might be introduced as an efficient application to partially overcome the negative effects expected from drought periods, and to ensure the right timing and dose of N fertilizer application, monitoring N status seems to be crucial. The normalized difference vegetation index (NDVI) is one of the widely-used, non-destructive methods for monitoring plant N nutrition in order to conveniently apply N fertilizer (Swain et al., 2011; Naohiro et al., 2016). For this goal, using the handheld equipment seems to be very efficient. The handheld NDVI equipment uses the following simple concept; certain wavelengths (visible light from 400 to 700 nm) of sunlight's spectrum are absorbed by chlorophylls for photosynthesis process, whereas other wavelengths (near-infrared (NIR) light from 700 to 1300 nm) are reflected; NDVI represents the difference between NIR and red reflectance divided by their sum. The index ranges from (-1) to (+1), and the closer to (+1) the number is, the better the plant's vigor and greenness are (Thapa et al., 2019).

Another common way to investigate N status in the leaves and to, consequently, measure the relative content of chlorophylls in the leaf is by using SPAD handheld equipment; this nondestructive device gives instant SPAD readings on the basis of quantifying the light intensity absorbed by the leaf using two wavelengths; 650 nm (red) and 940 nm (infrared) simultaneously emitted (Minolta Camera Co. Ltd., 1989).

Phosphorus (P), after nitrogen, is also one of the most important mineral nutrients for plant development and energy conservation and transfer (Abel et al., 2002; Elser et al., 2007). In addition, P has a vital role in photosynthesis and chloroplast composition (Hernández and Munné-Bosch, 2015). Considerable amounts of P, in the form of ATP, are needed for biological N₂-fixation process by the nodules in legume plants (Xavier and Germida, 2002), and increasing P rate resulted in adequate increase in seed-N resulting from N₂-fixation stimulation as reported by Ogoke et al. (2003). It was previously reported that P application increased the dry matter, biomass and, consequently, the yield of soybean plants (Andraski et al., 2003; Dong, 2009). Not only quantity, but also seed quality was reported to be improved by P application (Shahid et al. 2009).

Although soil might have high concentrations of P, yet most of it can be unavailable for plants due to its poor solubility and fixation (Smith et al., 2011; Mahanta et al., 2014). As a result, N₂-fixation rate in legumes and, consequently, the advantage of this ecologically friendly process can be decreased (Sulieman et al., 2013). P deficiency can also decrease seedling vigor and root development (Jin et al., 2006). As such, soybean plants that were subjected to drought stress conditions during reproductive stages but received P fertilizer had better root morphology, better P uptake and, as a result, better yield (Jin et al., 2005).

Like N, soybean has high requirements of available P (10-15 mg kg⁻¹ soil) (Aune and Lal, 1995), and low soil-P availability limits soybean yields (Qingping et al., 2003). However, excessive amounts of P resulted in growth inhibition in soybean (Cai et al., 2004), in addition to the fact that only 10%–45% of P- fertilizer added to the soil is readily usable (Adesemoye and Kloepper, 2009), so it's of high importance to determine the best P-rate application that can be optimally used by plants.

P application was reported to enhance drought stress tolerance (Gutiérrez-Boem and Thomas, 1998; Singh and Sale, 2000). Jin et al. (2006) shortlisted 3 explanations for this enhancement; 1) energy produced by photosynthesis and carbohydrate metabolism is stored in P compounds, and this stored energy has a role in drought tolerance (Jones, 2003); 2) P enhances water extraction by roots (Singh et al., 1997) and water conservation in the plant tissues (Garg et al., 2004); 3) P increases the soluble proteins under drought stress conditions by enhancing nitrogen metabolism (Al-Karaki et al., 1996).

2. OBJECTIVES

The current and the predicted climatic changes are and will certainly affect the yields of plants, which means putting food production for the growing world population under serious challenges, especially those species which can not properly tolerate abiotic stresses. Moreover, using the chemical fertilizers to re-enrich soils with nutrients is not without consequences on the environment, in addition to the higher costs of the production process. Hence, understanding the mechanisms that susceptible crops utilize to cope with changing climate can provide a more-clear idea on on-field applications that can lead to the optimum production.

As soybean is one of the most important food legumes, and with the two facts that soybean plants are susceptible to drought stress, in addition to the high demand of nutrients, especially nitrogen and phosphorus, our research aimed at:

- 1- revealing the sole effect of on-field drought stress on 7 soybean genotypes;
- 2- evaluating the sole and combined influence of drought stress and nitrogen fertilizer application on 2 soybean genotypes; '*Pannonia Kincse*', where only mineral nitrogen fertilizer was applied, and '*Boglár*', where nitrogen was applied from 2 different sources; fixed-N₂ through inoculation with *Bradyrhizobium japonicum* bacterium, and mineral nitrogen fertilizer;
- 3- revealing the effects of applying different N-fertilizer rates under natural drought on some physiological traits, namely; relative chlorophyll content (SPAD), leaf area index (LAI) and normalized difference vegetative index (NDVI) of the 2 soybean genotypes;
- 4- monitoring the sole and combined effects of P fertilization and drought stress on the 2 soybean genotypes, in addition to
- 5- revealing the probable positive effects of exogenously spraying H₂O₂ at early bloom (R1) stage on the physiology and the seed yield of the 2 soybean genotypes.
- 6- besides the on-field experiments, we studied the influence of PEG-induced drought stress on the germination parameters and the physiology of 2 soybean genotypes; '*ES Mentor*' and '*Pedro*' under controlled environment (climate chamber) conditions.

3. LITERATURE REVIEW

Determination of vegetative and reproductive Stages in soybean requires node identification. Nodes, not leaves, are used for stage determination because they are permanent. The two unifoliolate nodes are located directly opposite each other, immediately above the cotyledonary nodes. All nodes above the unifoliolate nodes have trifoliolate leaves. The trifoliolate nodes alternate from one side to the other up the main stem. To determine when the leaf is fully developed, leaf development is examined at the node immediately above. A leaf is considered fully developed (node is counted) when the leaf at the node above has unrolled sufficiently so that the two edges of each leaflet are not touching. Vegetative stages are described from the time the plant emerges from the soil. Only nodes on the main stem are counted. For vegetative stages, the letter V, followed by the number of the node that has a fully-developed leaf, is used. For example, V1 (first node) stage represents the vegetative stage when the leaves on the unifoliolate nodes are fully developed, whereas V2 (second node) stage is represented by a fully developed trifoliolate at the node above the unifoliolate nodes, and so on. For determining the reproductive stages, flowers, pods and seeds are used. Each reproductive stage is represented by the letter (R), followed by the suitable number based on the development stage. For example, R1 (beginning bloom) is the reproductive stage when any open flower on the main stem is noticed, whereas R2 (full bloom) represents the reproduct when an open flower on one of the two uppermost nodes on the main stem with a fully developed leaf is noticed (Fehr and Caviness, 1977).

Certain periods of soybean lifecycle are more susceptible to drought than others (Frederick et al., 2001; Aminifar et al., 2012; Ku et al., 2013). It was reported that soybean plants have low water demands at vegetative stages, whereas these demands increase during reproductive stages (Mian et al., 1996). As such, early drought, during vegetative stages, might not affect soybean final seed yield (Foroud et al., 1993; Turner, 1996; Melvin et al., 2005). Jumrani et al. (2017) reported that soybean plants subjected to drought at vegetative stages had less leaf area, less photosynthesis rate and less biomass; however, it was possible for the stressed plants to partially recover during post-stress period. On the other hand, drought occurred later during reproductive stages resulted in higher flower-abortion rate and reduced developed pods and seeds, with less opportunity to recover, resulting in noticeable yield loss (Bhatia and Jumrani 2016). Moreover, it was

reported that even at early reproductive stages (particularly flowering stages), drought did not measurably affect yield, whereas drought during pod filling stages significantly decreased the yield (Momen et al., 1979; Korte et al., 1983a; Yan et al., 2013). Lozovaya et al. (2005) reported that relative drought stress during seed formation (R6) stage enhanced the seeds' quality by increasing many isoflavones; however, seed formation was negatively affected.

Not only the stage, but also both the duration of the drought occurrence and the genotype have a role in the damage level (Farooq et al., 2014, Senapati et al., 2019).

Drought stress decreases stomatal conductance (Ruppenthal et al., 2016). Flexas et al. (2004) reported that drought stress level might be estimated by measuring stomatal conductance; if its value $\geq 0.2 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ then there is no drought stress, and if it falls between 0.1 and $0.2 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ then the plants are subjected to a moderate drought stress, and if it is $\leq 0.1 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ then severe drought stress is present.

Under drought stress conditions, leaf photosynthetic performance is also changed and can be inspected by observing the changes in the thylakoid membrane organization and function by measuring chlorophyll fluorescence. Chlorophyll fluorescence can also be considered as an indicator to the energy absorbed by chlorophyll being used by PS II (Baker et al., 2004). The quantum efficiency of PS II ($\Phi_{\text{PS II}}$), on the other hand, can be considered as an indication of overall photosynthesis as it measures the proportion of light absorbed by chlorophyll associated with PS II that is used in photochemistry; this trait also can be altered under certain stress conditions like drought (Fryer et al., 1998; Maxwell and Johnson, 2000).

Many other changes were also reported as a result of drought stress occurrence during the life cycle of soybean plants like plant height (Lee et al., 2015), leaf area (Garcia et al., 2010), seed yield (Kokubun, 2001; Bajaj et al., 2008), protein concentration (Rotundo and Westgate, 2010; Wang and Frei, 2011) and oil concentration (Boydak et al., 2002; Bellaloui and Mengistu, 2008).

Under laboratory conditions, aqueous substances with different osmotic potential levels are used in order to conduct experiments on drought stress's effects on the plants (Machado et al., 2001). The most two widely-used substances are mannitol, which is non-

toxic for plants, and polyethylene glycol (PEG), which can't enter the cells due to its molecular weight (Avila et al., 2007); PEG is considered as an effective method for drought stress simulation with its limited metabolic interferences, as plants are less likely to absorb it, besides, it is not phytotoxic (Lawlor 1970).

3.1. GERMINATION PARAMETERS AS AFFECTED BY DROUGHT, SEED SIZE AND H₂O₂ APPLICATION

Germination is one of the most critical periods in the life cycle of the plants. Under water stress, low water potential is a determining factor inhibiting seed germination (Wang et al., 2002). Avila et al. (2007) reported that drought stress is considered as one of the major limiting factors of germination and early seedling development, as it plays a key role in activating different metabolic processes directly related to seed germination. Particularly, drought stress reduces germination rate by decreasing enzyme activity, and consequently, reducing meristem development (Avila et al., 2007). Marcos (2005) reported that as the drought severity increases, the germination rate decreases and more inhibitors may fluctuate the developments of the germinated seedlings; i.e. below the ideal available-water amount, germination can be suppressed (Maraghni et al., 2010). Mengistu and Heatherly (2006) used a total of 10 cultivars from maturity group IV and 8 cultivars from maturity group V in their study to investigate the effect of irrigation on the germination ratio on soybean. They reported that the average germination ratio for total irrigated plots during the 4 years of the experiment was 71% compared to 65% for non-irrigated counterparts. Drought reduces germination ratio and finally delays establishment of plantlets (Prisco et al., 1992). Hellal et al. (2018) reported that the increase in PEG-6000 concentrations dramatically decreased germination percentage of overall studied cultivars (10 barley cultivars different in drought tolerance) as the increase in PEG by 5% decreased germination percentage by 19% relative to the untreated one, whereas increasing PEG from 5% to 10% decreased germination percentage by 9%, and increased PEG from 10% to 20% decreased germination percentage by 30%. The results directly affected the total germination percentage. Salehi (2010) reported reduction of germination percentage and increase of osmotic potential produced by polyethylene glycol on bean seedlings. Previously, many researchers concluded that increasing drought stress levels progressively delayed and reduced germination (Wiggans and Gardner 1959; Parmar and Moore 1968; Mcwilliam and Phillips, 1971; Pandya et al., 1972). However,

treating seeds before sowing with H₂O₂ or applying it as a foliar spray can enhance abiotic stress tolerance in plants. For example, a stimulation in the germination was reported when seeds of *Pseudotsuga menziesii*, *Sorghum nutum*, *Andropogon gerardii* and *Panicum virgatum* (Sarath et al., 2007), and *Zinnia elegans* (Ogawa and Iwabuchi, 2001) were pre-treated with H₂O₂. Similarly, enhanced germination rates were recorded in maize seeds pre-soaked in 140 mM of H₂O₂ (Ashraf et al., 2015). Jubany-Marí et al. (2009) reported that H₂O₂ is involved in the acclimation of *Cistus albidus* to summer drought.

Another factor affecting germination ratio is seed size (Longer et al., 1986). Burris et al. (1973) grouped soybean seeds in four groups depending on the size; they reported significantly lower germination ratio for the smallest-size group relative to the other groups. Longer et al. (1986) reported the bigger-sized seeds of two soybean cultivars to better germinate as compared to smaller counterparts, which also was previously reported (Haskins and Gorz, 1975; Goyal et al., 1980).

It was previously reported that increasing PEG concentration was accompanied by a reduction in the ultimate germination of 10 barley cultivars, regardless of their different drought tolerance potentials (Hellal et al., 2018). Kafi et al. (2005) stated that as the water potential of lentil (*Lens culinaris* Medik.) decreased, both germination percentage and root length decreased, which was supported later by the conclusion of Hellal et al. (2018) that root length of barley (*Hordeum vulgare* L.) cultivars under high PEG concentrations (10 and 20%) was noticeably less than control counterparts, regardless of cultivar. Similar findings were reported by Pandya et al. (1972) that early seedling development in terms of root length also declined with increasing water stress. In another experiment where the mannitol was used to induce drought stress on soybean, the germination ratio was significantly decreased to (39.5%) compared to (72.5%) for the non-stressed control. Also, the root length significantly decreased from 8.72 cm in the control to 2.86 cm when drought stress was applied (Braga et al., 2017).

3.2. NODULATION AND N₂ FIXATION AS AFFECTED BY DROUGHT AND N APPLICATION

Nodulation in soybean is negatively affected by drought stress (Abaidoo et al., 2007). Smith and Nelson (1986) reported substantial decreases in nodule mass in drought-

stressed soybean. Herrmann et al. (2014) explained the low soybean nodulation of their two-year experiment to be resulted by drought stress, which was also reported earlier by Zahran (1999). Moreover, some promiscuous soybean cultivars showed a correlation between nodulation and water availability (Mpeperekki et al., 2000; Musiyiwa et al., 2005a,b). In addition, soybean, among other legumes, is known to improve soil fertility by symbiotic N₂-fixation (Vanlauwe et al., 2010). Hence, both establishment and activity of symbiosis relationship are reported to be extremely sensitive to drought stress (Weber, 1966; Pankhurst and Sprent, 1975; Sprent, 1976; Patterson et al., 1979; Peterson and LaRue, 1983; Kirda et al., 1989; Chalk et al., 2010) as a result of nodulation reduction, consequently, N₂ in leaves decreases and the capacity of photosynthesis is reduced (Huang et al., 1975; Finn and Brun, 1980; Durand et al., 1987; Djekoun and Planchon, 1991). However, Sinclair et al. (1988) noticed a decrease in nodule number and dry weight only after a severe drought. Minguez and Sau (1989) reported that soybean plants accounting on N₂ fixation are more drought-susceptible compared to soybean plants accounting on N fertilizer, which was previously reported by Obaton et al. (1982). Fixed-N₂ decreases under drought stress, resulting in decreased N content in the leaves which, in part, leads to decreased photosynthetic capacity (Minguez and Sau, 1989; Djekoun and Planchon, 1991; Kao and Forseth, 1992).

It was previously reported that high rates of N-fertilizer inhibit N₂-fixation process, whereas a relatively-low dose at the early stages of soybean development can be beneficial as N₂-fixation process will not be initiated by that time yet (Miransari, 2016). Applying N fertilizer decreased nodule number and dry weight (Herrmann et al., 2014; Kaschuk et al., 2016) in a linear trend (Chen et al., 1992), consequently, N fertilizer resulted in less N₂ fixation (Salvagiotti et al., 2008). Applying N fertilizer at rates more than 45 kg ha⁻¹ on two soybean cultivars different in nodulation potential resulted in same nodule reductions for the two cultivars, which means that the advantage of the better-nodulated cultivar was eliminated by N fertilizer (Chen et al., 1992). Salvagiotti et al. (2008) reported that using foliar fertilization to prevent nodulation prohibition by soil N returned less agronomic efficiency.

3.3 STOMATAL CONDUCTANCE AS AFFECTED BY DROUGHT AND REACTIVE OXYGEN SPECIES (ROS) APPLICATION

Drought stress induces stomatal closure, limits gas exchange and photosynthesis (Yordanov et al., 2000). Ohashi et al. (2006) reported that stomatal conductance (g_s) of soybean plants significantly decreased under drought stress conditions; similar result was concluded by Zhang et al. (2016) who reported a 98.8% decrease in g_s under drought; they concluded that this reduction in g_s was a result of the reduced ratio of open stomata and stomatal aperture size in the plants subjected to drought stress. Hao et al. (2013) reported a significant reduction in stomatal conductance from 0.25 to 0.10 mol H₂O m⁻¹ s⁻¹ as a result of drought applied on soybean plants. Mathobo et al. (2017) justified the reduction in g_s in their experiment on dry beans (*Phaseolus vulgaris* L.) by the prevention of CO₂ from entering the leaf by stomatal closure. Similarly, Rosales et al. (2012) reported a 70% reduction of g_s after 22 days of drought Stress application. Tang et al. (2017) concluded that PEG 6000-induced water stress on soybean significantly reduced g_s by 73%. Ishibashi et al. (2011) compared g_s of two groups of soybean seedlings under drought stress conditions; one group was sprayed with H₂O₂ and the other group with distilled water (DW); they reported that g_s was significantly higher in H₂O₂-treated plants than in DW-treated plants. After two days of spraying, g_s levels in H₂O₂-treated and DW-treated plants were 508 and 323 mmol m⁻² s⁻¹, respectively. They concluded that H₂O₂ spraying reduced stomatal closure caused by drought stress; i.e. H₂O₂ treatment reduced soybean sensitivity to drought stress. In another experiment, maize leaves pretreated with 10 mM H₂O₂ significantly enhanced g_s (by about 50%) as compared to drought-stressed leaves (Terzi et al., 2014); they concluded that spraying leaves with H₂O₂ can reduce water loss under drought stress conditions by increasing the concentrations of metabolites that are involved in osmotic adjustment (like proline, polyamines and soluble sugars). Other ROS species were also reported to have a role in alleviating drought stress; Razmi et al. (2017) reported that water stress reduced stomatal conductance of three soybean leaves compared to well-watered counterparts, and foliar spray of 0.4 mM of Salicylic Acid (SA) significantly reversed drought-induced stomatal closure and increased it. Low concentrations of ROS play the role of signaling molecules that can ease several processes like germination and growth (Dowling and Simmons, 2009). Hydrogen peroxide (H₂O₂) regulates many physiological mechanisms such as growth and development under both normal and stressed conditions, playing a major role

in activating various signal molecules in plants leading to inducing different mechanisms of tolerance (Wendehenne et al., 2004; Bright et al., 2006; Foyer and Noctor, 2009). It was suggested that the mechanism by which plants exogenously-sprayed with H₂O₂ better tolerate stress could be by ROS-detoxification modification or by the regulation of multi-pathways that respond to stress (Hossain et al., 2015); other papers reported that H₂O₂ alleviated the negative stress effects by either a regulated stomatal closure (Kolla et al., 2007; Quan et al., 2008; Wang and Song, 2008) or by promoting the biosynthesis of the oligosaccharides and, accordingly, maintaining the leaf water content (Ishibashi et al., 2011).

3.4. CAROTENOIDS AS AFFECTED BY DROUGHT

Carotenoid can protect chlorophylls from damage by dissipating excess light energy around Photosystem II (PS II) through xanthophylls cycle (Carol and Kuntz, 2001; Aluru et al., 2006). Therefore, it is an important safeguard of photosynthetic mechanism, and its content can reflect the adaptive ability of plant to environment (Tang et al., 2017). Previously, Zhang et al. (2016) reported carotenoids content to be significantly reduced under drought stress conditions compared to the well-watered control, which was supported later by Tang et al. (2017), with their conclusion that exposing plants to water stress led to a significant decline in carotenoid content (from 3.4 to 2.1 mg/g dry weight).

3.5. QUANTUM YIELD OF PS II (Φ PS II) AND MAXIMUM QUANTUM YIELD OF PS II (F_v/F_m) AS AFFECTED BY DROUGHT

Zhang et al. (2016) reported maximum quantum yield of PS II (F_v/F_m) to be approximately 0.78–0.80 in control treatment, however, this parameter decreased in response to drought stress, but was not significantly different. Additionally, drought stress resulted in a reduction in quantum yield of PS II (Φ PS II) (from 0.53 to 0.13); they suggested that the reduced Φ PS II was a result of a decrease in the excitation energy trapping efficiency of PS II reaction centers. Similar conclusion was reported by Zlatev and Yordanov (2004) in bean plants. Hao et al. (2013) reported the decrease to be significant (from 0.83 to 0.66), whereas Mathobo et al. (2017) concluded that the reduction was insignificant after 93 days of planting between control plants and plants suffered from drought stress for 24 days in early stages; however, later in the same experiment (100 days after planting) the difference was significant. Decrease in F_v/F_m

was concluded to be an indication of down regulation of photosynthesis (Zlatev and Lidon, 2012). Liu et al. (2012) also observed a decline in F_v/F_m ratio in drought stressed plants of two maize cultivars. This occurrence of chronic photo-inhibition was justified as a result of photo-inactivation of PS II centers (Zlatev and Yordanov, 2004). Compared with the control, water stress markedly decreased F_v/F_m (from 0.80 to 0.76) and $\Phi PS II$ (from 0.69 to 0.58) (Tang et al., 2017). Water stress treatment reduced total chlorophyll content and chl_a/chl_b , indicating the decreased capacity of absorbing and conversion of light energy, which may be the reason of reduced $\Phi PS II$ (Tang et al., 2017). On the contrary, drought stress did not have an effect on F_v/F_m in dry bean (Terzi et al., 2010).

3.6. CHLOROPHYLL CONTENT AS AFFECTED BY DROUGHT, N FERTILIZATION AND ROS APPLICATION

Chlorophylls are the main pigments of light absorption, transport and conversion of light energy, and chlorophyll content is an important parameter indicating photosynthetic performance (Liu et al., 2007). Dong et al. (2015) concluded that light absorption was reduced by drought stress which resulted in changing both leaf area index and leaf chlorophyll content. Zhang et al. (2016) concluded that Chl_a was significantly reduced under drought conditions compared to the non-droughted counterpart, whereas Chl_b increased when plants suffered from drought stress. Exposing plants to drought stress led to a significant decline in chl_{a+b} (from 19.5 to 13.0 mg g⁻¹ DW), indicating the decreased capacity of absorbing and conversion of light energy (Tang et al., 2017). Total chlorophyll (chl_{a+b}) decreased by 42.5% under drought stress conditions imposed at flowering stage, whereas the reduction ratio was 15.7% when soybean plants suffered from drought stress at pod filling stage (Sapanlo et al., 2014). Both chlorophylls (chl_{a+b}) were reduced under drought stress (Farooq et al., 2010). Atti et al. (2004) reported that drought stress reduced relative chlorophyll content (SPAD) value by 11%. Previous studies reported that chlorophyll decreases under drought stress conditions (e.g. Cui et al., 2004; Pagter et al., 2005). Inamullah and Isoda (2005) reported reductions in chlorophyll content when soybean plants were subjected to continuous drought stress starting from early seed filling stage. Moreover, Hao et al. (2013) reported significant decrease (by 32.2%) in chlorophyll content as a result of drought stress, whereas Cerezini et al. (2016) reported a non-significant reduction in the chlorophyll content when drought stress was applied at R2 stage. Mathobo et al. (2017) subjected bean plants to drought stress for 24 days in

different stages; the reduction of chlorophyll content was higher when drought occurred at later stages as compared to earlier stages, and control plants were always the highest in chlorophyll content; they suggested that the reduction in chlorophyll content might have resulted from leaves being damaged and turning yellowish due to drought stress. In another study, SPAD values significantly decreased from 35.48 to 22.38 under drought stress applied 30 days after R5.5 stage (Ergo et al., 2018). These results are in agreement with the general chlorophyll drops that occur when soybean plants are subjected to continuous water stress from early seed filling (De Souza et al., 1997). Many papers reported a decrease in total chlorophyll content due to drought stress in many legumes like chickpea (Mafakheri et al., 2010), pea (Inaki-Iturbe et al., 1998) in addition to soybean (Makbul et al., 2011). Smirnoff (1995) indicated that the decrease in total chlorophyll content is resulting from the damage to the chloroplasts caused by reactive oxygen species (ROS) as drought stress leads to the production of reactive oxygen species (ROS) such as O_2^- and H_2O_2 , which lead to chlorophyll destruction (Foyer et al., 1994). This conclusion was supported later by Liu et al. (2007) who also reported that chl_a is more sensitive to ROS than chl_b . An evaluation of the effects of H_2O_2 on leaf chlorophyll content during adventitious rooting under drought conditions showed that drought stress resulted in a decline in chlorophyll content after 72 h of its application, producing a 39.1% decrease in the chl_a content compared to control. However, applying exogenous H_2O_2 in certain concentration retarded chlorophyll degradation, especially chl_a (Liao et al., 2012). Maize leaves had higher levels of both chlorophylls *a* and *b* when seeds were soaked in 140 mM H_2O_2 before sowing (Ashraf et al., 2015). Sun et al. (2016) reported that the exogenous application of certain concentration of H_2O_2 significantly increased the leaf chlorophyll content of cucumber plants exposed to medium drought conditions. Enhanced chlorophyll levels induced by hydrogen peroxide treatment were justified by H_2O_2 -stimulated antioxidant enzyme activities (Azevedo Neto et al., 2005; Gao et al., 2010). In their experiment, Razmi et al. (2017) reported that drought significantly reduced both chl_a and b contents in soybean leaves; however, significant increases (by 15% in chl_a and 19% in chl_b) were resulted from foliar application of 0.4 mM SA compared to control treatment (no SA). Moreover, Gavili et al. (2019) reported that both moderate and severe drought conditions significantly increased soybean's relative chlorophyll content (SPAD) values by 11 and 20%, respectively. The authors justified this increase by the increased N concentrations caused by the decreased fresh or dry matter, and the enhanced N

concentration will, in turn, enhance the chlorophyll content. Bredemeier (2005) reported similar conclusion on maize.

Cerezini et al. (2016) concluded that the application of 200 kg ha⁻¹ of N insignificantly enhanced chlorophyll content (by 1.8% under non-stressed conditions and by 3% when drought stress occurred at R2 stage). Islam et al. (2017) reported that leaf-SPAD values after 3, 8, 13, 17 and 23 days of applying different nitrogen concentrations in the nutrient solution (5, 25, 100 and 200 mg l⁻¹) were increased with increasing N concentration in both years of their experiment on soybean plants. In another study, SPAD values in the three studied stages; V4, R1 and R3 of soybean plants were positively influenced by increasing N levels (0, 20, 40 and 60 kg ha⁻¹); moreover, 60 N treatment was significantly better than the control (0 N) treatment at both V4 and R1 stages (Shafagh-Kolvanagh et al., 2008). The authors also reported that SPAD value showed a decreasing trend with the progress of soybean growing stages for all levels of N. Similar conclusion was reported by Shafagh-Kolvanagh et al. (2008). De Almeida et al. (2017) concluded that N deficiency significantly reduced the relative chlorophyll content in soybean plants by 84.4%. Cerezini et al. (2016) reported that chlorophyll content was higher in non-inoculated plants than inoculated counterparts when soybean did not suffer from drought stress.

3.7. RELATIVE WATER CONTENT (RWC) AS AFFECTED BY DROUGHT AND ROS APPLICATION

It was previously reported that drought stress reduced the relative water content (RWC) of soybean leaves (Razmi et al., 2017). In their experiment, Ishibashi et al. (2011) reported that RWC in H₂O₂-treated and DW-treated (a control treatment that was treated with distilled water only) plants was 60 and 40%, respectively after 4 days of drought stress application, and was also higher in H₂O₂-treated plants than in DW-treated plants after 6 days of drought stress imposition; they concluded that H₂O₂ spraying enabled the leaves to maintain high levels of RWC by regulating the osmolality in the leaves, consequently ameliorating the negative effects of drought stress. Similar results on cucumber seedlings were reported later by Sun et al. (2016). The exogenous application of salicylic acid (SA) on common bean improved RWC under drought stress conditions (Sadeghipour and Aghaei, 2012).

3.8. LEAF AREA INDEX (LAI) AS AFFECTED BY DROUGHT, FERTILIZATION AND ROS APPLICATION

Leaf area index (LAI) expresses the canopy density of a crop population, and has an important effect on the yield (Dong and Xie, 1999). It was previously concluded that high yield could be achieved by increased LAI, and also by greater photosynthetic rate (Chang, 1981). Generally, LAI reaches its maximum value at R5 stage and then gradually decreases, regardless of the soybean cultivar (Liu et al., 2005). Typically, increased LAI is correlated with increased yield until optimal LAI value is reached (Zhang and Song, 1979; Dong and Xie, 1999). Previously, Dong et al. (1979) reported LAI to be positively correlated with biomass and grain yield of eight soybean cultivars. Growth stage plays a role in the relationship between LAI and yield; Jin et al. (2004a,b,c,d) concluded that high LAI during reproductive stages was correlated with high soybean yield. Chang (1981) recorded a significant correlation coefficient ($r = 0.603$) between total LAI at R2, R4, R5 and R6.5 stages and the final yield in a 7-year experiment. Later, it was demonstrated that yield of soybean and LAI are positively correlated at the R5 stage (Wells et al., 1982; Koutroubas et al., 1998; Shimada et al., 1992; Board and Tan, 1995; Kumudi, 2002). Soybean genotype also plays a role in the LAI value and the corresponded yield; Liu et al. (2005) concluded that higher LAI in late maturity genotypes of soybean, compared to early and middle maturity group genotypes, increased solar energy interception and, consequently, CO₂-fixing ability which resulted in more assimilates accumulation.

Although high LAI causes mutual shading of lower leaves which, in part, causes some LAI value reductions on the lower plant level, yet drought stress decreases the LAI to values much less than those resulting from mutual shading (Liu et al., 2008). Sinclair and Serraj (1995) and Liu et al. (2008) reported drought stress to reduce leaf area, consequently, protein synthesis was decreased and yield was less (Purcell and King, 1996). Li et al. (2013) reported significant decreases in LAI (by 40, 33.8 and 36.4%) when plants were subjected to drought stress conditions at flowering, podding and seed-filling stages, respectively. Nagasuga et al. (2014) reported significant reduction of LAI by 19.76 and 31.7% in two soybean cultivars; Fukuyutaka and Misatozairai, respectively as a result of drought stress application. Gavili et al. (2019) reported that moderate and severe drought (corresponding to 70 and 55% FC, respectively) significantly decreased plant leaf area by 29 and 35% at V10 stage, 23 and 31% at V3 stage and 26 and 36% at R6 stage.

Karam et al. (2005) concluded that LAI decreased by 52% under drought stress conditions imposed at R2 stage. LAI was significantly different when moderate drought stress was applied early during vegetative stages (with a value of $5.7 \text{ m}^2 \text{ m}^{-2}$), compared to severe drought stress ($3.78 \text{ m}^2 \text{ m}^{-2}$) and control plants ($6.81 \text{ m}^2 \text{ m}^{-2}$) (Garcia et al., 2010). Pagter et al. (2005) explained the decreased LAI under drought stress conditions to be the result of less newly-produced leaves with a smaller size and a higher falling rate. Severe drought stress imposed at R4 stage resulted in 61.4% less leaf area in soybean (Wei et al., 2018). Moosavi et al. (2014) reported decreased leaf area in canola plants as a result of drought stress application. Çakir (2004) also reported a 23.5% decrease in leaf area when maize plants were subjected to drought stress conditions during the tasseling period. Atti et al. (2004) concluded that two drought stress severities; W1 and W2 (corresponding to 25 and 50% of crop evapotranspiration E_{Tc}) reduced soybean leaf area by 74.5 and 52.7%, respectively. Drought stress decreased soybean LAI at both flowering (by 48%) and maturity (by 47%) (He et al., 2019).

Ashraf et al. (2015) reported that seeds soaked in 20, 80, 100, and 140 mM of H_2O_2 later formed plants with higher leaf area under drought stress conditions compared to non-treated seeds. Using (SA), other reports concluded that treatments with this ROS species could improve LAI in different plants including soybean (Kuchlan et al., 2017; Razmi et al., 2017), strawberry (Ghaderi et al., 2015) and lemongrass (Idrees et al., 2010); this was attributed to increased accumulation of certain proteins (like proline) and soluble sugars which, in part, enhances cell turgor pressure (Razmi et al., 2017).

Caliskan et al. (2008) concluded that soybean LAI linearly increased with increased N rates. DeMooy et al. (1973) and Watanabe et al. (1986) reported that adding N fertilizer before reproductive stages enhances growth and LAI, consequently flowering and yield. Buttery (1969) concluded that LAI values were increased by N application, and Dadson and Acquah (1984) reported an increase in LAI values when N was applied 9 weeks after sowing. De Almeida et al. (2017) found out that the deficiency of N in soybean plants significantly decreased LAI by 87.5%, and Virk et al. (2018) reported that the application of N fertilizer significantly increased LAI in soybean. He et al. (2019) experimented 2 soybean genotypes different in yield and water use; Huandsedadou (HD) and Zhonghuang 30 (ZH). They imposed both genotypes, 15 days after sowing, to cyclic water stress by withholding irrigation until soil water capacity reached 30% of pot

capacity and then re-watered the plants again, whereas control plants were kept under 85-100% pot capacity. Each water treatment received either 60 or 120 kg ha⁻¹ of P fertilizer. The authors concluded that P enhanced LAI at both flowering and maturity stages. Averaged over the two genotypes, 60P, under drought stress conditions, increased LAI by 100 and 43% at flowering and maturity, respectively. 120P increased this trait by 113 and 48% at flowering and maturity, respectively. Under well-watered conditions, 138 and 46% increases in LAI at flowering and maturity, respectively were recorded in 60P, and 192 and 49% in 120P, respectively.

3.9. NORMALIZED DIFFERENCE VEGETATION INDEX (NDVI) AS AFFECTED BY DROUGHT AND FERTILIZATION

Crusiol et al. (2017) applied drought stress at two different stages on two soybean cultivars; BR 16 (sensitive to drought) and Embrapa 48 (less sensitive) using rainout shelters that automatically close under rainy conditions in 2012/2013 and 2013/2014 cropping years. They reported that NDVI was higher in both cultivars when drought stress was applied at vegetative stages compared to the control (non-stressed) counterparts, whereas it measurably dropped when drought was applied at reproductive stages. Camoglu et al. (2018) concluded that reducing irrigation by 25% as compared to the control (non-stressed) treatment insignificantly reduced NDVI by 2.4% in pepper plants, whereas a 50% reduction in irrigation water amount resulted in a significant 9.5% NDVI reduction. Some previous papers reported significant correlations between precipitation amounts and NDVI (e.g. Suzuki et al., 2000; Wang et al., 2001), however, that relationship differs depending on NDVI measurement timing and other factors (Al-Bakri and Suleiman, 2004).

Cerezini et al. (2016) reported that NDVI decreased by 5.4% in inoculated soybean plants compared to non-inoculated counterparts under drought stress conditions. Mupangwa et al. (2018) concluded that nitrogen application increased NDVI in maize; they attributed it to the increase in soil N supply. In their experiment, Saleem et al. (2010) concluded that NDVI value was higher in the wheat plots that received 150 kg N ha⁻¹ as compared to the other N rates (0, 50 and 100 N ha⁻¹) at both booting and flowering stages.

3.10. PLANT HEIGHT AS AFFECTED BY DROUGHT, FERTILIZATION AND ROS APPLICATION

Drought stress reduced mainstem nodes of soybean (Korte et al., 1983b; Kadhemi et al., 1985; Muchow, 1985), consequently, leaf area was decreased, resulting finally in seed yield loss (Sinclair et al., 1981; Monteith and Scott, 1982); this was confirmed later by Frederick et al. (1989) who studied four soybean cultivars. Moreover, Jordan (1983) concluded that the growth stage at which the drought stress was applied had a role in the number of nodes. Board (1987) reported that the genotype also plays a role, as the intermediate genotypes can produce more stem nodes than the determinate ones, which, in part, will increase the duration of the reproductive stages and, consequently, the yield.

Soybean seedling height decreased 4.3% under drought stress (Navari-Izzo et al., 1990). Both Newark (1991) and El Kheir et al. (1994) reported decreased plant height under drought stress conditions. Gavili et al. (2019) reported a 33 and 60% plant height reduction in their experiment under 70 and 55% FC conditions, respectively. Soybean plants had 22.4% shorter plants when severe drought stress occurred at R4 stage, whereas only 9% reduction was reported when same severe drought occurred at R6 stage (Wei et al., 2018). Drought reduced soybean plant height by 31.1% (Freitas et al., 2016). An indeterminate soybean cultivar (OAC Bayfield) was put under two drought stress severities; W1 and W2 (corresponding to 25 and 50% of crop evapotranspiration (ET_c), respectively as compared to control, 100% ET_c) at R1 stage (Atti et al., 2004). Plant height decreased by 33 and 28% in W1 and W2 treatments, respectively after 9 days of stress application. Furthermore, drought imposition resulted in 56 and 47% reduction in plant height in W1 and W2 treatments, respectively after 16 days. Sepanlo et al. (2014) also reported that soybean plants had 29.6% shorter plants under drought stress imposed at flowering stage. Many other papers reported a reduction in plant height under drought stress conditions (Pang, 1964; Brady et al., 1974; Kadhemi et al., 1985; Atti et al., 2004; Demirtas et al., 2010; Hao et al., 2013; Mak et al., 2014), similarly, Garcia et al. (2010) reported a significant difference in plant height of drought-stressed soybean genotypes compared to control counterparts; they also reported the different examined genotypes to be significantly different in plant height, which was demonstrated later by Hossain et al. (2014) who studied the effect of drought stress on the plant height of three soybean genotypes; one drought-susceptible and two drought-tolerant genotypes. The authors

reported plant height to be shortened as a result of drought stress in the three genotypes; however, the drought-susceptible genotype had a height valued 44.3% of the control plants, whereas it was 56.7% and 59.1% in the two drought-tolerant genotypes. The authors attributed this reduction to a drought tolerance mechanism, as cell swelling, cell wall and synthesis enzymes are reduced, consequently, growth and plant height are decreased (Levitt, 1980; Austin, 1989). Banon et al. (2006), on the other hand, justified this decrease by a reduction in cell elongation caused by inhibited growth promoting hormones which, in part, led to decreasing cell turgor, cell volume and eventually cell growth and/or by a restriction of xylem and phloem vessels (Lovisolo and Schubert, 1998). Another explanation was that drought results in a decrease in the rate at which the stem nodes are produced (Frederick et al., 1989), whereas Neilson and Nelson (1998) explained this reduction in plant height under drought by the delayed stem elongation caused by shortened distance among nodes. Iqbal et al. (2018) concluded that decreasing available water at R4 stage from 100 to 50% FC slightly increased plant height in soybean. However, further reduction to 20% FC resulted in shorter plants compared to both 10 and 50% FC.

Abass and Mohamed (2011) conducted an experiment on common bean (*Phaseolus vulgaris* L.) seeds where half of the seeds were soaked in hydrogen peroxide (2%) for 4 hours and then air dried, and the other half of the seeds were soaked in distilled water for 4 hours and then air dried. Their results showed an increase by 43.6% in the H₂O₂-treated seedling height under a drought level of 60% of field capacity, moreover, increasing the drought severity (to reach only 40% of field capacity) decreased the seedling height of both treatments. However, H₂O₂-treated seedlings had 38.4% more height.

Plant height was enhanced by N fertilization as reported by Hanway and Weber (1971) and Dadson and Acquah (1984). 30.4% significant reduction in plant height as a result of N deficiency was reported (de Almeida et al., 2017). Virk et al. (2018) reported that soybean plant height was insignificantly enhanced by N application. Abera et al. (2019) compared soybean plants using 7 rhizobia isolates and a non-inoculated control in an experiment conducted in 2 different sites. The authors reported that plant height of all inoculated treatments was higher than non-inoculated control at both experimental sites. Similar conclusion was also reported earlier by Bekere and Hailemaria (2012). Significant

increases in soybean plant height (by 21.1 and 23.7%) as a result of inoculation were reported by Adeyemi et al. (2020) in pot and field experiments, respectively.

Adjei-Nsiah et al. (2019) tested the effect of 2 different sources of P fertilizer; triple superphosphate (TSP) (46% P₂O₅) and Morocco phosphate rock (MPR) (30% P₂O₅) on 3 soybean genotypes. Fertilization rate was applied at 30 kg P ha⁻¹. They concluded that P fertilization from both sources significantly increased the plant height; by 10.5% in MPR treatment, and by 21.1% in TSP treatment.

3.11. FLOWER AND POD NUMBER PER PLANT AS AFFECTED BY DROUGHT AND FERTILIZATION

He et al. (2017a) reported that cyclic drought (where water was withheld from V5-V6 stage until reaching 30% field capacity (FC), and then pots were re-watered to 100% FC, and again water was withheld in order to reach 30% FC) resulted in 53.8% decreased flower number per plant, whereas terminal drought (where no irrigation was applied after V5-V6 stage) further increased that ratio of flower loss to 72.5%. In their experiment, Atti et al. (2004) reported that flower number per plant decreased by 79.4 and 58.8% in W1 and W2 treatments, respectively. The authors explained this decrease by both reduced node number and increased flower abortion as a result of drought stress application. Drought stress negatively affects pollination process, leading to increased flower and pod abortion (Desclaux et al., 2000; Fang et al., 2010). Irrigation enhanced pod number per plant as reported by Pookpakdi et al. (1990) and Pawar et al. (1992) and later by He et al. (2017a) who concluded that cyclic and terminal drought stress resulted in 42.3 and 90.4% less pods per plant. Westgate and Peterson (1993) concluded that drought stress during flowering caused a 70% reduction in pod number per plant. Exposing soybean plants to drought at pod filling stages decreased pod number per plant by 36.6%, whereas a 42.6% reduction was recorded when drought was imposed at flowering stage (Sapanlo et al., 2014). Pod number decreased from 25 to 15 pods per plant when available water decreased from 100 to 70% FC, and further reduction to 55% FC further decreased pod number to 14 pods per plant (Gavili et al., 2019). In their experiment, Iqbal et al. (2018) decreased FC from 100 to 50% at R4 stage to study the effect of drought at this stage on soybean; they reported that pod number per plant significantly decreased by 21.4% as a consequence of drought imposition, and when FC was further reduced to 20%, another significant reduction (by 34.7% compared to 100% FC treatment) was recorded for this

trait. Atti et al. (2004) found out that both drought stress treatments (W1 and W2) had caused a 92.7 and a 67.3% reduction in pod number per plant, respectively at the beginning of pod formation, and a 81.6 and a 39.5% reduction, respectively at the pod lengthening stage. Jumrani and Bhatia (2018) found out that soybean plants that were subjected to drought stress early at V4 stage had a very similar pod number per plant as compared to non-stressed control (45 and 47 pods per plant, respectively), whereas drought at R5 stage significantly decreased this number to 30 pods per plant. The authors concluded that these reductions were caused by decreased flower number, reduced pod formation, increased pod abortion and decreased pod lengthening. Pod number per plant decreased by 49 and 43% in HD and ZH, respectively as a result of drought stress application (He et al., 2019).

Purcell and King (1996) reported that applying N fertilizer increased flower number in plants by reducing flower abortion rate. Earlier, Brevedan et al. (1978) reported similar conclusion under both greenhouse and field conditions. Virk et al. (2018) concluded that N fertilization resulted in 15.2% increase in soybean pod number per plant. The Authors attributed this increase to enhanced vegetative growth caused by fertilization. 21.1% higher pod number per plant was achieved by N fertilization (Abera et al., 2019). He et al. (2019) reported that pod number per plant increased (by 13 and 140% in HD and ZH, respectively) in 60P treatment under drought, whereas 120P did not further increase this trait. They also reported that under well-watered treatment, pod number per plant increased by 74 and 89% in 60P treatment for HD and ZH, respectively, whereas 120P treatment further increased this trait for HD, but not for ZH. Kamara et al. (2007) conducted field experiments to evaluate the response of four soybean cultivars to P application (0, 20, and 40 kg P ha⁻¹). Their results demonstrated that pod number per plant increased by 42.5% when 20 kg ha⁻¹ of P fertilizer was applied, whereas 40 kg ha⁻¹ P increased this trait by 56.0%. Adjei-Nsiah et al. (2019) found out that both P-fertilizer sources did not enhance pod number per plant in the pot experiment, whereas 8.3 and 22.3% more pod per plant were recorded when P was applied from MRP and TSP sources, respectively in the field experiment. Moreover, they concluded that P-fertilizer from TSP source had significantly greater number of pods than both P-fertilizer treatment from MRP source and the non-fertilized control. Similar results were reported earlier by Rani (1999).

3.12. 100-SEED WEIGHT AS AFFECTED BY DROUGHT AND FERTILIZATION

Wei et al. (2018) concluded that moderate drought at R4 or at R6 stage decreased the 100-seed weight of soybean plants by 2.7 and 19.7%, respectively, whereas severe drought caused 26.1 and 44.4% decrease, respectively. Drought at R5 and R6 stages resulted in reduced seed size (Krivosudská and Filová 2013). Soybean plants subjected to drought stress conditions at either flowering or pod filling stage had 10.7 and 13.7% decrease in 100-seed weight, respectively (Sepanlo et al., 2014). Imposing drought stress at R4 stage by reducing available water from 100 to 50% FC slightly reduced 100-seed weight by 3.3%, whereas a significant 14.3% reduction in this trait was recorded when FC was further reduced to 20% (Iqbal et al., 2018). Freitas et al. (2016) reported that drought significantly reduced the average 100-seed weight from 16.5 to 14.5 g; similar conclusion was reported earlier by Popović et al. (2012) who concluded that drought stress resulted in a 21% decrease in 100-seed weight. Subjecting soybean plants to drought stress conditions at V4 stage resulted in a 9% decrease in 100-seed weight, whereas drought stress at R5 stage caused a 36% reduction in this trait (Jumrani and Bhatia, 2018). On the other hand, Gavili et al. (2019) concluded that reducing irrigation water from 100 to 70 and 55% FC was accompanied by a 5.4% 2.9% increase in 100-seed weight; however, the increase was insignificant.

A 3.6% increase in 100-seed weight as a result of N fertilization was reported by Virk et al. (2018). Abera et al. (2019) also reported a 2.7% increase in the 100-seed weight as a result of N fertilization. Inoculation, using two different inocula, significantly increased the 100-seed weight by 33.9% (using TAL 377 inoculum) and by 38.2% (using Isolate-2) (Elsheikh et al., 2009), whereas Temesgen (2017) concluded that inoculation had no significant effect on the 100-seed weight.

3.13. YIELD AS AFFECTED BY DROUGHT, FERTILIZATION AND ROS APPLICATION

A reduction in soybean biomass due to drought stress was early reported (Read and Bartlett, 1972), and confirmed lately (Khan and Setsuko Komatsu, 2016). Particularly, the biomass was significantly decreased when drought stress was applied at R4 stage (Demirtas et al., 2010) more than V4 stage (Maleki et al., 2013). The latter researchers reported also that the effect of drought stress on the harvest index was significant as

drought occurrence at R5 stage reduced it by 27.9% compared to control. Earlier, Ashley and Ethridge, (1978) suggested that the harvest index was reduced due to the loss of flowers and the decrease in seed number per plant.

Soybean seed yield decreases under drought stress conditions as reported by many researchers (Doss et al., 1974; Heatherly and Elmore, 1986; Rose, 1988; Kokubun, 2001; Liu et al., 2003; Dogan et al., 2007; Bajaj et al., 2008; Liu et al., 2008; Sincik et al., 2008; Behtari and Abadiyyan, 2009; Gercek et al., 2009; Manavalan et al., 2009; Masoumi et al., 2011; Sadeghipour and Abbasi, 2012; Li et al., 2013; He et al., 2017b). Moreover, seed yield was reduced by 57.4 and 95.3% as a result of cyclic and terminal drought stress, respectively (He et al., 2017a). 63.7 and 57.1% reduction in soybean seed yield was reported by Sepanlo et al. (2014) in their experiment where drought was imposed at flowering or at pod filling stage, respectively. Drought stress significantly decreased the seed yield in soybean by 35.7% (Freitas et al., 2016). Legume productivity can be greatly reduced both by moderate and severe drought (Saxena et al., 1993; Subbarao et al., 1995). Severe drought stress reduced the seed yield of soybean more than moderate drought stress (Dornbos and Mullen, 1992). Moderate drought at R4 stage reduced soybean seed yield by 31.2%, whereas severe drought at the same stage resulted in 77.7% less seed yield (Wei et al., 2018). The same researchers also reported that subjecting soybean plants to moderate and severe drought at R6 stage decreased the final seed yield by 33.4 and 62.4%, respectively. Many studies concluded that drought stress during the vegetative stages does not measurably affect the yield (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, Doss et al. (1974) and Sionit and Kramer (1977) found that drought stress during R3 and R4 stages resulted in greater yield reduction than that occurred during R1 and R2 stages. 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 (Xie et al., 1994; Jin et al., 2005). Turner et al. (2005) reported a yield reduction by 20% when drought was applied during seed filling. Similar conclusions were presented by many researchers (e.g. Ashley and Ethridge 1978; Huck et al., 1983; Eck et al., 1987; Foroud et al., 1993; Karam et al., 2005; Demirtas et al., 2010; Maleki et al., 2013). Jumrani and Bhatia (2018) subjected soybean plants to drought stress at two different stages; V4 and R5. They reported that the seed yield was decreased by 28 and 74%, respectively compared to control treatment

where no drought stress was imposed, concluding that drought had much higher effect when it was imposed at reproductive stage R5 as compared to vegetative stage V4. Garcia et al. (2010) reported that the genotypes significantly differ in yield production under drought stress conditions and also within the interaction between the drought stress and the genotype; similar conclusion were reported (Brown et al., 1985; Bellaloui and Mengistu, 2008; Maleki et al., 2013; He et al., 2017a). Different explanations for yield decrease under drought stress conditions were suggested; Smiciklas et al. (1992) reported that drought stress shortens the seed-filling period which results in yield loss; others suggested it to be due to the reduction of seeds number (Dornbos et al., 1989), seeds weight (Samarah et al., 2006; Demirtas et al., 2010) and pod number per plant (Atti et al., 2004; Khatun et al., 2016). Seed yield was found to have a significantly positive correlation with flower and pod abortion (Liu et al., 2003), plant height, number of pods and seeds per plant, seed weight and harvest index (Georgiev, 2004; Maleki et al., 2013). When drought was imposed at R4 stage, soybean plants had 32.0 and 48.7% less seed yield under 50 and 20% FC, respectively compared to 100 FC control (Iqbal et al., 2018). The authors concluded that the decrease in seed yield was mainly caused by increased number of empty pods, decreased number of seeds per plant, decreased 100-seed weight and decreased number of pods per plant. Seed yield was decreased by 41 and 64% when available irrigation water was reduced from 100% to 70 and 55% FC, respectively (Gavili et al., 2019). The authors concluded that the decreased seed yield was caused by reduced number of seeds per pot. Drought negatively affects N₂-fixation process and, eventually, the final yield (Purcell et al., 2004; Sinclair et al., 2007). A 10% increase in seed yield was recorded with the application of 200 kg ha⁻¹ N under drought stress imposed at R2 stage, whereas the same application decreased the yield by 1.5% under drought-free conditions (Cerezini et al., 2016).

Hungria et al. (2006) reported that the application of 200 kg ha⁻¹ of N-fertilizer did not increase the yield with the absence of drought, whereas 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. Other reports also concluded that N-fertilizer application resulted in better seed yield under drought stress conditions (e.g. Ray et al., 2006; Salvagiotti et al., 2008). Both fertilization and inoculation significantly increased the yield by 85 and 98%, respectively (Seneviratne et al., 2000). Hungria et al. (2013) reported an average of 8% yield enhancement as a result of inoculation treatment in the areas where *Bradyrhizobium* is

well-established. Silva et al. (2013) concluded that inoculation process increased soybean seed yield by 18%. Similar conclusion was reported by Couto et al. (2011). Adeyemi et al. (2020) reported that inoculation significantly increased soybean yield in both pot (by 55%) and field (by 82%) experiments. Seneviratne et al. (2000) reported that a relatively-small amount of N fertilizer (46 kg/ha) significantly increased the seed yield by 84.7%. Fertilization significantly increased the yield of soybean by 18.3% (Virk et al., 2018) and by 15.1% (Abera et al., 2019); the latter authors attributed this increase to enhancements in growth traits which have led to better carbohydrate synthesis and, consequently, better yield. Salvagiotti et al. (2009) also reported increased yield as a result of N application. Not only N fertilization, but also P fertilization could partially mitigate drought stress; He et al. (2019) concluded that under drought stress conditions, the yield increased by 10 and 50% in 60P, and by 30 and 63% in 120P for HD and ZH, respectively compared to 0P counterpart. Under well-watered conditions, however, 60P increased the yield by 143 and 41% for HD and ZH, respectively, whereas 120P did not have measurable effect on the final yield (He et al., 2019). The authors attributed the yield improvement by P application to the improved filled-pod number and grain number, whereas Belanger et al. (2002) concluded that P application enhanced the shoot biomass and, consequently, the seed yield. In their experiment, Jin et al. (2006) examined the effect of drought stress at R1 and R4 stages on 2 soybean genotypes; Heisheng 101 (genotype with high protein concentration in the seeds) and Dongnong 46 (a genotype with low protein concentration in the seeds) under 3 P-fertilizer rates; 0, 15 and 30 mg kg⁻¹ soil. The authors reported that in Heisheng 101, 15P increased yield by 1.4% when there was no drought, and by 9.3 and 16.5% when drought occurred at R1 and R4 stages, respectively. 30P increased yield by 12.1% compared to 15P under no-drought, but reduced it by 5.9 and 3.4% under drought at R1 and R4, respectively but it was still higher than 0P. In Dongnong 46, only 30P increased yield compared to 0P under no-drought, but both 15P and 30P increased yield by 1.1 and 5.0% when drought happened at R1, and by 52.1 and 68.9% when drought happened at R4 (Jin et al., 2006). They also reported that the application of P fertilizer could mitigate the negative effect of drought stress on the yield of both genotypes and that seed yield was significantly associated with P accumulation before and after the initial pod filling (R5) stage and also with the total P accumulation. Other researchers reported similar effect in soybean (He et al., 2017a), moth bean (Garg et al., 2004) and malting barley (Jones, 2003). Soil available-P deficiency is an important limiting factor in the development and the final yield of soybean (Wissuwa, 2003). Zheng

et al. (2009) studied an area consisting of 43 soybean fields in China in 2007 when soybean plants suffered from severe drought stress. The authors reported that P-fertilizer rate was the highest effecting factor (by 60.6%) that was attributed to differences in the final yield. Adjei-Nsiah et al. (2019) reported that yield was enhanced by P fertilization from both sources (by 10.0 and 8.6% in MPR and TSP treatments, respectively); however, the increases were insignificant. 52 and 63% higher seed yields were recorded in 20P and 40P treatments, respectively compared to 0P counterpart (Kamara et al., 2007). The authors reported that seed yield was strongly associated with pod number per plant and seed weight. Similar conclusions on yield enhancement by P application was also reported by Lampitey et al. (2014) and Ronner et al. (2016). The application of P fertilizer in the recommended rate (35 kg ha⁻¹) significantly increased the yield by 71% (Mahanta et al., 2014).

Exogenous application of H₂O₂ has improved plant biomass in wheat under drought stress (He et al., 2009), and (SA) application improved the grain yield of common bean under drought stress conditions (Sadeghipour and Aghaei, 2012). Horvath et al. (2007) reported that (SA) can enhance metabolite stream to the developing grains, resulting in reducing abortion rate which, in part, can significantly increase pod number per plant and seed number pod⁻¹ in soybean (Khatun et al., 2016). Not only yield, but also yield components (number of grains m⁻², pod per plant) were enhanced with the application of (SA) on soybean leaves under drought stress conditions (Razmi et al., 2017). The authors attributed the increase of grain yield due to (SA) application to Improved RWC, reduced restrictions of stomatal conductance and the enhanced biosynthesis of photosynthetic pigments in the leaves.

3.14. PROTEIN CONCENTRATION AS AFFECTED BY DROUGHT AND FERTILIZATION

Sepanlo et al. (2014) concluded that drought stress imposed on soybean plants at pod filling stage resulted in 15.5% reduction in protein concentration in the seeds. Reduced protein concentration under drought stress conditions was also reported by other researchers (e.g. Rose, 1988; Specht et al., 2001; Boydak et al., 2002; Turner et al., 2005; Carrera et al., 2009). However, few studies showed no effect of drought stress on seed protein concentration (e.g. Sionit and Kramer, 1977). On the other hand, increased protein concentration under drought stress was also reported (e.g. Dornbos and Mullen, 1992,

Kumar et al., 2006; Bellaloui and Mengistu, 2008; Wang and Frei, 2011). This was explained by drought stress rapidly remobilizing nitrogen from leaves to seeds which leads to increasing protein concentration (DeSouza et al., 1997; Brevedan and Egli, 2003). Borrás et al. (2004), on the other hand, attributed this increase to the reduced seed number with increased seed size. The relationship between drought stress and soybean seed composition remains controversial (Medic and Atkinson 2014). Differences among the reported conclusions were suggested to be due to the timing and intensity of the drought stress during the different stages (Carrera et al., 2009). Bellaloui and Mengistu (2008) suggested that the plant's response to drought stress might be cultivar-dependent as well.

In general, protein concentration is increased when N is increased (Ham et al., 1975), whether the source of N is mineral fertilization or N₂ fixation. Fabre and Planchon (2000) reported protein concentration to be positively associated with N₂ fixation, especially during pod filling stages; similar results were reported by Leffell et al. (1992). Rotundo and Westgate (2009) reported that the addition of N fertilizer during the vegetative stages has led to about 2% increase in protein concentration, whereas the increase ratio was 3% when N was added at early reproductive stages. The authors also concluded, from their meta-analysis study, that adding N fertilizer increased protein concentration about 27% in all study environments; particularly, the increase was about 8% in field studies. N fertilizer dose has a significant effect on the seed protein concentration; the dose of 100 kg ha⁻¹ increased seed protein just 2%, whereas the dose of 200 kg ha⁻¹ resulted in 14% increase in seed protein (Miransari, 2016). Bloom (2006) reported that increasing applied-N rate was accompanied by enhanced protein concentration. N Fertilization increased % seed-N by 8.3% (Seneviratne et al., 2000). Abera et al. (2019) also reported that N fertilization increased protein concentration in soybean seeds by 4.1%. P-fertilizer also has an effect on soybean seed protein as reported by Jin et al. (2006) who demonstrated that both 15P and 30P treatments increased seed protein compared to 0P, however, 15P was higher than 30P in most cases (Jin et al., 2006).

3.15. OIL CONCENTRATION AS AFFECTED BY DROUGHT AND FERTILIZATION

Sepanlo et al. (2014) reported that drought at flowering stage increased oil concentration in soybean seeds by 5.7%, and further increased it (by 19.7%) when drought was imposed at pod filling stage. Boydak et al. (2002) also concluded that drought stress enhanced oil

concentration. However, results of many other studies indicated that drought stress reduced oil concentration in soybean seeds (e.g. Rose, 1988; Bellaloui and Mengistu, 2008; Rotundo and Westgate, 2009; Maleki et al., 2013). However, Gao et al. (2009) reported that drought stress had little effect on the oil content. The timing of drought stress was concluded 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 by 35% of oil content (Rotundo and Westgate, 2009). The effect of drought stress on oil content was different at different stages, and the lowest oil percentage was obtained when drought stress was applied at V5 stage (Dornbos and Mullen, 1992; Smiciklas et al., 1992; Maleki et al., 2013). In general, soybean seed protein content is negatively correlated with the amount of seed oil (Chung et al., 2003).

Silva et al. (2013) reported that the fatty acid content in the soybean seeds inoculated with *Bradyrhizobium japonicum* was significantly higher than non-inoculated counterparts. Brechenmacher et al. (2010) reported that the inoculation process with *B. japonicum* has a role in fatty acid production which, in part, increases the cell membrane's fluidity, and helps the bacteria successfully colonize the cells. Moreover, this increase in the cell membrane's fluidity enhances abiotic stress tolerance (Brechenmacher et al., 2010). Similarly, Elsheikh et al. (2009) reported that 49.8 and 56.5% more fat concentration could be achieved by inoculating soybean seeds (with TAL 377 and Isolate-2 inocula, respectively). However, mineral N-Fertilizer reduced oil concentration in soybean seeds by 5.4% as reported by Abera et al. (2019). As for P-fertilizer application, Costache and Nica (1968) and Dadson and Acquaaah (1984) concluded that increasing P rate significantly increased oil concentration in the seeds. Also, Win et al. (2010) reported that adding 1.0 mmol l⁻¹ of P (in the form of KH₂PO₄) to Hoagland solution (1 mM P) increased oil concentration in three soybean cultivars by 7.1%, whereas further increasing P concentration to 2 mM P reduced oil concentration by 3.3% compared to 1 mM P treatment, yet it was still higher than non-fertilized control by 3.6%.

4. MATERIALS AND METHODS

4.1. FIELD EXPERIMENTS

4.1.1. Location of Field Experiments

All field experiments were carried out in the experimental station of the University of Debrecen (Látókép) (N. latitude 47° 33', E. longitude 21° 27') during 2017, 2018 and 2019 growing seasons. Soil type of the site is calcareous chernozem. Table 3 shows the outcome of the soil chemical analysis before sowing in 2017 growing season.

Table 3. Soil chemical analysis in Látókép, Debrecen in 2017.

Depth (cm)	pH (KCl)	K _A	CaCO ₃ %	OM %	Total N %	NO ₃ + NO ₂ ppm	P ₂ O ₅ ppm	K ₂ O ppm	Mg ppm	Na ppm	Zn ppm	So ₄ ppm
0-25	6.46	43.0	0	2.76	0.15	6.20	133.4	239.8	332.4	38.0	2.80	9.25
25-50	6.36	44.6	0	2.16	0.12	1.74	48.0	173.6	405.4	66.2	0.80	9.13
50-75	6.58	47.6	0	1.52	0.09	0.60	40.4	123.0	366.6	55.4	0.58	10.80
75-100	7.27	46.6	10.25	0.90	0.08	1.92	39.8	93.6	249.0	67.8	0.48	7.95
100-125	7.36	45.4	12.75	0.59	0.08	1.78	31.6	78.0	286.6	62.6	0.84	22.98

4.1.2. Agro-technical applications in Field Experiments

Table 4 shows the different agro-technical applications during 2017, 2018 and 2019 growing seasons.

Table 4. Agro-technical applications during 2017, 2018 and 2019 growing seasons in Látókép, Debrecen.

2016/2017 growing season	
September 26 th , 2016	Maize stem-crush
October 5 th , 2016	Disk plouing and rolling (Güttler)
November 7 th , 2016	Ploughing (32-35 cm)
March 7 th , 2017	Softening
March 31 st , 2017	Plowing (after manual fertilizer application)
April 13 th , 2017	Plowing
April 26 th , 2017	Plowing + sowing + rolling
2017/2018 growing season	
October 3 rd , 2017	Maize stem-crush
October 16 th , 2017	Disk plouing and rolling (Güttler)
November 17 th , 2017	Ploughing (32-35 cm)
April 10 th , 2018	Plowing
April 13 th , 2018	Plowing (after manual fertilizer application)
April 22 nd , 2018	Plowing
April 23 rd , 2018	Plowing + sowing + rolling
2018/2019 growing season	
September 12 th , 2018	Maize stem-crush
September 13 th , 2018	Disk plouing and rolling (Güttler)
October 5 th , 2018	Ploughing (32-35 cm)
March 4 th , 2019	Plowing
April 2 nd , 2019	Plowing (after manual fertilizer application)
April 24 th , 2019	Plowing + sowing + rolling

In addition, chemicals were applied as needed throughout the experiments period as shown in table 5.

Table 5. Chemicals applied during 2017, 2018 and 2019 growing seasons in Látókép, Debrecen.

2016/2017 growing season	
April 27 th , 2017	Stomp Super 5 l/ha Pre-emergence weed control
June 1 st , 2017	Pantera 40 EC 1l/ha Post-emergence weed control
June 28 th , 2017	Bellis 0,8 kg/ha, Nissuron 0,5 kg/ha Treatment with fungicide and insecticide
July 14 th , 2017	Ridomil Gold Plus 4kg/ha Fungicide treatment
September 1 st , 2017	Total 4l/ha, Elastiq ultra 1l/ha Desiccation
2017/2018 growing season	
May 15 th , 2018	Pulsar 40 SI 1,2 l/ha Post-emergence weed control
July 2 nd , 2018	Ridomil Golg Plus 4 kg/ha Nissoran 0,5 kg/ha Fungicide and insecticide treatment
August 31 st , 2018	Figaró 5 l/ha Desiccation
2018/2019 growing season	
April 26 th , 2019	Stomp 5 l/ha Pre-emergence weed control
May 15 th , 2019	Pulsar Post-emergence weed control
September 16 th , 2019	Figaró 5 l/ha Desiccation

4.1.3. Weather Conditions of Field Experiments

The mean temperature during the period before sowing was characterized by colder levels in January during 2017 and 2019 compared to the average of the past 10 years before the experiment (2007 – 2016), followed by very close levels from February to April, whereas it followed an opposite trend in 2018 (Fig. 2).

During the vegetative period of soybean (from May till September), the mean temperature during 2017 and 2019 was very close to average, whereas 2018 had higher levels during

the early vegetative stages in May, and also during the late reproductive stages in July and August (Fig. 2).

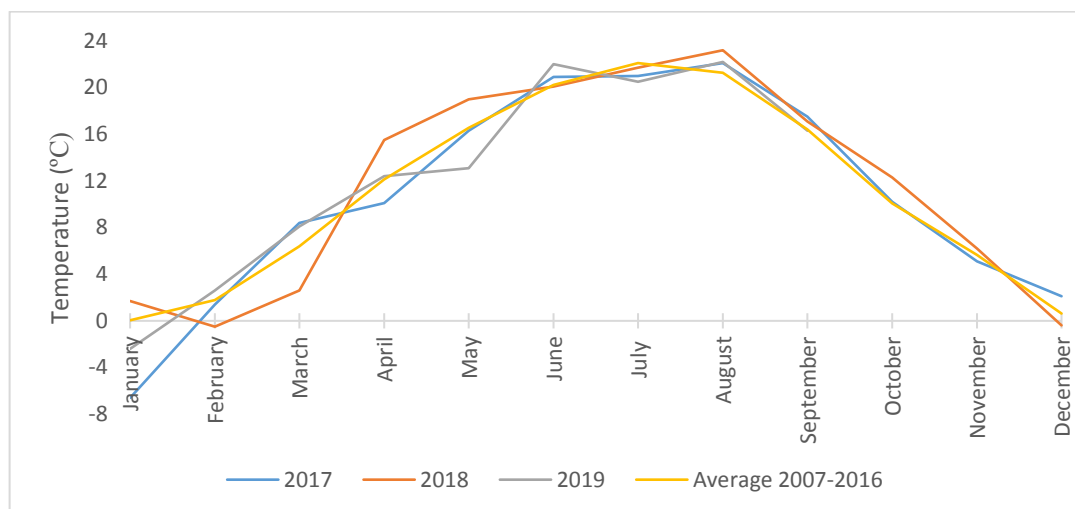


Fig. 2. Mean temperature in 2017, 2018 and 2019 compared to the average 2007-2016 in Látókép, Debrecen, Hungary. Source: meteorological station in the experimental site.

As for precipitation distribution and amounts, the early months of the year before sowing (from January till March) experienced relatively less precipitation amounts in 2017 compared to 2007-2016 average, whereas April had above-average precipitation. In 2018, the precipitation in both February and March was measurably higher than the average, whereas April received an equivalent amount. 2019, on the other hand, followed an opposite trend compared to 2018 as February and March were the months where significant low precipitation was recorded, whereas April was slightly above the average (Fig. 3).

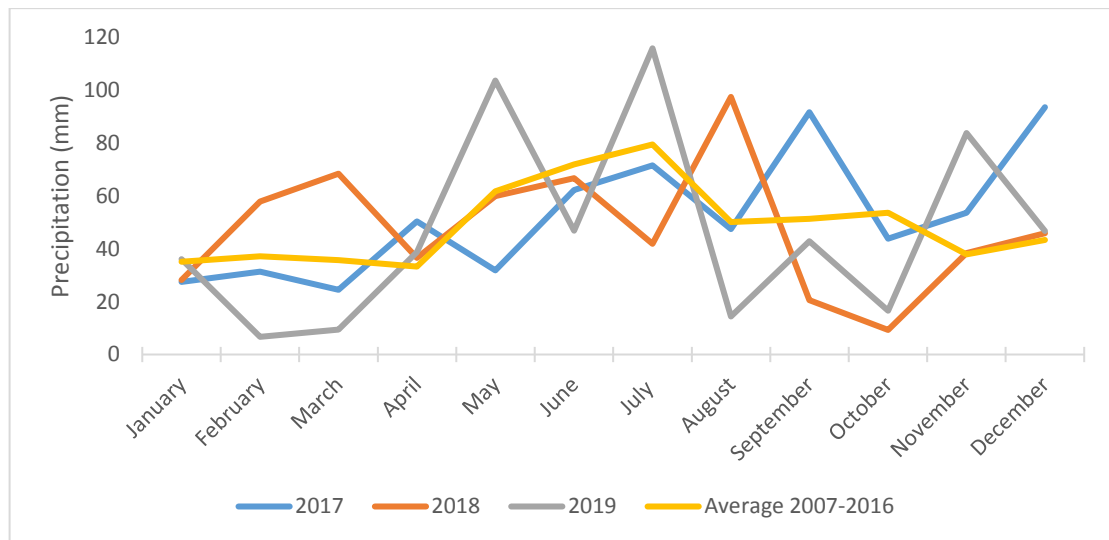


Fig. 3. Precipitation amounts in 2017, 2018 and 2019 compared to the average 2007-2016 in Látókép, Debrecen, Hungary. Source: meteorological station in the experimental site.

During the vegetative period of soybean plants, precipitation in May was less than the average in 2017, close to the average in 2018 and higher than the average in 2019. The two years of 2017 and 2018 were close to the 10-year average precipitation amount in June, whereas 2019 was the year where precipitation was noticeably less than the average. In July, precipitation amount in 2017 was close to the average, whereas 2018 had lower, and 2019 had higher precipitation amount compared to the average. On the contrary in August, 2017 was also close to average, whereas 2018 was higher, and 2019 was lower than the average (Fig. 3).

4.1.4. The Effect of Drought Stress on The Morpho-physiology, Yield Components and Seed Quality of 7 Soybean Genotypes

A field experiment was carried out on 7 soybean genotypes; Bokréta, Bólyi 612, ES Pallador, ES Mentor, Pannonia Kincse, Coraline and Ananda. The reason of choosing these genotypes was because they are well used in the different soybean growing regions in Hungary. In addition, these genotypes belong to different maturity groups, enabling for different comparisons by means of morpho-physiological, yield components and quality traits. Sowing dates were April 26th in 2017, April 23rd in 2018 and April 24th in 2019, and harvest dates were September 15th in 2017, September 16th in 2018 and September 23rd in 2019.

The experimental design was split-plot design, with 2 irrigation regimes; drought-stressed regime (DS) and fully-irrigated regime (FI) being the main plots and the 7 genotypes being the sub-plots with 4 replications. The final plot number was 56 (7 genotypes * 2 irrigation regimes * 4 replications). The plot area was 23.4 m², with 6 rows in each plot (annex 1).

DS treatment received only precipitation as water irrigation amount, whereas FI treatment received, in addition to precipitation, a total of 80mm of irrigation water in 2017 and 100 mm in 2018 and 2019.

4.1.5. The Effects of Drought Stress and Nitrogen Fertilization on The Morphophysiology, Yield Components and Seed Quality of 2 Soybean Genotypes

2 field experiments were carried out on 2 soybean genotypes; '*Pannonia Kincse*' and '*Boglár*' during 2017, 2018 and 2019 growing seasons. Both genotypes are considered among the leading varieties in the Hungarian market. '*Pannonia Kincse*' is a middle maturity group (I) genotype, whereas '*Boglár*' is an early maturity group (0) genotype, with a tendency of drought tolerance.

In the first experiment, '*Pannonia Kincse*' genotype was sown in a split-plot design; 3 irrigation regimes; non-irrigated, half-irrigated and fully irrigated (NI, HI and FI, respectively) represented the main plots, and 3 N-fertilizer rates (applied with sowing as a single application in the form of NH₄NO₃); 0, 35 and 105 kg ha⁻¹ N (0N, 35N and 105N, respectively) represented the sub-plots with 4 replications each. Phosphorus (P) and Potassium (K) fertilizers were applied adequately at the time of sowing. Final plot number for this experiment was 36 (3 irrigation regimes * 3 fertilization rates * 4 replications). The plot area was 27 m² with 12 rows in each plot (Annex 2).

In the second experiment, '*Boglár*' genotype was sown in a split-split-plot design; the same 3 irrigation regimes represented the main plots, and 2 inoculation treatments; inoculated with *Bradyrhizobium japonicum* inoculant (+) and non-inoculated (-) represented the sub-plots, and the same 3 N-fertilizer rates represented the sub-sub-plots. Phosphorus (P) and Potassium (K) fertilizers were applied adequately at the time of sowing. Final plot number for this experiment was 72 (3 irrigation regimes * 2 inoculation treatments * 3 fertilization rates * 4 replications). The plot area was 27 m² with 12 rows in each plot (Annex 3).

In both experiments, NI treatment received only precipitation as water irrigation amount, whereas HI treatment received, in addition to precipitation, a total of 40 mm of irrigation water in 2017 and 50 mm in 2018 and 2019. FI treatment, on the other hand, received, in addition to precipitation, a total of 80 mm of irrigation water in 2017 and 100 mm in 2018 and 2019 (Fig. 4).

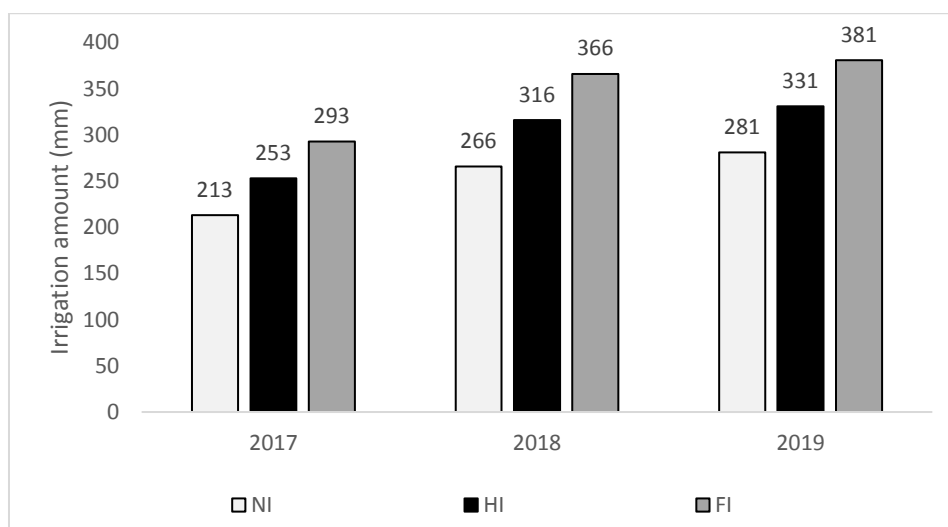


Fig. 4. Irrigation amounts during the vegetative period of soybean genotypes in 2017, 2018 and 2019 in Látókép, Debrecen. NI: non-irrigated, HI: half-irrigated, FI: fully-irrigated.

4.1.6. The Effects of Drought Stress and Phosphorus Fertilization on The Morphophysiology, Yield Components and Seed Quality of 2 Soybean Genotypes

Two soybean genotypes; ‘*Pannonia Kincse*’ and ‘*Boglár*’ were sown on April 23rd and 24th, and were harvested on September 15th and 23rd in 2018 and 2019, respectively. The experimental design was split-split plot design, with genotypes being the main plots, irrigation regimes being the sub-plots and P fertilization rates being the sub-sub-plots. Three P-fertilizer rates; 0, 45 and 90 kg ha⁻¹ P₂O₅ (0P, 45P and 90P, respectively) were applied under two irrigation regimes; drought stress regime (accounting only on the precipitation as the only source of water supply) and fully-irrigated regime (where, in addition to precipitation, a total of 100 mm of irrigation water was applied). Each treatment consisted of three replications. Final plot number was 36 (2 genotypes * 2 irrigation regimes * 3 fertilization rates * 3 replications). The plot area was 10 m² with 6 rows in each plot (Annex 4).

4.1.7. The Effects of Drought Stress and Exogenous Application of Hydrogen Peroxide (H₂O₂) on The Physiology and The Yield of 2 Soybean Genotypes

Two soybean genotypes; '*Pannonia Kincse*' and '*Boglár*' were sown on April 23rd and 24th and were harvested on September 15th and 23rd in 2018 and 2019, respectively. The experimental design was split-plot design, with genotypes representing the main plots and irrigation treatments being the sub-plots. Three irrigation treatments were applied, each of which in three replications; fully-irrigated (FI) treatment where, in addition to precipitation, a total of 100 mm of irrigation water was applied, drought-stressed treatment (counting only on precipitation) with the application of 1mM of Hydrogen Peroxide (H₂O₂) as a foliar spray at R1 stage (Fehr and Caviness, 1977) (HP) and drought-stressed treatment with distilled-water foliar spray at R1 stage (DW). The final number of plots was 18 (2 genotypes * 3 irrigation treatments * 3 replications), with a plot area of 10 m². Each plot had 6 rows (Annex 5).

All traits were measured at R2 stage (1-2 weeks after H₂O₂ application).

4.1.8. Measurements of Field Experiments

To calculate the relative water content, ten fully-matured leaves were collected and fresh weight (FW) of each leaf was measured immediately. Dry weight (DW) was determined via drying the sample leaves at 80 °C to constant weight, and turgid weight (TW) was obtained after floating the leaves in distilled water at 4 °C for 48 h. RWC was calculated as $RWC(\%) = (FW - DW) / (TW - DW) \times 100\%$ (Weatherley, 1950).

Stomatal conductance (g_s) was measured using AP4 porometer (Delta-t devices, UK).

Leaf Area Index (LAI) values were recorded using SS1 – SunScan canopy analysis system (Delta- T Devices, UK). Relative chlorophyll content (in the form of Soil Plant Analysis Development; SPAD) was measured using SPAD-502Plus (Konica Minolta, Japan). Normalized Difference Vegetation Index (NDVI) values were recorded using Trimble Greenseeker Handheld (AS Communications Ltd, UK). 10 randomly-selected plants from the middle rows of each plot were used for the mentioned traits, and 3 measurements from the second most developed trifoliate (1 measurement for each leaflet) were taken and then averaged. All traits were measured at four different stages of

soybean's life cycle (Fehr and Caviness, 1977); fourth node (V4), full bloom (R₂), full pod (R4) and full seed (R6).

Flower number per plant was determined at R2 stage. Pod number per plant was determined at R4 stage. Plant height was measured at R6 stage using a standard ruler. 10 randomly-selected plants from the middle rows of each plot were used for these traits.

Seed yield was calculated by harvesting the middle 4 meters of each plot and adjusting the yield to 13% moisture content. 100-seed weight was determined after oven-drying the seeds at 65 °C until constant weight. Both protein and oil concentrations were determined using NIR analyser Granolyser (Pfeuffer, Germany).

4.2. CLIMATE CHAMBER EXPERIMENTS

4.2.1. The Effects of PEG-induced Drought Stress on The Germination Parameters of 2 Soybean Genotypes

The experiment was conducted on a two-stage basis at the Institute of Crop Sciences, University of Debrecen in 2018.

In a preliminary field experiment, 20 soybean genotypes were subjected to continuous drought stress conditions by withholding irrigation water throughout the vegetative period of soybean plants. After the harvest, a cluster analysis was conducted to group the genotypes based on their reactions to drought. Based on the results of the cluster analysis, two genotypes; ES Mentor (100-seed weight = 201 g) and 'Pedro' (100-seed weight = 161 g) were chosen for this experiment.

In both stages, the 2 soybean genotypes were surface-sterilized using 6% (v/v) H₂O₂ for 20 minutes, rinsed extensively with deionized water and germinated geotropically between moisten filter papers at 22 °C. Each roll contained 30 seeds. PEG 6000 (VWR International bvba Geldenaaksebaan, Leuven, Belgium) was used in both stages to induce drought stress.

In the first stage, PEG concentrations of 0, 10, 15, 20, 25 and 30% were applied, whereas in the second stage, and based on the results of the first stage, 0, 2.5, 5, 7.5, 10, 12.5 and 15% concentrations were applied. Each treatment (concentration) had 3 replications in both stages.

Germinated seeds were counted every day, and the daily-associated root elongation was measured. Each stage was considered finished when the average hypocotyl of the control treatment reached 3 cm long.

Germination energy (GE) was expressed as the percentage of germinated seeds after five days from the beginning of each stage of the experiment.

From the germination counts the following germination parameters were determined (Saxena et al., 1996);

(1) Ultimate Germination (UG): The maximum number of seeds that germinated during the experiment.

(2) Mean Period of Ultimate Germination (MPUG) = $\frac{\sum_{i=1}^d N_i D_i}{UG}$

(3) Percentage Inhibition or Stimulation = $\frac{UG \text{ in aqueous extracts (\%)}}{UG \text{ in distilled water (\%)}}$

where,

N is the daily increase in seedling number, and D is the number of days from seed placement.

4.2.2. *The Effects of PEG-induced Drought Stress on The Physiology of 2 Soybean Genotypes*

Seedlings of the two soybean genotypes were germinated following the above-mentioned method under no-stress conditions (0% PEG) for using them in this experiment. Seedlings with good vigor were then planted in 5-liter pots. Each pot contained 10 seedlings. Each pot received 50 ml of dicot nutrient solution that consisted of the following substances: 2.0 mM Ca(NO₃)₂, 0.7 mM K₂SO₄, 0.5 mM MgSO₄, 0.1 mM KH₂PO₄, 0.1 mM KCl, 10 μM H₃BO₃, 0.5 μM MnSO₄, 0.5 μM ZnSO₄ and 0.2 μM CuSO₄. Iron was supplied in the form of 10⁻⁴M Fe-EDTA (Cakmak and Marschner 1990), in addition to corresponding PEG solution. Nutrient solution of each pot was replaced with fresh alternative every 3 days. PEG 6000 (VWR International bvba Geldenaaksebaan, Leuven, Belgium) was used to induce drought stress. PEG concentrations were as follows; 0, 2.5, 5, 7.5 and 10% (0%PEG, 2.5%PEG, 5%PEG, 7.5%PEG and 10%PEG, respectively).

Relative chlorophyll content (SPAD values) was recorded using SPAD-502Plus (Konica Minolta, Japan). Stomatal conductance (g_s) was measured using AP4 porometer (Delta-t devices, UK). Both SPAD and (g_s) were calculated by averaging 10 values per leaf of the second most recently developed trifoliate.

Chl-fluorescence was determined on dark-adapted leaves (20 min of dark adaptation) by attaching light exclusion clips to the central region of each leaf. Chl-fluorescence parameters were measured using a portable chlorophyll fluorometer-PAM-2100 (WALZ, Germany) as described by Schreiber et al. (1986). The fluorescence parameters included the minimal fluorescence (F_0) when PS II centres are open (open state) and increases the maximum fluorescence (F_m) when PS II centres are closed (closed state), the variable fluorescence (F_v), the potential photosynthetic capacity (F_v/F_0) which reflects the efficiency of electron donation to PS II and the ratio $(F_m - F_0)/F_m$, also known as F_v/F_m (potential/maximum photochemical efficiency of PS II) which is calculated from fluorescence values F_0 and F_m . The F_v/F_m ratio is one of the most common parameters used in fluorescence that reflects the capacity to trap electron by the PS II reaction centre. The actual photochemical efficiency of PS II (Yield) was also recorded. All of the fluorescence parameters were recorded from the second last fully-developed trifoliate of one seedling in every pot (replication).

Chlorophylls a and b and total carotenoids concentrations were calculated using the method described by Wellburn (1994); 50 mg of each leaf was blended with 5ml N,N-Dimethylformamide (N,N-DMF). This solution was cooled down at 4°C for 72 hours and finally, the extract content of the pigment was determined using UV–VIS spectrophotometry (Metertech SP-830 PLUS, Taiwan) at three wavelengths; 480, 647 and 664 nm (Moran and Porath 1980). The following equations were used for quantifying chl $_a$ and b and total carotenoids contents (Wellburn, 1994):

$$\text{Chl}_a (\mu\text{g ml}^{-1}) = (11.65 A_{664} - 2.69 A_{647})$$

$$\text{Chl}_b (\mu\text{g ml}^{-1}) = (20.81 A_{647} - 4.53 A_{664}).$$

$$\text{Chl}_{x+c} (\mu\text{g ml}^{-1}) = (1000 A_{480} - 0.89 \text{Chl}_a - 52.02 \text{Chl}_b)/245$$

Each treatment had 3 replications in a split-plot design where the genotype represented the main plots and PEG concentrations represented the sub-plots. The total number of pots was 30 (2 genotypes x 5 PEG treatments x 3 replicates).

All measurements were made at 4 different stages of each genotype (Fehr and Caviness, 1977); second node (V2), fourth node (V4), full bloom (R2) and full pod (R4).

4.3. STATISTICAL ANALYSIS

For germination parameters experiment, the statistical analysis was conducted using “Repeated Measurement” method (IBM SPSS ver.26, USA software) software. For all other experiments, SPSS software was run to analyze and compare the means (ANOVA) and to indicate the effect size (by means of Partial Eta Squared), followed by Tukey post-hoc test to indicate the statistically-different means, and Pearson’s correlation to indicate correlation coefficient (IBM SPSS ver.26, USA software). All data presented and analyzed in all field experiments are means of the three years of experiment.

5. RESULTS AND DISCUSSION

5.1. FIELD EXPERIMENTS

5.1.1. *The Effect of Drought Stress on The Morpho-physiology, Yield Components and Seed Quality of 7 Soybean Genotypes*

5.1.1.1. *The Effect of Drought Stress on The Relative Chlorophyll Content (SPAD), Normalized Difference Vegetation Index (NDVI) and Leaf Area Index (LAI) of 7 Soybean Genotypes*

Irrigation had no significant effect on SPAD values; moreover, it did not have a steady trend through genotypes or stages. In most genotypes, drought reduced SPAD in certain stages but increased it in other stages, whereas in genotype Bólyi 612, drought resulted in better SPAD readings throughout the whole stages. Therefore, drought resulted, averaged over the whole season, in decreased SPAD in some genotypes (ES Pallador and 'Pannonia Kincse') while it enhanced this trait in the others (Table 6). This result can lead to a conclusion that SPAD can not be considered as a reliable trait when evaluating the effect of drought stress on soybean, or at least on the studied genotypes, in the study area.

On the other hand, the stage at when the measurement was taken had a significant effect ($p < 0.05$) on SPAD trait, with also different trends among genotypes. Regardless of irrigation regime, SPAD had the highest value at the early V4 stage in both Bokréta and Bólyi 612, whereas it was the highest at the late R6 stage in the case of ES Pallador, 'Pannonia Kincse' and Ananda, and at R4 stage in both Coraline and ES Mentor (Table 6).

Genotypes had highly significant effect ($p < 0.01$) on SPAD values, which can be logically concluded based on the different trends mentioned above in the different genotypes. Genotype effect on this trait was estimated as 39.7% (Table 7), supporting the conclusion of SPAD trait being different from a genotype to another.

Although its effect was not significant, yet drought decreased NDVI in all genotypes at all studied stages.

Table 6. SPAD, NDVI and LAI of 7 soybean genotypes at different stages under drought-stressed (DS) and fully-irrigated (FI) treatments in Látókép, Debrecen averaged over 2017, 2018 and 2019.

Genotype	Stage	SPAD		NDVI		LAI (m ² m ⁻²)	
		FI	DS	FI	DS	FI	DS
Bokréta	V4	38.2	38.0	75.7	73.1	2.1	1.7
	R2	35.8	35.6	81.8	80.6	5.1	4.8
	R4	37.1	36.7	80.2	79.5	8.4	7.1
	R6	29.2	31.3	66.7	64.3	4.0	4.4
	Average	35.1	35.4	76.1	74.3	4.9	4.5
Bólyi 612	V4	39.9	40.1	76.5	71.8	2.6	2.1
	R2	38.5	39.6	84.2	83.4	6.2	5.5
	R4	36.8	36.8	81.0	81.0	9.5	7.3
	R6	37.4	37.5	77.7	74.8	5.9	6.9
	Average	38.2	38.5	80.2	77.3	6.1	5.4
ES Pallador	V4	43.2	44.1	73.5	70.1	1.9	1.5
	R2	44.1	43.5	84.7	83.9	4.6	3.8
	R4	41.7	42.8	83.8	83.5	9.9	7.6
	R6	49.2	46.4	81.3	79.1	7.0	6.2
	Average	44.5	44.2	80.8	79.2	5.9	4.8
ES Mentor	V4	41.6	42.4	75.8	72.2	2.2	1.6
	R2	40.2	40.3	84.3	83.8	4.9	4.5
	R4	44.4	43.9	83.2	82.6	6.9	5.6
	R6	27.8	32.3	63.4	61.1	2.6	2.9
	Average	38.5	39.7	76.7	74.9	4.1	3.7
Pannonia Kincse	V4	43.3	43.1	77.4	75.4	2.3	2.1
	R2	42.8	43.0	84.3	84.2	5.9	5.0
	R4	41.6	42.5	82.6	81.3	9.9	7.2
	R6	46.4	43.5	80.4	77.4	6.0	5.8
	Average	43.6	43.0	81.2	79.6	6.0	5.0
Coraline	V4	39.3	40.8	77.3	71.2	2.4	1.8
	R2	38.9	39.1	82.9	82.0	5.4	4.4
	R4	41.5	43.3	82.1	81.7	7.2	6.2
	R6	33.5	32.2	66.3	60.7	3.5	3.1
	Average	38.3	38.9	77.1	73.9	4.6	3.9
Ananda	V4	41.8	41.8	76.6	75.6	2.4	2.0
	R2	40.7	43.0	83.9	83.8	5.4	4.7
	R4	38.3	39.8	83.0	82.1	9.7	8.0
	R6	47.6	45.2	80.9	80.3	6.6	6.0
	Average	42.1	42.4	81.3	80.2	6.0	5.2

Table 7. The effect size of genotype, irrigation regime and stage on SPAD, NDVI and LAI traits.

Factor	Trait	F value	Sig.	Effect size (%)
Genotype	SPAD	12.273	.000	39.7
	NDVI	2.207	.047	10.6
	LAI	4.562	.000	19.6
Irrigation regime	SPAD	.157	.693	0.01
	NDVI	1.957	.165	1.7
	LAI	8.107	.005	6.7
Stage	SPAD	2.762	.045	6.9
	NDVI	18.683	.000	33.4
	LAI	86.682	.000	69.9

The effect of the stage was highly significant; NDVI values were at their maximum at R2 stage, regardless of irrigation regime and genotype. On the other hand, some genotypes had the minimum NDVI at the earlier studied stage (V4) where the plants were still developing their vegetative growth, whereas others had its minimum value at the late season (R6) stage when plant were getting ready for converting into maturity stages (Table 6).

Genotype's effect on NDVI values was also significant, with Ananda having the highest average NDVI (80.8) and Bokréta with the lowest (75.2) (Table 7).

In all genotypes, drought decreased the average LAI. In certain genotypes, LAI was lower in the drought-stressed treatments throughout the season (i.e. at all stages), whereas in other genotypes, LAI of drought-stressed treatment could maintain slightly higher value at the late R6 stage but without affecting the average seasonal decrease caused by drought (Table 6).

Regardless of irrigation and genotype, LAI gradually increased through stages, starting with a minimum value at V4 stage and reaching its peak at R4 stage (Table 6).

Highly significant effect of genotype factor on LAI trait was found out and estimated at 19.6%. The highest LAI throughout the experiment period was recorded in Bólyi 612 (5.8), and the lowest was in ES Mentor (3.9) (Table 6).

All the three factors; irrigation, stage and genotype had highly significant effect on LAI trait, which might suggest this trait to be the most reliable physiological trait to count on in evaluating soybean's performance in the study area (Table 7).

5.1.1.2. The Effect of Drought Stress on The Plant height of 7 Soybean Genotypes

Table 8. Plant height (cm) of 7 soybean genotypes under drought-stressed (DS) and fully-irrigated (FI) treatments in Látókép, Debrecen averaged over 2017, 2018 and 2019.

Genotype	DS	FI	Average
ES Mentor	80.1 ^{bD}	92.1 ^{aB}	86.1
Bokréta	89.2 ^{bBC}	95.3 ^{aAB}	92.2
Coraline	90.1 ^{bB}	96.7 ^{aA}	93.4
Bólyi 612	85.9 ^{bC}	91.6 ^{aB}	88.7
Ananda	93.6 ^A	96.5 ^A	95.1
ES Pallador	91.0 ^{bAB}	97.0 ^{aA}	94.0
Pannonia Kincse	90.2 ^B	92.4 ^B	91.3
Average	88.6	94.5	91.6

- Different small letters in each genotype indicate significant difference at .05 level between irrigation treatments.
- Different capital letters in each irrigation treatment indicate significant difference at .05 level among genotypes.

In all genotypes, drought decreased plant height; the average reduction of all genotypes was calculated as 6.2%. Moreover, the decrease was significant in Bokréta, Coraline, Bólyi 612 and ES Pallador, whereas it was not significant in the other genotypes (Table 8).

There were also significant differences among genotypes under both irrigation regimes. Under fully-irrigated treatment, ES Pallador plants showed the highest value of this trait, whereas Bólyi 612 plants showed the lowest value. Under drought-stressed treatment, Ananda had the tallest plants, whereas ES Mentor was significantly shorter than all other genotypes (Table 8).

5.1.1.3. The Effect of Drought Stress on The Flower number per plant of 7 Soybean Genotypes

Drought decreased the flower number per plant in all genotypes; the decrease was, on average, 13.9%, being significant in Bokréta, Coraline, Bólyi 612 and ES Pallador (Table 9).

Regardless of irrigation regime, significant differences in this trait were recorded among genotypes, with Bólyi 612 having the highest flower number and Bokréta having the lowest flower number under both irrigation regimes compared to the other genotypes (Table 9).

Table 9. Flower number per plant of 7 soybean genotypes under drought-stressed (DS) and fully-irrigated (FI) treatments in Látókép, Debrecen averaged over 2017, 2018 and 2019.

Genotype	DS	FI	Average
ES Mentor	45.8 ^B	48.3 ^C	47.0
Bokréta	36.0 ^{bC}	46.7 ^{aC}	41.3
Coraline	39.8 ^{bC}	47.7 ^{aC}	43.7
Bólyi 612	55.0 ^{bA}	70.4 ^{aA}	62.7
Ananda	48.3 ^B	50.8 ^C	49.5
ES Pallador	47.8 ^{bB}	58.2 ^{aB}	53.0
Pannonia Kincse	49.5 ^B	51.8 ^C	50.6
Average	46.0	53.4	49.7

- Different small letters in each genotype indicate significant difference at .05 level between irrigation treatments.
- Different capital letters in each irrigation treatment indicate significant difference at .05 level among genotypes.

5.1.1.4. The Effect of Drought Stress on The Pod number per plant of 7 Soybean Genotypes

Both irrigation and genotype factors followed the same trend of the previous trait (flower number per plant), as Bólyi 612 and Bokréta, under both irrigation treatments, had the highest and the lowest pod number, respectively (Table 10).

Table 10. Pod number per plant of 7 soybean genotypes under drought-stressed (DS) and fully-irrigated (FI) treatments in Látókép, Debrecen averaged over 2017, 2018 and 2019.

Genotype	DS	FI	Average
ES Mentor	29.3 ^{bc}	34.6 ^{aD}	31.9
Bokréta	29.2 ^{bc}	34.4 ^{aD}	31.8
Coraline	30.3 ^{bc}	36.9 ^{aCD}	33.6
Bólyi 612	47.4 ^{bA}	54.6 ^{aA}	51.0
Ananda	37.8 ^B	40.5 ^C	39.2
ES Pallador	40.2 ^{bb}	46.2 ^{aB}	43.2
Pannonia Kincse	42.2 ^B	46.1 ^B	44.1
Average	36.6	41.9	39.3

- Different small letters in each genotype indicate significant difference at .05 level between irrigation treatments.
- Different capital letters in each irrigation treatment indicate significant difference at .05 level among genotypes.

5.1.1.5. The Effect of Drought Stress on the 100-seed weight of 7 Soybean Genotypes

Table 11. 100-seed weight (g) of 7 soybean genotypes under drought-stressed (DS) and fully-irrigated (FI) treatments in Látókép, Debrecen averaged over 2017, 2018 and 2019.

Genotype	DS	FI	Average
ES Mentor	19.3 ^A	18.8 ^A	19.0
Bokréta	15.7 ^{bD}	16.4 ^{aD}	16.0
Coraline	17.6 ^B	17.7 ^{BC}	17.6
Bólyi 612	18.7 ^A	18.8 ^A	18.8
Ananda	18.6 ^A	18.6 ^A	18.6
ES Pallador	16.7 ^C	17.3 ^C	17.0
Pannonia Kincse	17.5 ^{bb}	18.3 ^{aAB}	17.9
Average	17.7	18.0	17.9

- Different small letters in each genotype indicate significant difference at .05 level between irrigation treatments.
- Different capital letters in each irrigation treatment indicate significant difference at .05 level among genotypes.

Except for ES Mentor and Ananda, the 100-seed weight decreased under drought stress conditions; the decrease was significant in both Bokréta and *Pannonia Kincse*. Interestingly, the 100-seed weight of Ananda did not change under both irrigation regimes, and that of ES Mentor was higher under drought stress conditions (Table 11), which might draw a conclusion that these two genotypes could be adopted under drought stress conditions in the study area.

As for comparing among genotypes, Bokréta had the lowest value of this trait, whereas ES Mentor had the highest value under both irrigation regimes (Table 11), supporting the initial conclusion of the suitability of ES Mentor for cultivation in the study area even under drought stress conditions in case the 100-seed weight (i.e. seed size) was the target.

5.1.1.6. The Effect of Drought Stress on The Seed Yield of 7 Soybean Genotypes

Drought stress resulted in less seed yield in all genotypes; however, only in ES Mentor and Coraline were the reductions significant. Averaged over all genotypes, 5.9% less seed yield was recorded under drought stress conditions (Fig. 5).

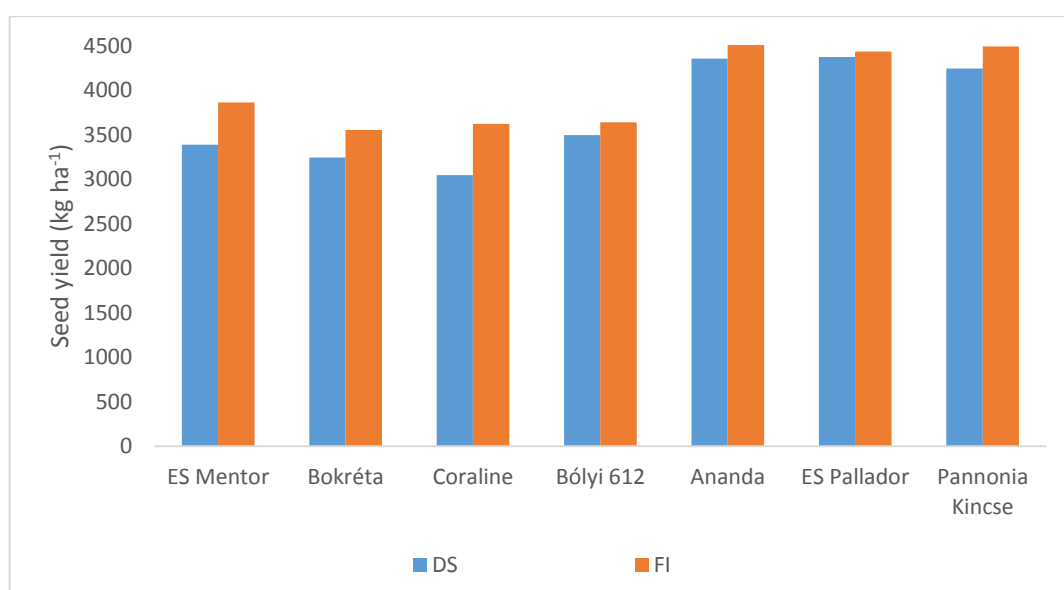


Fig. 5. Seed yield (kg ha⁻¹) of 7 soybean genotypes under drought-stressed (DS) and fully-irrigated (FI) treatments in Látókép, Debrecen averaged over 2017, 2018 and 2019.

Under both irrigation regimes, Ananda, ES Pallador and *Pannonia Kincse* genotypes had significantly higher seed yield compared to the other genotypes, with ES Pallador

resulting in the highest seed yield under drought (4372 kg ha⁻¹), whereas Ananda resulted in the best seed yield under fully-irrigated regime (4511 kg ha⁻¹) (Fig. 5). These results suggest that Ananda, ES Pallador and '*Pannonia Kincse*' genotypes might be more suitable for cultivation in the study area, especially under drought conditions (Fig. 5).

5.1.1.7. The Effect of Drought Stress on The Protein Concentration of 7 Soybean Genotypes

Drought differently affected the protein concentration in the produced seeds; it resulted in increasing this concentration in ES Mentor, Bokréta and ES Pallador genotypes, whereas it decreased it in Coraline, Ananda and '*Pannonia Kincse*' and did not affect it in Bólyi 612 genotype. All differences were insignificant. This alteration resulted in very similar average protein concentration among genotypes under both irrigation regimes (Table 12).

Significant differences among genotypes were recorded for this trait under both irrigation regimes, with ES Mentor resulting in the highest protein concentration and Bólyi 612 resulting in the lowest (Table 12).

Table 12. Protein concentration (%) (PC) and protein yield (t ha⁻¹) (PY) in the seeds of 7 soybean genotypes under drought-stressed (DS) and fully-irrigated (FI) treatments in Látókép, Debrecen averaged over 2017, 2018 and 2019.

Genotype	DS		FI		Average	
	PC (%)	PY (t ha ⁻¹)	PC (%)	PY (t ha ⁻¹)	PC (%)	PY (t ha ⁻¹)
ES Mentor	40.0 ^A	1.355	39.8 ^A	1.536	39.9	1.446
Bokréta	37.1 ^{BC}	1.203	35.8 ^C	1.272	36.5	1.238
Coraline	39.3 ^A	1.197	39.8 ^A	1.441	39.6	1.319
Bólyi 612	36.1 ^C	1.262	36.1 ^C	1.313	36.1	1.288
Ananda	38.0 ^B	1.654	39.2 ^A	1.768	38.6	1.711
ES Pallador	37.0 ^{BC}	1.618	36.7 ^{BC}	1.626	36.9	1.622
Pannonia Kincse	37.0 ^{BC}	1.570	38.1 ^{AB}	1.710	37.5	1.640
Average	37.8	1.408	37.9	1.524	37.9	1.466

- Different small letters in each genotype indicate significant difference at .05 level between irrigation treatments.
- Different capital letters in each irrigation treatment indicate significant difference at .05 level among genotypes.

5.1.1.8. The Effect of Drought Stress on The Oil Concentration of 7 Soybean Genotypes

Also for this trait, drought had different effects among genotypes; it increased the oil concentration in the produced seeds of ES Mentor, Coraline, Ananda and 'Pannonia Kincse', whereas reduced it in Bokréta, Bólyi 612 and ES Pallador; however, all increases and reductions were insignificant, and the average oil concentration among all genotypes was very similar under both irrigation regimes (Table 13).

The genotype Coraline resulted in the highest oil concentration in the produced seeds under both irrigation regimes (23.9 and 23.7% under DS and FI regimes, respectively), whereas Ananda had the lowest oil concentration under both regimes (21.5 and 21.1% under DS and FI regimes, respectively) as well (Table 13).

Table 13. Oil concentration (%) (OC) and oil yield (t ha⁻¹) (OY) in the seeds of 7 soybean genotypes under drought-stressed (DS) and fully-irrigated (FI) treatments in Látókép, Debrecen averaged over 2017, 2018 and 2019.

Genotype	DS		FI		Average	
	OC (%)	OY (t ha ⁻¹)	OC (%)	OY (t ha ⁻¹)	OC (%)	OY (t ha ⁻¹)
ES Mentor	22.8 ^B	0.772	22.2 ^B	0.752	22.5	0.762
Bokréta	22.7 ^B	0.736	23.1 ^A	0.749	22.9	0.743
Coraline	23.9 ^A	0.728	23.7 ^A	0.722	23.8	0.725
Bólyi 612	21.7 ^C	0.759	21.8 ^{BC}	0.762	21.7	0.761
Ananda	21.5 ^C	0.936	21.1 ^C	0.918	21.3	0.927
ES Pallador	21.7 ^C	0.949	21.8 ^{BC}	0.953	21.8	0.951
Pannonia Kincse	22.3 ^{BC}	0.946	21.7 ^{BC}	0.921	22.0	0.934
Average	22.4	0.832	22.2	0.825	22.3	0.829

- Different small letters in each genotype indicate significant difference at .05 level between irrigation treatments.
- Different capital letters in each irrigation treatment indicate significant difference at .05 level among genotypes.

5.1.2. The Effects of Drought Stress and Nitrogen Fertilization on The Morphophysiology, Yield Components and Seed Quality of 2 Soybean Genotypes

5.1.2.1. The Effect of Natural Drought at Different Stages on The Physiology of 2 Soybean Genotypes under Different N-fertilizer Rates (0, 35 and 105 kg ha⁻¹) in 2017, 2018 and 2019

To illustrate the effect of drought stress occurring naturally in the study area on the physiology of soybean plants, we analyzed the data collected from the non-irrigated plots of both '*Pannonia Kincse*' and '*Boglár*' genotypes under the three fertilization rates (0N, 35N and 105N).

5.1.2.1.1. Drought Occurrence during The Different Stages in 2017, 2018 and 2019

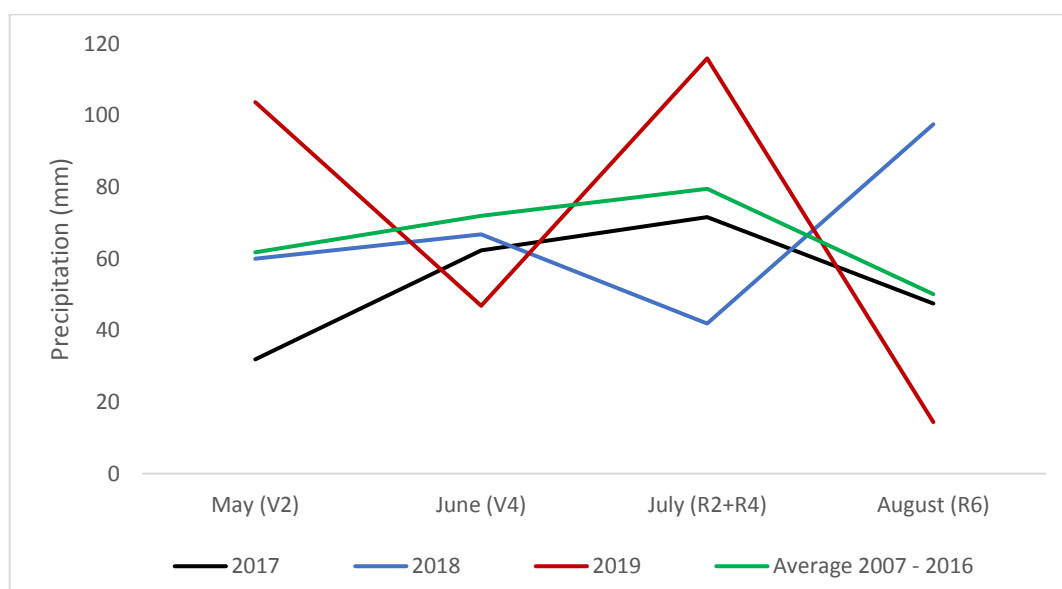


Fig. 6. Precipitation amounts during the vegetative period of soybean genotypes '*Pannonia Kincse*' and '*Boglár*' in Látókép, Debrecen in 2017, 2018 and 2019 compared to the previous 10-year average.

At V4 stage, the two years of 2017 and 2018 were close to the 10-year average precipitation amount, whereas 2019 was the year with drought occurrence. At both R2 and R4 stages, precipitation amount in 2017 was close to the average, whereas 2018 represented the drought-stressed year and 2019 represented the rainy year compared to the average. At R6 stage, 2017 was also close to average, whereas 2018 represented the

rainy year and 2019 was the year when plants encountered drought stress conditions (Fig. 6).

5.1.2.1.2. The Effect of Natural Drought at Different Stages on The Physiology of 2 Soybean Genotypes under Different N-fertilizer Rates (0, 35 and 105 kg ha⁻¹) in 2017, 2018 and 2019

5.1.2.1.2.1. The Effect of Natural Drought at Different Stages on The Relative Chlorophyll Content (SPAD) of 2 Soybean Genotypes under Different N-fertilizer Rates (0, 35 and 105 kg ha⁻¹) in 2017, 2018 and 2019

In ‘Pannonia Kincse’, and regardless of N rate, drought at V4 stage (in 2019) did not result in any significant decrease/increase in SPAD values, however, drought had a significant effect (of 69.6% in terms of calculated partial eta squared) when the high rate of N fertilizer (105N) was applied; i.e. 69.6% of changes of SPAD values at this N fertilizer rate was resulted from the different precipitation amounts. At R2 and R4 stages, both drought and rainy conditions did not result in significant differences regardless of N rate (except for a significant increase in 2019 at 0N rate where the effect size was 75.5%). However, in 2018 (drought conditions), SPAD recorded the least values compared to both other years. Light absorption is influenced by drought stress that results in changes in both leaf area index and leaf chlorophyll content (Dong et al. 2015). Previous studies reported chlorophyll decreases under drought stress conditions (e.g. Cui et al. 2004; Pagter et al. 2005). Inamullah and Isoda (2005) reported reductions in chlorophyll content when soybean plants were subjected to continuous drought stress starting from early seed filling stage. Cerezini et al. (2016) reported a non-significant reduction in the chlorophyll content when drought stress was applied at R2 stage. At R6 stage, drought in 2019 did not significantly affect SPAD values compared to the average year of 2017, moreover, it resulted in better SPAD values compared to the rainy year of 2018 (Table 14).

SPAD values of ‘Pannonia Kincse’ did not majorly vary among stages in 2017 (when no drought occurred), whereas the reduction of this trait, as a result of drought stress, at R2 stage in 2018 was sustained at R4 stage, and the above-average precipitation during R6 stage couldn’t recover SPAD value. In 2019, plants encountered drought early at V4 stage, followed by above-average precipitation at R2 stage, resulting in enhanced SPAD value, whereas late drought at R6 stage did not affect this trait (Fig. 7).

The effect of N fertilizer rate in ‘Pannonia Kincse’ was insignificant at V4 stage in the three years of experiment except for significantly higher SPAD value in 2017 for the high rate of N fertilizer (105N) where the effect value was 79.6%. Similarly, at R2 and R4 stages fertilization rate did not result in any significant differences in all three years; however, (105N) treatment resulted in better SPAD values compared to (0N) treatment at both stages, whereas (35N) treatment did not in most cases. No significant effect of fertilization rate was recorded at R6 stage as well, however, (105N) treatment resulted in better SPAD values compared to both (0N) and (35N) treatments (Table 14). These findings are in agreement with the findings of Cerezini et al. (2016) who reported a non-significant increase in the chlorophyll content (by 1.8% under non-stressed conditions and by 3% when drought stress occurred at R2 stage) when 200 kg ha⁻¹ of N fertilizer was applied.

Table 14. Relative chlorophyll content (SPAD) at the studied stages of soybean genotype ‘Pannonia Kincse’ under different N fertilizer rates (0, 35 and 105 kg ha⁻¹) in Látókép, Debrecen in 2017, 2018 and 2019.

Stage	N fertilizer rate (kg ha ⁻¹)	Year		
		2017	2018	2019
V4	0N	41.2 ^{ba}	43.0 ^{aA}	41.9 ^{aA}
	35N	41.3 ^{ba}	43.2 ^{aA}	42.3 ^{aA}
	105N	46.0 ^{aA}	40.4 ^{aB}	44.3 ^{aA}
R2	0N	40.1 ^{aB}	41.2 ^{aB}	44.9 ^{aA}
	35N	43.5 ^{aA}	40.8 ^{aA}	44.1 ^{aA}
	105N	42.5 ^{aA}	41.8 ^{aA}	44.9 ^{aA}
R4	0N	43.4 ^{aA}	41.2 ^{aA}	42.5 ^{aA}
	35N	41.7 ^{aA}	41.0 ^{aA}	43.7 ^{aA}
	105N	44.9 ^{aA}	41.3 ^{aA}	44.6 ^{aA}
R6	0N	41.5 ^{aAB}	40.5 ^{aB}	45.9 ^{aA}
	35N	43.8 ^{aA}	40.7 ^{aA}	42.3 ^{aA}
	105N	44.9 ^{aA}	42.6 ^{aA}	46.8 ^{aA}

- in certain stage, same small letters indicate no significant differences at .05 level among fertilization rates within the same year
- in certain stage, same capital letters indicate no significant differences at .05 level among years within the same fertilization rate

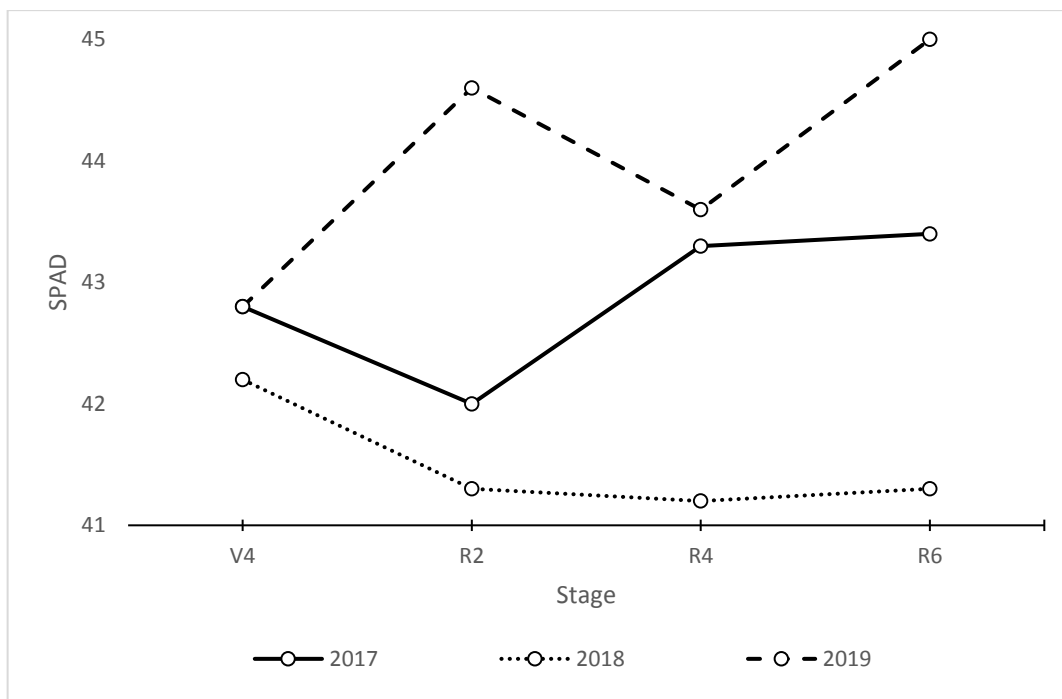


Fig. 7. Precipitation (drought) effects on SPAD values during different stages of the vegetative period of soybean genotype '*Pannonia Kincse*' in Látókép, Debrecen in 2017, 2018 and 2019.

In 'Boglár', drought at V4 stage insignificantly increased SPAD of both (0 N) and (35 N) treatments, whereas reduced it in (105 N) treatment. The effect of drought was not significant; however, it was higher than fertilization's effect. Drought resulted in significant decrease of SPAD trait, regardless of N fertilizer application or rate, at both R2 and R4 stages, whereas rainy conditions significantly increased this trait at R2 but not at R4 stage. Year's effect (drought in 2018 and over-average precipitation in 2019 as compared to normal-average precipitation in 2017) was significant in all three fertilization treatments (82, 88.6 and 89.9% at R2, and 62.4, 59.8 and 78.3% at R4 for 0 N, 35 N and 105 N, respectively), and was higher than fertilization's effect. Late drought at R6 stage resulted in noticeable increases in SPAD values of all fertilization treatments, and similar effect was recorded when plants got over-average precipitation (in 2018) (Table 15).

Table 15. Relative chlorophyll content (SPAD) at the studied stages of soybean genotype '*Boglár*' under different N fertilizer rates (0, 35 and 105 kg ha⁻¹) in Látókép, Debrecen in 2017, 2018 and 2019.

Stage	Fertilization rate (kg ha ⁻¹)	2017	2018	2019
V4	0N	38.0 ^{aA}	38.9 ^{aA}	39.5 ^{aA}
	35N	37.5 ^{aA}	38.1 ^{aA}	40.2 ^{aA}
	105N	38.1 ^{aA}	41.6 ^{aA}	40.4 ^{aA}
R2	0N	35.0 ^{aB}	29.9 ^{bC}	40.9 ^{aA}
	35N	35.8 ^{aB}	32.6 ^{abC}	41.9 ^{aA}
	105N	38.9 ^{aB}	33.9 ^{aC}	42.1 ^{aA}
R4	0N	40.2 ^{bA}	32.6 ^{bB}	37.4 ^{aAB}
	35N	41.5 ^{abA}	36.4 ^{abB}	37.2 ^{aB}
	105N	44.5 ^{aA}	39.1 ^{aB}	39.8 ^{aB}
R6	0N	31.7 ^{aB}	34.2 ^{bAB}	42.1 ^{aA}
	35N	34.8 ^{aA}	36.9 ^{bA}	42.2 ^{aA}
	105N	35.8 ^{aB}	41.6 ^{aA}	44.4 ^{aA}

- in certain stage, same small letters indicate no significant differences at .05 level among fertilization rates within the same year
- in certain stage, same capital letters indicate no significant differences at .05 level among years within the same fertilization rate

SPAD values were close to each other in '*Boglár*' at V4 stage, but drought in 2018 during R2 stage resulted in measurably decrease in this trait whereas over-average precipitation in 2019 enhanced it. Drought's effect was embedded at R4 stage where drought-stressed treatment had less SPAD value compared to the over-average counterpart, whereas the average precipitation reached its peak resulting in the best SPAD value, followed by a sharp degradation at late reproductive stage (R6), probably as a result of the re-allocation of N from the leaves to the formed seeds, whereas both late drought in 2019 and late ultra-precipitation in 2018 enhanced this trait during this stage (Fig. 8).

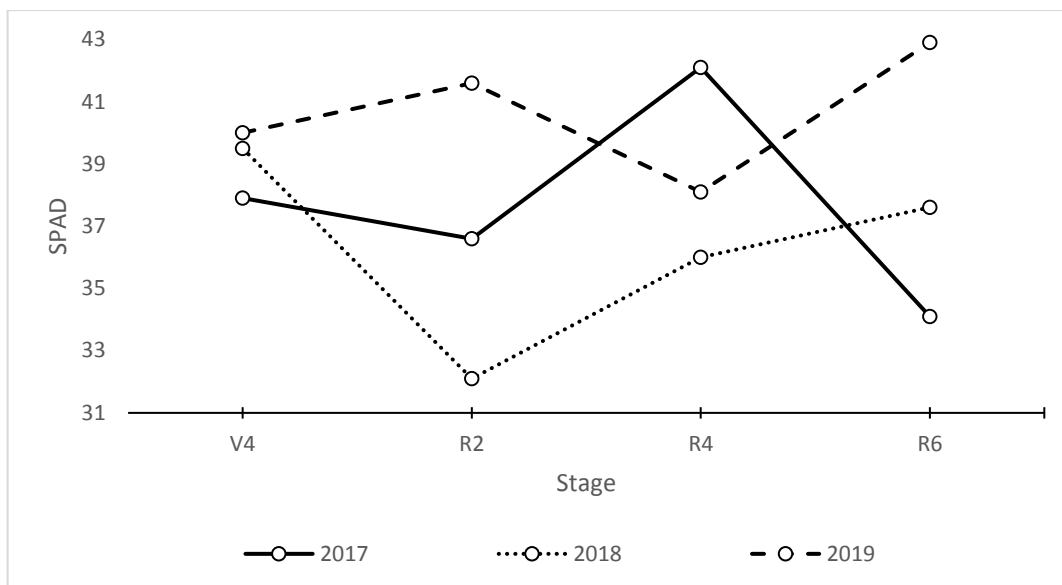


Fig. 8. Precipitation (drought) effects on SPAD values during different stages of the vegetative period of soybean genotype '*Boglár*' in Látókép, Debrecen in 2017, 2018 and 2019.

In the three years of experiment, increasing fertilization rate in '*Boglár*' had no significant effect on SPAD values at V4 stage, however, the high rate (105 N) resulted in the best SPAD values compared to the other two fertilization treatments. Under drought stress conditions (in 2018), 35 N treatment relatively enhanced SPAD values at both early reproductive stages (R2 and R4); furthermore, 105 N resulted in significant increase at both stages. On the other hand, increasing N rate did not significantly increase SPAD values in the over-precipitation year of 2019. Even in the average year of 2017 fertilization had significantly increased SPAD only at R4 stage with the high rate (105 N). Islam et al. (2017) reported that leaf-SPAD values after 3, 8, 13, 17 and 23 days of applying different nitrogen concentration in the nutrient solution (5, 25, 100 and 200 mg/l) were increased with increasing N concentration in both years of their experiment on soybean plants. In another study, SPAD values in the three studied stages; V4, R1 and R3 of soybean were positively influenced by increasing N levels (0, 20, 40 and 60 kg ha⁻¹); moreover, 60 N treatment was significantly better than the control (0 N) treatment at both V4 and R1 stages. The authors also reported that SPAD value showed a decreasing trend with the progress of soybean growing stages for all levels of N (Shafagh-Kolvanagh et al., 2008). Fertilization enhanced SPAD values at the late reproductive stage (R6) in all three years, with a significant increase only under over-precipitation conditions and

only for the high N rate. Moreover, the fertilization's effect was much less than the precipitation's effect, except for the year 2018 (Table 15).

5.1.2.1.2.2. The Effect of Natural Drought at Different Stages on The Leaf Area Index (LAI) of 2 Soybean Genotypes under Different N-fertilizer Rates (0, 35 and 105 kg ha⁻¹) in 2017, 2018 and 2019

In 'Pannonia Kincse', drought stress in 2019 at V4 stage resulted in reduced LAI values compared to both average years of 2017 and 2018; the reduction was significant when no N fertilizer (0N) was applied with an effect size of 51%. Drought's effect was also measurable at both R2 and R4 stages, regardless of fertilization rate; it significantly decreased LAI values at R2 stage (with an effect size of 84, 81.8 and 84.8% for 0N, 35N and 105N treatments, respectively) compared to the average year of 2017, whereas the decrease was insignificant at R4 stage. The rainy year of 2019 also resulted in significantly less LAI values at both stages compared to the average year of 2017 (except for 105N treatment at R4 stage where the reduction was insignificant) and also less LAI values compared to the drought year of 2018 (Table 16), leading to the conclusion that LAI is vulnerable to both drought and rainy conditions during these stages. Leaf area index (LAI) expresses the canopy density of a crop population (Dong and Xie 1999), and drought stress reduces this trait (Sinclair and Serraj 1995). Li et al. (2013) reported significant decreases in LAI (by 40, 33.8 and 36.4%) when plants were subjected to drought stress conditions at flowering, podding and seed-filling stages, respectively. At R6 stage, drought in 2019 reduced LAI values compared to the average year of 2017, however, the reduction was not significant; the rainy year of 2018, on the other hand, significantly increased LAI values (Table 16).

LAI values in 2017 increased with growth progress, peaking at R4 stage, followed by degradation at R6 stage. Drought in the following years did not change this trend; however, it reduced LAI values when occurred at early reproductive stages (R2 and R4) and it further decreased it when occurred early at V4 stage at 2019 as compared to the normal year of 2017 (Fig. 9).

Table 16. Leaf area index (LAI) ($\text{m}^2 \text{m}^{-2}$) at the studied stages of soybean genotype '*Pannonia Kincse*' under different N fertilizer rates (0, 35 and 105 kg ha^{-1}) in Látókép, Debrecen in 2017, 2018 and 2019.

Stage	N fertilizer rate (kg ha^{-1})	Year		
		2017	2018	2019
V4	0N	2.28 ^{bA}	2.1 ^{aAB}	1.8 ^{aB}
	35N	2.53 ^{abA}	2.2 ^{aA}	1.9 ^{aA}
	105N	3.05 ^{aA}	2.4 ^{aA}	2.1 ^{aA}
R2	0N	6.1 ^{aA}	4.6 ^{aB}	3.4 ^{aC}
	35N	6.9 ^{aA}	4.9 ^{aB}	2.9 ^{aC}
	105N	7.5 ^{aA}	5.4 ^{aB}	3.5 ^{aC}
R4	0N	8.5 ^{aA}	7.3 ^{aAB}	6.8 ^{aB}
	35N	8.2 ^{aA}	7.8 ^{aA}	6.8 ^{aB}
	105N	10.1 ^{aA}	8.2 ^{aA}	7.1 ^{aA}
R6	0N	5.3 ^{aB}	8.6 ^{aA}	5.0 ^{aB}
	35N	5.2 ^{aB}	8.1 ^{aA}	5.0 ^{aB}
	105N	5.4 ^{aB}	7.5 ^{aA}	4.5 ^{aB}

- in certain stage, same small letters indicate no significant differences at .05 level among fertilization rates within the same year
- in certain stage, same capital letters indicate no significant differences at .05 level among years within the same fertilization rate

Increasing N fertilizer rate in was accompanied by higher LAI values at V4 stage in the three years of experiment; the increase was significant in 2017 with an effect size of 61.1%. Applying higher rate of N fertilizer (105N) resulted in enhancing LAI values at both R2 and R4 stages compared to the non-fertilized treatment (0N) regardless of precipitation amount in the three years, however, the increase was not statistically significant. Previously, Buttery (1969) concluded that LAI values were increased by N application, and Dadson and Acquaah (1984) reported an increase in LAI values when N was applied 9 weeks after sowing. Later, it was reported that increasing N rates linearly increased LAI values (Caliskan et al. 2008). N fertilizer application did not result in any measurable enhancements in LAI values at R6 stage in all three years of experiment; it even resulted in decreasing LAI values in most cases (Table 16).

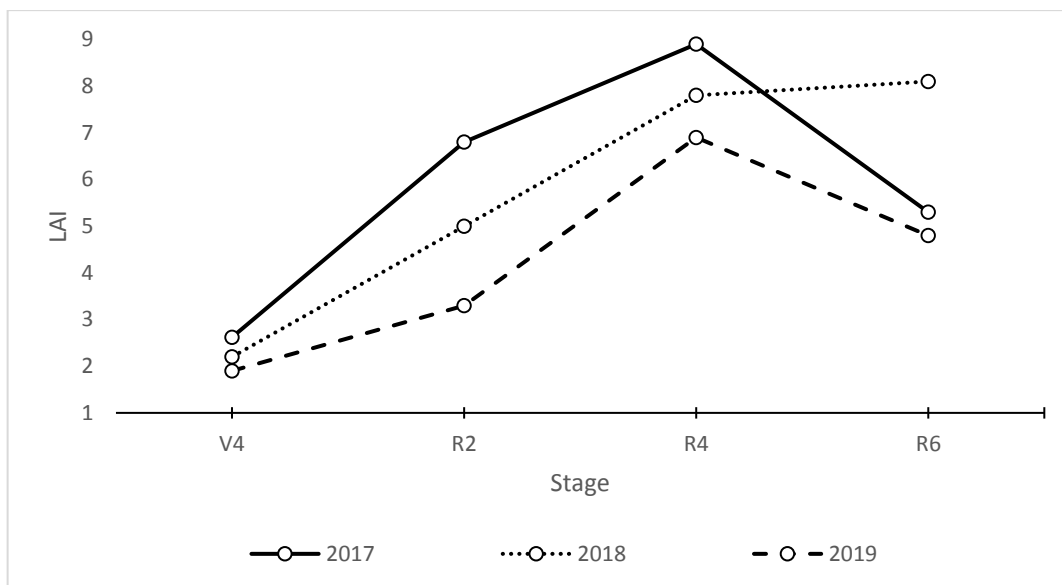


Fig. 9. Precipitation (drought) effects on LAI ($\text{m}^2 \text{m}^{-2}$) values during different stages of the vegetative period of soybean genotype '*Pannonia Kincse*' in Látókép, Debrecen in 2017, 2018 and 2019.

In 'Boglár', and regardless of fertilization application and rate, early drought at V4 stage considerably reduced LAI values; the reduction was significant in both 0N and 105N treatments compared to their counterparts in the average year of 2017. The effect of drought at early reproductive stages on LAI was even more measurable; the reduction was significant in most treatments. In addition, over-average precipitation also resulted in noticeably-reduced LAI values (except for a slight increase in 0 N at R4 stage compared to drought-stressed counterpart; however, it was still less than average-year counterpart). Nagasuga et al. (2014) reported significant reduction of LAI by 19.76 and 31.7% in two soybean cultivars; Fukuyutaka and Misatozairai, respectively as a result of drought stress application. Late drought slightly enhanced LAI values at R6 stage as compared to normal year counterparts, whereas over-average precipitation significantly increased LAI, regardless of fertilization application and rate (Table 17).

Early drought resulted in the lowest LAI value at V4 stage, but after that LAI gradually increased in all treatments during early reproductive stages (both R2 and R4); however, both drought and ultra-precipitation resulted in lower LAI values at both stages compared to the normal-average precipitation in 2017. Similar to NDVI trait, LAI values started degrading during R6 stage under both normal and drought stress conditions (without

reaching the minimum of the early V4 stage), whereas ultra-precipitation during this stage in 2019 further enhanced NDVI value (Fig. 10).

Table 17. Leaf area index (LAI) ($\text{m}^2 \text{m}^{-2}$) at the studied stages of soybean genotype ‘*Boglár*’ under different N fertilizer rates (0, 35 and 105 kg ha^{-1}) in Látókép, Debrecen in 2017, 2018 and 2019.

Stage	Fertilization rate (kg ha^{-1})	2017	2018	2019
V4	0N	2.8 ^{aA}	1.6 ^{aB}	1.2 ^{aB}
	35N	2.1 ^{aA}	1.8 ^{aA}	1.5 ^{aA}
	105N	3.1 ^{aA}	1.9 ^{aB}	1.6 ^{aB}
R2	0N	4.5 ^{bA}	3.7 ^{aA}	3.4 ^{aA}
	35N	6.2 ^{abA}	3.8 ^{aB}	3.0 ^{aB}
	105N	7.9 ^{aA}	4.3 ^{aB}	3.2 ^{aB}
R4	0N	7.6 ^{aA}	6.1 ^{bA}	6.3 ^{bA}
	35N	8.1 ^{aA}	6.3 ^{abB}	6.1 ^{bB}
	105N	9.0 ^{aA}	7.8 ^{aA}	7.4 ^{aA}
R6	0N	4.4 ^{aB}	7.0 ^{aA}	5.4 ^{aB}
	35N	4.3 ^{aB}	6.9 ^{aA}	5.5 ^{aB}
	105N	3.9 ^{aB}	7.3 ^{aA}	5.2 ^{aB}

- in certain stage, same small letters indicate no significant differences at .05 level among fertilization rates within the same year
- in certain stage, same capital letters indicate no significant differences at .05 level among years within the same fertilization rate

Apart from a non-significant decrease in 35 N treatment in 2017, fertilization enhanced LAI values at R4 stage, regardless of precipitation; however, the increases were insignificant. In both normal and drought-stressed years, fertilization increased LAI values at both R2 and R4 stages, moreover, 105 N treatment had higher values compared to 35 N counterparts. Under over-average conditions, however, 35 N decreased LAI values in both stages as compared to normal-average counterparts, whereas 105 N decreased LAI at R2 stage but increased it at R4 stage. Fertilization had no measurable effects on LAI trait at R6 stage, regardless of precipitation (Table 17).

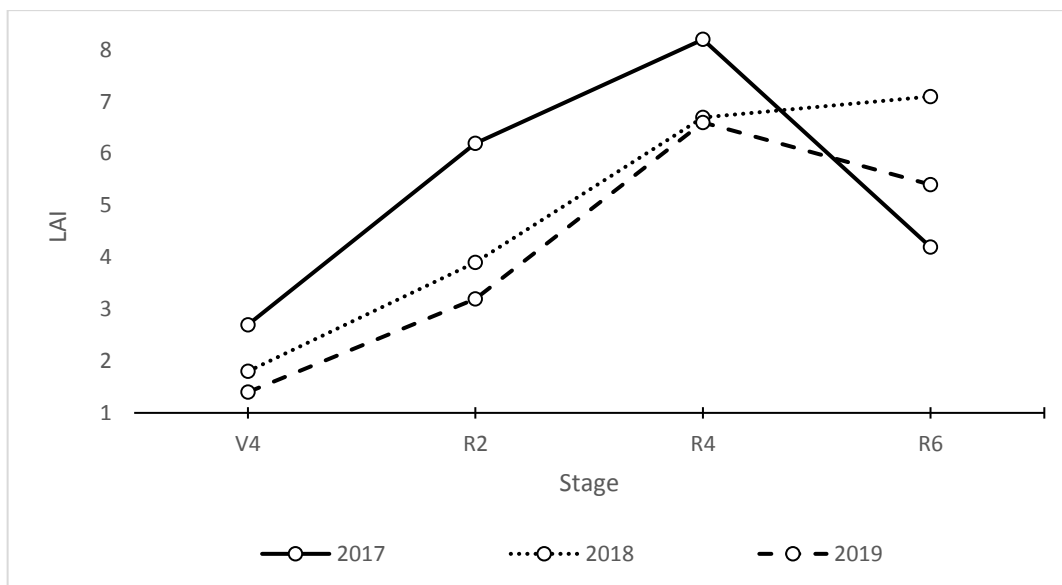


Fig. 10. Precipitation (drought) effects on LAI ($\text{m}^2 \text{m}^{-2}$) values during different stages of the vegetative period of soybean genotype 'Boglár' in Látókép, Debrecen in 2017, 2018 and 2019.

5.1.2.1.2.3. The Effect of Natural Drought at Different Stages on The Normalized Difference Vegetation Index (NDVI) of 2 Soybean Genotypes under Different N-fertilizer Rates (0, 35 and 105 kg ha^{-1}) in 2017, 2018 and 2019

In 'Pannonia Kincse', drought stress at V4 stage resulted in significantly enhanced NDVI values, regardless of N rate; the effect size was 81.2, 61.2 and 74.4% for (0N), (35N) and (105N) treatments, respectively. At R2 stage, drought in 2018 positively affected NDVI values compared to the average year of 2017, regardless of N rate, whereas it negatively affected this trait at R4 stage except for the higher N rate (105N) (Table 18). Camoglu et al. (2018) concluded that reducing irrigation by 25% as compared to the control (non-stressed) treatment insignificantly reduced NDVI by 2.4%, whereas a 50% reduction in irrigation water amount resulted in a significant 9.5% NDVI reduction. On the other hand, the rainy year of 2019 measurably increased the NDVI values at both stages and regardless of N rate; the increase was significant in most cases. At R6 stage, drought in 2019 insignificantly enhanced NDVI values compared to the average year of 2017, and the rainy year of 2018 resulted in measurably higher NDVI values; the increase was even significant in N-fertilized treatments (with an effect size of 52.8 and 62.8% for 35N and 105N treatments, respectively) (Table 18), leading to a conclusion that rainy conditions can enhance NDVI values at any stage. Some previous papers reported significant

correlations between precipitation amounts and NDVI (e.g. Suzuki et al. 2000; Wang et al. 2001), however, that relationship differs depending on NDVI measurement timing and other factors (Al-Bakri and Suleiman 2004).

Table 18. Normalized difference vegetation index (NDVI) at the studied stages of soybean genotype '*Pannonia Kincse*' under different N fertilizer rates (0, 35 and 105 kg ha⁻¹) in Látókép, Debrecen in 2017, 2018 and 2019.

Stage	N fertilizer rate (kg ha ⁻¹)	Year		
		2017	2018	2019
V4	0N	69.1 ^{aB}	75.3 ^{aB}	82.95 ^{abA}
	35N	72.0 ^{aB}	76.4 ^{aAB}	81.15 ^{bA}
	105N	77.1 ^{aB}	78.7 ^{aB}	84.75 ^{aA}
R2	0N	83.6 ^{aB}	84.8 ^{aAB}	85.2 ^{aA}
	35N	83.4 ^{aA}	84.9 ^{aA}	84.8 ^{aA}
	105N	83.7 ^{aB}	85.5 ^{aA}	85.5 ^{aA}
R4	0N	81.0 ^{aAB}	79.7 ^{aB}	86.9 ^{aA}
	35N	81.2 ^{aB}	81.0 ^{aB}	86.4 ^{aA}
	105N	80.7 ^{aC}	83.1 ^{aB}	87.0 ^{aA}
R6	0N	76.7 ^{aA}	85.0 ^{aA}	82.8 ^{aA}
	35N	76.8 ^{aB}	84.8 ^{aA}	83.2 ^{aAB}
	105N	78.0 ^{aB}	88.3 ^{aA}	79.5 ^{aB}

- in certain stage, same small letters indicate no significant differences at .05 level among fertilization rates within the same year
- in certain stage, same capital letters indicate no significant differences at .05 level among years within the same fertilization rate

As for precipitation effects during progressive stages, NDVI value in 2017 increased after V4 stage reaching the peak at R2 stage, followed by reductions at the following stages without reaching the value of the first measurement at V4. In 2018, precipitation amounts were better compared to 2017 during V4 stage, which resulted in better NDVI value, and even when drought occurred after that stage, plants could maintain higher NDVI value at R2 stage, but when drought continued during R4 stage, NDVI value decreased to the similar value of that in 2017, and later when the precipitation was better during R6 stage, NDVI value increased accordingly. Also, in 2019 when drought occurred early at V4 stage, plants could maintain a high NDVI level, most probably because of the above-average precipitation earlier that year, and kept increasing with better precipitation

amounts during both R2 and R4 stages, however, late drought at R6 stage resulted in decreasing this trait's value (Fig. 11).

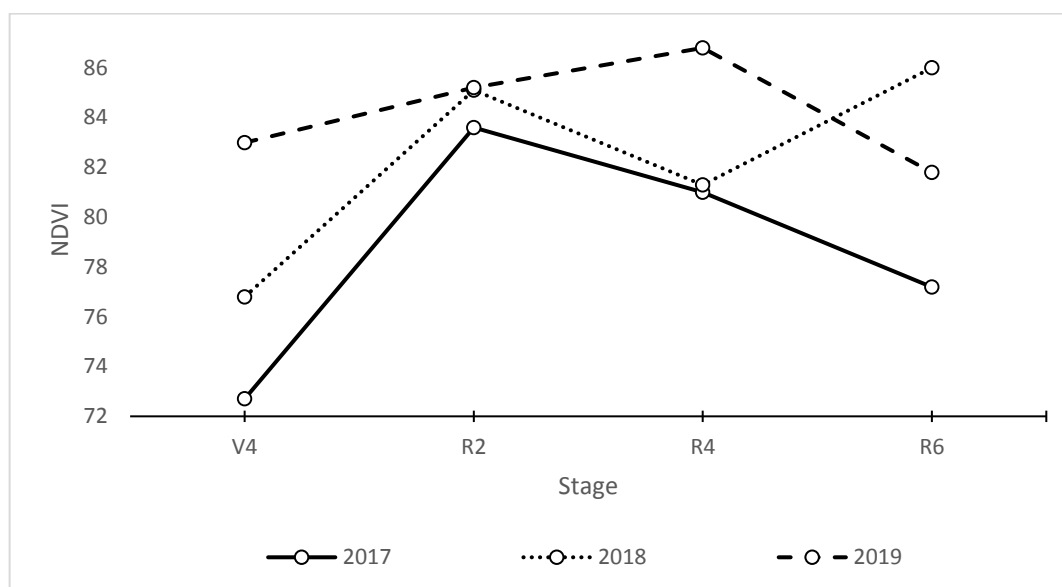


Fig. 11. Precipitation (drought) effects on NDVI values during different stages of the vegetative period of soybean genotype '*Pannonia Kincse*' in Látókép, Debrecen in 2017, 2018 and 2019.

Apart from a slight decrease in (35N) treatment in 2019, fertilization enhanced this trait at V4 stage in all years of experiment. Saleem et al. (2010) concluded that NDVI value was higher in the wheat plots that received 150 kg N ha⁻¹. Mupangwa et al. (2018) concluded that nitrogen application increased NDVI in maize; they attributed it to the increase in soil N supply. At all reproductive stages (R2, R4 and R6 stages), the effect of fertilization was slight and insignificant in all three years of experiment (Table 18). Cerezini et al. (2016) reported similar conclusion on soybean.

In 'Boglár', drought occurred early at V4 stage resulted in significantly enhanced NDVI values in all three fertilization treatments; the effect size was also significant with ratios of 61.5, 64.7 and 69.8% in 0 N, 35 N and 105 N treatments, respectively. Crusiol et al. (2017) reported that NDVI was higher in both cultivars when drought stress was applied at vegetative stages compared to the control (non-stressed) counterparts, whereas it measurably dropped when drought was applied at reproductive stages. At R2 and R4 stages, NDVI trait was not measurably affected by drought in both 0 N and 35 N treatments, whereas 105 N had significantly higher values in the drought-stressed year of

2018 compared to the average year of 2017. Over-average precipitation had neglectable changes in R2 stage, whereas it had significantly higher NDVI values compared to the other two precipitation amounts (the average one in 2017 and the below-average one in 2018) at R4 stage. Late drought at R6 stage significantly enhanced NDVI trait, regardless of fertilization, and over-precipitation had led to a similar result (Table 19).

Table 19. Normalized difference vegetation index (NDVI) at the studied stages of soybean genotype '*Boglár*' under different N fertilizer rates (0, 35 and 105 kg ha⁻¹) in Látókép, Debrecen in 2017, 2018 and 2019.

Stage	Fertilization rate (kg ha ⁻¹)	2017	2018	2019
V4	0N	70.3 ^{aAB}	67.9 ^{aB}	77.9 ^{aA}
	35N	68.6 ^{aB}	71.5 ^{aAB}	76.7 ^{aA}
	105N	75.5 ^{aAB}	71.6 ^{aB}	80.1 ^{aA}
R2	0N	80.2 ^{bA}	82.6 ^{bA}	82.3 ^{aA}
	35N	82.2 ^{aA}	84.2 ^{aA}	82.0 ^{aA}
	105N	82.5 ^{aB}	84.6 ^{aA}	83.6 ^{aAB}
R4	0N	76.9 ^{aB}	76.7 ^{bB}	86.5 ^{aA}
	35N	77.6 ^{aB}	78.3 ^{abB}	85.9 ^{aA}
	105N	78.7 ^{aC}	81.4 ^{aB}	87.6 ^{aA}
R6	0N	67.3 ^{aB}	81.8 ^{bA}	83.1 ^{aA}
	35N	64.9 ^{aB}	85.8 ^{abA}	82.7 ^{aA}
	105N	62.4 ^{aB}	87.0 ^{aA}	80.9 ^{aA}

- in certain stage, same small letters indicate no significant differences at .05 level among fertilization rates within the same year
- in certain stage, same capital letters indicate no significant differences at .05 level among years within the same fertilization rate

NDVI values noticeably increased between V4 and R2 stages, reaching a very similar values at R2 stage, regardless of precipitation amount. However, over-average precipitation measurably enhanced this trait at R4 stage, allowing it to reach the peak point, whereas it started degrading under both normal and drought conditions. At R6 stage, NDVI values decreased under both normal and drought conditions as compared to the previous stage, whereas the late over-precipitation in 2018 could re-enhance this trait reaching its maximum value (Fig. 12).

Whether precipitation amount was within the average (in both 2017 and 2018) or below-average (in 2019), 105 N treatment resulted in higher NDVI values compared to 0 N and 35 N treatments at V4 stage; however, the increase was insignificant, and the effect of fertilization was less than that of precipitation. At both R2 and R4 stages, fertilization enhanced NDVI in all three years of experiment (except for a slight decrease in 35 N in 2019). Applying N fertilizer negatively affected NDVI values at R6 stage in both average and drought-stressed year, whereas enhanced this trait under over-precipitation conditions only. In this stage as well the effect of precipitation was higher than that of fertilization (Table 19).

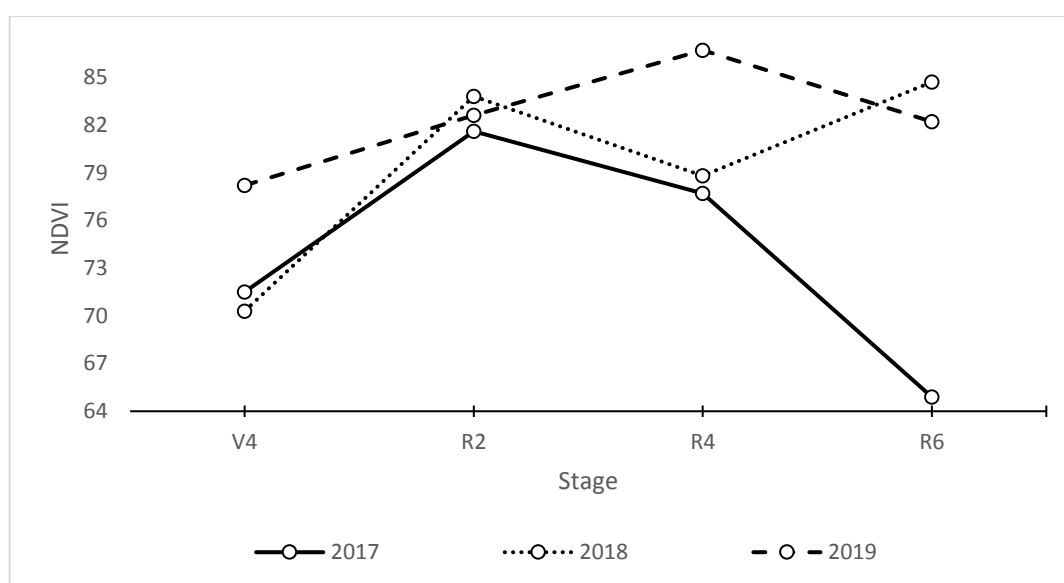


Fig. 12. Precipitation (drought) effects on NDVI values during different stages of the vegetative period of soybean genotype ‘Boglár’ in Látókép, Debrecen in 2017, 2018 and 2019.

5.1.2.2. The Effect of Chemical N-fertilizer on The Morpho-physiology, Yield Components and Seed Quality of Soybean Genotype ‘Pannonia Kincse’ under Different Irrigation Regimes

5.1.2.2.1. The Effect of Chemical N-fertilizer on The Relative Chlorophyll Content (SPAD) of Soybean Genotype ‘Pannonia Kincse’ under Different Irrigation Regimes

Except for an insignificant decrease in 105N treatment compared to 35N counterpart, increasing fertilization rate was accompanied by increases in SPAD values in all fertilization treatments; the increase was significant in 105N treatment at both V4 and R6

stages as compared to 0N counterparts. However, no significances were estimated when fertilization rate was increased from 35 to 105 kg ha⁻¹. On average, 35N and 105N resulted in 1.4 and 2.4% increase in SPAD values, respectively compared to 0N counterpart (Table 20). Fertilization was positively correlated with SPAD trait at all stages, but more obviously at V4 and R6 stages where the correlation was significant (Table 21).

Table 20. The effect of different fertilization rates (0, 35 and 105 kg ha⁻¹) on SPAD at different stages during the vegetative period of soybean genotype '*Pannonia Kincse*' in Látókép, Debrecen averaged over 2017, 2018 and 2019.

Stage	0N	35N	105N
V4	41.5 ^b	42.0 ^{ab}	42.7 ^a
R2	42.2 ^a	43.1 ^a	42.7 ^a
R4	41.8 ^a	42.3 ^a	42.4 ^a
R6	44.4 ^b	44.8 ^{ab}	46.2 ^a
Average	42.5	43.1	43.5

- Same letter indicates no significant difference at .05 level among fertilization treatments within a certain stage.

Regardless of fertilization treatment, SPAD values increased with plants reached early reproductive stage (R2), then a relatively-slight decrease was recorded at R4 stage, followed by rapid increase at R6 stage (Table 20).

Table 21. Correlation coefficient of SPAD, NDVI and LAI traits at different stages with fertilization (0, 35 and 105 kg ha⁻¹) treatments.

Stage	SPAD	NDVI	LAI
V4	.229 [*]	.229 [*]	.357 ^{**}
R2	.088	.006	.300 ^{**}
R4	.106	.088	.236 [*]
R6	.233 [*]	.053	-.012
Overall	.150 ^{**}	.090	.092

- **. Correlation is significant at the 0.01 level (2-tailed).
- *. Correlation is significant at the 0.05 level (2-tailed).

Drought at both vegetative (V4) and early reproductive (R2 and R4) stages enhanced SPAD trait; the enhancement was even significant at R4 stage; however, drought resulted

in significantly less SPAD values at late reproductive stage (R6) (Table 22). Gavili et al. (2019) reported that both moderate and severe drought conditions significantly increased soybean SPAD values by 11 and 20%, respectively. The authors justified this increase by the increased N concentrations caused by the decreased fresh or dry matter, and the enhanced N concentration will, in turn, enhance the chlorophyll content. Bredemeier (2005) reported similar conclusion on maize.

Table 22. The effect of different irrigation regimes on SPAD at different stages during the vegetative period of soybean genotype '*Pannonia Kincse*' in Látókép, Debrecen averaged over 2017, 2018 and 2019.

Stage	Non-Irrigated	Half-Irrigated	Fully-Irrigated
V4	42.6 ^a	41.7 ^a	41.9 ^a
R2	42.7 ^a	42.4 ^a	43.0 ^a
R4	42.7 ^a	42.4 ^{ab}	41.4 ^b
R6	43.2 ^c	45.3 ^b	46.9 ^a
Average	42.8	43.0	43.3

- Same letter indicates no significant difference at .05 level among irrigation regimes within a certain stage.

Under all irrigation regimes, SPAD values increased along with plants' life stage development (except for a reduction at R4 stage under fully-irrigated system) (Table 22). The correlation coefficient, with irrigation treatments, was negative at both V4 and R4 stages, but positive at both R2 and R6 stages (Table 23).

Table 23. Correlation coefficient of SPAD, NDVI and LAI traits at different stages with irrigation treatments.

Stage	SPAD	NDVI	LAI
V4	-.128	-.033	-.226 [*]
R2	.056	-.282 ^{**}	.114
R4	-.233 [*]	.103	.272 ^{**}
R6	.474 ^{**}	.211 [*]	.091
Overall	.073	.031	.057

- **, Correlation is significant at the 0.01 level (2-tailed).
- *, Correlation is significant at the 0.05 level (2-tailed).

5.1.2.2.2. The Effect of Chemical N-fertilizer on The Normalized Difference Vegetation Index (NDVI) of Soybean Genotype 'Pannonia Kincse' under Different Irrigation Regimes

Fertilization relatively enhanced NDVI values at all studied stages; however, all enhancements were insignificant. On average, NDVI was 0.5 and 1.2% higher in 35N and 105N treatments, respectively compared to 0N treatment (Table 24). NDVI was positively correlated with fertilization treatments at all stages, being significant only at V4 stage (Table 21).

Regardless of fertilization treatment, NDVI was measurably increased when plants entered early reproductive stage (R2), followed by gradual, slight reductions at the next reproductive stages (Table 24).

Table 24. The effect of different fertilization rates (0, 35 and 105 kg ha⁻¹) on NDVI at different stages during the vegetative period of soybean genotype 'Pannonia Kincse' in Látókép, Debrecen averaged over 2017, 2018 and 2019.

Stage	0N	35N	105N
V4	75.7 ^a	76.7 ^a	78.6 ^a
R2	84.2 ^a	84.2 ^a	84.3 ^a
R4	83.2 ^a	83.5 ^a	83.8 ^a
R6	82.8 ^a	83.3 ^a	83.4 ^a
Average	81.5	81.9	82.5

- Same letter indicates no significant difference at .05 level among fertilization treatments within a certain stage.

At both V4 and R2 stages, drought enhanced NDVI values, whereas it resulted in lower NDVI values at the later stages (R4 and R6). Fully-irrigated regime could increase NDVI values while plants were developing from stage to stage, whereas NDVI peaked at R2 stage under both non-and half-irrigated regimes and started degrading after that stage (Table 25). Correlation with irrigation was negative at the first-two studied stages (V4 and R2), but positive at later stages (R4 and R6) (Table 23).

Table 25. The effect of different irrigation regimes (non-irrigated, half-irrigated and fully-irrigated) on NDVI at different stages during the vegetative period of soybean genotype '*Pannonia Kincse*' in Látókép, Debrecen averaged over 2017, 2018 and 2019.

Stage	Non-Irrigated	Half-Irrigated	Fully-Irrigated
V4	77.5 ^a	76.4 ^a	77.1 ^a
R2	84.6 ^a	84.6 ^a	83.5 ^b
R4	83.0 ^a	83.7 ^a	83.8 ^a
R6	81.7 ^a	83.7 ^a	83.9 ^a
Average	81.7	82.1	82.1

- Same letter indicates no significant difference at .05 level among irrigation regimes within a certain stage.

5.1.2.2.3. The Effect of Chemical N-fertilizer on The Leaf Area Index (LAI) of Soybean Genotype '*Pannonia Kincse*' under Different Irrigation Regimes

Compared to 0N counterpart, 105N treatment significantly increased LAI value at all stages except the late reproductive stage R6 where the LAI values were very close in all fertilization treatments. DeMooy et al. (1973) and Watanabe et al. (1986) reported that adding N fertilizer before reproductive stages enhances growth and LAI, consequently flowering and yield.

LAI values rapidly increased with plants' development in life cycle, peaking at R4 stage, followed by reducing LAI values in all fertilization treatments (Table 26). The correlation coefficient decreased with the development of plants through stages, being significant and positive at all stages except for the late R6 stage (Table 21).

Drought significantly-positively affected LAI at V4 stage; however, it resulted in reducing LAI values at all reproductive stages, especially at R4 stage where the reduction was significant. Similarly, Gavili et al. (2019) reported that moderate and severe drought (corresponding to 70 and 55% FC, respectively) significantly decreased plant leaf area by 29 and 35% at V10 stage, 23 and 31% at V3 stage and 26 and 36% at R6 stage. Karam et al. (2005) concluded that LAI decreased by 52% under drought stress conditions imposed at R2 stage. Pagter et al. (2005) explained the decreased LAI under drought stress conditions to be the result of less newly-produced leaves with a smaller size and a higher falling rate. Severe drought stress imposed at R4 stage resulted in 61.4% less leaf area in soybean (Wei et al., 2018). Moosavi et al. (2014) reported decreased leaf area in canola

plants as a result of drought stress application. Çakir (2004) also reported a 23.5% decrease in leaf area when maize plants were subjected to drought stress conditions during the tasseling period.

Table 26. The effect of different fertilization rates (0, 35 and 105 kg ha⁻¹) on LAI (m² m⁻²) at different stages during the vegetative period of soybean genotype '*Pannonia Kincse*' in Látókép, Debrecen averaged over 2017, 2018 and 2019.

Stage	0N	35N	105N
V4	1.9 ^b	2.0 ^b	2.4 ^a
R2	4.6 ^b	5.2 ^{ab}	5.8 ^a
R4	8.1 ^b	8.4 ^{ab}	9.0 ^a
R6	6.4 ^a	6.5 ^a	6.5 ^a
Average	5.3	5.5	5.9

- Same letter indicates no significant difference at .05 level among fertilization treatments within a certain stage.

Regardless of irrigation regime, gradual enhancements in LAI values with plants' development were recorded until the peak at R4 stage, where LAI started degrading after (Table 27). The correlation coefficient gradually increased from stage to another until R4 stage; after that it started decreasing with staying positive (Table 23).

Table 27. The effect of different irrigation regimes (non-irrigated, half-irrigated and fully-irrigated) on LAI (m² m⁻²) at different stages during the vegetative period of soybean genotype '*Pannonia Kincse*' in Látókép, Debrecen averaged over 2017, 2018 and 2019.

Stage	Non-Irrigated	Half-Irrigated	Fully-Irrigated
V4	2.3 ^a	2.0 ^{ab}	1.9 ^b
R2	5.0 ^a	5.1 ^a	5.4 ^a
R4	7.9 ^b	8.8 ^a	8.9 ^a
R6	6.1 ^a	6.9 ^a	6.4 ^a
Average	5.3	5.7	5.7

- Same letter indicates no significant difference at .05 level among irrigation regimes within a certain stage.

5.1.2.2.4. The Effect of Chemical N-fertilizer on The Plant Height (cm) of Soybean Genotype 'Pannonia Kincse' under Different Irrigation Regimes

Both irrigation and fertilization had highly significant effect on the plant height, whereas their interaction had no significant effect. However, slightly-positive correlation coefficient was estimated with both irrigation and fertilization (Tables 28 and 29).

Regardless of irrigation regime, increasing fertilization rate was accompanied by insignificant enhancement in plant height; 35N and 105N treatments resulted in 2.4 and 3.9% taller plants, respectively (averaged among all three irrigation regimes) (Table 28). Fertilization's effect on this trait was 16.4%. Similar conclusion was reported earlier by Hanway and Weber (1971) and Dadson and Acquah (1984).

Table 28. The effect of different fertilization rates (0, 35 and 105 kg ha⁻¹) on plant height (cm) of soybean genotype 'Pannonia Kincse' under different irrigation regimes (non-irrigated, half-irrigated and fully-irrigated) in Látókép, Debrecen averaged over 2017, 2018 and 2019.

Treatment	0N	35N	105N	Average
Non-Irrigated	87.8 ^{aA}	88.7 ^{bA}	90.4 ^{bA}	89.0
Half-Irrigated	90.3 ^{aA}	92.8 ^{abA}	95.0 ^{aA}	92.7
Fully-Irrigated	91.9 ^{aA}	95.0 ^{aA}	95.2 ^{aA}	94.0
Average	90.0	92.2	93.5	91.9

- Same small letter indicates no significant difference at .05 level among irrigation regimes within certain fertilization treatment.
- Same capital letter indicates no significant difference at .05 level among fertilization treatments within certain irrigation regime.

Plant height was positively affected by irrigation; however, the ratios of enhancement differed between half- and fully-irrigated regimes. Half-irrigated regimes resulted in 2.8, 4.6 and 5.1% taller plants, whereas fully-irrigated regime resulted in 4.7, 7.1 and 5.3% taller plants in 0N, 35N and 105N treatments, respectively. Moreover, the difference was significant in both 35N and 105N treatments under fully-irrigated regime as compared to non-irrigated counterparts but only for 105N treatment under half-irrigated regime (Table 28). 28.6% of the differences in this trait were caused by the different irrigation regimes applied. Both Newark (1991) and El Kheir et al. (1994) reported decreased plant height under drought stress conditions. Gavili et al. (2019) reported a 33 and 60% plant height

reduction in their experiment under 70 and 55% FC conditions, respectively. Soybean plants had 22.4% shorter plants when severe drought stress occurred at R4 stage, whereas only 9% reduction was reported when same severe drought occurred at R6 stage (Wei et al., 2018). Drought reduced soybean plant height by 31.1% (Freitas et al., 2016). Neilson and Nelson (1998) explained this reduction in plant height under drought by the delayed stem elongation caused by shortened distance among nodes. Atti et al. (2004) reported that the plant height of an indeterminate soybean cultivar (OAC Bayfield) decreased by 33 and 28% in W1 and W2 treatments, respectively after 9 days of stress application. Furthermore, after 16 days of drought imposition resulted in 56 and 47% reduction in plant height in W1 and W2 treatments, respectively.

Table 29. Correlation coefficient of yield and yield components traits with fertilization (0, 35 and 105 kg ha⁻¹) treatments.

Protein Concentration	Oil Concentration	Yield	Plant Height	Flower Number	Pod Number	100-seed Weight
.286**	-.120	.195*	.090	.229*	.259**	.286**

- **. Correlation is significant at the 0.01 level (2-tailed).
- *. Correlation is significant at the 0.05 level (2-tailed).

5.1.2.2.5. The Effect of Chemical N-fertilizer on The Flower Number Per plant of Soybean Genotype 'Pannonia Kincse' under Different Irrigation Regimes

Both irrigation and fertilization highly-significantly influenced this trait, and their interaction had significant effect as well. This trait was significantly correlated with fertilization (Table 29) and highly-significantly correlated with irrigation (Table 31).

Under all irrigation regimes, increasing fertilization rate resulted in higher flower number per plant (except 105N under fully-irrigated regime as compared to 35N counterpart). On average, 35N treatment enhanced this trait by 6.5% compared to 0N, and 105N had a slight, additional enhancement by 1.4% (Table 30). The effect size of fertilization on this trait was estimated as 9.3%. Purcell and King (1996) reported that applying N fertilizer increased flower number in plants by reducing flower abortion rate. Brevedan et al. (1978) reported similar conclusion under both greenhouse and field conditions.

Table 30. The effect of different fertilization rates (0, 35 and 105 kg ha⁻¹) on flower number per plant of soybean genotype '*Pannonia Kincse*' under different irrigation regimes (non-irrigated, half-irrigated and fully-irrigated) in Látókép, Debrecen averaged over 2017, 2018 and 2019.

Treatment	0N	35N	105N	Average
Non-Irrigated	49.3 ^{ba}	51.0 ^{ba}	52.5 ^{ba}	50.9
Half-Irrigated	57.4 ^{aB}	58.3 ^{aAB}	64.4 ^{aA}	60.1
Fully-Irrigated	56.0 ^{abB}	63.7 ^{aA}	58.6 ^{abAB}	59.4
Average	54.2	57.7	58.5	56.8

- Same small letter indicates no significant difference at .05 level among irrigation regimes within certain fertilization treatment.
- Same capital letter indicates no significant difference at .05 level among fertilization treatments within certain irrigation regime.

Half-irrigated regime significantly increased flower number per plant in all fertilization treatments compared to non-irrigated counterparts; the average increase was 18.1%. However, further water irrigation amounts (fully-irrigated regime) did not further enhance this trait except in 35N treatment, yet still average increase, compared to non-irrigated counterpart, was 16.7% (Table 30). Irrigation explained 34.2% of differences in flower number per plant. He et al. (2017) reported that cyclic drought resulted in 53.8% decreased flower number per plant, whereas terminal drought further increased that ratio to 72.5%. In their experiment, Atti et al. (2004) reported that flower number per plant decreased by 79.4 and 58.8% in W1 and W2 treatments, respectively. The authors explained this decrease by both reduced node number and increased flower abortion as a result of drought stress application. Flower number in chickpea was reported to have an important role on the final yield (Fang et al., 2010).

Table 31. Correlation coefficient of yield and yield components traits with irrigation treatments.

Protein Concentration	Oil Concentration	Yield	Plant Height	Flower Number	Pod Number	100-seed Weight
.244 [*]	-.368 ^{**}	.151	.116	.456 ^{**}	.419 ^{**}	0.000

- **. Correlation is significant at the 0.01 level (2-tailed).
- *. Correlation is significant at the 0.05 level (2-tailed).

5.1.2.2.6. The Effect of Chemical N-fertilizer on The Pod Number Per plant of Soybean Genotype 'Pannonia Kincse' under Different Irrigation Regimes

The sole effect of irrigation and fertilization, in addition to their interaction, on pod number per plant were highly significant. Moreover, highly-significant correlation coefficient was estimated for this trait with both fertilization and irrigation (Tables 29 and 32).

Fertilization influence was very similar to flower number per plant trait (Table 32), and 15.8% of changes in this trait were explained by fertilization effect.

Table 32. The effect of different fertilization rates (0, 35 and 105 kg ha⁻¹) on pod number per plant of soybean genotype 'Pannonia Kincse' under different irrigation regimes (non-irrigated, half-irrigated and fully-irrigated) in Látókép, Debrecen averaged over 2017, 2018 and 2019.

Treatment	0N	35N	105N	Average
Non-Irrigated	40.5 ^{ba}	42.3 ^{ba}	44.8 ^{ba}	42.5
Half-Irrigated	49.3 ^{aB}	50.7 ^{aAB}	54.5 ^{aA}	51.5
Fully-Irrigated	45.6 ^{aB}	52.8 ^{aA}	47.4 ^{bB}	48.6
Average	45.1	48.6	48.9	47.5

- Same small letter indicates no significant difference at .05 level among irrigation regimes within certain fertilization treatment.
- Same capital letter indicates no significant difference at .05 level among fertilization treatments within certain irrigation regime.

Similar trend was recorded in this trait under the different irrigation regimes as flower number per plant trait; the average increase was 21.2 and 14.4% in half- and fully-irrigated regimes, respectively compared to non-irrigated counterpart (Table 32). 47.3% of changes in this trait were caused by irrigation. Similar conclusion was reported by Pookpakdi et al. (1990) and Pawar et al. (1992) and later by He et al. (2017); the authors concluded that cyclic and terminal drought stress resulted in 42.3 and 90.4% less pods per plant. Westgate and Peterson (1993) concluded that drought stress during flowering caused a 70% reduction in pod number per plant. Exposing soybean plants to drought at pod filling stages decreased pod number per plant by 36.6%, whereas a 42.6% reduction was recorded when drought was imposed at flowering stage (Sepanlo et al., 2014). Pod number decreased from 25 to 15 when available water decreased from 100 to 70% FC,

and further reduction to 55% FC further decreased pod number to 14 (Gavili et al., 2019). In their experiment, Iqbal et al. (2018) decreased FC from 100 to 50% at R4 stage to study the effect of drought at this stage on soybean; they reported that pod number per plant significantly decreased by 21.4% as a consequence of drought imposition, and when FC was further reduced to 20%, another significant reduction (by 34.7% compared to 100% FC treatment) was recorded in this trait. Leport et al. (2006) concluded that decreased pod number majorly affects the seed yield of chickpea.

5.1.2.2.7. The Effect of Chemical N-fertilizer on the 100-seed Weight (g) of Soybean Genotype 'Pannonia Kincse' under Different Irrigation Regimes

Only fertilization had a highly-significant effect on this trait, whereas irrigation, solely and in interaction with fertilization, had no significant effect. Moreover, the correlation with fertilization was highly significant (Table 29), whereas no correlation with irrigation could be estimated (Table 31).

Fertilization enhanced the 100-seed trait, regardless of irrigation regime. The average enhancement was 10.6 and 11.2% in 35N and 105N, respectively compared to 0N counterpart (Table 33).

Table 33. The effect of different fertilization rates (0, 35 and 105 kg ha⁻¹) on 100-seed weight (g) of soybean genotype 'Pannonia Kincse' under different irrigation regimes (non-irrigated, half-irrigated and fully-irrigated) in Látókép, Debrecen averaged over 2017, 2018 and 2019.

Treatment	0N	35N	105N	Average
Non-Irrigated	17.3 ^{aB}	18.7 ^{aAB}	20.6 ^{aA}	18.8
Half-Irrigated	18.9 ^{aA}	20.3 ^{aA}	20.6 ^{aA}	19.9
Fully-Irrigated	17.6 ^{aA}	20.3 ^{aA}	18.7 ^{aA}	18.8
Average	17.9	19.8	19.9	19.2

- Same small letter indicates no significant difference at .05 level among irrigation regimes within certain fertilization treatment.
- Same capital letter indicates no significant difference at .05 level among fertilization treatments within certain irrigation regime.

Both half- and fully-irrigated regimes could insignificantly enhance this trait, compared to non-irrigated counterpart, in both 0N and 35N treatments, but not 105N (Table 33).

5.1.2.2.8. *The Effect of Chemical N-fertilizer on The Seed Yield (kg ha⁻¹) of Soybean Genotype 'Pannonia Kincse' under Different Irrigation Regimes*

Only fertilization had significant effect on yield, whereas both sole irrigation and its interaction with fertilization had no significant effects on this trait. Yield correlation with irrigation and, to a higher extent, with fertilization was positive (Table 29).

Fertilization increased the yield under all three irrigation regimes, again except 105N treatment under fully-irrigated regime, introducing a conclusion that moderate fertilization is an advisable practice under all irrigation regimes, whereas high rates of N are only recommended under relative drought conditions. On average, 35N treatment resulted in a 6.3% increase (4813 kg ha⁻¹), whereas 105N had 6.9% higher yield (4839 kg ha⁻¹) as compared to non-fertilized (0N) counterpart (4527 kg ha⁻¹) (Fig. 13). Hungria et al. (2006) also reported that the application of 200 kg ha⁻¹ of N-fertilizer did not increase the yield. Other reports also concluded that N-fertilizer application resulted in better seed yield under drought stress conditions (e.g. Ray et al., 2006; Salvagiotti et al., 2008). The application of N fertilizer increased soybean 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 (Purcell and King, 1996). Under severe drought stress, every 1 kg ha⁻¹ of N fertilizer resulted in extra 1.2 kg ha⁻¹ seeds (Chen et al., 1992). Seneviratne et al. (2000) reported that a relatively-small amount of N fertilizer (46 kg/ha) significantly increased the seed yield by 84.7%. Similar conclusion was reported by Purcell and King (1996) that N fertilizer significantly increased the yield to 2798 kg ha⁻¹ compared to 2373 kg ha⁻¹ without N fertilizer; they associated this increase to increased seed number because of decreased flower and pod abortion.

Although it was not statistically significant, yet irrigation could enhance the yield under all fertilization rates except for fully-irrigated treatment in 105N rate where a reduction in the yield was recorded compared to non-and half-irrigated counterparts. Averaged among the three fertilization rates, half-irrigation regime increased the yield by 4.6% (4781 kg ha⁻¹), whereas fully-irrigated regime had increased it by 5.6% (to 4827 kg ha⁻¹) compared to non-irrigated counterpart (4571 kg ha⁻¹) (Fig. 13). Previously, Foroud et al. (1993) reported that drought decreased the seed yield of soybean plants. Reductions in seed yield under drought stress conditions were also reported by Liu et al. (2003) and

Masoumi et al. (2011). Moreover, seed yield was reduced by 57.4 and 95.3% as a result of cyclic and terminal drought stress, respectively (He et al., 2017). Moderate drought at R4 stage reduced soybean seed yield by 31.2% (averaged on both years of experiment), whereas severe drought at the same stage resulted in 77.7% less seed yield (Wei et al., 2018). The same researchers also reported that subjecting soybean plants to moderate and severe drought at R6 stage decreased the final seed yield by 33.4 and 62.4%, respectively. When drought was imposed at R4 stage, soybean plants had 32.0 and 48.7% less seed yield under 50 and 20% FC, respectively compared to 100 FC control (Iqbal et al., 2018). The authors concluded that the decrease in seed yield was mainly caused by increased number of empty pods, decreased number of seeds per plant, decreased 100-seed weight and decreased number of pods per plant. Jumrani and Bhatia (2018) subjected soybean plants to drought stress at two different stages; V4 and R5. They reported that the seed yield was decreased by 28 and 74%, respectively compared to control treatment where no drought stress was imposed, concluding that drought had much higher effect when it was imposed at reproductive stage R5 as compared to vegetative stage V4.

Table 34. Correlation coefficient of yield components, fertilization treatments and irrigation regimes with the final seed yield.

100-seed Weight	Pod Number	Flower Number	Plant Height	NDVI	Fertilization Rate	Irrigation Regime	Protein Concentration	SPAD	LAI	Oil Concentration
.521**	.471**	.462**	.447**	.436**	.195*	.151	-.034	-.048	-.061	-.245*

- **. Correlation is significant at the 0.01 level (2-tailed).
- *. Correlation is significant at the 0.05 level (2-tailed).

100-seed weight, pod number per plant, flower number per plant and NDVI were highly-significantly correlated with the final seed yield. Yield was also correlated with both fertilization and irrigation, whereas it was negatively correlated with protein concentration, SPAD, LAI and oil concentration (Table 34).

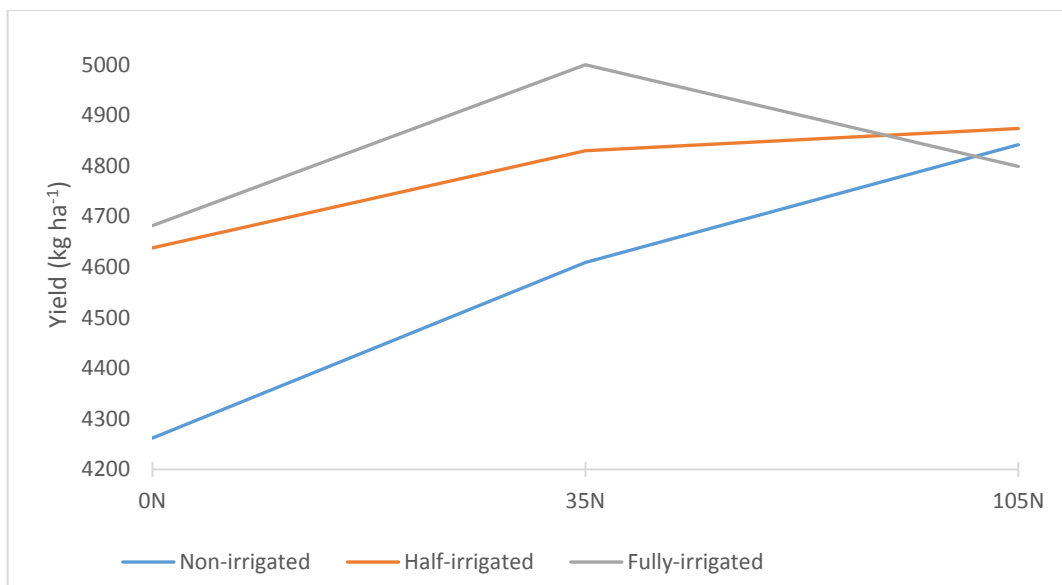


Fig. 13. The effect of different fertilization rates (0, 35 and 105 kg ha⁻¹) on the yield (kg ha⁻¹) of soybean genotype '*Pannonia Kincse*' under different irrigation regimes (non-irrigated, half-irrigated and fully-irrigated) in Látókép, Debrecen averaged over 2017, 2018 and 2019.

5.1.2.2.9. The Effect of Chemical N-fertilizer on The Protein Concentration (%) of Soybean Genotype '*Pannonia Kincse*' under Different Irrigation Regimes

Irrigation and fertilization solely and also their interaction (irrigation*fertilization) had highly significant ($p < 0.01$) effects on protein concentration. Moreover, the correlation between this trait and the fertilization rate was highly significant (Table 29), and the correlation with irrigation regimes was significant as well (Table 31).

Fertilization, regardless of irrigation regime, could enhance protein concentration only when applied in a high rate (105N). Protein concentration was 1.9 and 1.1% higher in 105N treatment in half- and fully-irrigated, respectively; moreover, it was significantly higher (by 7.3%) under non-irrigated regime, and this high fertilization rate, under non-irrigated regime, resulted in the best protein concentration compared to all other N rates and irrigation regimes (Table 35), which implies the importance of N application in relatively high rates under drought stress conditions as it could alleviate the effect of drought in reducing protein concentration recorded in both 0N and 35N treatments, and even resulting in the highest protein concentration. Previously, Bloom (2006) reported that increasing applied-N rate was accompanied by enhanced protein concentration.

Rotundo and Westgate (2009) reported that the addition of N fertilizer during the vegetative stages has led to about 2% increase in protein concentration, they also concluded, from 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. N fertilizer dose also has a significant effect on the seed protein content; the dose of 100 kg/ha increased seed protein just 2%, whereas the dose of 200 kg/ha resulted in 14% increase in seed protein (Miransari, 2016).

The effects size (calculated as partial Eta squared) of fertilization (28.4%) was higher than that of irrigation (15.4%); i.e. 28.4% of the differences among protein concentration values can be explained by the changes in fertilization rates, whereas 15.4% can be explained by different irrigation regimes.

Table 35. The effect of different fertilization rates (0, 35 and 105 kg ha⁻¹) on the seed protein concentration (%) of soybean genotype '*Pannonia Kincse*' under different irrigation regimes (non-irrigated, half-irrigated and fully-irrigated) in Látókép, Debrecen averaged over 2017, 2018 and 2019.

Treatment	0N	35N	105N	Average
Non-Irrigated	35.8 ^{bb}	35.8 ^{bb}	38.4 ^{aa}	36.7
Half-Irrigated	37.0 ^{abA}	36.6 ^{abA}	37.7 ^{aa}	37.1
Fully-Irrigated	37.7 ^{aa}	37.4 ^{aa}	38.1 ^{aa}	37.7
Average	36.8	36.6	38.1	37.2

- Same small letter indicates no significant difference at .05 level among irrigation regimes within certain fertilization treatment.
- Same capital letter indicates no significant difference at .05 level among fertilization treatments within certain irrigation regime.

Irrigation increased protein concentration in both 0N and 35N; moreover, the increase in fully-irrigated treatment (by 5.3 and 4.5% in 0N and 35N, respectively) was significant compared to non-irrigated counterpart. On the other hand, non-irrigated treatment resulted in relatively higher protein concentration in 105N treatment as compared to the other two irrigation regimes, however, the difference was slight and insignificant (Table 35). Similarly, Sepanlo et al. (2014) concluded that drought stress imposed on soybean plants at pod filling stage resulted in 15.5% reduction in protein concentration in the

seeds. Reduced protein concentration under drought stress conditions was also reported by Turner et al. (2005) and Carrera et al. (2009).

5.1.2.2.10. The Effect of Chemical N-fertilizer on The Oil Concentration (%) of Soybean Genotype 'Pannonia Kincse' under Different Irrigation Regimes

Oil concentration was highly-significantly affected ($p < 0.01$) by irrigation, significantly affected ($p < 0.05$) by fertilization whereas their interaction had no significant effect. However, the correlation coefficient was negative in relation with fertilization and, to a higher level, with irrigation (Tables 29 and 31); i.e. increasing fertilization or irrigation water amount resulted, in most cases, in reducing oil concentration, which can be confirmed by the values in table 36.

Fertilization, especially applied in higher (105N) rate decreased oil concentration, regardless of irrigation regime; however, all reductions were slight and insignificant (Table 36). Overall fertilization effect on oil concentration was 7.5%.

Table 36. The effect of different fertilization rates (0, 35 and 105 kg ha⁻¹) on the seed oil concentration (%) of soybean genotype 'Pannonia Kincse' under different irrigation regimes (non-irrigated, half-irrigated and fully-irrigated) in Látókép, Debrecen averaged over 2017, 2018 and 2019.

Treatment	0N	35N	105N	Average
Non-Irrigated	22.5 ^{aA}	22.5 ^{aA}	22.0 ^{aA}	22.3
Half-Irrigated	21.8 ^{abA}	21.8 ^{bA}	21.6 ^{aA}	21.7
Fully-Irrigated	21.6 ^{bA}	21.5 ^{bA}	21.5 ^{aA}	21.5
Average	22.0	21.9	21.7	21.9

- Same small letter indicates no significant difference at .05 level among irrigation regimes within certain fertilization treatment.
- Same capital letter indicates no significant difference at .05 level among fertilization treatments within certain irrigation regime.

Oil concentration was reversely affected by increasing irrigation water amounts. The reduction ratios in fully-irrigated, compared to non-irrigated, regime (by 4.0 and 4.4%) were significant in both 0N and 35N treatments, respectively but not in 105N treatment (where the reduction ratio was 2.3%) (Table 36). The effect size of irrigation on this trait was 36.1%. Sepanlo et al. (2014) reported that drought at flowering stage increased oil

concentration in soybean seeds by 5.7%, and further increased it (by 19.7%) when drought was imposed at pod filling stage. Boydak et al. (2002) also concluded that drought stress enhanced oil concentration.

5.1.2.3. The Effect of Nitrogen and Inoculation on The Morpho-physiology, Yield Components and Seed Quality of Soybean Genotype 'Boglár' under Different Irrigation Regimes

5.1.2.3.1. The Effect of Nitrogen and Inoculation on The Relative Chlorophyll content (SPAD) of Soybean Genotype 'Boglár' under Different Irrigation Regimes

In inoculated plants at all studied stages, increased SPAD values could be recorded with increasing fertilization rates, with the high fertilization rate being significantly higher at late reproductive stages (R4 and R6) compared to 0N counterpart. On average, SPAD value was 3.5 and 6.4% in 35N and 105N treatments, respectively compared to 0N treatment (Table 37). Significant correlation between fertilization and SPAD trait at all stages was estimated (Table 38). A very similar conclusion was recorded in non-inoculated plants, and the enhancement rate was 2.6 and 6.6% for 35N and 105N treatments, respectively compared to 0N treatment (Table 37). de Almeida et al. (2017) concluded that N deficiency significantly reduced the relative chlorophyll content in soybean plants by 84.4%. Increasing N rate resulted in better SPAD values at different stages in soybean (Islam et al., 2017). Similar conclusion was reported by Kolvanagh et al. (2008). Correlation coefficient with fertilization was positive and significant at all stages except for R2 stage (Table 38).

Drought had vulnerable and insignificant effect on SPAD values at the studied stages in inoculated plants, but had significant negative effect at R6 stage, where 7.7 and 11.8% reduction in SPAD value was recorded compared to half- and fully-irrigated treatments, respectively. On average, irrigation increased SPAD values by 1.0 and 2.9% under half- and fully-irrigated regimes, respectively compared to non-irrigated counterpart (Table 39). Only at R6 stage was the correlation between irrigation and SPAD significant (Table 40). In non-inoculated plants also, drought decreased SPAD value by 5.4 and 10.8% compared to half- and fully-drought regimes, respectively (Table 39). Similar conclusion was recorded regarding correlation (Table 40). Fixed-N₂ decreases under drought stress,

resulting in decreased N content in the leaves which, in part, leads to decreased photosynthetic capacity (Minguez and Sau, 1989; Djekoun and Planchon, 1991; Kao and Forseth, 1992). Drought stress reduced SPAD value by 11% (Atti et al., 2004). Total chlorophyll (chl a+b) decreased by 42.5% under drought stress conditions imposed at flowering stage, whereas the reduction ratio was 15.7% when soybean plants suffered from drought stress at pod filling stage (Sepanlo et al., 2014).

Table 37. The effect of different fertilization rates (0, 35 and 105 kg ha⁻¹) on SPAD at different stages during the vegetative period of soybean genotype '*Boglár*' in Látókép, Debrecen averaged over 2017, 2018 and 2019.

Inoculation	Stage	0N	35N	105N
Inoculated	V4	38.3	38.5	39.6
	R2	35.9	37.5	37.9
	R4	36.2 ^b	38.1 ^a	39.6 ^a
	R6	39.4 ^b	41.2 ^{ab}	42.3 ^a
	Average	37.5	38.8	39.9
Non-inoculated	V4	38.0 ^b	38.9 ^{ab}	40.2 ^a
	R2	36.6	37.8	38.5
	R4	36.8 ^b	38.1 ^b	40.5 ^a
	R6	40.3	40.7	42.6
	Average	37.9	38.9	40.4

- Different letters indicate significant differences at .05 level among fertilization treatments within a certain stage.

Interestingly, non-inoculated plants had higher SPAD values than inoculated counterparts in all fertilization treatments and under all irrigation regimes (Tables 37 and 39). Cerezini et al. (2016) reported that chlorophyll content was higher in non-inoculated plants than inoculated counterparts when soybean did not suffer from drought stress.

Table 38. Correlation coefficient of SPAD, NDVI and LAI traits at different stages with fertilization (0, 35 and 105 kg ha⁻¹) treatments.

Inoculation	Stage	SPAD	NDVI	LAI
Inoculated	V4	.205*	.316**	.324**
	R2	.221*	.383**	.383**
	R4	.386**	.167	.468**
	R6	.269**	-.005	-.006
	Overall	.251**	.121*	.139**
Non-inoculated	V4	.312**	.117	.269**
	R2	.181	.017	.280**
	R4	.456**	.144	.194*
	R6	.192*	.003	.069
	Overall	.381**	.098	.292**

- *. Correlation is significant at the 0.05 level (2-tailed).
- **. Correlation is significant at the 0.01 level (2-tailed).

Table 39. The effect of different irrigation regimes (non-irrigated, half-irrigated and fully-irrigated) on SPAD at different stages during the vegetative period of soybean genotype '*Boglár*' in Látókép, Debrecen averaged over 2017, 2018 and 2019.

Inoculation	Stage	Non-Irrigated	Half-Irrigated	Fully-Irrigated
Inoculated	V4	39.1	38.5	38.7
	R2	36.8	37.1	37.4
	R4	38.7	37.5	37.6
	R6	38.2 ^b	41.4 ^a	43.3 ^a
	Average	38.2	38.6	39.3
Non-inoculated	V4	39.4	39.3	38.5
	R2	37.1	38.0	37.9
	R4	38.7	38.6	38.1
	R6	38.9 ^b	41.1 ^{ab}	43.6 ^a
	Average	38.5	39.2	39.5

- Different letters indicate significant differences at .05 level among irrigation regimes within a certain stage.

Table 40. Correlation coefficient of SPAD, NDVI and LAI traits at different stages with irrigation.

Inoculation	Stage	SPAD	NDVI	LAI
Inoculated	V4	-.062	.107	-.009
	R2	.070	-.028	.143
	R4	-.130	.102	.456**
	R6	.472**	.240*	.194*
	Overall	.109*	.111*	.112*
Non-inoculated	V4	-.124	-.108	-.146
	R2	.069	-.201*	.012
	R4	-.075	.083	.252**
	R6	.397**	.126	.134
	Overall	.149	-.019	.132

- *. Correlation is significant at the 0.05 level (2-tailed).
- **. Correlation is significant at the 0.01 level (2-tailed).

5.1.2.3.2. *The Effect of Nitrogen and Inoculation on The Normalized Difference Vegetation Index (NDVI) of Soybean Genotype 'Boglár' under Different Irrigation Regimes*

Except for a slight, insignificant decrease in 105N compared to 35N counterpart, increased fertilization rate in inoculated plants was accompanied by increased NDVI values, with 105N treatment being significantly higher than 0N treatment at V4 stage, and significantly higher than both 0N and 35N treatments at R2 stage. Averaged over all stages, 1.3 and 2.2% higher NDVI values were recorded in 35N and 105N treatments, respectively compared to 0N counterpart. In all fertilization treatments, a rapid increase in NDVI was recorded between V4 and R2 stages, followed by gradual reduction through later stages (Table 41). The correlation coefficient was highly-significant at both V4 and R2 stages, but started decreasing after to become slightly negative at R6 stage (Table 38). Non-inoculated plants responded positively to fertilization; however, no significance was recorded. Similar trend was recorded among stages for non-inoculated plants (Table 41), and correlation coefficient was insignificantly positive throughout all stages (Table 38).

Table 41. The effect of different fertilization rates (0, 35 and 105 kg ha⁻¹) on NDVI at different stages during the vegetative period of soybean genotype '*Boglár*' in Látókép, Debrecen averaged over 2017, 2018 and 2019.

Inoculation	Stage	0N	35N	105N
Inoculated	V4	72.2 ^b	74.0 ^{ab}	76.1 ^a
	R2	81.9 ^b	82.8 ^a	83.5 ^a
	R4	80.6	81.4	82.1
	R6	79.7	80.2	79.6
	Average	78.6	79.6	80.3
Non-Inoculated	V4	73.7	75.0	75.0
	R2	82.2	82.2	82.5
	R4	81.2	81.7	82.5
	R6	79.7	79.7	79.7
	Average	79.2	79.6	79.9

- Different letters indicate significant differences at .05 level among fertilization treatments within a certain stage.

In general, irrigation enhanced this trait in inoculated plants (except at R2 stage, where also both irrigation regimes had higher NDVI value than non-irrigated counterpart, but half-irrigated regime had higher NDVI than did fully-irrigated regime). Moreover, drought significantly reduced (by 5.3% compared to fully-irrigated counterpart) NDVI value at R6 stage. On average, drought reduced NDVI value by 1.5 and 2.0% compared to half- and fully-irrigated regimes, respectively. The effect of irrigation on NDVI values through stages was similar to that of fertilization (Table 42). The correlation with irrigation was positive at all stages except for R2 stage (Table 40). Irrigation's effect on non-inoculated plants was more measurable at late reproductive stages (R4 and R6), but only half-irrigated regime, on average, resulted in better NDVI than drought-stressed counterpart. NDVI values reached their maximum at R2 stage under both non- and half-irrigated regimes, whereas it reached the maximum at R4 stage under fully-irrigated regime, but without reaching the maximum value of the other two regimes (Table 42). Correlation with irrigation was negative at both V4 and R2 stages, but positive later at R4 and R6 stages (Table 40).

Table 42. The effect of different irrigation regimes (non-irrigated, half-irrigated and fully-irrigated) on NDVI at different stages during the vegetative period of soybean genotype ‘*Boglár*’ in Látókép, Debrecen averaged over 2017, 2018 and 2019.

Inoculation	Stage	Non-Irrigated	Half-Irrigated	Fully-Irrigated
Inoculated	V4	73.3	74.4	74.6
	R2	82.7	83.1	82.5
	R4	81.0	81.1	81.9
	R6	77.3 ^b	80.6 ^{ab}	81.6 ^a
	Average	78.6	79.8	80.2
Non-inoculated	V4	74.7	75.6	73.4
	R2	82.9	82.9	80.9
	R4	81.4	81.7	82.2
	R6	78.7	79.7	80.6
	Average	79.4	80.0	79.3

- Different letters indicate significant differences at .05 level among irrigation regimes within a certain stage.

A very close average value of NDVI was recorded for both inoculated and non-inoculated plants (Tables 41 and 42), however, non-inoculated plants had higher NDVI under drought stress conditions (Table 42). Similarly, Cerezini et al. (2016) reported that NDVI decreased by 5.4% in inoculated plants compared to non-inoculated counterparts under drought stress conditions.

5.1.2.3.3. The Effect of Nitrogen and Inoculation on The Leaf Area Index (LAI) of Soybean Genotype ‘Boglár’ under Different Irrigation Regimes

Enhanced LAI values could be recorded at all stages with increasing fertilization rate in both inoculated and non-inoculated plants, with the high rate (105N treatment) having significantly higher values at both V4 and R2 stages and an average 18.8 and 14% higher LAI values compared to 0N and 35N treatments, respectively in inoculated plants, and 14.9 and 8.0% in non-inoculated plants. Regardless of inoculation, gradual increases in LAI values through plants’ development were recorded, with a peak at R4 stage in all fertilization treatments (Table 43). De Almeida et al. (2017) found out that the deficiency of N in soybean plants significantly decreased LAI by 87.5%. Significant correlation at all studied stages, except for the late R6 stage, was estimated, regardless of inoculation (Table 38).

Table 43. The effect of different fertilization rates (0, 35 and 105 kg ha⁻¹) on LAI (m² m⁻²) at different stages during the vegetative period of soybean genotype '*Boglár*' in Látókép, Debrecen averaged over 2017, 2018 and 2019.

Inoculation	Stage	0N	35N	105N
Inoculated	V4	1.7 ^b	1.8 ^b	2.2 ^a
	R2	4.0 ^b	4.5 ^b	5.4 ^a
	R4	7.4	7.7	9.1
	R6	6.0	6.1	6.1
	Average	4.8 ^b	5.0 ^b	5.7 ^a
Non-inoculated	V4	1.7 ^b	1.8 ^{ab}	2.1 ^a
	R2	3.9 ^b	4.6 ^{ab}	5.3 ^a
	R4	7.3	7.6	8.0
	R6	5.8	6.0	6.1
	Average	4.7	5.0	5.4

- Different letters indicate significant differences at .05 level among fertilization treatments within a certain stage.

In inoculated plants, half-irrigated regime did not result in better LAI values at both V4 and R2 stages, but did at later stages. Fully-irrigated regime, on the other hand, had higher LAI values at all stages compared to both other regimes. Irrigation increased LAI by 8.3 and 14.9% under half- and fully-irrigated regimes, respectively compared to non-irrigated counterpart. Similar conclusion could be recorded in non-inoculated plants in all stages except for V4 stage, where fully-irrigated regime, in addition to half-irrigated regime, couldn't enhance LAI. In this trait as well, irrigation followed similar trend to fertilization effect throughout plants' development, regardless of inoculation (Table 44). Atti et al. (2004) concluded that two drought stress severities; W1 and W2 (corresponding to 25 and 50% of crop evapotranspiration ET_c) reduced soybean leaf area by 74.5 and 52.7%, respectively. The correlation coefficient gradually increased through stages to reach a highly-significant peak at R4 stage, followed by a reduction at R6 stage that, however, kept it significant in inoculated plants, but not in non-inoculated counterparts (Table 40).

Inoculated plants were, on average, 4% higher in LAI compared to non-inoculated counterparts, but the difference was insignificant (Tables 43 and 44).

Table 44. The effect of different irrigation regimes (non-irrigated, half-irrigated and fully-irrigated) on LAI (m² m⁻²) at different stages during the vegetative period of soybean genotype ‘*Boglár*’ in Látókép, Debrecen averaged over 2017, 2018 and 2019.

Inoculation	Stage	Non-Irrigated	Half-Irrigated	Fully-Irrigated
Inoculated	V4	1.8	1.8	1.9
	R2	4.5	4.5	5.0
	R4	7.2 ^c	8.2 ^b	8.8 ^a
	R6	5.6	6.2	6.3
	Average	4.8	5.2	5.5
Non-inoculated	V4	2.0	1.8	1.7
	R2	4.6	4.5	4.7
	R4	7.2 ^b	7.5 ^{ab}	8.1 ^a
	R6	5.6	6.2	6.1
	Average	4.9	5.0	5.2

- Different letters indicate significant differences at .05 level among irrigation regimes within a certain stage.

5.1.2.3.4. The Effect of Nitrogen and Inoculation on The Plant Height (cm) of Soybean Genotype ‘*Boglár*’ under Different Irrigation Regimes

Both irrigation and fertilization, but not their interaction, had highly significant effect on the plant height of inoculated plants, whereas both treatments, in addition to their interaction, had no significant effect on non-inoculated plants. Correlation coefficient was positive, yet not significant, with both treatments, regardless of inoculation treatment (Table 46).

In inoculated plants, both half- and fully-irrigated regimes resulted in significantly taller plants compared to non-irrigated counterpart, regardless of fertilization treatment. Compared to half-irrigated, however, fully-irrigated regime could enhance this trait only in 0N treatment, resulting in similar enhancement average of 7.5% as compared to non-irrigated regime. 46.0% of differences in plant height were resulted from the different irrigation regimes. In non-inoculated plants, similar enhancement, as a result of irrigation application, was recorded; however, no significant differences were recorded. Moreover, half-irrigated regime resulted in taller plants than did fully-irrigated regime, regardless of fertilization treatment (Table 45). Iqbal et al. (2018) concluded that decreasing available water at R4 stage from 100 to 50% FC slightly increased plant height in soybean;

however, further reduction to 20% FC resulted in shorter plants compared to both 10 and 50% FC. Sepanlo et al. (2014) also reported that soybean plants had 29.6% shorter plants under drought stress imposed at flowering stage.

Table 45. The effect of different fertilization treatments (0, 35 and 105 kg ha⁻¹) on plant height (cm) of soybean genotype '*Boglár*' under different irrigation regimes (non-irrigated, half-irrigated and fully-irrigated) in Látókép, Debrecen averaged over 2017, 2018 and 2019.

Inoculation	Irrigation regime	0N	35N	105N	Average
Inoculated	Non-Irrigated	82.5 ^b	85.1 ^b	88.6 ^b	85.4
	Half-Irrigated	86.9 ^a	93.1 ^a	95.4 ^a	91.8
	Fully-Irrigated	89.8 ^a	91.4 ^a	94.2 ^a	91.8
	Average	86.4	89.9	92.7	89.7
Non-inoculated	Non-Irrigated	80.7	84.8	85.9	83.8
	Half-Irrigated	87.5	91.6	93.3	90.8
	Fully-Irrigated	86.9	90.6	91.6	89.7
	Average	85.0	89.0	90.2	88.1

- In each inoculation treatment, different letters indicate significant differences at .05 level among irrigation regimes within a certain fertilization treatment.

Although not statistically significant, yet measurable enhancements in this trait were accompanied with increasing fertilization rate in inoculated plants. On average, 4.1 and 7.3% taller plants were resulted from 35N and 105N treatments, respectively as compared to 0N treatment. Fertilization was responsible for 38.7% of differences in plant height. Similar enhancements by fertilization treatments were recorded in non-inoculated plants (Table 45). 30.4% significant reduction in plant height as a result of N deficiency was reported (de Almeida et al., 2017). Virk et al. (2018) reported that soybean plant height was insignificantly enhanced by N application.

Inoculation had no significant effect on this trait; however, inoculated plants were, on average, 1.8% taller than non-inoculated plants (Table 45). Abera et al. (2019) compared soybean plants using 7 rhizobia isolates and a non-inoculated control in an experiment conducted in 2 different sites. The authors reported that plant height of all inoculated treatments was higher than non-inoculated control at both experimental sites. Similar conclusion was also reported earlier by Bekere and Hailemaria (2012). Significant increases in soybean plant height (by 21.1 and 23.7%) as a result of inoculation were reported by Adeyemi et al. (2020) in pot and field experiments, respectively.

5.1.2.3.5. The Effect of Nitrogen and Inoculation on The Flower Number Per plant of Soybean Genotype 'Boglár' under Different Irrigation Regimes

Flower number per plant was differently affected in terms of inoculation; in inoculated plants, Irrigation had highly significant effect on this trait, whereas fertilization had no significant effect. In non-inoculated plants, irrigation had significant, and fertilization had highly-significant effects. However, the interaction between fertilization and irrigation had no significant effect, regardless of inoculation, whereas the correlation was positive, and in most cases significant, with both fertilization and irrigation (Table 46).

Table 46. Correlation coefficient of flower number per plant (FN), pod number per plant (PN), 100-seed weight (100-SW), yield, protein concentration and oil concentration traits with fertilization treatments and irrigation regimes.

Correlation	Inoculation Treatment	FN per plant	PN per plant	100-SW	Yield	Protein Concentration	Oil Concentration
with fertilization	Inoculated	.170	.120	.207*	.080	.423**	-.243*
	Non-inoculated	.351**	.340**	.405**	.253**	.427**	-.267**
with irrigation	Inoculated	.498**	.507**	.237*	.433**	.530**	-.402**
	Non-inoculated	.224*	.225*	.039	.219*	.452**	-.244*

- *. Correlation is significant at the 0.05 level (2-tailed).
- **. Correlation is significant at the 0.01 level (2-tailed).

Compared to non-irrigated regime, both half-and fully-irrigated regimes in inoculated plants significantly increased flower number per plant in all fertilization treatments (23.7 and 22.5% on average, respectively). Similar conclusion could be made in non-inoculated plants, where the differences were not significant, yet drought stress decreased flower number per plant by 13.5 and 12.6% compared to half- and fully-irrigated regimes, respectively. The effect size of irrigation on this trait was estimated as 36.3% and 8.7% in inoculated and non-inoculated plants, respectively (Table 47).

Increasing fertilization rate in inoculated plants was accompanied by relevant increases in flower number per plant under all irrigation regimes; average increase was 1.7 and 6.8% in 35N and 105N, respectively compared to 0N counterpart. All increases were insignificant. Similarly, 5.2 and 22.3% increased flower number per plant, on average,

were obtained from 35N and 105N treatments, respectively compared to 0N counterpart, with an effect size of 15.1% (Table 47).

2.8% higher flower number (47.2 flower per plant) was achieved in inoculated plants compared to non-inoculated counterparts (45.9 flower per plant) (Table 47).

Table 47. The effect of different fertilization treatments (0, 35 and 105 kg ha⁻¹) on flower number per plant of soybean genotype ‘*Boglár*’ under different irrigation regimes (non-irrigated, half-irrigated and fully-irrigated) in Látókép, Debrecen averaged over 2017, 2018 and 2019.

Inoculation	Treatment	0N	35N	105N	Average
Inoculated	Non-Irrigated	39.5 ^b	39.7 ^b	43.7 ^b	40.9
	Half-Irrigated	49.3 ^a	50.3 ^a	52.3 ^a	50.6
	Fully-Irrigated	48.9 ^a	50.2 ^a	51.1 ^a	50.1
	Average	45.9	46.7	49.0	47.2
Non-inoculated	Non-Irrigated	35.3 ^B	40.2 ^{AB}	49.9 ^A	41.8
	Half-Irrigated	45.5	48.5	50.8	48.3
	Fully-Irrigated	45.4	44.2	53.8	47.8
	Average	42.1	44.3	51.5	45.9

- In each inoculation treatment, different small letters indicate significant differences at .05 level among irrigation regimes within a certain fertilization treatment.
- In each inoculation treatment, different capital letters indicate significant differences at .05 level among fertilization treatments within a certain irrigation regime.

5.1.2.3.6. The Effect of Nitrogen and Inoculation on The Pod Number Per plant of Soybean Genotype ‘*Boglár*’ under Different Irrigation Regimes

Similar effects of fertilization and irrigation were estimated on soybean plants compared to flower number per plant trait, with similar correlation trend as well (Table 46).

Apart from a slight decrease under fully-irrigated regime in 105N treatment, compared to half-irrigated regime, increasing irrigation water amount in inoculated plants resulted in increasing pod number per plant. Moreover, the increase was significant under half-irrigated regime with an average increase of 22.0%. Non-inoculated plants, on the other hand, followed the same trend as of flower number per plant. The differences in pod number per plant were 33.8% in inoculated plants, and 7.9% in non-inoculated plants, caused by irrigation regimes (Table 48). Atti et al. (2004) found out that both drought

stress treatments (W1 and W2) had caused a 92.7 and a 67.3% reduction in pod number per plant, respectively at the beginning of pod formation, and an 81.6 and a 39.5% reduction, respectively at the pod lengthening stage. In another experiment, soybean plants that were subjected to drought stress at V4 stage had a very similar pod number per plant as compared to non-stressed control (45 and 47 pod per plant, respectively), whereas drought at R5 stage significantly decreased this number to 30 pod per plant (Jumrani & Bhatia 2018). The authors concluded that these reductions were caused by decreased flower number, reduced pod formation, increased pod abortion and decreased pod lengthening.

Table 48. The effect of different fertilization treatments (0, 35 and 105 kg ha⁻¹) on pod number per plant of soybean genotype '*Boglár*' under different irrigation regimes (non-irrigated, half-irrigated and fully-irrigated) in Látókép, Debrecen averaged over 2017, 2018 and 2019.

Inoculation	Treatment	0N	35N	105N	Average
Inoculated	Non-Irrigated	32.9 ^b	30.2 ^b	35.4 ^a	32.8
	Half-Irrigated	38.9 ^a	39.9 ^a	41.2 ^a	40.0
	Fully-Irrigated	39.5 ^a	41.9 ^a	40.3 ^a	40.6
	Average	37.1	37.3	38.9	37.8
Non-inoculated	Non-Irrigated	29.8 ^B	35.3 ^{AB}	39.5 ^A	34.8
	Half-Irrigated	37.2	40.3	40.5	39.3
	Fully-Irrigated	36.5	37.7	43.8	39.3
	Average	34.5	37.8	41.3	37.8

- In each inoculation treatment, different small letters indicate significant differences at .05 level among irrigation regimes within a certain fertilization treatment.
- In each inoculation treatment, different capital letters indicate significant differences at .05 level among fertilization treatments within a certain irrigation regime.

On average, pod number per plant in inoculated plants was enhanced by increasing fertilization rate; this trait was 0.5 and 4.9% higher in 35N and 105N treatments, respectively compared to 0N counterpart. This conclusion could also be reported in non-inoculated plants with higher differences among fertilization treatments; 9.6 and 19.7% average increase in this trait was recorded in 35N and 105N treatments, respectively compared to 0N counterpart (Table 48). The effect size of fertilization on this trait was estimated as 12.8%. Virk et al. (2018) concluded that N fertilization resulted in 15.2% increase in soybean pod number per plant. The Authors attributed this increase to

enhanced vegetative growth caused by fertilization. 21.1% higher pod number per plant was achieved by fertilization (Abera et al. 2019).

Inoculation did not result in changing the average pod number per plant (37.8 pod per plant) (Table 48).

5.1.2.3.7. The Effect of Nitrogen and Inoculation on the 100-seed Weight (g) of Soybean Genotype 'Boglár' under Different Irrigation Regimes

In inoculated plants, only irrigation had highly significant effect on the 100-seed weight, whereas only fertilization did in non-inoculated plants. However, the interaction between fertilization and irrigation had no significant effect, regardless of inoculation. Both treatments were significantly correlated with this trait, regardless of inoculation (except for correlation with irrigation in non-inoculated plants, where the correlation was positive but insignificant) (Table 46).

Increased 100-seed weight values were recorded in all three fertilization treatments in inoculated plants under half-irrigated compared to non-irrigated regime (12.8% on average), whereas fully-irrigated regime increased the value of this trait in 0N and 35N treatments but not in 105N treatment. This trait did not follow a particular trend in non-inoculated plants; however, irrigation, on average, enhanced this trait by 1.9 and 3.2% in half- and fully-irrigated regimes, respectively compared to drought-stressed counterpart (Table 49). Wei et al. (2018) concluded that moderate drought at R4 or at R6 stage decreased the 100-seed weight of soybean plants by 2.7 and 19.7%, respectively, whereas severe drought caused 26.1 and 44.4% decrease, respectively. Drought at R5 and R6 stages resulted in reduced seed size (Krivosudská and Filová 2013). Soybean plants subjected to drought stress conditions at either flowering or pod filling stage had 10.7 and 13.7% decrease in 100-seed weight, respectively (Sepanlo et al., 2014). Imposing drought stress at R4 stage by reducing available water from 100 to 50% FC slightly reduced 100-seed weight by 3.3%, whereas a significant 14.3% reduction in this trait was recorded when FC was further reduced to 20% (Iqbal et al. 2018). Freitas et al. (2016) reported that drought significantly reduced the average 100-seed weight from 16.5 to 14.5 g. Similar conclusion was reported by Popović et al. (2012) who concluded that drought stress resulted in a 21% decrease in 100-seed weight. Subjecting soybean plants to drought stress conditions at V4 stage resulted in a 9% decrease in 100-seed weight, whereas

drought stress at R5 stage caused a 36% reduction in this trait (Jumrani & Bhatia 2018). Reducing irrigation water from 100 to 70 and 55% FC was accompanied by a 5.4% 2.9% increase in 100-seed weight; however, the increase was insignificant (Gavili et al. 2019).

Table 49. The effect of different fertilization treatments (0, 35 and 105 kg ha⁻¹) on 100-seed weight (g) of soybean genotype '*Boglár*' under different irrigation regimes (non-irrigated, half-irrigated and fully-irrigated) in Látókép, Debrecen averaged over 2017, 2018 and 2019.

Inoculation	Treatment	0N	35N	105N	Average
Inoculated	Non-Irrigated	13.8 ^a	13.8 ^b	16.8 ^a	14.8
	Half-Irrigated	16.0 ^a	17.1 ^{ab}	17.1 ^a	16.7
	Fully-Irrigated	15.6 ^a	18.1 ^a	16.2 ^a	16.6
	Average	15.1	16.3	16.7	16.1
Non-inoculated	Non-Irrigated	12.4 ^B	15.8 ^{AB}	19.3 ^A	15.8
	Half-Irrigated	14.3	16.8	17.1	16.1
	Fully-Irrigated	15.3	14.8	18.7	16.3
	Average	14.0	15.8	18.3	16.0

- In each inoculation treatment, different small letters indicate significant differences at .05 level among irrigation regimes within a certain fertilization treatment.
- In each inoculation treatment, different capital letters indicate significant differences at .05 level among fertilization treatments within a certain irrigation regime.

Regardless of inoculation, average fertilization enhanced the 100-seed weight; the enhancement ratio was 7.9 and 10.6% in 35N and 105N, respectively compared to 0N counterpart in inoculated plants, and 12.9 and 30.7%, respectively in non-inoculated plants (Table 49). A 3.6% increase in 100-seed weight as a result of N fertilization was reported by Virk et al. (2018).

No measurable average difference in 100-seed weight was estimated by inoculation process (Table 49). Temesgen (2017) also concluded that inoculation had no significant effect on this trait, whereas Abera et al. (2019) reported a 2.7% increase in 100-seed weight as a result of fertilization. Inoculation, using two different inocula, significantly increased the 100-seed weight by 33.9% (using TAL 377 inoculum) and by 38.2% (using Isolate-2) (Elsheikh et al., 2009).

5.1.2.3.8. *The Effect of Nitrogen and Inoculation on The Seed Yield (kg ha^{-1}) of Soybean Genotype 'Boglár' under Different Irrigation Regimes*

In inoculated plants, only irrigation had highly significant effect on the yield, whereas both irrigation and fertilization had significant effect on the final yield of the non-inoculated plants. However, interaction between irrigation and fertilization had no significant effect on this trait, regardless of inoculation. Moreover, highly-significant correlation coefficient in relation with irrigation was estimated for inoculated plants, whereas both correlation coefficients with irrigation and fertilization were significant in non-inoculated plants (Table 46).

In inoculated plants, measurable increases were recorded in all fertilization treatments under half-irrigated regime as compared to non-irrigated counterpart; the increase ratio was, on average, 15.8% (being significant in both 0N and 35N treatments). Under fully-irrigated regime, however, further enhancements (by 1.9 and 4.6%) were recorded in 0N and 35N treatments, respectively, whereas yield was reduced by 2.8% in 105N treatment under this regime (Fig. 14). Overall irrigation effect on yield was calculated as 35.6%. In non-inoculated plants, on the other hand, average yield was reduced by drought. However, increasing the fertilization rate was accompanied with reducing the yield gap between drought-stressed treatments and the other two irrigated treatments (Fig. 15), leading to a conclusion of the importance of N fertilizer application under drought stress conditions in case the plants are not inoculated. 7.0% of changes in yield were caused by different irrigation regimes. Cerezini et al. (2016) concluded that plants subjected to drought stress at (V2–V4 and R1–R5) stages yielded 68% less than non-stressed counterparts, and fertilization partly ameliorated that effect and enhanced the yield to some extent. Drought negatively affects N_2 -fixation process and, eventually, the final yield (Purcell et al. 2004; Sinclair et al. 2007). Seed yield was decreased by 41 and 64% when available irrigation water was reduced from 100% to 70 and 55% FC, respectively (Gavili et al. 2019). The authors concluded that the decreased seed yield was caused by reduced number of seeds per pot. 63.7 and 57.1% reduction in soybean seed yield was reported by Sepanlo et al. (2014) in their experiment where drought was imposed at flowering or at pod filling stage, respectively. Drought stress significantly decreased the seed yield in soybean by 35.7% (Freitas et al. 2016). Liu et al. (2008) also reported that drought decreased soybean seed yield.

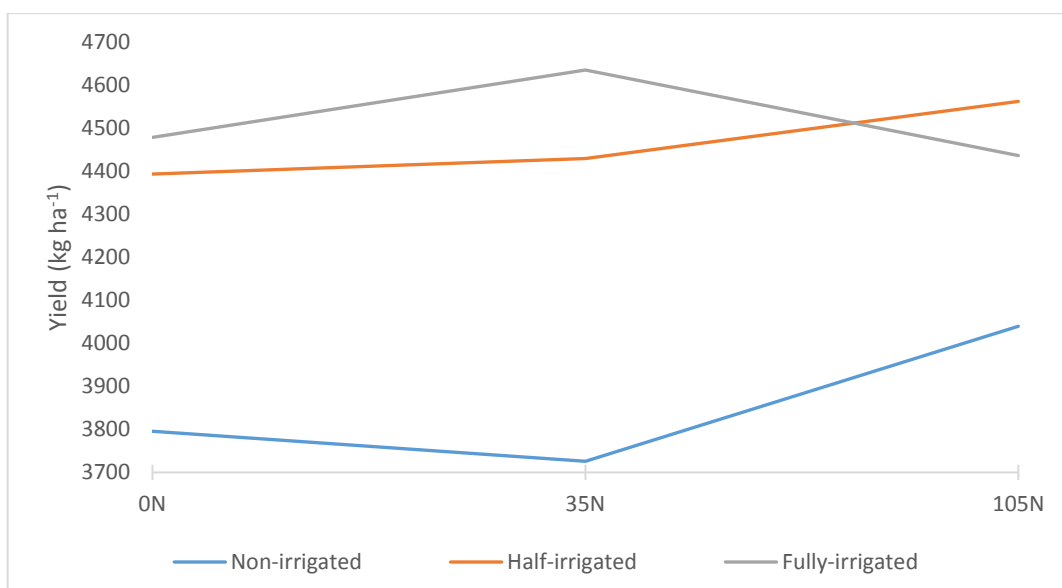


Fig. 14. The effect of different fertilization treatments (0, 35 and 105 kg ha⁻¹) on seed yield (kg ha⁻¹) of inoculated soybean genotype '*Boglár*' under different irrigation regimes (non-irrigated, half-irrigated and fully-irrigated) in Látókép, Debrecen averaged over 2017, 2018 and 2019.

In the presence of drought, whether severe (non-irrigated regime) or moderate (half-irrigated regime), 35N treatment did not have a measurable role on the yield in the case of inoculated plants, whereas 105N increased the yield by 6.4 and 3.8% under non- and half-irrigated regimes, respectively (Fig. 14), emphasizing the importance of N fertilization application in inoculated soybean under drought stress conditions. However, under fully-irrigated regime, 105N treatment resulted in the lowest yield compared to both 0N and 35N treatments, whereas 35N treatment increased the yield by 3.5 and 4.5% compared to 0N and 105N, respectively (Fig. 14). In total, the effect size of fertilization was much lower than that of irrigation and was estimated as only 1.6%. In non-inoculated plants, fertilization, in general, enhanced the final yield; 4.9 and 13.8% increased yield was achieved under half- and fully-irrigated regimes, respectively compared to drought-stressed counterpart (Fig. 15). The estimated effect size on the yield was 7.2%. Fertilization significantly increased the yield of soybean by 18.3% (Virk et al. 2018) and by 15.1% (Abera et al. 2019). The authors attributed this increase to enhancements in growth traits which have led to better carbohydrate synthesis and, consequently, better yield. Salvagiotti et al. (2009) also reported increased yield as a result of N application.

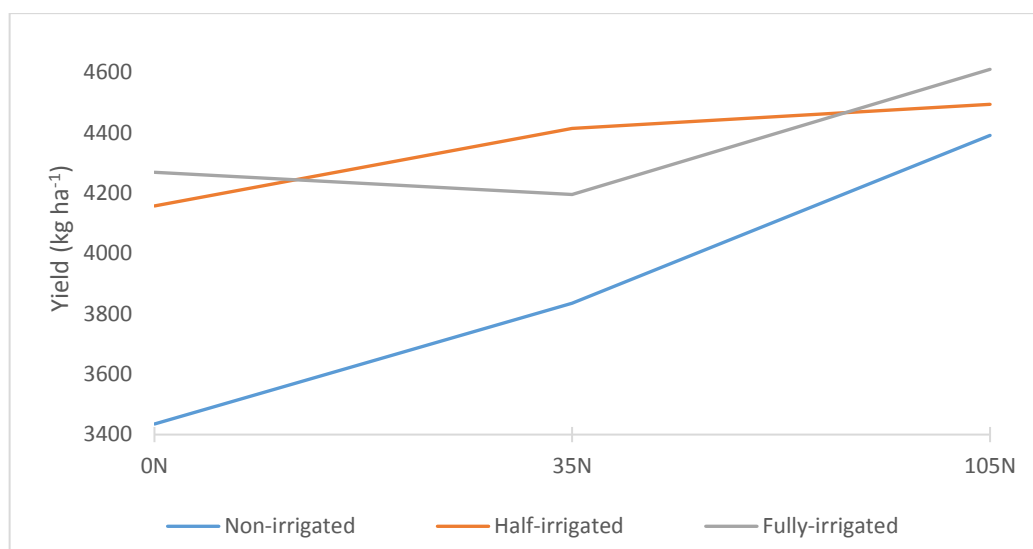


Fig. 15. The effect of different fertilization treatments (0, 35 and 105 kg ha⁻¹) on seed yield (kg ha⁻¹) of non-inoculated soybean genotype '*Boglár*' under different irrigation regimes (non-irrigated, half-irrigated and fully-irrigated) in Látókép, Debrecen averaged over 2017, 2018 and 2019.

On average, the inoculated plants resulted in 1.8% higher yield than the non-inoculated counterparts. However, the application of fertilization (35N and 105N treatments) under drought stress conditions (non-irrigated regime) resulted in higher yields in the non-inoculated plants, most probably because of the negative influence of mineral N fertilizer on the symbiotic process. Moreover, relatively-high fertilization rate (105N) resulted in better yield in non-inoculated plants, compared to inoculated counterparts, when drought was waived off. Hungria et al. (2013) reported an average of 8% yield enhancement as a result of inoculation treatment in the areas where *Bradyrhizobium* is well-established. Silva et al. (2013) concluded that inoculation process increased soybean seed yield by 18%. Similar conclusion was reported by Couto et al. (2011). Adeyemi et al. (2020) reported that inoculation significantly increased soybean yield in both pot (by 55%) and field (by 82%) experiments. Both fertilization and inoculation significantly increased the yield by 85 and 98%, respectively (Seneviratne et al. 2000).

5.1.2.3.9. The Effect of Nitrogen and Inoculation on The Protein Concentration (%) of Soybean Genotype 'Boglár' under Different Irrigation Regimes

Irrigation, fertilization and their interaction had highly significant effect on Protein concentration in both inoculated and non-inoculated plants. Moreover, highly-significant correlation with both treatments was estimated (Table 46).

In inoculated plants, protein concentration significantly increased with increasing irrigation water amount in both 0N and 35N treatments, whereas only fully-irrigated regime resulted in better protein concentration in 105N treatment. Drought resulted, on average, in 2.5% and 5.7% reduction in protein concentration compared to half- and fully-irrigated regimes, respectively (from 37.1% in fully-irrigated and 35.9% in half irrigated regimes to 35.0% in non-irrigated regime). Irrigation was responsible for 58.9% of changes in this trait. Drought significantly reduced protein concentration of non-inoculated plants in both 0N and 35N treatments, whereas it slightly enhanced this trait in 105N treatment. On average, 3.0 and 4.8% reductions were resulted from drought-stressed regime compared to half- and fully-irrigated regimes, respectively (Table 50). 34.1% of changes in this trait could be explained by changes in irrigation regimes.

Table 50. The effect of different fertilization treatments (0, 35 and 105 kg ha⁻¹) on seed protein concentration (%) of soybean genotype 'Boglár' under different irrigation regimes (non-irrigated, half-irrigated and fully-irrigated) in Látókép, Debrecen averaged over 2017, 2018 and 2019.

Inoculation	Treatment	0N	35N	105N	Average
Inoculated	Non-Irrigated	34.2 ^{cb}	33.7 ^{cb}	37.1 ^{abA}	35.0
	Half-Irrigated	35.5 ^b	35.6 ^b	36.7 ^b	35.9
	Fully-Irrigated	36.7 ^a	37.0 ^a	37.7 ^a	37.1
	Average	35.5	35.4	37.2	36.0
Non-inoculated	Non-Irrigated	34.5 ^{bb}	33.9 ^{bb}	37.6 ^A	35.4
	Half-Irrigated	36.0 ^{ab}	36.2 ^{ab}	37.5 ^A	36.5
	Fully-Irrigated	36.9 ^a	37.3 ^a	37.4	37.2
	Average	35.8	35.8	37.5	36.4

- In each inoculation treatment, different small letters indicate significant differences at .05 level among irrigation regimes within a certain fertilization treatment.
- In each inoculation treatment, different capital letters indicate significant differences at .05 level among fertilization treatments within a certain irrigation regime.

Regardless of inoculation, and apart from a slight, insignificant reduction in 35N under non-irrigated regime compared to 0N counterpart, fertilization enhanced protein concentration under all three irrigation regimes. Moreover, this increase was significant in 105N treatment under non-irrigated regime in inoculated plants, and under both non- and half-irrigated regimes in non-inoculated plants (Table 50). The effect size of fertilization treatments on protein concentration was 55.3 and 37.5% in inoculated and non-inoculated plants, respectively. Fertilization increased % seed N by 8.3% (Seneviratne et al. 2000). Abera et al. (2019) also reported that fertilization increased protein concentration in soybean seeds by 4.1%.

Interestingly, non-inoculated plants had higher protein concentration than inoculated counterparts under all irrigation regimes and in all fertilization treatments (except in 105N treatment under fully-irrigated regime), with an average increase of 1.1% (Table 50).

5.1.2.3.10. The Effect of Nitrogen and Inoculation on The Oil Concentration (%) of Soybean Genotype 'Boglár' under Different Irrigation Regimes

Table 51. The effect of different fertilization treatments (0, 35 and 105 kg ha⁻¹) on seed oil concentration (%) of soybean genotype 'Boglár' under different irrigation regimes (non-irrigated, half-irrigated and fully-irrigated) in Látókép, Debrecen averaged over 2017, 2018 and 2019.

Inoculation	Treatment	0N	35N	105N	Average
Inoculated	Non-Irrigated	23.1 ^{aAB}	23.2 ^{aA}	22.1 ^{aB}	22.8
	Half-Irrigated	22.2 ^b	22.0 ^b	21.7 ^a	22.0
	Fully-Irrigated	21.9 ^b	21.8 ^b	21.6 ^a	21.8
	Average	22.4	22.3	21.8	22.2
Non-inoculated	Non-Irrigated	22.4	22.5 ^a	21.6	22.2
	Half-Irrigated	21.8	21.8 ^{ab}	21.4	21.7
	Fully-Irrigated	22.0	21.4 ^b	21.4	21.6
	Average	22.1	21.9	21.5	21.8

- In each inoculation treatment, different small letters indicate significant differences at .05 level among irrigation regimes within a certain fertilization treatment.
- In each inoculation treatment, different capital letters indicate significant differences at .05 level among fertilization treatments within a certain irrigation regime.

Both irrigation and fertilization had highly significant effect on this trait in inoculated plants, and significant effect in non-inoculated plants, whereas their interaction did not. The correlation was negative with both treatments, regardless of inoculation (Table 46).

In inoculated plants, on the contrary to protein concentration, irrigation resulted in reduced oil concentration, regardless of fertilization treatment. Applied half-irrigated regime resulted in significantly reduced oil concentration in both 0N and 35N treatments, whereas the reduction was insignificant in 105N treatment. Increasing the irrigation water amount (fully-irrigated regime) further reduced oil concentration in all three fertilization treatments but in a much lower ratio. Similarly, in non-inoculated plants drought, on average, resulted in 2.3 and 2.8% increase in oil concentration compared to half- and fully-irrigated regimes, respectively (Table 51). 48.7% of changes in this trait were resulted from the different irrigation regimes applied in inoculated plants, and 8.1% in the case of non-inoculated plants.

On average, Oil concentration was reduced with increasing fertilization rate, regardless of inoculation. The average reduction resulted from higher fertilization rate (105N) was 2.7 and 2.2% compared to 0N and 35N treatments, respectively in inoculated plants, and 2.7 and 1.8%, respectively in non-inoculated plants (Table 51). The effect size of fertilization was calculated as 27.6% for inoculated, and 8.5% for non-inoculated plants. Fertilization reduced oil concentration in soybean seeds by 5.4% (Abera et al. 2019).

Inoculated plants had, on average, 1.8% higher oil concentration than non-inoculated counterparts (Table 51). In their experiment, Silva et al. (2013) reported that the fatty acid content in the inoculated soybean seeds was significantly higher than non-inoculated counterparts. Brechenmacher et al. (2010) reported that the inoculation process with *B. japonicum* has a role in fatty acid production which, in part, increases the cell membrane's fluidity, and helps the bacteria successfully colonize the cells. Moreover, this increase enhances abiotic stress tolerance (Brechenmacher et al. 2010). Similarly, Elsheikh et al. (2009) reported that 49.8 and 56.5% more fat concentration could be achieved by inoculating soybean seeds (with TAL 377 and Isolate-2 inocula, respectively).

5.1.3 The Effects of Drought Stress and Phosphorus Fertilization on The Morpho-physiology, Yield Components and Seed Quality of 2 Soybean Genotypes

5.1.3.1 The Effects of Drought Stress and Phosphorus Fertilization on The Stomatal Conductance (g_s) ($\text{mmol m}^{-2} \text{s}^{-1}$) of 2 Soybean Genotypes

Table 52. The effect of drought stress on stomatal conductance (g_s) ($\text{mmol m}^{-2} \text{s}^{-1}$) of 2 soybean genotypes under different P-fertilizer rates (0, 45 and 90 kg ha⁻¹) in Látókép, Debrecen averaged over 2017 and 2018 years.

Genotype	Irrigation regime	0P	45P	90P	Average
Pannonia Kincse	Drought-stressed	201.7 ^B	227.8 ^B	221.8 ^B	217.1
	Irrigated	393.3 ^{abA}	420.7 ^{aA}	389.2 ^{bA}	401.1
	Average	297.5	324.3	305.5	309.1
Boglár	Drought-stressed	176.8 ^B	194.7 ^B	195.5 ^B	189.0
	Irrigated	375.7 ^A	391.2 ^A	383.7 ^A	383.5
	Average	276.3	292.9	289.6	286.3

- For each genotype, different small letters indicate significant differences at .05 level among fertilization treatments within certain irrigation regime.
- For each genotype, different capital letters indicate significant differences at .05 level between irrigation regimes within certain fertilization treatment.

In the two studied genotypes, both irrigation and fertilization treatments had highly-significant effect on g_s , whereas their interaction did not.

In both genotypes, and regardless of irrigation regime, 45P treatment increased g_s (by 9.0 and 6.0% for '*Pannonia Kincse*' and '*Boglár*', respectively) compared to 0P. 90P treatment, on the other hand, had higher g_s than 0P, but not 45P (Tables 52 and 53). The effect size of fertilization on g_s in '*Pannonia Kincse*' genotype was estimated at 34.1; i.e. 34.1% of changes in g_s are the result of the different fertilization rates. In '*Boglár*' genotype, on the other hand, the effect size was estimated as 29.9%. However, the correlation between g_s and fertilization was slight and insignificant (Table 52).

Drought significantly decreased g_s in all fertilization treatments of both genotypes. The average reduction was 45.9 and 50.7% for '*Pannonia Kincse*' and '*Boglár*', respectively (Table 52). Irrigation was responsible for 97.2 and 98.7% of changes in g_s in '*Pannonia*

Kincse’ and *’Boglár*’, respectively. In addition, the correlation coefficient between g_s and irrigation was highly significant in both genotypes (Tables 56 and 57).

5.1.3.2 The Effects of Drought Stress and Phosphorus Fertilization on The Relative Chlorophyll Content (SPAD) of 2 Soybean Genotypes

The effect of fertilization was highly-significant on *’Pannonia Kincse*’ and significant on *’Boglár*’ genotype, whereas the effect of irrigation was only highly-significant on *’Pannonia Kincse*’. The interaction of fertilization and irrigation had no significant effect on both genotypes.

Table 53. The effect of drought stress on relative chlorophyll content (SPAD) of 2 soybean genotypes under different P-fertilizer rates (0, 45 and 90 kg ha⁻¹) in Látókép, Debrecen averaged over 2017 and 2018 years.

Genotype	Irrigation regime	0P	45P	90P	Average
Pannonia Kincse	Drought-stressed	42.2	43.4	43.0	42.9
	Irrigated	43.0	44.6	44.4	44.0
	Average	42.6	44.0	43.7	43.4
Boglár	Drought-stressed	36.8	37.9	37.7	37.5
	Irrigated	36.9	38.6	38.4	38.0
	Average	36.8	38.2	38.0	37.7

- For each genotype, different small letters indicate significant differences at .05 level among fertilization treatments within certain irrigation regime.
- For each genotype, different capital letters indicate significant differences at .05 level between irrigation regimes within certain fertilization treatment.

45P enhanced SPAD values in both genotypes compared to 0P, regardless of irrigation regime; however, the differences were insignificant. 90P did not further enhance SPAD values compared to 45P counterparts for both genotypes and under both irrigation regimes (Table 53). 29.6 and 21.4% of differences in SPAD were attributed to fertilization effect in *’Pannonia Kincse*’ and *’Boglár*’, respectively. The correlation with fertilization was significant in both genotypes (Tables 56 and 57).

Drought stress decreased SPAD values by an average of 2.5 and 1.3% for *’Pannonia Kincse*’ and *’Boglár*’, respectively; however, the reductions were insignificant (Table 53). 28.0% of differences in this trait were a result of drought stress in *’Pannonia Kincse*’, but only 4.0% in the case of *’Boglár*’ genotype.

5.1.3.3. The Effects of Drought Stress and Phosphorus Fertilization on The Leaf Area Index (LAI) of 2 Soybean Genotypes

Both fertilization and irrigation treatments significantly affected this trait in '*Pannonia Kincse*', whereas fertilization highly-significantly affected this trait in '*Boglár*' genotype, however, the irrigation effect was insignificant. Moreover, both genotypes were not affected by the interaction of fertilization and irrigation.

Table 54. The effect of drought stress on leaf area index (LAI) ($\text{m}^2 \text{m}^{-2}$) of 2 soybean genotypes under different P-fertilizer rates (0, 45 and 90 kg ha^{-1}) in Látókép, Debrecen averaged over 2017 and 2018 years.

Genotype	Irrigation regime	0P	45P	90P	Average
Pannonia Kincse	Drought-stressed	5.3	6.1	6.0	5.8
	Irrigated	5.9	6.7	6.8	6.5
	Average	5.6	6.4	6.4	6.1
Boglár	Drought-stressed	4.7	5.5	5.6	5.2
	Irrigated	5.1	5.9	6.2	5.7
	Average	4.9	5.7	5.9	5.5

- For each genotype, different small letters indicate significant differences at .05 level among fertilization treatments within certain irrigation regime.
- For each genotype, different capital letters indicate significant differences at .05 level between irrigation regimes within certain fertilization treatment.

Except for a slight decrease in 90P of '*Pannonia Kincse*' plants compared to 45P under drought stress, increasing P fertilizer rate was accompanied with increasing LAI values for both genotypes, regardless of irrigation regime. All the differences, however, were insignificant (Table 54). The effect size of the fertilization on LAI was estimated at 21.5 and 29.1% in '*Pannonia Kincse*' and '*Boglár*', respectively. He et al. (2019) reported that P enhanced LAI at both flowering and maturity stages. Averaged over the two genotypes, 60P, under drought stress conditions, increased LAI by 100 and 43% at flowering and maturity, respectively. 120P increased this trait by 113 and 48% at flowering and maturity, respectively. Under well-watered conditions, 138 and 46% increases in LAI at flowering and maturity, respectively were recorded in 60P, and 192 and 49% in 120P, respectively.

LAI values were reduced as a result of drought stress application, regardless of genotype and fertilization treatment. The average reduction caused by drought was 10.8 and 8.8%

for *'Pannonia Kincse'* and *'Boglár'*, respectively. In this trait as well the differences between the two irrigation regimes were insignificant (Table 54). 17.9 and 11.4% of changes in LAI were resulted from drought stress in *'Pannonia Kincse'* and *'Boglár'*, respectively. Only in *'Pannonia Kincse'* was the correlation coefficient between LAI and irrigation significant (Tables 56 and 57). He et al. (2019) also reported that drought stress decreased LAI at both flowering (by 48%) and maturity (by 47%).

5.1.3.4. The Effects of Drought Stress and Phosphorus Fertilization on The Plant Height (cm) of 2 Soybean Genotypes

Highly significant effects of both fertilization and irrigation were estimated in both genotypes, whereas the fertilization*irrigation effect was significant in *'Boglár'* only.

Table 55. The effect of drought stress on plant height (cm) of 2 soybean genotypes under different P-fertilizer rates (0, 45 and 90 kg ha⁻¹) in Látókép, Debrecen averaged over 2017 and 2018 years.

Genotype	Irrigation regime	0P	45P	90P	Average
Pannonia Kincse	Drought-stressed	70.7 ^{b2}	82.3 ^{a2}	79.8 ^{a2}	77.6
	Irrigated	82.0 ^{b1}	97.3 ^{a1}	95.7 ^{a1}	91.7
	Average	76.3	89.8	87.8	84.6
Boglár	Drought-stressed	69.5 ^{bB}	74.7 ^{aB}	75.8 ^{aB}	73.3
	Irrigated	76.8 ^{bA}	85.5 ^{aA}	88.8 ^{aA}	83.7
	Average	73.2	80.1	82.3	78.5

- For each genotype, different small letters indicate significant differences at .05 level among fertilization treatments within certain irrigation regime.
- For each genotype, different capital letters indicate significant differences at .05 level between irrigation regimes within certain fertilization treatment.

P-fertilizer application, under both irrigation regimes, significantly increased plant height in both genotypes compared to non-fertilized counterpart. However, increasing the fertilization rate (90P) had no significant effect on this trait compared to the lower rate (45P); it slightly increased the plant height of *'Boglár'* genotype, but decreased it in *'Pannonia Kincse'* genotype (Table 55). 88.3 and 79.3% of differences in plant height in *'Pannonia Kincse'* and *'Boglár'*, respectively were attributed to different fertilization rates, with a highly significant correlation coefficient (Tables 56 and 57). Adjei-Nsiah et al. (2019) tested the effect of 2 different sources of P fertilizer; triple superphosphate (TSP) (46% P₂O₅) and Morocco phosphate rock (MPR) (30% P₂O₅) on 3 soybean

genotypes. Fertilization rate was applied at 30 kg P ha⁻¹. They concluded that P fertilization from both sources significantly increased the plant height; by 10.5% in MPR treatment, and by 21.1% in TSP treatment.

Regardless of fertilization treatment, drought stress significantly decreased the plant height of both genotypes; the average reduction was 15.4 and 12.4% in '*Pannonia Kincse*' and '*Boglár*', respectively (Table 55). Drought stress was responsible for 91.4 and 87.2% changes in the plant height of '*Pannonia Kincse*' and '*Boglár*', respectively. In addition, the plant height of both genotypes was highly-significantly correlated with irrigation treatments (Tables 56 and 56).

Table 56. Correlation coefficient of irrigation and fertilization treatments with stomatal conductance (g_s), relative chlorophyll content (SPAD), leaf area index (LAI), plant height (PH), pod number per plant (PN), yield, protein concentration (PC) and oil concentration (OC) of soybean genotype '*Pannonia Kincse*'.

Treatment	g_s	SPAD	LAI	PH	PN	Yield	PC	OC
Irrigation	.977**	.461**	.382*	.740**	.848**	.752**	-.534**	.188
Fertilization	.035	.364*	.376*	.491**	.339*	.505**	.015	.815**

- **. Correlation is significant at the 0.01 level (2-tailed).
- *. Correlation is significant at the 0.05 level (2-tailed).

5.1.3.5. The Effects of Drought Stress and Phosphorus Fertilization on The Pod Number Per plant of 2 Soybean Genotypes

The effect of fertilization on this trait was highly significant in both genotypes, whereas irrigation's effect was highly significant in the case of '*Pannonia Kincse*', and significant in the case of '*Boglár*'. However, the interaction of irrigation and fertilization did not have any significance, regardless of genotype.

Under both irrigation regimes, pod number per plant in both genotypes was lower in non-fertilized plots compared to fertilized counterparts; however, the reduction was insignificant (except for drought-stressed, non-fertilized treatment of '*Boglár*', where the reduction was significant) (Table 58). Fertilization rates had an effect percentage of 48.2 and 59.4% of the pod number per plant of '*Pannonia Kincse*' and '*Boglár*', respectively. The correlation coefficient of this trait with fertilization was significant, and higher for '*Boglár*' compared to '*Pannonia Kincse*' (Tables 56 and 57). He et al. (2019) reported

that pod number per plant increased (by 13 and 140% in HD and ZH, respectively) in 60P under drought, whereas 120P did not further increase this trait. They also reported that under well-watered treatment, pod number per plant increased by 74 and 89% in 60P for HD and ZH, respectively, whereas 120P further increased this trait for HD, but not for ZH. Kamara et al. (2007) conducted field experiments to evaluate the response of four soybean cultivars to P application (0, 20, and 40 kg P ha⁻¹). Their results demonstrated that pod number per plant increased by 42.5% when 20 kg ha⁻¹ of P fertilizer was applied, whereas 40 kg ha⁻¹ P increased this trait by 56.0%. Adjei-Nsiah et al. (2019) found out that both P-fertilizer sources did not enhance pod number per plant in the pot experiment, whereas 8.3 and 22.3% more pod per plant were recorded when P was applied from MRP and TSP sources, respectively. Moreover, they concluded that P-fertilizer from TSP source had significantly greater number of pods than both P-fertilizer treatment from MRP source and the non-fertilized control. Similar results were reported earlier by Rani (1999).

Table 57. Correlation coefficient of irrigation and fertilization treatments with stomatal conductance (g_s), relative chlorophyll content (SPAD), leaf area index (LAI), plant height (PH), pod number per plant (PN), yield, protein concentration (PC) and oil concentration (OC) of soybean genotype '*Boglár*'.

Treatment	g_s	SPAD	LAI	PH	PN	Yield	PC	OC
Irrigation	.991**	.176	.288	.753**	.230	.637**	-.913**	.577**
Fertilization	.055	.357*	.488**	.543**	.661**	.569**	.015	.669**

- **. Correlation is significant at the 0.01 level (2-tailed).
- *. Correlation is significant at the 0.05 level (2-tailed).

Although drought reduced pod number per plant in both genotypes, yet its effect was more measurable on '*Pannonia Kincse*', where the reduction was significant, regardless of fertilization treatment (Table 58). In '*Boglár*', however, pod number per plant was significantly lower in 0P treatment, whereas the difference was slight and insignificant in both 45P and 90P treatments (Table 58), leading to a conclusion that P-fertilizer application could partly ameliorate the negative effect of drought stress on this trait by decreasing the reduction level of pods resulting from exposure to drought. 83.4 of differences in this trait were attributed to drought stress application on '*Pannonia Kincse*' genotype, which was considerably higher than the effect of drought stress application on

'Boglár' genotype where the effect size was estimated as 12.9%. This conclusion was supported by the higher correlation coefficient of this trait with irrigation treatments in the case of 'Pannonia Kincse' compared to 'Boglár' genotype (Tables 56 and 57). It was previously reported that drought stress negatively affects pollination process, leading to increased flower and pod abortion (Desclaux et al., 2000; Fang et al., 2010). Pod number per plant decreased by 49 and 43% in HD and ZH, respectively as a result of drought stress application (He et al., 2019).

Table 58. The effect of drought stress on pod number (per plant) of 2 soybean genotypes under different P-fertilizer rates (0, 45 and 90 kg ha⁻¹) in Látókép, Debrecen averaged over 2017 and 2018 years.

Genotype	Irrigation regime	0P	45P	90P	Average
Pannonia Kincse	Drought-stressed	39.0 ^B	40.7 ^B	40.8 ^B	40.1
	Irrigated	43.2 ^{bA}	45.3 ^{aA}	45.9 ^{aA}	44.8
	Average	41.1	43.0	43.3	42.5
Boglár	Drought-stressed	36.2 ^{bB}	39.9 ^a	40.3 ^a	38.8
	Irrigated	38.4 ^A	40.1	40.5	39.7
	Average	37.3	40.0	40.4	39.2

- For each genotype, different small letters indicate significant differences at .05 level among fertilization treatments within certain irrigation regime.
- For each genotype, different capital letters indicate significant differences at .05 level between irrigation regimes within certain fertilization treatment.

5.1.3.6. The Effects of Drought Stress and Phosphorus Fertilization on The Seed Yield (kg ha⁻¹) of 2 Soybean Genotypes

Regardless of genotype, both irrigation and fertilization treatments, but not their interaction, had highly significant effects on the final seed yield. The correlation of both treatments with the yield was also highly significant in both genotypes.

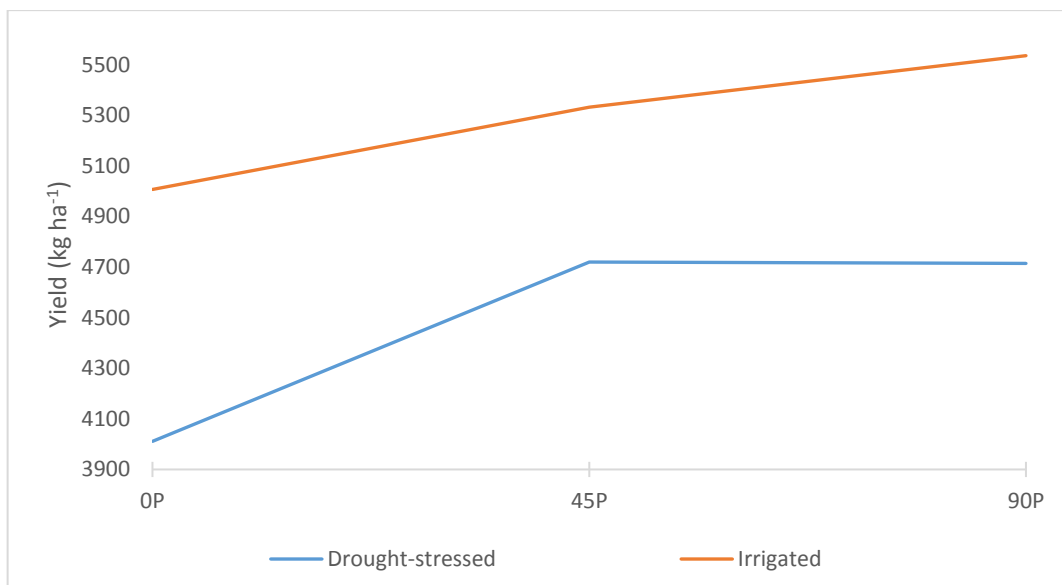


Fig. 16. The effect of drought stress on the seed yield (kg ha^{-1}) of soybean genotype '*Pannonia Kincse*' under different P-fertilizer rates (0, 45 and 90 kg ha^{-1}) in Látókép, Debrecen averaged over 2017 and 2018 years.

Fertilization, regardless of rate, significantly increased the final seed yield of both genotypes and under both irrigation regimes. However, 90P did not result in any further yield increase compared to 45P counterpart under drought stress conditions, whereas it slightly increased the yield under irrigated regime in both genotypes (Table 59, Fig. 16 and 17). 73.3 and 67.6% of changes in the final seed yield were attributed to the different rate of fertilization in '*Pannonia Kincse*' and '*Boglár*', respectively. Soil available-P deficiency is an important limiting factor in the development and the final yield of soybean (Wissuwa, 2003). In their experiment, Jin et al. (2006) reported that in Heisheng 101, 15P increased yield by 1.4% when there was no drought, and by 9.3 and 16.5% when drought occurred at R1 and R4, respectively. 30P increased yield by 12.1% compared to 15P under no-drought, but reduced it by 5.9 and 3.4% under drought at R1 and R4, respectively but still higher than 0P. In Dongnong 46, only 30P increased yield compared to 0P under no-drought, but both 15P and 30P increased yield by 1.1 and 5.0% when drought happened at R1, and by 52.1 and 68.9% when drought happened at R4 (Jin et al., 2006). The authors also reported that seed yield was significantly associated with P accumulation before and after the initial pod filling (R5) stage and also with the total P accumulation. Zheng et al. (2009) studied an area consisting of 43 soybean fields in China in 2007 when soybean plants suffered from severe drought stress. The authors reported that P-fertilizer rate was the highest effecting factor (by 60.6%) that was attributed to

differences in the final yield. Adjei-Nsiah et al. (2019) reported that yield was enhanced by P fertilization from both sources (by 10.0 and 8.6% in MPR and TSP treatments, respectively); however, the increases were insignificant. 52 and 63% higher seed yields were recorded in 20P and 40P treatments, respectively compared to 0P counterpart (Kamara et al., 2007). The authors reported that seed yield was strongly associated with pod number per plant and seed weight. Similar conclusions on yield enhancement by P application was also reported by Lamptey et al. (2014) and Ronner et al. (2016). The application of P fertilizer in the recommended rate (35 kg ha⁻¹) significantly increased the yield by 71% (Mahanta et al., 2014). Under drought stress conditions, the yield increased by 10 and 50% in 60P, and by 30 and 63% in 120P for HD and ZH, respectively. Under well-watered conditions, however, 60P increased the yield by 143 and 41% for HD and ZH, respectively, whereas 120P did not have measurable effect on the final yield (He et al., 2019). The authors attributed the yield improvement by P application to the improved filled-pod number and grain number, whereas Belanger et al. (2002) concluded that P application enhanced the shoot biomass and, consequently, the seed yield.

Table 59. The effect of drought stress on seed yield (kg ha⁻¹) of 2 soybean genotypes under different P-fertilizer rates (0, 45 and 90 kg ha⁻¹) in Látókép, Debrecen averaged over 2017 and 2018 years.

Genotype	Irrigation regime	0P	45P	90P	Average
Pannonia Kincse	Drought-stressed	4011 ^{bB}	4720 ^{aB}	4715 ^{aB}	4482
	Irrigated	5007 ^{bA}	5332 ^{aA}	5536 ^{aA}	5292
	Average	4509	5026	5126	4887
Boglár	Drought-stressed	3721 ^{bB}	4422 ^{aB}	4412 ^{aB}	4185
	Irrigated	4409 ^{bA}	5038 ^{aA}	5124 ^{aA}	4857
	Average	4065	4729	4768	4521

- For each genotype, different small letters indicate significant differences at .05 level among fertilization treatments within certain irrigation regime.
- For each genotype, different capital letters indicate significant differences at .05 level between irrigation regimes within certain fertilization treatment.

The final seed yield was significantly decreased by drought, regardless of genotype and fertilization treatment. On average, ‘*Pannonia Kincse*’ and ‘*Boglár*’ had 13.5 and 12.5% less yield, respectively as a result of drought stress (Table 59). Drought stress was estimated to be responsible for 83.6 and 68.0% of the differences of the final seed yield of ‘*Pannonia Kincse*’ and ‘*Boglár*’, respectively. Similar conclusion was reported by Jin

et al. (2006) who also reported that the application of P fertilizer could mitigate the negative effect of drought stress on yield in both cultivars. Other researchers reported similar effect in soybean (He et al., 2017a) and in other crops [moth bean (Garg et al., 2004) and malting barley (Jones et al., 2003)]. Drought stress decreased the yield of both genotypes (by 60 and 50% in HD and ZH, respectively) (He et al., 2019). Many previous papers reported similar negative effect of drought stress on soybean seed yield (e.g. Manavalan et al., 2009; Masoumi et al., 2011; Behtari and Abadiyyan, 2009; He et al., 2017b).

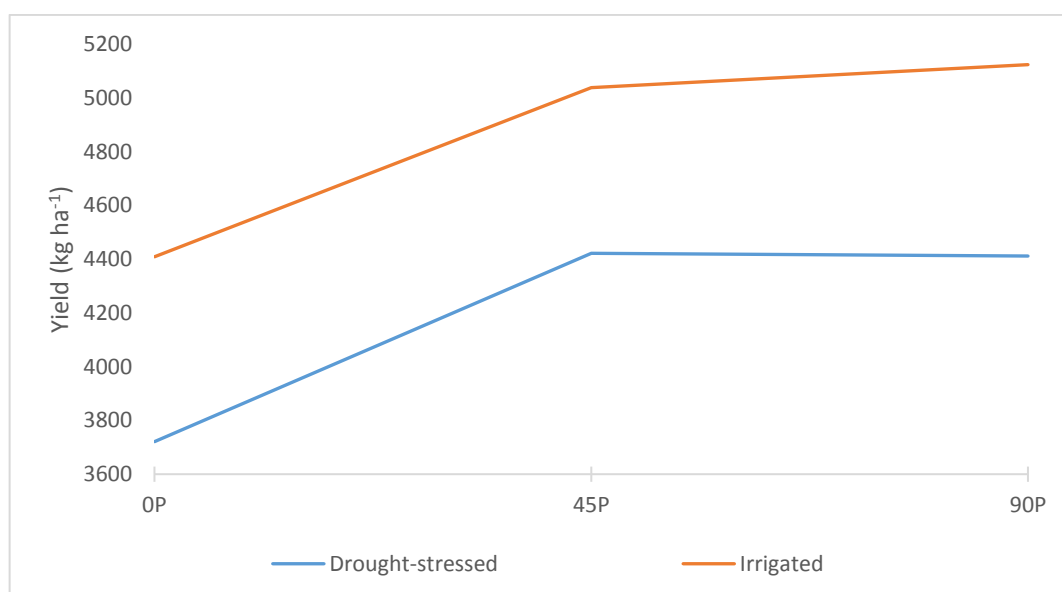


Fig. 17. The effect of drought stress on the yield (kg ha^{-1}) of soybean genotype 'Boglár' under different P-fertilizer rates (0, 45 and 90 kg ha^{-1}) in Látókép, Debrecen averaged over 2017 and 2018 years.

5.1.3.7. The Effects of Drought Stress and Phosphorus Fertilization on The Protein Concentration (%) of 2 Soybean Genotypes

Irrigation had highly significant effect on protein concentration in both genotypes; moreover, the correlation between protein concentration and irrigation treatments was significantly negative, i.e. increasing irrigation water amount was accompanied by decreasing protein concentration (Tables 56 and 57). In other words; drought stress increased protein concentration, which is demonstrated in table 60. Fertilization, on the other hand, had relatively low effect on this trait, with a non-significant correlation (Tables 56 and 57).

Table 60. The effect of drought stress on protein concentration (%) of 2 soybean genotypes under different P-fertilizer rates (0, 45 and 90 kg ha⁻¹) in Látókép, Debrecen averaged over 2017 and 2018 years.

Genotype	Irrigation regime	0P	45P	90P	Average
Pannonia Kincse	Drought-stressed	39.1	39.2	40.0 ¹	39.5
	Irrigated	37.8	38.4	37.0 ²	37.7
	Average	38.5	38.8	38.5	38.6
Boglár	Drought-stressed	39.6 ^A	40.9 ^A	39.9 ^A	40.1
	Irrigated	34.3 ^B	34.5 ^B	34.2 ^B	34.3
	Average	36.9	37.7	37.1	37.2

- For each genotype, different small letters indicate significant differences at .05 level among fertilization treatments within certain irrigation regime.
- For each genotype, different capital letters indicate significant differences at .05 level between irrigation regimes within certain fertilization treatment.

Compared to 0P treatment, 45P treatment resulted in relatively higher protein concentration, regardless of genotype and irrigation regime. 90P treatment, on the other hand, resulted in higher protein concentration only under drought stress conditions, but not under irrigated conditions. However, all differences were insignificant (Table 60). Jin et al. (2006) tested 2 soybean genotypes different in seed protein concentration; their results demonstrated that regardless of water availability, in both genotypes both 15P and 30P increased seed protein compared to 0P, however, 15P was higher than 30P in most cases (Jin et al., 2006).

Drought stress resulted in significantly higher protein concentration in both genotypes, regardless of fertilization treatment. The average protein concentration was 4.8 and 16.9% higher of drought-stressed '*Pannonia Kincse*' and '*Boglár*' plants, respectively compared to their irrigated counterparts (Table 60). 31.2% of increased protein concentrations were attributed to drought stress in '*Pannonia Kincse*', and drought had even higher (84.7%) attribution in the case of '*Boglár*' genotype. Increased protein contents under drought stress were reported earlier (e.g. Rotundo and Westgate, 2009; Wang and Frei, 2011) and were explained by drought stress rapidly remobilizing nitrogen from leaves to seeds (Brevedan and Egli, 2003) which leads to increasing protein concentration, or by reducing seed number with increased seed size (Borras et al., 2004).

5.1.3.8. The Effects of Drought Stress and Phosphorus Fertilization on The Oil Concentration (%) of 2 Soybean Genotypes

Fertilization had highly significant effect on the oil concentration in both genotypes, and irrigation had significant effect on this trait in '*Pannonia Kincse*' genotype, and even highly significant effect in the case of '*Boglár*' genotype.

Table 61. The effect of drought stress on oil concentration (%) of soybean genotype '*Pannonia Kincse*' under different P-fertilizer rates (0, 45 and 90 kg ha⁻¹) in Látókép, Debrecen averaged over 2017 and 2018 years.

Genotype	Irrigation regime	0P	45P	90P	Average
Pannonia Kincse	Drought-stressed	20.4 ^c	21.6 ^b	23.1 ^a	21.7
	Irrigated	21.2 ^b	22.4 ^a	22.7 ^a	22.1
	Average	20.8	22.0	22.9	21.9
Boglár	Drought-stressed	21.2 ^{cB}	22.6 ^{bB}	23.7 ^{aB}	22.5
	Irrigated	23.0 ^{bA}	24.3 ^{aA}	24.9 ^{aA}	24.1
	Average	22.1	23.4	24.3	23.3

- For each genotype, different small letters indicate significant differences at .05 level among fertilization treatments within certain irrigation regime.
- For each genotype, different capital letters indicate significant differences at .05 level between irrigation regimes within certain fertilization treatment.

Both fertilization treatments (45P and 90P) significantly increased oil concentration in both genotypes and under both irrigation regimes. Moreover, 90P treatment had significantly higher oil concentration than 45P treatment in both genotypes under drought stress conditions, but not under irrigated conditions. Compared to 0P treatment, 45P and 90P treatments resulted, on average, in 5.8 and 10.1% higher oil concentration, respectively in '*Pannonia Kincse*', and 5.9 and 10.0%, respectively in '*Boglár*' (Table 61). Fertilization rates were responsible for 74.8 and 69.3% of differences in this trait in '*Pannonia Kincse*' and '*Boglár*', respectively, with a highly significant correlation of this trait with fertilization treatments (Tables 56 and 57). Our results are consistent with those of Costache and Nica (1968) and Dadson and Acquaah (1984) who concluded that increasing P rate significantly increased oil concentration in the seeds. Also, Win et al. (2010) reported that adding 1.0 mmol l⁻¹ of P (in the form of KH₂PO₄) to Hoagland solution (1 mM P) increased oil concentration in three soybean cultivars by 7.1%, whereas further increasing P concentration to 2 mM P reduced oil concentration by 3.3% compared to 1 mM P treatment, yet it was still higher than non-fertilized control by 3.6%.

Drought, on average, resulted in reducing the oil concentration in both genotypes, with more measurable effect in '*Boglár*', where the difference was significant, regardless of fertilization treatment (with and average reduction of 6.6%) (Table 61). Similar to its effect on the protein concentration, drought affected '*Boglár*' genotype by a higher ratio (62.2%) than did on '*Pannonia Kincse*' genotype (13.6), which is further supported by the correlation coefficient, as it was highly significant in '*Boglár*', but not in '*Pannonia Kincse*' (Tables 56 and 57). Results of many studies indicated that drought stress reduced oil concentration in soybean seeds (e.g. Bellaloui and Mengistu, 2008; Maleki et al., 2013).

5.1.4. The Effects of Drought Stress and Exogenous Application of Hydrogen Peroxide (H_2O_2) on The Physiology and The Yield of 2 Soybean Genotypes

5.1.4.1. The Effects of Drought Stress and Exogenous Application of Hydrogen Peroxide (H_2O_2) on The Stomatal Conductance (g_s) ($mmol\ m^{-2}\ s^{-1}$) of 2 Soybean Genotypes

Table 62. Stomatal conductance (g_s) of soybean genotypes '*Boglár*' and '*Pannonia Kincse*' under three irrigation treatments; fully-irrigated (FI), drought-stressed with H_2O_2 foliar spray (HP) and drought-stressed (DW) in Látókép, Debrecen averaged over 2017 and 2018 years.

Trait	Treatment	' <i>Boglár</i> '	' <i>Pannonia Kincse</i> '
g_s ($mmol\ m^{-2}\ s^{-1}$)	DW	190.0 ^c	218.3 ^c
	HP	307.7 ^b	336.3 ^b
	FI	392.7 ^a	417.7 ^a

- The same letter indicates no significant differences at .05 level among the treatments within the same genotype.

In both genotypes, g_s was significantly higher when irrigation (FI) was applied; however, H_2O_2 -sprayed plots were significantly higher than drought-stressed counterparts in terms of g_s value. Drought application reduced g_s by 51.6 and 47.7% compared to irrigated counterparts, whereas H_2O_2 spraying decreased the reduction ratio to 21.6 and 19.5% in '*Boglár*' and '*Pannonia Kincse*', respectively (Table 62). Correlation between g_s and irrigation treatment was highly significant ($<.01$) (Table 63), and the effect size of H_2O_2 application (calculated as partial Eta squared) was 81.6 and 90.5% in '*Boglár*' and '*Pannonia Kincse*', respectively; in other words, H_2O_2 application was responsible for

81.6 and 90.5% of g_s changes in '*Boglár*' and '*Pannonia Kincse*', respectively. Drought stress induces stomatal closure, limits gas exchange and photosynthesis (Yordanov et al., 2000). Ishibashi et al. (2011) reported that g_s was significantly higher in H_2O_2 -treated plants than in DW-treated plants. After two days of spraying, g_s levels in H_2O_2 -treated and DW-treated plants were 508 and 323 $mmol\ m^{-2}\ s^{-1}$, respectively. They concluded that H_2O_2 spraying reduced stomatal closure caused by drought stress; i.e. H_2O_2 treatment reduced soybean sensitivity to drought stress. In another experiment, maize leaves pretreated with 10 mM H_2O_2 significantly enhanced g_s (by about 50%) as compared to drought-stressed leaves (Terzi et al., 2014); they concluded that spraying leaves with H_2O_2 can reduce water loss under drought stress conditions by increasing the concentrations of metabolites that are involved in osmotic adjustment (like proline, polyamines and soluble sugars). Other ROS species were also reported to have a role in alleviating drought stress; Razmi et al. (2017) reported that water stress reduced stomatal conductance of three soybean leaves compared to well-watered counterparts, and foliar spray of 0.4 mM Salicylic Acid (SA) significantly reversed drought induced stomatal closure and increased it.

Table 63. Correlation coefficient of irrigation treatments with the studied traits.

Trait	' <i>Boglár</i> '	' <i>Pannonia Kincse</i> '
g_s	.959**	.975**
SPAD	.758*	.926**
RWC	.929**	.948**
LAI	.668*	.720*
Plant Height	.908**	.755*
Yield	.861**	.912**

- *. Correlation is significant at the 0.05 level (2-tailed).
- **. Correlation is significant at the 0.01 level (2-tailed).

5.1.4.2. The Effects of Drought Stress and Exogenous Application of Hydrogen Peroxide (H_2O_2) on The Relative Chlorophyll Content (SPAD) of 2 Soybean Genotypes

In '*Boglár*', drought significantly decreased SPAD trait by 26.7% (to 26.4) compared to the irrigated counterpart (36.0), whereas H_2O_2 spraying resulted in better SPAD (37.2) than both (FI) and (DW) counterparts. In '*Pannonia Kincse*', on the other hand, irrigation resulted in the highest SPAD value (43.3); it was significantly higher (by 22.4%) than drought-stressed counterpart (33.6). However, (HP) treatment enhanced this trait (by

13.1%) compared to (DW), without reaching the same level of (FI) treatment as in 'Boglár' (Table 64). The correlation with irrigation was significant ($<.05$) and highly significant ($<.01$) in 'Boglár' and 'Pannonia Kincse' genotypes, respectively (Table 63), and the effect size of H_2O_2 application was noticeably higher (85.7%) in 'Boglár' compared to 'Pannonia Kincse' (59.1%). Similarly, Ergo et al. (2018) reported that SPAD values significantly decreased from 35.5 to 22.4 under drought stress applied 30 days after R5.5 stage. Subjecting plants to drought stress resulted in a significant decline in chl $a+b$ (from 19.5 to 13.0 mg g⁻¹ DW), indicating a reduced capacity of absorbing and converting light energy (Tang et al., 2017). Similarly, Dong et al. (2015) concluded that light absorption was reduced by drought stress which resulted in changing both leaf area index and leaf chlorophyll content. Both chlorophylls a and b were reduced under drought stress (Farooq et al., 2010). Other papers also reported that chlorophyll content was decreased because of drought in soybean (Makbul et al., 2011), chickpea (Mafakheri et al., 2010) and pea (Inaki-Iturbe et al., 1998). That reduction was attributed to induced destruction of the chloroplasts and to the instability of the chlorophyll protein complex (Khan et al., 2015). Sun et al. (2016) reported that the application of H_2O_2 significantly increased the leaf chlorophyll content of cucumber plants exposed to medium drought conditions. An evaluation of the effects of H_2O_2 on leaf chlorophyll content during adventitious rooting under drought conditions showed that drought stress resulted in a decline in chlorophyll content after 72 h of its application, producing a 39.1% decrease in the chlorophyll a content compared to control; however, applying exogenous H_2O_2 retarded chlorophyll degradation, especially chlorophyll a (Liao et al., 2012). Maize leaves had higher levels of both chlorophylls a and b When seeds were soaked in 140 mM H_2O_2 before sowing (Ashraf et al., 2015). Enhanced chlorophyll levels induced by hydrogen peroxide treatment was justified by H_2O_2 -stimulated antioxidant enzyme activities (Azevedo Neto et al., 2005; Gao et al., 2010). In their experiment, Razmi et al. (2017) reported that drought significantly reduced both chlorophyll a and b contents in soybean leaves; however, significant increases (by 15% in chlorophyll a and 19% in chlorophyll b) resulted from foliar application of 0.4 mM SA compared to control treatment (no SA).

Table 64. Relative chlorophyll content (SPAD) of soybean genotypes '*Boglár*' and '*Pannonia Kincse*' under three irrigation treatments; fully-irrigated (FI), drought-stressed with H₂O₂ foliar spray (HP) and drought-stressed (DW) in Látókép, Debrecen averaged over 2017 and 2018 years.

Trait	Treatment	' <i>Boglár</i> '	' <i>Pannonia Kincse</i> '
SPAD	DW	26.4 ^b	33.6 ^b
	HP	37.2 ^a	38.0 ^b
	FI	36.0 ^a	43.3 ^a

- The same letter indicates no significant differences at .05 level among the treatments within the same genotype.

5.1.4.3. The Effects of Drought Stress and Exogenous Application of Hydrogen Peroxide (H₂O₂) on The Relative Water Content (RWC) (%) of 2 Soybean Genotypes

In '*Boglár*', drought significantly decreased RWC (to 57.3%) compared to the irrigated counterpart (72.7%). H₂O₂-sprayed treatment, however, significantly increased RWC (to 69.3%) compared to drought-stressed treatment and had very close value to (FI) treatment. In '*Pannonia Kincse*', applying H₂O₂ significantly increased RWC (to 79.7%) compared to the drought-stressed treatment (61.7%); however, irrigation treatment had significantly higher RWC (68.3%) compared to (HP) and (DW) treatments (Table 65).

H₂O₂ application had a significant effect size on RWC (by 95.9 and 97.3% in '*Boglár*' and '*Pannonia Kincse*', respectively) with a highly significant correlation coefficient (Table 63). It was previously reported that drought stress reduced RWC of soybean leaves (Razmi et al., 2017). In their experiment, Ishibashi et al. (2011) reported that RWC in H₂O₂-treated and DW-treated (control, treated with distilled water only) plants was 60 and 40%, respectively after 4 days of drought stress application, and was also higher in H₂O₂-treated plants than in DW-treated plants after 6 days of drought stress imposition; they concluded that H₂O₂ spraying enabled the leaves to maintain high levels of RWC by regulating the osmolality in the leaves, consequently ameliorating the negative effects of drought stress. Similar results on cucumber seedlings were reported later by Sun et al. (2016). Application of (SA) on common bean improved RWC under drought stress conditions (Sadeghipour and Aghaei, 2012).

Table 65. Relative water content (RWC) of soybean genotypes '*Boglár*' and '*Pannonia Kincse*' under three irrigation treatments; fully-irrigated (FI), drought-stressed with H₂O₂ foliar spray (HP) and drought-stressed (DW) in Látókép, Debrecen averaged over 2017 and 2018 years.

Trait	Treatment	' <i>Boglár</i> '	' <i>Pannonia Kincse</i> '
RWC (%)	DW	57.3 ^b	61.7 ^c
	HP	69.3 ^a	79.7 ^b
	FI	72.7 ^a	86.3 ^a

- The same letter indicates no significant differences at .05 level among the treatments within the same genotype.

5.1.4.4. The Effects of Drought Stress and Exogenous Application of Hydrogen Peroxide (H₂O₂) on The Leaf Area Index (LAI) of 2 Soybean Genotypes

Both genotypes followed the same trend; LAI was significantly lower in (DW) treatment (by 13.0 and 17.5% in '*Boglár*' and '*Pannonia Kincse*', respectively) compared to (FI) counterpart. (HP) treatment resulted in the highest LAI in both genotypes; LAI was 21.3 and 28.8% higher compared to (DW) counterparts in '*Boglár*' and '*Pannonia Kincse*', respectively (Table 66). Ashraf et al. (2015) reported that seeds soaked in 20, 80, 100, and 140 mM of H₂O₂ later formed plants with higher leaf area under drought stress conditions compared to non-treated seeds. Using (SA), other reports concluded that treatments with this ROS species could improve LAI in different plants including soybean (Kuchlan et al., 2017; Razmi et al., 2017), strawberry (Ghaderi et al., 2015) and lemongrass (Idrees et al., 2010); this was attributed to increased accumulation of certain proteins (like proline) and soluble sugars which, in part, enhances cell turgor pressure (Razmi et al., 2017).

Table 66. Leaf area index (LAI) of soybean genotypes '*Boglár*' and '*Pannonia Kincse*' under three irrigation treatments; fully-irrigated (FI), drought-stressed with H₂O₂ foliar spray (HP) and drought-stressed (DW) in Látókép, Debrecen averaged over 2017 and 2018 years.

Trait	Treatment	' <i>Boglár</i> '	' <i>Pannonia Kincse</i> '
LAI (m ² m ⁻²)	DW	4.7 ^b	5.2 ^b
	HP	5.7 ^a	6.7 ^a
	FI	5.4 ^a	6.3 ^a

- The same letter indicates no significant differences at .05 level among the treatments within the same genotype.

Significant correlation was recorded between irrigation and LAI (Table 63); the effect size of H₂O₂ application was also significant in both genotypes (with ratios of 92.8 and 95.1% in ‘Boglár’ and ‘Pannonia Kincse’, respectively) as well.

5.1.4.5. The Effects of Drought Stress and Exogenous Application of Hydrogen Peroxide (H₂O₂) on The Plant Height (cm) of 2 Soybean Genotypes

Drought significantly reduced plant height in both genotypes compared to irrigated counterpart; the reduction ratio was 13.2 and 7.1% in ‘Boglár’ and ‘Pannonia Kincse’, respectively. Applying H₂O₂ significantly increased plant height in both genotypes; plant height was 3.6% less in ‘Boglár’, whereas it was only 0.2% less in ‘Pannonia Kincse’ compared to irrigated counterparts (Table 67). H₂O₂ application had an effect size of 84.2 and 82.8% in ‘Boglár’ and ‘Pannonia Kincse’, respectively. Abbas and Mohamed (2011) conducted an experiment on common bean (*Phaseolus vulgaris* L.) seeds where half of the seeds were soaked in hydrogen peroxide (2%) for 4 hours and then air dried, and the other half of the seeds were soaked in distilled water for 4 hours and then air dried. Their results showed an increase by 43.6% in the H₂O₂-treated seedling height under a drought level of 60% of field capacity, moreover, increasing the drought severity (to reach only 40% of field capacity) decreased the seedling height of both treatments; however, H₂O₂-treated seedlings showed better height by 38.4%.

Table 67. Plant height of soybean genotypes ‘Boglár’ and ‘Pannonia Kincse’ under three irrigation treatments; fully-irrigated (FI), drought-stressed with H₂O₂ foliar spray (HP) and drought-stressed (DW) in Látókép, Debrecen averaged over 2017 and 2018 years.

Trait	Treatment	‘Boglár’	‘Pannonia Kincse’
Plant Height (cm)	DW	86.9 ^b	94.1 ^b
	HP	96.5 ^a	101.1 ^a
	FI	100.1 ^a	101.3 ^a

- The same letter indicates no significant differences at .05 level among the treatments within the same genotype.

5.1.4.6. The Effects of Drought Stress and Exogenous Application of Hydrogen Peroxide (H₂O₂) on The Seed Yield (kg ha⁻¹) of 2 Soybean Genotypes

Irrigation resulted in the best yield in both genotypes; the yield significantly increased by 27.3 and 24.3% in irrigated treatment compared to drought-stressed counterpart in ‘Boglár’ and ‘Pannonia Kincse’, respectively. H₂O₂ spraying also increased the yield of

both genotypes compared to drought-stressed treatment; the increase ratio was 21.2 and 13.5% in '*Boglár*' and '*Pannonia Kincse*', respectively (Table 68, Fig. 18). The effect size of H₂O₂ application was bigger (78.9%) in '*Pannonia Kincse*' compared to '*Boglár*' (72.1%). Correlation of irrigation with yield was highly significant in both genotypes (Table 63). Exogenous application of H₂O₂ had improved plant biomass in wheat under drought stress (He et al., 2009), and (SA) application improved the grain yield of common bean under drought stress conditions (Sadeghipour and Aghaei, 2012). Horvath et al. (2007) reported that (SA) can enhance metabolite stream to the developing grains which reduces the abortion rate which, in part, can significantly increase pod number per plant and seed number pod⁻¹ in soybean (Khatun et al., 2016). Not only yield, but also yield components (number of grains m⁻², pods per plant) were enhanced with the application of (SA) on soybean leaves under drought stress conditions (Razmi et al., 2017). The authors attributed the increase of grain yield due to (SA) application to Improved RWC, reduced restrictions of stomatal conductance and the enhanced biosynthesis of photosynthetic pigments in the leaves.

Table 68. Seed yield of soybean genotypes '*Boglár*' and '*Pannonia Kincse*' under three irrigation treatments; fully-irrigated (FI), drought-stressed with H₂O₂ foliar spray (HP) and drought-stressed (DW) in Látókép, Debrecen averaged over 2017 and 2018 years.

Trait	Treatment	' <i>Boglár</i> '	' <i>Pannonia Kincse</i> '
Seed yield (kg ha ⁻¹)	DW	3321 ^b	3730 ^b
	HP	4029 ^a	4225 ^{ab}
	FI	4207 ^a	4631 ^a

- The same letter indicates no significant differences at .05 level among the treatments within the same genotype.

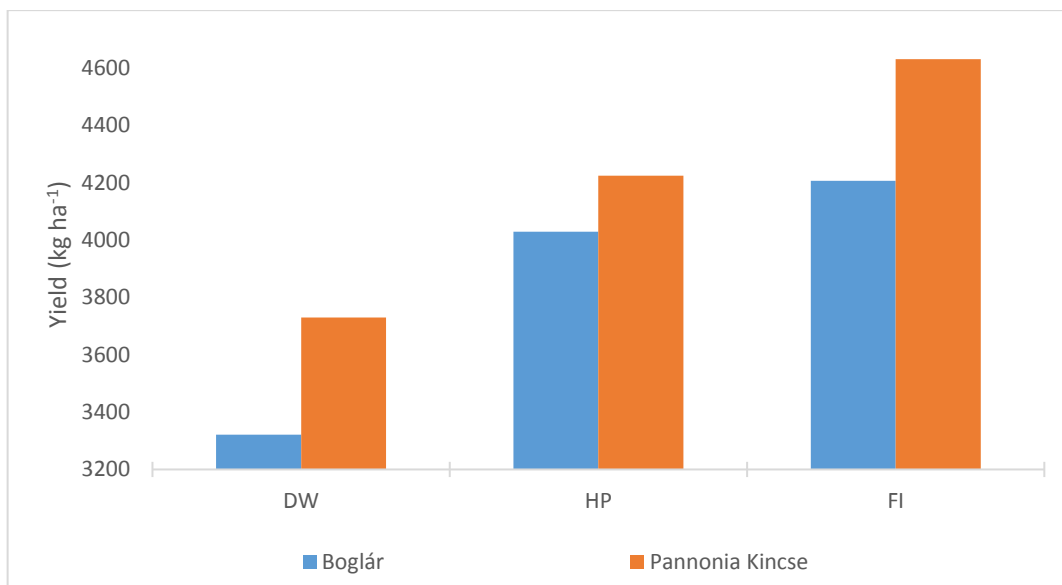


Fig. 18. The seed yield (kg ha^{-1}) of soybean genotypes '*Boglár*' and '*Pannonia Kincse*' under three irrigation treatments; fully-irrigated (FI), drought-stressed with H_2O_2 foliar spray (HP) and drought-stressed (DW) in Látókép, Debrecen averaged over 2017 and 2018 years.

5.2. CLIMATE CHAMBER EXPERIMENTS

5.2.1. The Effects of PEG-induced Drought Stress on The Germination Parameters of 2 Soybean Genotypes

5.2.1.1. First-stage Experiment

5.2.1.1.1. The Effects of PEG-induced Drought Stress on The Germination Ratio (%) of 2 Soybean Genotypes

Significant differences were recorded among PEG concentrations of both genotypes.

For genotype '*ES Mentor*', germination started at the second day after seeding for both 0 (control) and 10% of PEG concentration with significant differences between the two ratios (82.5 and 15.4%, respectively). After that, the control kept a very similar ratio of germinated seeds, whereas (10%) treatment gradually increased until peaking at the fifth day after seeding, where the germination ratio (72.6%) was very close to the peak of the control (83.6%), then a degradation in the germinated seeds was recorded at the sixth day after seeding, followed by continuous increasing without reaching the same peak (Fig. 19a).

(15%) treatment started germination at the fifth day after seeding with a low ratio (8.8%) compared to both control and (10%) treatment, and gradually increased till the eighth day after seeding, reaching (22%), thereafter a very little decrease was recorded (Fig. 19a).

(20%) treatment could not germinate until the last day of the experiment, with a very low ratio (7.7%) compared to both control and (10%) treatment (Fig. 19a).

For genotype '*Pedro*', only the control could germinate in the second day after seeding with a relatively-high ratio (46%), then further increase (to 81%) at the third day, but after that, a peak with a very small increase (to 83%) was recorded in the fourth day, then in the following days, a fluctuation in germination was recorded; however, it was not extreme. The germination ratio of the control was significantly higher than all of the other concentrations' ratios, regardless of the day-after-seeding (Fig. 19b).

(10%) treatment started to germinate in the third day (7.7%), and increased gradually till reaching the peak (37.4%) at the fifth day, then a slight reduction (to 31.9%) was recorded at the next day, followed by an increase (to 33.0%) and then a peak (of 41.8%) at the eighth day (Fig. 19b).

Both (15%) and (20%) treatments had very low germination ratios compared to both control and (10%) treatments (Fig. 19b). Germination is one of the most critical periods in the life cycle of the plants. Under water stress, low water potential is a determining factor inhibiting seed germination (Wang et al. 2002). It was previously reported that drought stress reduces germination rate by decreasing enzyme activity, and consequently, reducing meristem development (Avila et al. 2007). Mengistu and Heatherly (2006) reported that the average germination ratio for total irrigated plots during the 4 years of the experiment was 71% compared to 65% for non-irrigated counterparts. Drought reduces germination ratio and finally delays establishment of plantlets (Prisco et al. 1992). Hellal et al. (2018) reported that the increase in PEG concentrations dramatically decreased germination percentage of overall studied cultivars (10 barley cultivars different in drought tolerance) as the increase in PEG by 5% decreased germination percentage by 19% relative to the untreated one, whereas increasing PEG from 5% to 10% decreased germination percentage by 9%, and increased PEG from 10% to 20% decreased germination percentage by 30%. The results directly affected the total germination percentage. Salehi (2010) reported reduction of germination percentage and

increase of osmotic potential produced by polyethylene glycol on bean seedlings. Previously, many researchers concluded that increasing drought stress levels progressively delayed and reduced germination (Wiggans and Gardner 1959; Parmar and Moore 1968; Mcwilliam and Phillips 1971; Pandya et al. 1972).

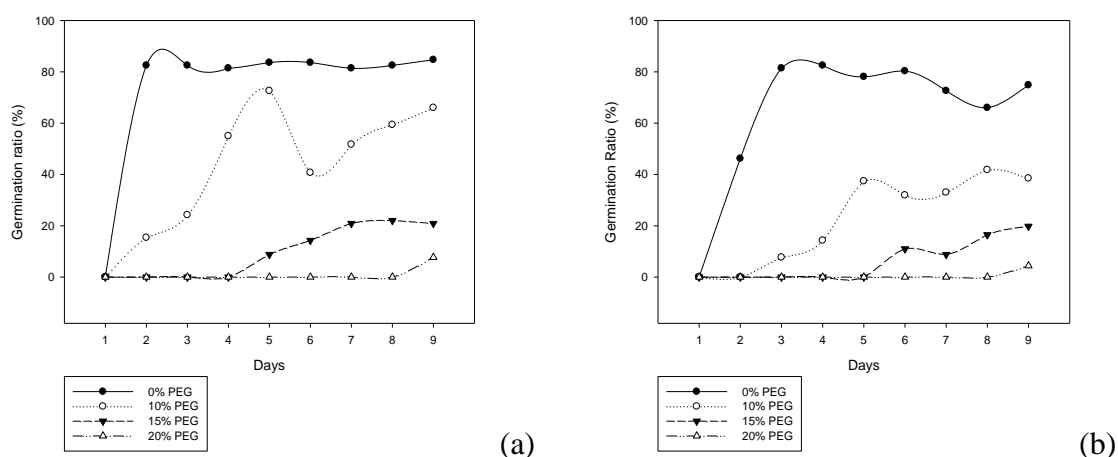


Fig. 19. Germination ratio (GR) (%) of soybean genotypes '*ES Mentor*' (a) and '*Pedro*' (b) under different polyethylene glycol (PEG) concentrations

5.2.1.1.2. The Effects of PEG-induced Drought Stress on The Germination Energy (GE) (%) of 2 Soybean Genotypes

The germination energy of '*ES Mentor*' was significantly different among concentrations; the higher the concentration, the lower the germination energy, taking into consideration that (20%) was not germinated when calculating this trait (Table 69).

The germination energy of '*Pedro*' followed the same trend of '*ES Mentor*'; however, both (15%) and (20%) treatments were not germinated by the time of measurement (Table 69).

Comparing between the two genotypes, there was no significant difference between the two control treatments; however, '*ES Mentor*' was significantly higher in all other treatments (Table 69).

Table 69. Germination energy (GE) of soybean genotypes ‘*ES Mentor*’ and ‘*Pedro*’ under different polyethylene glycol (PEG) concentrations.

PEG Concentration (%)	Germination Energy (%)	
	‘ <i>ES Mentor</i> ’	‘ <i>Pedro</i> ’
0%	83.6 ^{aA}	78.1 ^{aA}
10%	72.6 ^{bA}	37.4 ^{bB}
15%	8.8 ^{cA}	0 ^{cB}

- Same small letter indicates no significant differences at .05 level among PEG concentrations within certain genotype.
- Same capital letter indicates no significant differences at .05 level between genotypes within certain PEG concentration.

5.2.1.1.3. The Effects of PEG-induced Drought Stress on The Root elongation (RE) (cm) of 2 Soybean Genotypes

For genotype ‘*ES Mentor*’, root elongation of control treatment increased rapidly starting from the first day of germination (second day after seeding) (1.7 cm) till the seventh day (16.1 cm), followed by another rapid increase between the eighth and the ninth days (15.5 and 21.6 cm, respectively). The average root elongation was significantly higher than those of the other concentrations starting from the third day (Fig. 20a).

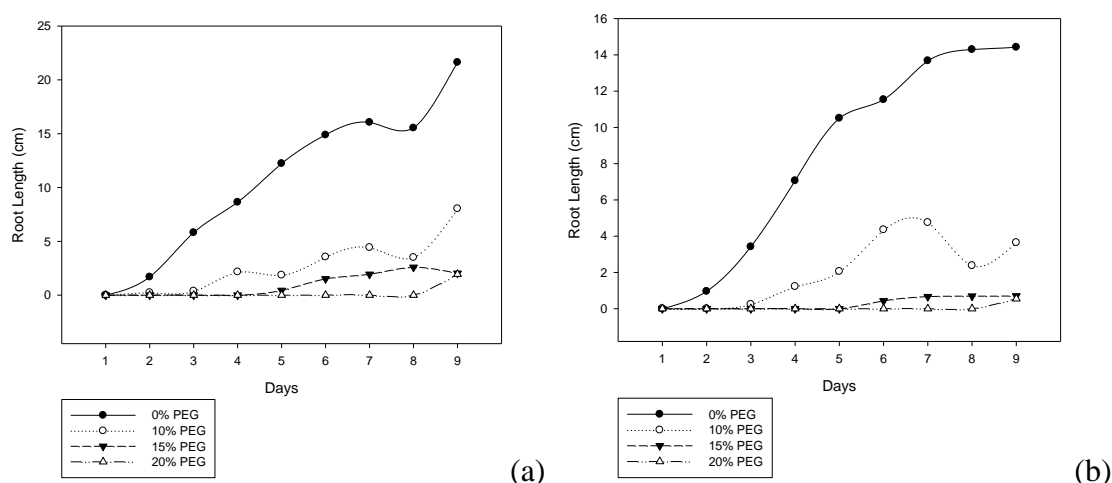


Fig. 20. Root elongation (RE) (cm) of soybean genotypes ‘*ES Mentor*’ (a) and ‘*Pedro*’ (b) under different polyethylene glycol (PEG) concentrations

The root elongation of (10%) concentration slowly increased day after day, with slight decreases at days 5 and 8 after seeding, reaching the peak (8.0 cm) at day 9 with a

noticeable increase compared to the previous days. Similarly, (15%) treatment slowly increased during the days after germination, peaking at day 8 (2.6 cm), and slightly decreasing at day 9 (2.0 cm) (Fig. 20a).

(20%) treatment did not give noticeable values, as it started root elongation only at the final day of the experiment (Fig. 20a).

For genotype '*Pedro*', the root elongation of the control treatment rapidly (and significantly compared to the other concentrations) increased till day 7 (reaching 13.7 cm), and then slightly increased, until peaking (14.4 cm) at day 9 (Fig. 20b).

The root elongation of (10%) treatment slowly increased during the first few days after germination till day 5 (reaching 2.1 cm), thereafter, a relatively rapid increase was recorded at day 6 (to 4.4 cm), followed by alterations during the next days (Fig. 20b).

Both (15%) and (20%) concentration treatments acted very similar to genotype '*ES Mentor*', indicating that under severe drought stress conditions, both genotypes responded similarly to drought stress by means of root elongation (Fig. 20b). Kafi et al. (2005) stated that as the water potential of lentil (*Lens culinaris* Medik.) decreased, both germination percentage and root length decreased, which was supported later by the conclusion of Hellal et al. (2018) that root length of barley (*Hordeum vulgare* L.) cultivars under high PEG concentrations (10 and 20%) was noticeably less than control counterparts, regardless of cultivar. Similar findings were reported before by Pandya et al. (1972) that early seedling development in terms of root length also declined with increasing water stress. In another experiment where the mannitol was used to induce drought stress on soybean, the germination ratio was significantly decreased to (39.5%) compared to (72.5%) for the non-stressed control. Also, the root length significantly decreased from (8.72 cm) in the control to (2.86 cm) when drought stress was applied (Braga et al. 2017).

5.2.1.1.4. The Effects of PEG-induced Drought Stress on The Ultimate germination (UG) of 2 Soybean Genotypes

Both genotypes followed the same trend; increasing PEG concentration was accompanied by significant decrease in (UG). Moreover, '*ES Mentor*' was significantly higher, compared to '*Pedro*', in UG under both 0 and 10%, whereas it was insignificantly higher under both 15 and 20% PEG concentrations (Table 70). It was previously reported that

increasing PEG concentration was accompanied by a reduction in UG of 10 barley cultivars, regardless of their different drought tolerance potentials (Hellal et al. 2018).

Table 70. Ultimate germination (UG), mean period of ultimate germination (MPUG) and percentage inhibition or stimulation of soybean genotypes '*ES Mentor*' and '*Pedro*' under different polyethylene glycol (PEG) concentrations.

Trait	PEG Concentration	' <i>ES Mentor</i> '	' <i>Pedro</i> '
Ultimate germination (UG)	0%	25.67 ^{aA}	22.67 ^{aB}
	10%	19 ^{bA}	11.67 ^{bB}
	15%	6.33 ^{cA}	6.00 ^{cA}
	20%	2.33 ^{dA}	1.33 ^{dA}
Mean period of ultimate germination (MPUG)	0%	2.03 ^{cA}	2.73 ^{bA}
	10%	4.32 ^{bcA}	5.31 ^{abA}
	15%	5.84 ^{abA}	5.67 ^{aA}
	20%	7.72 ^{aA}	6.75 ^{aA}
Percentage inhibition or stimulation	10%	99.26 ^{aA}	99.49 ^{aA}
	15%	99.74 ^{aA}	99.75 ^{aA}
	20%	99.91 ^{aA}	99.94 ^{aA}

- Same small letter indicates no significant differences at .05 level among PEG concentrations within certain trait.
- Same capital letter indicates no significant differences at .05 level between the two genotypes under the same PEG concentration level within certain trait.

5.2.1.1.5. The Effects of PEG-induced Drought Stress on The Mean period of ultimate germination (MPUG) of 2 Soybean Genotypes

For both genotypes, MPUG increased with increasing PEG concentration. Compared to control, MPUG insignificantly increased in (10%) treatment, whereas it was significantly higher in both (15 and 20%) treatments. MPUG was insignificantly lower for '*ES Mentor*' in both control and (10%) treatments, whereas it was higher under the higher concentrations (Table 70), reflecting the ability of '*Pedro*' to reach the ultimate germination in relatively shorter period of time under drought conditions which, in turn, provides another evidence of '*Pedro*' to tolerate higher water stress levels compared to '*ES Mentor*'; this conclusion is supported by the results of root elongation, which lead to the conclusion that the germinated seeds of '*Pedro*' were able to better tolerate water stress.

5.2.1.1.6. The Effects of PEG-induced Drought Stress on The Percentage inhibition or stimulation (%) of 2 Soybean Genotypes

For both genotypes, the germination inhibition increased with increasing PEG concentration, however, the differences were insignificant. Under each PEG concentration, the inhibition was insignificantly higher for 'Pedro' compared to 'ES Mentor' (Table 70), which is consistent with the results of germination ratio, as 'Pedro' showed less germination ratio than did 'ES Mentor' under water stress conditions.

There was significant negative correlation between PEG concentration and root elongation, germination ratio, germination energy and ultimate germination; i.e. increasing drought severity (by increasing PEG concentration) significantly decreases these traits, whereas both mean period of ultimate germination and inhibition percentage were positively correlated with increasing drought severity (Table 71).

Table 71. Correlations between PEG concentration and germination parameters of soybean genotypes 'ES Mentor' and 'Pedro'.

	UG	MPUG	Inh/Sti	GE	GR	RL
<i>'ES Mentor'</i>						
PEG Concentration	-.978**	.928**	.308	-.944**	-.976**	-.906**
<i>'Pedro'</i>						
PEG Concentration	-.973**	.906**	.219	-.941**	-.976**	-.878**

- **. Correlation is significant at the 0.01 level (2-tailed).

5.2.1.2. Second-stage Experiment

5.2.1.2.1. The Effects of PEG-induced Drought Stress on The Germination Ratio (%) of 2 Soybean Genotypes

For both genotypes, the higher PEG concentrations significantly affected both germination ratio and root length; however, the effects were relatively different on each genotype.

For genotype 'ES Mentor', PEG treatments of (0, 2.5 and 5%) started germinating at the second day after seeding; the germination ratio was lower as PEG concentration (drought stress) was higher (74.8, 44.0 and 14.3% for 0, 2.5 and 5%, respectively). However,

(2.5%) treatment resulted in very close ratios compared to control after day 2, whereas (5%) treatment reached that only after day 4 (Fig. 21a).

Both (7.5 and 10%) treatments started germination at day 3; however, (10%) treatment could reach a higher final germination ratio (90%) than did (7.5%) treatment (83.6%). (12.5%) treatment started germination at day 4 with a very low ratio (7.7%), and gradually increased till day 8 where a rapid increase (to 73%) was achieved, followed by a final ratio of (77.3%). 15% treatment started germination at day 4 with the same ratio as (12.5%) treatment, however, the daily increase was noticeably less, reaching a final ratio of (23.7%) (Fig. 21a).

For '*Pedro*', control, (2.5 and 5%) treatments started germination at the second day, but both control and (2.5%) treatments reached the same final ratio of (85.1%), whereas (5%) could reach a slightly higher ratio (87.3%); the difference, however, was insignificant. (7.5%) treatment, although it started germinating at day 3, could reach very close ratios after day 3 compared to (5%) treatment, and after day 5 compared to control and (2.5%) treatments. (10%) treatment could be considered as the middle trend; it started germinating at day 3 with a very low ratio (5.5%), then rapidly increased till day 6 (76.3%), after that, a measurable decrease was recorded at day 7 (to 47.1%), followed by rapid (to 81.4%), at day 8, and slight (to 82.7%) increase at day 9 (Fig. 21b).

(12.5%) and (15%) treatments followed a very similar trend at most days of the experiment; they started germinating at day 4 (11.0 and 8.8%, respectively), then increased gradually till day 9; however, (12.5%) treatment resulted in much better germination ratios starting from day 8 (Fig. 21b).

From a seed-size standpoint, and in both stages, '*ES Mentor*' (100-seed weight = 201 g) could achieve higher germination ratio compared to '*Pedro*' (100-seed weight = 161 g) (except for 15% at the second-stage experiment); this could lead to a conclusion that seed size plays a role in seed germination ratio under both no-drought and relatively moderate drought stress conditions. Another factor affecting germination ratio is seed size (Longer et al. 1986). Burris et al. (1973) grouped soybean seeds in four groups depending on the size; they reported significantly lower germination ratio for the smallest-size group relative to the other groups. Longer et al. (1986) reported the bigger-sized seeds of two

soybean cultivars to better germinate as compared to smaller counterparts, which also was previously reported (Haskins and Gorz 1975; Goyal et al. 1980).

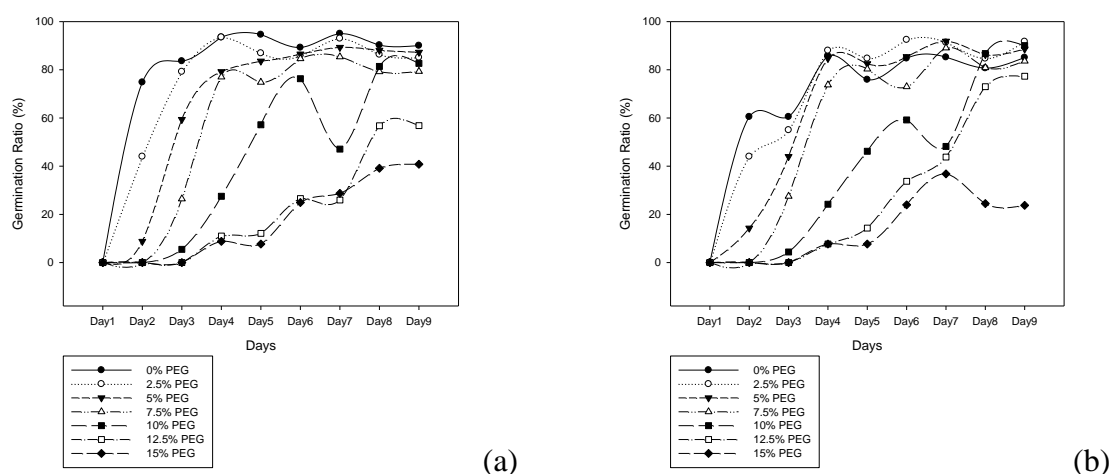


Fig. 21. Germination ratio (GR) (%) of soybean genotypes '*ES Mentor*' (a) and '*Pedro*' (b) under different polyethylene glycol (PEG) concentrations

5.2.1.2.2. The Effects of PEG-induced Drought Stress on The Germination Energy (GE) (%) of 2 Soybean Genotypes

The germination energy of '*ES Mentor*' decreased as PEG concentration increased; the control was higher than other treatments. (2.5, 5 and 7.5%) treatments were not significantly different compared to each other and to the control; however, they were significantly higher than (10%) treatment. (15%) treatment was close to (12.5%) treatment and insignificantly lower (Table 72).

The germination energy followed completely different trend for '*Pedro*'; (2.5%) treatment resulted in the best germination energy, followed by (5%) treatment, and though they were not significantly different, yet they were significantly higher than control which, in part, was significantly higher than (10%) treatment. (12.5%) treatment resulted in significantly lower germination energy compared to the lower concentration treatments; however, it was higher than (15%) treatment (Table 72).

Table 72. Germination energy (GE) of soybean genotypes ‘*ES Mentor*’ and ‘*Pedro*’ under different polyethylene glycol (PEG) concentrations.

PEG Concentration (%)	Germination Energy (%)	
	‘ <i>ES Mentor</i> ’	‘ <i>Pedro</i> ’
0	94.6 ^{aA}	75.9 ^{bB}
2.5	84.7 ^{aA}	86.9 ^{aA}
5	82.5 ^{abA}	83.6 ^{aA}
7.5	80.3 ^{abA}	74.8 ^{bA}
10	46.2 ^{cB}	57.2 ^{cA}
12.5	14.3 ^{dA}	12.1 ^{dA}
15	7.7 ^{dA}	7.7 ^{dA}

- Same small letter indicates no significant differences at .05 level among PEG concentrations within certain genotype.
- Same capital letter indicates no significant differences at .05 level between genotypes within certain PEG concentration.

5.2.1.2.3. The Effects of PEG-induced Drought Stress on The Root elongation (RE) (cm) of 2 Soybean Genotypes

For ‘*Pedro*’, control treatment was significantly higher than the other PEG-concentration treatments starting from the third day after seeding, peaking at day 6 (16.8 cm), whereas all other concentrations gradually increased root elongation day after day (peaking at day 9) (Fig. 22b).

Both (2.5%) and (5%) treatments had very close root elongation values all days long, whereas (7.5%) and (10%) could be considered as the middle trend, though the root elongation was significantly higher for (7.5%) treatment starting from day 6 compared to counterpart values of (10%) treatment (Fig. 22b).

Both (12.5%) and (15%) treatments had very close values till day 7, where (12.5%) treatment had measurably higher values (Fig. 22b).

For ‘*ES Mentor*’, relatively similar results were obtained as compared to ‘*Pedro*’, except that the differences between (2.5%) and (5%) treatments were relatively less. In addition, (10%), (12.5%) and (15%) treatments had very close values all days long (Fig. 22a).

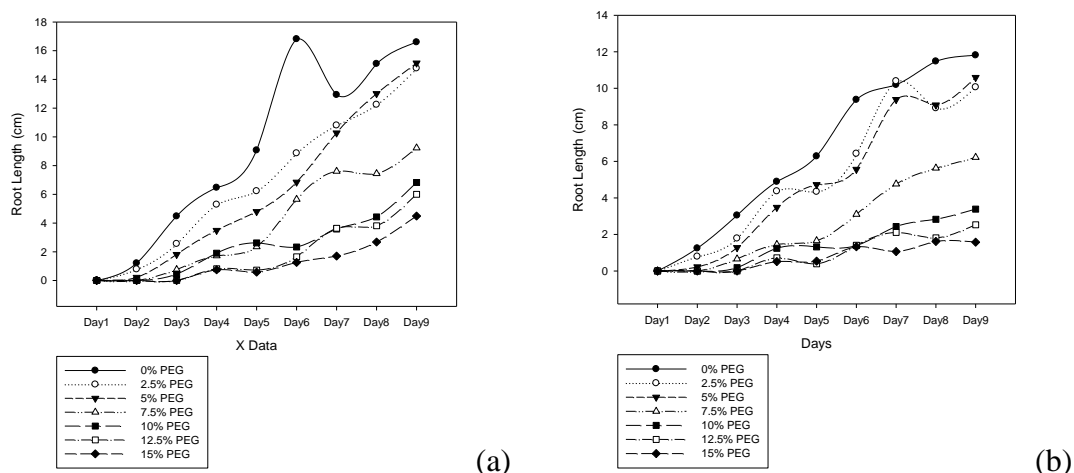


Fig. 22. Root elongation (RE) (cm) of soybean genotypes ‘*ES Mentor*’ (a) and ‘*Pedro*’ (b) under different polyethylene glycol (PEG) concentrations

5.2.1.2.4. The Effects of PEG-induced Drought Stress on The Ultimate germination (UG) of 2 Soybean Genotypes

For ‘*ES Mentor*’, (UG) was similar in control, (2.5 and 5%) treatments; however, it significantly decreased in both (12.5 and 15%) treatments. For ‘*Pedro*’ also, same UG (with a value of 26) was recorded for control, (2.5 and 5%) treatments. However, UG decreased under higher PEG concentrations; the reduction was significant in both (12.5 and 15%) treatments (Table 65).

5.2.1.2.5. The Effects of PEG-induced Drought Stress on The Mean period of ultimate germination (MPUG) of 2 Soybean Genotypes

Both genotypes tended to increase MPUG with increasing PEG concentration, moreover, the increase was significant under the high concentrations (12.5 and 15%) compared to the control and the low concentrations (2.5 and 5%) (Table 73).

5.2.1.2.6. The Effects of PEG-induced Drought Stress on The Percentage inhibition or stimulation (%) of 2 Soybean Genotypes

Similarly, for both genotypes increasing PEG concentration was accompanied by an increase in inhibition percentage; however, it was insignificant for both genotypes (Table 73).

Same correlation trends were recorded for both genotypes as compared to the first-stage experiment (Table 74).

Table 73. Ultimate germination (UG), mean period of ultimate germination (MPUG) and percentage inhibition or stimulation of soybean genotypes '*ES Mentor*' and '*Pedro*' under different polyethylene glycol (PEG) concentrations.

Trait	PEG Concentration (%)	' <i>ES Mentor</i> '	' <i>Pedro</i> '
Ultimate germination (UG)	0	27 ^{aA}	26 ^{aA}
	2.5	27 ^{aA}	26 ^{aA}
	5	27 ^{aA}	26 ^{aA}
	7.5	25 ^{abA}	25 ^{aA}
	10	25 ^{abA}	25 ^{aA}
	12.5	23 ^{bA}	17 ^{bB}
	15	7 ^{cB}	12 ^{cA}
Mean period of ultimate germination (MPUG)	0	2.48 ^{cdA}	2.54 ^{dA}
	2.5	3.11 ^{cdA}	2.88 ^{cA}
	5	3.78 ^{bcdA}	3.5 ^{bcA}
	7.5	4.48 ^{bcdA}	4.25 ^{abcA}
	10	5.89 ^{bcA}	5.12 ^{abcA}
	12.5	6.78 ^{abA}	6.65 ^{aA}
	15	9.43 ^{aA}	6.75 ^{aB}
Percentage inhibition or stimulation	2.5	96.26 ^{aA}	97.48 ^{aA}
	5	97.33 ^{aA}	97.52 ^{aA}
	7.5	97.68 ^{aA}	97.94 ^{aA}
	10	98.24 ^{aA}	98.29 ^{aA}
	12.5	98.51 ^{aA}	98.59 ^{aA}
	15	98.78 ^{aA}	98.84 ^{aA}

- Same small letter indicates no significant differences at .05 level among PEG concentrations within certain trait.
- Same capital letter indicates no significant differences at .05 level between the two genotypes under the same PEG concentration level within certain trait.

Table 74. Correlations between PEG concentration and germination parameters of soybean genotypes '*ES Mentor*' and '*Pedro*'.

	UG	MPUG	Inh/Sti	GE _n	GR	RL
<i>'ES Mentor'</i>						
PEG Concentration	-.859 ^{**}	.855 ^{**}	.542 [*]	-.937 ^{**}	-.867 ^{**}	-.957 ^{**}
<i>'Pedro'</i>						
PEG Concentration	-.685 ^{**}	.823 ^{**}	.694 ^{**}	-.880 ^{**}	-.675 ^{**}	-.956 ^{**}

- **, Correlation is significant at the 0.01 level (2-tailed).

5.2.2. The Effects of PEG-induced Drought Stress on The Physiology of 2 Soybean Genotypes

Both genotypes could not survive after V2 stage in 10% PEG treatment, moreover, both 7.5% PEG and 5% PEG treatments caused ‘*ES Mentor*’ plants to die starting from the stage after V4, whereas only 7.5% PEG treatment had a similar effect on Pedro plants.

5.2.2.1. The Effects of PEG-induced Drought Stress on The Relative Chlorophyll Content (SPAD) of 2 Soybean Genotypes

For ‘*ES Mentor*’ and similar to the total chlorophyll content trait, PEG treatments resulted in lower relative chlorophyll content than control treatment did in all studied stages. Also for this trait, increasing PEG concentration was accompanied with decreasing SPAD values (Fig. 23A).

Genotype Pedro followed a very similar trend except for a slight, insignificant increase in 2.5% PEG treatment at V2 stage as compared to control. However, differences were more measurable at later stages; both 5% PEG and 7.5% PEG treatments were significantly lower than control treatment at V4 stage, and both 2.5% PEG and 5% PEG treatments were significantly lower compared to control treatment at both R2 and R4 stages (Fig. 23B). SPAD values significantly decreased from 35.48 to 22.38 under drought stress applied 30 days after R5.5 stage (Ergo et al., 2018). These results are in agreement with the general chlorophyll drops that occur when soybean plants are subjected to continuous water stress from early seed filling (De Souza et al., 1997; Inamullah and Isoda, 2005).

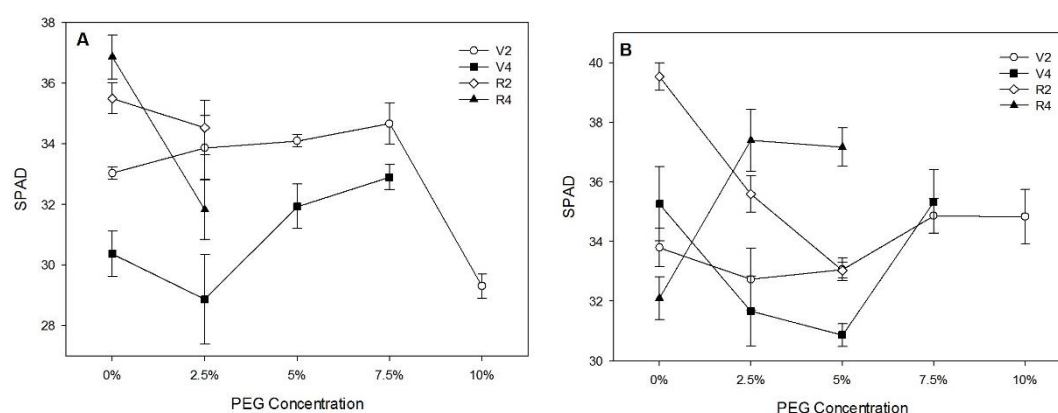


Fig. 23. SPAD of ‘*ES Mentor*’ (A) and Pedro (B) in different PEG concentrations at different stages.

5.2.2.2. The Effects of PEG-induced Drought Stress on The Total Chlorophyll Content ($Chl_{a,b}$) ($\mu g\ ml^{-1}$) of 2 Soybean Genotypes

For genotype '*ES Mentor*', both Chl_a and Chl_b decreased as PEG concentration increased at all 4 studied stages (Fig 24A and 25A); the reduction was insignificant at both vegetative stages (V2 and V4 stages), however, the reduction was significant at reproductive stages (R2 and R4).

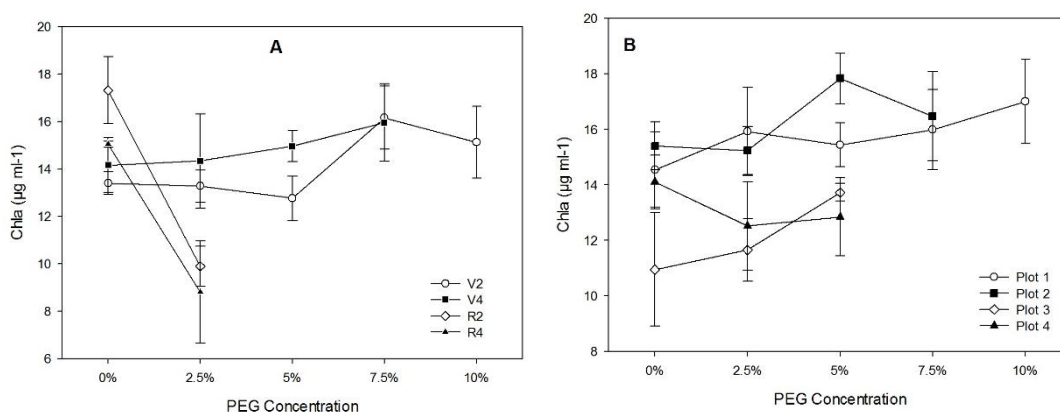


Fig. 24. Chl_a content of '*ES Mentor*' (A) and '*Pedro*' (B) in different PEG concentrations at different stages

For '*Pedro*', 2.5% PEG treatment had the best Chl_a content at V2 stage, and 5% PEG treatment was also better than control treatment. However at the following stages, control treatment could maintain the best Chl_a content, and the increase in PEG concentration was accompanied by a decrease in Chl_a content. All differences were insignificant (Fig. 24B). Chl_b , on the other hand, was significantly higher for 5% PEG treatment than control at V2 stage; it was also higher for 2.5 PEG treatment, whereas 7.5% PEG and 10% PEG treatments resulted in the least Chl_b content at this stage. At V4 stage, 2.5% PEG resulted in higher Chl_b content as compared to control treatment, and both 5% PEG and 7.5 PEG treatments were significantly lower. In the following stages (R2 and R4), Chl_b content insignificantly decreased with increasing PEG concentration (Fig. 25B). Similarly, Zhang et al. (2016) concluded that Chl_a was significantly reduced under drought conditions compared to the non-drought counterpart, whereas Chl_b increased when plants suffered from water deficit. Hao et al. (2013) reported significant decrease (by 32.2%) in chlorophyll content as a result of drought stress. Mathobo et al. (2017) subjected bean plants to drought stress for 24 days in different stages; the reduction of chlorophyll content was higher when drought occurred at later stages as compared to earlier stages,

and control plants were always the highest in chlorophyll content; they suggested that the reduction in chlorophyll content might have resulted from leaves being damaged and turning yellowish due to drought stress. Previously, many papers reported a decrease in total chlorophyll content due to drought stress in other legumes like soybean (Makbul et al., 2011), chickpea (Mafakheri et al., 2010) and pea (Inaki-Iturbe et al., 1998). Moreover, Smirnoff (1995) indicated that the decrease in total chlorophyll content is resulting from the damage to the chloroplasts caused by reactive oxygen species (ROS) as drought stress leads to the production of reactive oxygen species (ROS) such as O_2^- and H_2O_2 , which lead to chlorophyll destruction (Foyer et al., 1994). Chlorophylls, as the main pigments of absorption, transport and conversion of light energy, its content is an important parameter indicating photosynthetic performance. ROS accumulated under environmental stresses will destroy chlorophylls, and chl_a is more sensitive to ROS than chl_b (Liu et al., 2007). Exposing plants to water stress led to a significant decline in chl_{a+b} (from 19.5 to 13.0 mg g⁻¹ DW), indicating the decreased capacity of absorbing and conversion of light energy (Tang et al., 2017). Similar results were reported (Cui et al., 2004; Pagter et al., 2005).

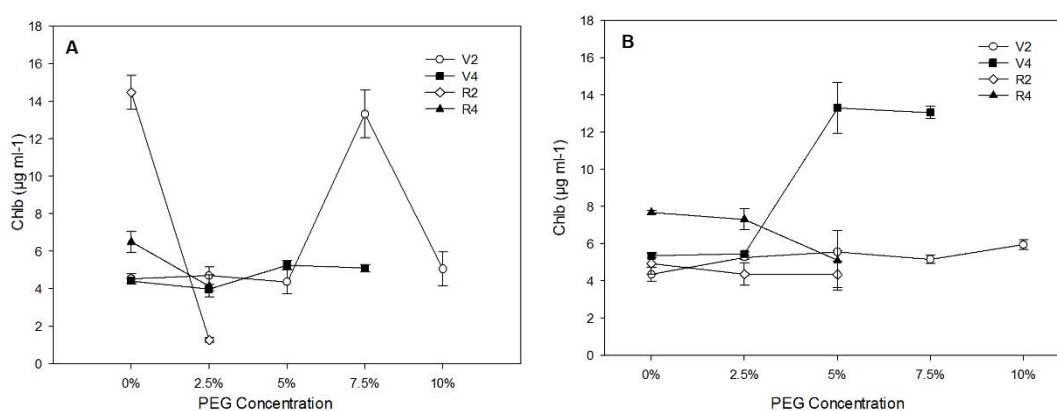


Fig. 25. Chl_b content of 'ES Mentor' (A) and 'Pedro' (B) in different PEG concentrations at different stages

5.2.2.3. The Effects of PEG-induced Drought Stress on The Total Carotenoids (Chl_{x+c}) (µg ml⁻¹) of 2 Soybean Genotypes

For 'ES Mentor', control treatment had the highest Chl_{x+c} content at all studied stages compared to PEG treatments; the higher PEG concentration, the lower Chl_{x+c} was, however, the differences were insignificant at all stages except for at R4 stage where control treatment was significantly higher than 2.5% PEG treatment (Fig. 26A).

For ‘*Pedro*’, both 2.5% PEG and 5% PEG treatments had higher Chl_{x+c} than control treatment (4.28 ± 1.4) at V2 stage, whereas Chl_{x+c} of both 7.5% PEG and 10% PEG treatments were lower. At the following stages, Chl_{x+c} content decreased as PEG concentration increased, but the differences were insignificant (Fig. 26B). Total carotenoids were reduced as a result of drought stress application at all stages in ES Mentor, and at V4, R2 and R4 stages in Pedro. Previously, Zhang et al. (2016) reported carotenoids content to be significantly reduced under drought stress conditions compared to the well-watered control, which was supported later by the conclusion that exposing plants to water stress led to a significant decline in carotenoid content (from 3.4 to 2.1 mg/g dry weight) (Tang et al., 2017).

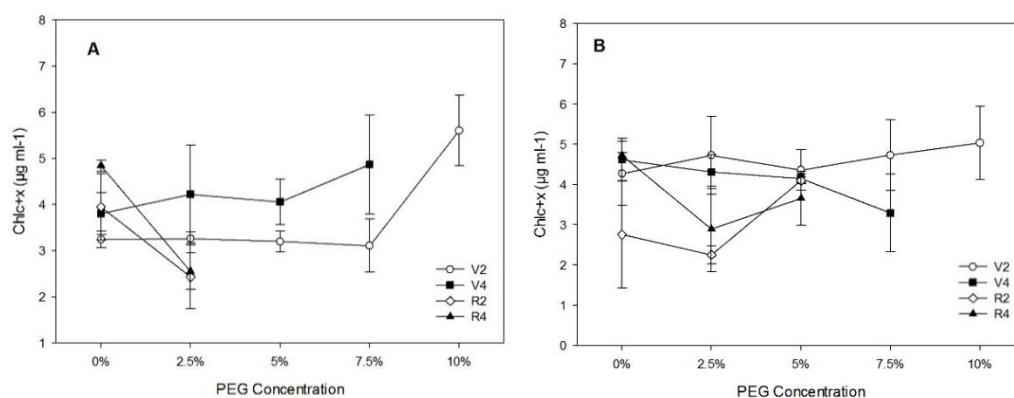


Fig. 26. Chl_{x+c} content of ‘*ES Mentor*’ (A) and ‘*Pedro*’ (B) in different PEG concentrations at different stages

5.2.2.4. The Effects of PEG-induced Drought Stress on The Maximum Photochemical Efficiency of PS II (F_v/F_m) of 2 Soybean Genotypes

For both genotypes, F_v/F_m followed one trend throughout the studied stages; it decreased with increasing PEG concentration (Fig. 27 A,B). Moreover, for ‘*ES Mentor*’, control and 2.5% PEG treatments were not significantly different in all stages, however, 5% PEG and 7.5% PEG treatments were significantly less at V4 stage compared to control, whereas the difference was significant only in 7.5% PEG treatment for ‘*Pedro*’.

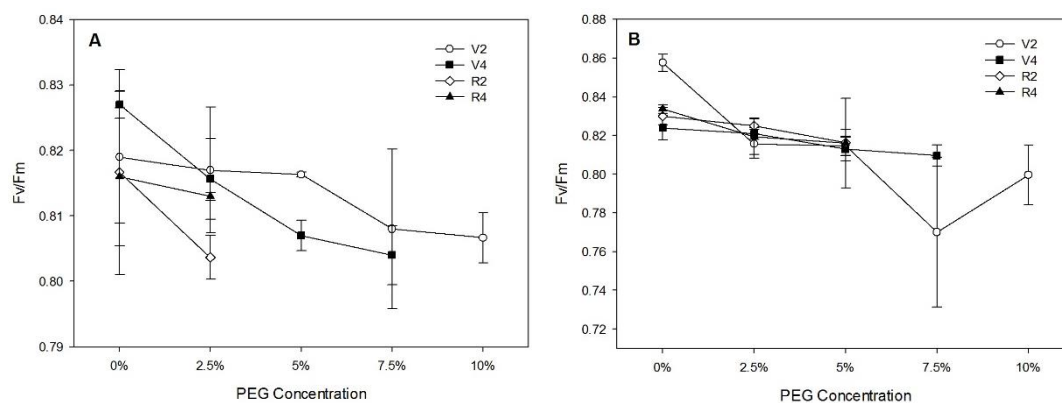


Fig. 27. Maximum photochemical efficiency of PS II (F_v/F_m) of 'ES Mentor' (A) and 'Pedro' (B) in different PEG concentrations at different stages

5.2.2.5. The Effects of PEG-induced Drought Stress on The Actual photochemical efficiency of PS II ($\Phi PS II$) of 2 Soybean Genotypes

Increasing PEG concentration was accompanied by a non-significant decrease in ($\Phi PS II$) of 'ES Mentor' in all stages (Fig. 28A). For 'Pedro' on the other hand, 2.5% PEG treatment resulted in better, yet not significant, ($\Phi PS II$) compared to control treatment at both vegetative stages (V2 and V4), however, control was the highest at later stages and ($\Phi PS II$) decreased with increasing PEG concentration (Fig. 28B). Zhang et al. (2016) reported maximum quantum yield of PS II (F_v/F_m) to be approximately 0.78–0.80 in control treatment, however, this parameter decreased in response to drought stress, but was not significantly different. Additionally, drought stress resulted in a reduction in quantum yield of PS II ($\Phi PS II$) (from 0.53 to 0.13); they suggested that the reduced $\Phi PS II$ was a result of a decrease in the excitation energy trapping efficiency of PS II reaction centers. Similar conclusion was reported by Zlatev and Yordanov (2004) in bean plants. Hao et al. (2013) reported the decrease to be significant (from 0.83 to 0.66), whereas Mathobo et al. (2017) concluded that the reduction was insignificant after 93 days of planting between control plants and plants suffered from drought stress for 24 days in early stages; however, later in the same experiment (100 days after planting) the difference was significant. Decrease in F_v/F_m was concluded to be an indication of down regulation of photosynthesis (Zlatev and Lidon, 2012). Liu et al. (2012) also observed a decline in F_v/F_m ratio in drought stressed plants of two maize cultivars. This occurrence of chronic photo-inhibition was justified as a result of photo-inactivation of PS II centers

(Zlatev and Yordanov, 2004). Compared with the control, water stress markedly decreased F_v/F_m (from 0.80 to 0.76) and $\Phi PS II$ (from 0.69 to 0.58) (Tang et al., 2017). Consistently with our results, water stress treatment reduced total chlorophyll content and chl_a/chl_b , indicating the decreased capacity of absorbing and conversion of light energy, which may be the reason of reduced $\Phi PS II$ (Tang et al., 2017). On the contrary, drought stress did not have an effect on F_v/F_m in dry bean (Terzi et al., 2010).

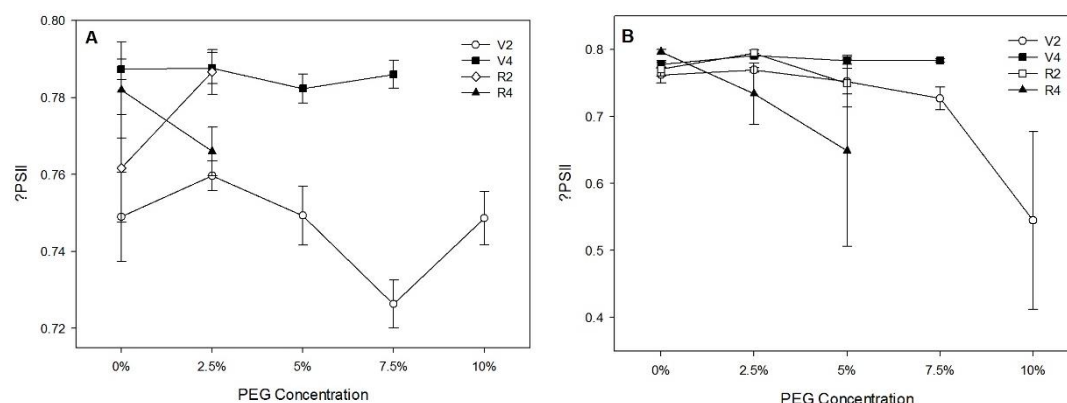


Fig. 28. Actual photochemical efficiency of PS II ($\Phi PS II$) of '*ES Mentor*' (A) and '*Pedro*' (B) in different PEG concentrations at different stages

5.2.2.6. The Effects of PEG-induced Drought Stress on The Stomatal Conductance (g_s) ($mmol m^{-2} s^{-1}$) of 2 Soybean Genotypes

Significant differences were recorded for both genotypes in response of stomatal conductance to PEG application; increasing PEG concentration resulted in lower stomatal conductance in all stages (except for a slight increase in 5% PEG treatment compared to 2.5% PEG treatment at V2 stage for '*Pedro*') (Fig. 29A,B). Control treatment was significantly higher than all other PEG treatments, and 2.5% PEG treatment was significantly better than higher PEG-concentration treatments for '*ES Mentor*'. Previously, Ohashi et al. (2006) reported that stomatal conductance of soybean plants significantly declined under water stress; similar results were reported by Zhang et al. (2016) who found a decrease in g_s by 98.8% under drought; they concluded that this decrease in g_s may be caused by the reduced open stomata ratio and stomatal aperture size in exposed water-stressed plants. Hao et al. (2013) also reported a significant reduction in stomatal conductance from 0.25 to 0.10 $mol H_2O m^{-1} s^{-1}$. Mathobo et al. (2017) justified the reduction in g_s in their experiment by the stomatal closure which prevented CO_2 from

entering the leaf. A 70% reduction of g_s after 22 days of drought stress was observed in dry bean (Rosales et al., 2012). Tang et al. (2017) concluded that PEG-induced water stress significantly decreased g_s by 73%.

Table 75. F_0 , F_m , F_v , F_v/F_0 and F_m/F_0 for the two studied genotypes in different PEG concentrations (%) at different stages.

%PEG	Stage	F_0		F_m		F_v		F_v/F_0		F_m/F_0	
		'ES Mentor'	'Pedro'	'ES Mentor'	'Pedro'	'ES Mentor'	'Pedro'	'ES Mentor'	'Pedro'	'ES Mentor'	'Pedro'
0%	V2	0.344	0.322	1.783	1.808	1.461	1.551*	4.263	4.818	5.199	5.616
2.5%		0.320	0.348	1.767	1.788	1.443	1.458	4.547	4.203	5.558	5.149
5%		0.313	0.329	1.715	1.779	1.400	1.449	4.472	4.418	5.478	5.421
7.5%		0.343	0.339	1.795	1.547	1.451	1.199*	4.249	3.622	5.250	4.644
10%		0.331	0.339	1.814	1.710	1.463	1.367	4.429	4.061	5.490	5.084
0%	V4	.314	.314	1.818*	1.787	1.503*	1.473	4.794*	4.689	5.797*	5.688
2.5%		.323	.328	1.756	1.841	1.432	1.512	4.447	4.601	5.450	5.604
5%		.328	.285	1.697	1.554	1.370*	1.264	4.182*	4.428	5.181*	5.445
7.5%		.309	.306	1.662*	1.698	1.336*	1.374	4.332	4.504	5.386	5.561
0%	R2	.320	.329	1.651	1.880	1.351	1.560	4.239	4.753	5.175	5.725
2.5%		.309	.308	1.687	1.806	1.355	1.489	4.400	4.853	5.475	5.881
5%		NA	.311	NA	1.700	NA	1.388	NA	4.473	NA	5.476
0%	R4	.314	.287	1.719	1.724	1.402	1.437	4.497	5.010	5.505	6.009
2.5%		.330	.288	1.759	1.605	1.430	1.317	4.336	4.558	5.334	5.558
5%		NA	.284	NA	1.563	NA	1.279	NA	4.605	NA	5.606

- *. Significant at 0.05 level among PEG concentrations within certain stage and genotype.

As shown in table 76, stomatal conductance showed significant negative correlation with drought application at all stages in both genotypes. SPAD value was also negatively affected by drought in both genotypes; the effect was more measurable at R4 stage. In additions, both genotypes showed reduced F_v/F_m value with increasing drought stress at all stages. However, both F_v and F_m were positively correlated with drought stress except at V4 stage in 'ES Mentor', whereas both traits were negatively affected by drought in 'Pedro', and the most negative effect occurred at R2 stage.

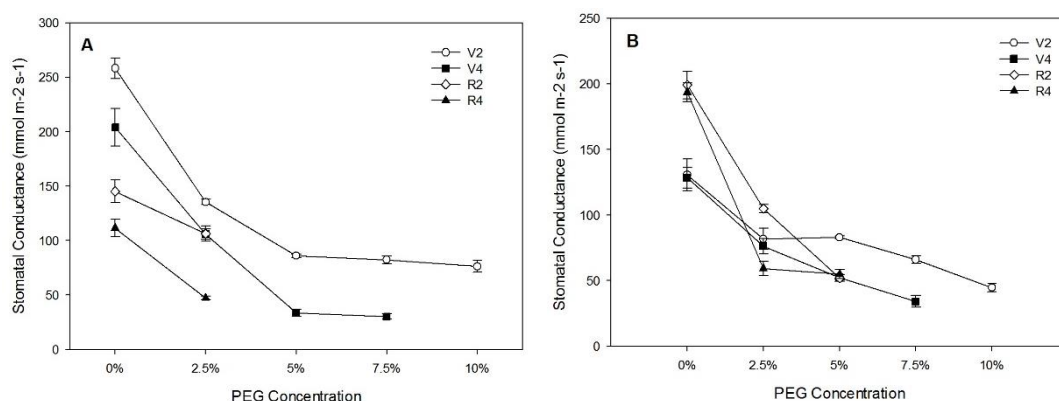


Fig. 29. Stomatal conductance of '*ES Mentor*' (A) and '*Pedro*' (B) in different PEG concentrations at different stages

In '*ES Mentor*' all chlorophylls were more affected by drought at reproductive as compared to vegetative stages, whereas in '*Pedro*' Chl_a was most-negatively affected by drought at R2 stage, whereas both Chl_b and Chl_{x+c} were most affected at V4 stage.

Table 76. Correlation between the studied traits and PEG concentrations.

genotype	'ES Mentor'				'Pedro'			
Stage	V2	V4	R2	R4	V2	V4	R2	R4
g _s	-.855**	-.924**	-.840*	-.969**	-.887**	-.946**	-.976**	-.873**
SPAD	-.449	-.591*	-.422	-.899*	-.430	-.027	-.964**	-.764*
F _v /F _m	-.366	-.814**	-.376	-.141	-.583*	-.594*	-.595	-.330
F _m	.145	-.783**	.188	.226	-.446	-.400	-.670*	-.414
F _v	.023	-.841**	.022	.221	-.562*	-.439	-.759*	-.422
F ₀	-.013	-.069	-.369	.282	.124	-.342	-.286	-.049
F _v /F ₀	.014	-.651*	.190	-.306	-.493	-.360	-.413	-.274
F _m /F ₀	.109	-.598*	.342	-.338	-.389	-.280	-.364	-.273
ΦPS II	-.316	-.170	.636	-.505	-.562*	.116	-.240	-.441
Chl _a	-.453	-.309	-.914*	-.823*	-.337	-.346	-.502	-.253
Chl _b	-.375	-.647*	-.991**	-.825*	-.697**	-.862**	-.256	-.584
Chl _{x+c}	-.559*	-.275	-.607	-.941**	-.179	-.478	-.408	-.344

- *. Correlation is significant at 0.05 level (2-tailed).
- **. Correlation is significant at 0.01 level (2-tailed).

Calculating the effect size also reflected the effect of PEG concentration on the different traits studied; except Chl_b at V4 stage and Fv/Fm at R2 stage, PEG concentration was higher and more significant compared to genotype effect (Table 77).

Table 77. Effect size of PEG concentration, genotype and PEG concentration* genotype on the studied traits.

	SPAD	Chl _a	Chl _b	Chl _{c+x}	F _v /F _m	ΦPSII	g _s
V2							
PEG concentration	22	17.8	42.2*	21.6	31.2*	24.1	67.5*
genotype	5.6	13.9*	4.5	13.7*	0.1	4.1	16.0*
PEG concentration* genotype	61.5*	8.6	81.5*	14.8	25.4	32.3	86.6*
V4							
PEG concentration	32.7*	12.7	35.6*	0.5	50.2*	15.0	83.6*
genotype	19.9*	10.4	39.1*	0.4	2.9	2.1	3.2
PEG concentration* genotype	37.5	5.7	84.5*	15.8	7.1	14.6	74.3*
R2							
PEG concentration	54.4*	24.7	48.2*	26.4	7.9	27.6	83.8*
genotype	4.9	5.3	12.6	0.3	20.5	0.2	0.5
PEG concentration* genotype	38.8*	49.9*	90.8*	4.5	2.3	0.0	57.1*
R4							
PEG concentration	14.0	35.8	19.9	54.2*	5.2	22.3	75.7*
genotype	4.4	4.2	14.2	0.1	5.0	4.6	4.2
PEG concentration* genotype	79.1*	21.2	12.1	1.4	2.1	1.2	79.1*

- *. The effect size (Partial Eta Squared) is significant at 0.05 level.

6. CONCLUSIONS AND RECOMMENDATIONS

6.1. FIELD EXPERIMENTS

6.1.1. The Effect of Drought Stress on The Morpho-physiology, Yield Components and Seed Quality of 7 Soybean Genotypes

Under both drought stress and irrigated regimes, the seed yield of Ananda, ES Pallador and Pannonia Kincse genotypes was significantly higher than the other genotypes, suggesting that these genotypes might be more suitable for cultivation in the study area, especially under drought conditions. Moreover, the 100-seed weight of Ananda did not differ under drought stress conditions, and that of ES Mentor was even higher, suggesting that these two genotypes could be adopted under drought stress conditions in the study area in case the seed size is a matter of concern.

SPAD is not recommended as a reliable trait when evaluating the effect of drought stress on soybean, at least on the studied genotypes, in the study area, as it showed different trends through stages of different genotypes. On the contrary, LAI trait presented a steady trend through stages and genotypes under both drought stress and irrigated conditions, which might suggest this trait to be the most reliable physiological trait to count on in evaluating soybean's performance in the study area.

6.1.2. The Effects of Drought Stress and Nitrogen Fertilization on The Morpho-physiology, Yield Components and Seed Quality of 2 Soybean Genotypes

In 'Pannonia Kincse' genotype, drought stress at V4 stage had no measurable effect on SPAD, whereas it decreased LAI values and increased NDVI values. Drought negatively affected SPAD values (whenever N fertilizer was applied) and LAI values (regardless of N fertilization and rate) but increased NDVI values at R2 stage, whereas negatively affected both SPAD and LAI at R4 stage whereas NDVI values did not follow a certain trend as fertilization played a role. At R6 stage SPAD and LAI values were not measurably affected by drought, whereas NDVI trait was enhanced under drought conditions; i.e. LAI was the mostly-affected trait when drought occurred at early reproductive stages (R2 and R4). Rainy conditions enhanced both SPAD and NDVI values but reduced LAI values at R2, and negatively affected LAI values at R4 stage; however, at R6 stage it reduced SPAD values but enhanced LAI and NDVI values. Moreover, drought effect was more measurable on LAI than on SPAD and NDVI through progressive stages of soybean plants. Fertilization did not have significant role on SPAD

and NDVI traits in most cases, whereas positively affected LAI trait except at R6 stage, supporting the conclusion that LAI measurements, particularly at early reproductive stages, can provide more effective data about soybean growth and development compared to SPAD and NDVI measurements in the study area.

N fertilization significantly affected plant height, flower and pod number per plant, 100-seed weight, and protein and oil concentrations and yield as well. Irrigation, on the other hand, had significant effects on plant height, flower and pod number per plant and protein and oil concentrations; however, no significant effect was recorded on 100-seed weight or yield.

Fertilization increased the yield under all irrigation regimes except when high rate was applied with the absence of drought, so a conclusion that low-rate fertilization is recommended under all irrigation regimes, whereas high rates of N are only recommended under relative drought conditions could be introduced.

In '*Boglár*' genotype, on the other hand, drought had a significant effect on SPAD trait at early reproductive stages (R2 and R4), where over-average irrigation noticeably enhanced SPAD values. However, drought effect at early vegetative stage (V4) was neglectable, and its late occurrence (at R6 stage) resulted in enhanced SPAD values. NDVI was positively affected by drought in most stages, and similar conclusion was obtained under over-average precipitation. LAI was measurably affected by drought at all stages except for late drought at R6 stage, where LAI was significantly enhanced by over-average precipitation.

Although fertilization had a small effect on SPAD trait early at V4 stage, yet it could enhance it at both R2 and R4 stages, with the high rate ($105 \text{ kg ha}^{-1} \text{ N}$) significantly increasing SPAD values under drought conditions. Moreover, fertilization could enhance SPAD trait even at late R6 stage. Fertilization increased NDVI values at R2 and R4 stages, and the high rate could enhance NDVI trait under drought stress conditions. The high rate also resulted in the highest NDVI values as compared to the other counterparts in all studied stages except for R6 where high rate increased NDVI only under over-average precipitation conditions. LAI was increased early at V4 stage by fertilization, and the latter had positive effect on LAI values under drought conditions. However, late season LAI values were relatively not affected by fertilization.

Both drought stress and mineral N-deficiency decreased flower and pod number per plant, 100-seed weight, seed yield and seed protein concentration; however, they increased oil concentration in the seeds. Inoculation, on the other hand, enhanced flower number per plant, seed yield and seed oil concentration, but reduced pod number per plant, 100-seed weight and, interestingly, seed protein concentration.

Regardless of inoculation, high mineral N application under drought stress conditions resulted in better yield, emphasizing the importance of relatively-high mineral N rates under drought stress conditions whether the seeds were pre-inoculated or not.

6.1.3 The Effects of Drought Stress and Phosphorus Fertilization on The Morpho-physiology, Yield Components and Seed Quality of 2 Soybean Genotypes

The morpho-physiology of both '*Pannonia Kincse*' and '*Boglár*' genotypes was measurably affected by P fertilization, with more significant effects on stomatal conductance and plant height traits. In addition, pod number per plant and, consequently, the final seed yield were noticeably affected by the application of P fertilizer, however, the high rate (90P) did not significantly increase these traits compared to the lower rate (45P). P application significantly increased the oil concentration in the produced seeds, with more significant effect under drought stress conditions, whereas it did not affect the protein concentration trait.

6.1.4. The Effects of Drought Stress and Exogenous Application of Hydrogen Peroxide (H₂O₂) on The Physiology and The Yield of 2 Soybean Genotypes

Treating drought-stressed soybean plants with 1 mM of H₂O₂ could alleviate the negative influence of drought and enhance both the morpho-physiology and the yield of both '*Pannonia Kincse*' and '*Boglár*' genotypes; its effect was more noticeable on the leaf area index (LAI) as H₂O₂-sprayed plants had higher (LAI) values than both drought-stressed and fully-irrigated counterparts. The effect size was also noticeable on the relative water content (RWC) of both genotypes.

6.2. CLIMATE CHAMBER EXPERIMENTS

6.2.1. The Effects of PEG-induced Drought Stress on The Germination Parameters of 2 Soybean Genotypes

Significant differences among PEG concentrations, between genotypes and their interaction were recorded. For both genotypes, germination ratio (GR) and root elongation (RE) decreased as the PEG concentration increased. Both germination energy

(GE) and ultimate germination (UG) decreased, whereas mean period of ultimate germination (MPUG) and percentage inhibition increased with increasing water stress. 'ES Mentor' could maintain higher (GR) than 'Pedro' under all PEG concentrations except 15%, whereas (RE) was lower under all concentrations.

'ES Mentor' could achieve higher germination ratio under different water deficiency levels; however, germinated seeds of 'Pedro' could tolerate relative water stress better, as the roots could elongate deeper searching for available water.

The seed size had a noticeable effect on germination ratio.

6.2.2. The Effects of PEG-induced Drought Stress on The Physiology of 2 Soybean Genotypes

For both genotypes, increasing PEG concentration was accompanied by decreasing SPAD values at all stages.

Concerning chlorophyll content, Chl_a , Chl_b and Chl_{x+c} decreased as PEG concentration increased at all stages of 'ES Mentor'; the reduction was insignificant at vegetative stages (V_2 and V_4 stages) and significant at reproductive stages (R_2 and R_4), whereas for 'Pedro' 2.5% PEG treatment had the best Chl_a and Chl_{x+c} contents at V_2 stage. However at the following stages, control treatment could maintain the best values, and the increase in PEG concentration was accompanied by a decrease in both contents. Chl_b , on the other hand, was significantly higher for 2.5% PEG treatment than control at both vegetative stages, whereas in the reproductive stages it insignificantly decreased with increasing PEG concentration.

Maximum photochemical efficiency of PSII (Fv/Fm) of both genotypes followed one trend throughout the studied stages; it decreased with increasing PEG concentration.

Increasing PEG concentration was accompanied by a non-significant decrease in the actual photochemical efficiency of PSII ($\Phi PSII$) of 'ES Mentor' in all stages, whereas for 'Pedro' 2.5% PEG treatment resulted in better $\Phi PSII$ compared to control treatment at both vegetative stages, however, control was the highest at later stages and $\Phi PSII$ decreased with increasing PEG concentration.

Significant differences were recorded for both genotypes in response of stomatal conductance to PEG application; increasing PEG concentration resulted in lower stomatal conductance in all stages (except for a slight increase in 5% PEG treatment compared to 2.5% PEG treatment at V_2 stage in 'Pedro' plants).

7. NEW SCIENTIFIC RESULTS

- 1- SPAD is not a reliable trait when evaluating the effect of drought stress on soybean in the study area, whereas LAI presented a steady trend through stages and genotypes, suggesting that it can be a reliable physiological trait to count on in evaluating soybean's performance in the study area.
- 2- Ananda and ES Mentor genotypes can be adopted under drought stress conditions in the study area in case optimum seed size is the target of cultivating soybean, whereas Ananda, ES Pallador and Pannonia Kincse genotypes are more suitable for cultivation in the study area, especially under drought conditions, in case the aim of cultivation is the maximum seed yield.
- 3- Regardless of fertilizer application (N, P) and rate, drought resulted in shorter plants in all field experiments, leading to a conclusion that this physio-morphological trait can be counted as an early-season indicator of the expected yield, taking into consideration that plant height had a positive correlation coefficient in all field experiments. As for yield component traits, both flower and pod number⁻¹ showed measurable reductions under drought stress conditions, which can also be taken into consideration for estimating the probable reductions in the final yield caused by this abiotic stress.
- 4- High protein concentration in the seeds can be achieved by applying high nitrogen fertilizer rates (regardless of its source whether from inoculation or chemical fertilizer); in our experiment, Pannonia Kincse had 3.5% and Boglár had 4.8 and 4.7% seed protein concentration (inoculated and non-inoculated, respectively) when 105 kg ha⁻¹ of mineral fertilizer was applied as compared to control treatment with no N fertilizer applied.
- 5- High oil concentration in the seeds can be achieved by the application of P fertilizer, regardless of water availability for the plants; in our experiment, 45 and 90 kg ha⁻¹ of P increased the oil concentration by 5.8 and 10.1% respectively in Pannonia Kincse, and by 5.9 and 10.0% respectively in Boglár as compared to the non-fertilized control.
- 6- Exogenously-sprayed plants with 1 mM H₂O₂ under drought stress conditions had 21.3% higher seed yield in the case of Pannonia Kincse genotype and 13.3% in the case of Boglár genotype, confirming that this treatment can be extremely beneficial under drought stress conditions.

- 7- Chl *a* consistently decreased under all drought stress severities and at all studied stages, whereas Chl *b* showed relatively higher levels under mild and moderate drought stress levels at early stages (V2 and V4); however, it followed the similar trend of Chl *a* at later stages (R2 and R4). This conclusion suggests counting on Chl *a* concentration measurement, rather than Chl *b*, to evaluate drought susceptibility when plants are subjected to drought stress early in the season.
- 8- Stomatal conductance trait showed significant reductions in both studied genotypes, even with mild drought stress application (2.5% PEG concentration) at all stages, suggesting that this trait could also be a suitable early alert of drought occurrence.

8. PRACTICAL UTILIZATION OF RESULTS

- 1- Low-rate N fertilization of soybean in the study area is recommended under all irrigation regimes, whereas high rates of N are only recommended under relative drought conditions.
- 2- Relatively-high mineral N-fertilizer rates under drought stress conditions in the study area are of much importance whether the seeds were pre-inoculated or not.
- 3- The final seed yield was noticeably affected by the application of P-fertilizer, however, the high rate (90P) did not significantly increase the yield compared to the lower rate (45P).
- 4- P application significantly increased the oil concentration in the produced seeds, with more significant effect under drought stress conditions, whereas it did not affect the protein concentration trait.
- 5- Treating drought-stressed soybean plants with 1 mM of H₂O₂ could alleviate the negative influence of drought and enhance both the morpho-physiology and the yield of soybean plants.

9. SUMMARY

Soybean (*Glycine max* (L.) Merrill) is one of the most important food legumes because of its high protein (about 40%) and oil (about 20%) concentrations, in addition to carbohydrates and minerals. Soybean has the highest average harvested area among all legumes, and it has the highest harvested area of all oilseed crops as well.

The current global climatic changes have put this crop under certain periods of drought stress during different stages of its vegetative growth, and soybean is reported to be sensitive to several abiotic stresses as compared to other legumes and crops. Moreover, soybean is currently sown as a rainfed crop in many regions, that's why drought is continuously affecting soybean production and quality, especially with the fact that drought intensively increased over the past decades, altering precipitation amounts and distribution, and is predicted to further increase in frequencies and intensities, putting the production of soybean, and other sensitive crops, under serious challenges and raising the concern about the world's food security, especially with the fact that global population is continuously increasing.

Understanding the influence of drought stress on crops becomes vital, as such understanding can be exploited in irrigation-scheduling practices which, in part, reduces drought-related fluctuations in food production. However, the response to drought stress is a very complex process that involves multiple mechanisms on different morphological, physiological and metabolic levels.

Under drought conditions, reactive oxygen species (ROS) are produced in higher concentrations, resulting in cellular damage and, eventually, cell death. However, despite the fact that high concentrations of ROS cause damages to the cells, yet low concentrations play the role of signaling molecules that can ease several processes like germination and growth. For example, ROS play noticeable role in regulating stomatal closure in order to optimize water use efficiency. Hydrogen peroxide (H₂O₂) is a compound that belongs to non-radical ROS; it regulates many physiological mechanisms such as growth and development under both normal and stressed conditions, playing a major role in activating various signal molecules in plants leading to inducing different mechanisms of tolerance.

Nitrogen (N) is one of the most important macronutrients for plant vegetative growth and development, affecting several functions and components such as enzymes, proteins and cell walls. In addition, N represents a major component of the chlorophyll; as such, it affects chlorophyll formation and, consequently, photosynthesis. Moreover, N is essentially needed for soybean in order to produce optimum biomass. Because of its high protein concentration in the seeds, soybean plants have high N requirements. The two main sources of nitrogen for soybean plants are biologically-fixed N₂ and mineral N fertilizer. N is particularly important under drought stress conditions for improving shoot nitrogen and shoot biomass accumulation. Accordingly, N fertilization might be introduced as an efficient application to partially overcome the negative effects expected from drought periods.

Phosphorus (P), after nitrogen, is also one of the most important mineral nutrients for plant development and energy conservation and transfer. In addition, P has a vital role in photosynthesis and chloroplast composition. Considerable amounts of P, in the form of ATP, are needed for biological N₂-fixation process by the nodules in legume plants. Although soil might have high concentrations of P, yet most of it can be unavailable for plants due to its poor solubility and fixation. As a result, N₂-fixation rate in legumes and, consequently, the advantage of this ecologically friendly process can be decreased. P deficiency can also decrease seedling vigor and root development. Like N, soybean has high requirements of available P (10-15 mg kg⁻¹ soil), and low soil-P availability limits soybean yields. However, excessive amounts of P resulted in growth inhibition in soybean, in addition to the fact that only 10%–45% of P- fertilizer added to the soil is readily usable, so it's of high importance to determine the best P-rate application that can be optimally used by plants. P application was reported to enhance drought stress tolerance.

The current and the predicted climatic changes are and will certainly affect the yields of plants, which means putting food production for the growing world population under serious challenges, especially those species which can not properly tolerate abiotic stresses. Moreover, using the chemical fertilizers to re-enrich soils with nutrients is not without consequences on the environment, in addition to the higher costs of the production process. Hence, understanding the mechanisms that susceptible crops utilize to cope with changing climate can provide a more-clear idea on on-field applications that

can lead to the optimum production. Thus, this study aimed at revealing the sole effect of on-field drought stress on 7 soybean genotypes, with evaluating the sole and combined influence of drought stress and nitrogen fertilizer application on 2 soybean genotypes; '*Pannonia Kincse*' and '*Boglár*'. This study also monitored the sole and combined effects of P fertilization and drought stress on the 2 soybean genotypes, in addition to revealing the probable positive effects of exogenously spraying H₂O₂ at early bloom (R1) stage on the physiology and the seed yield of the 2 soybean genotypes.

Besides the on-field experiments, this study illustrated the influence of PEG-induced drought stress on the germination parameters and the physiology of 2 soybean genotypes; '*ES Mentor*' and '*Pedro*' under controlled environment (climate chamber) conditions.

The results of the field experiments showed that under both drought stress and irrigated regimes, the seed yield of Ananda, ES Pallador and Pannonia Kincse genotypes was significantly higher than the other genotypes, suggesting that these genotypes might be more suitable for cultivation in the study area, especially under drought conditions. Moreover, the 100-seed weight of Ananda did not differ under drought stress conditions, and that of ES Mentor was even higher, suggesting that these two genotypes could be adopted under drought stress conditions in the study area in case the seed size is a matter of concern.

In '*Pannonia Kincse*' genotype, N fertilization significantly affected plant height, flower and pod number per plant, 100-seed weight, and protein and oil concentrations and yield as well. Irrigation, on the other hand, had significant effects on plant height, flower and pod number per plant and protein and oil concentrations; however, no significant effect was recorded on 100-seed weight or yield. Fertilization increased the yield under all irrigation regimes except when high rate was applied with the absence of drought, so a conclusion that low-rate fertilization is recommended under all irrigation regimes, whereas high rates of N are only recommended under relative drought conditions could be introduced.

In '*Boglár*' genotype, on the other hand, both drought stress and mineral N-deficiency decreased flower and pod number per plant, 100-seed weight, seed yield and seed protein concentration; however, they increased oil concentration in the seeds. Inoculation, on the other hand, enhanced flower number per plant, seed yield and seed oil concentration, but

reduced pod number per plant, 100-seed weight and, interestingly, seed protein concentration. Regardless of inoculation, high mineral N application under drought stress conditions resulted in better yield, emphasizing the importance of relatively-high mineral N rates under drought stress conditions whether the seeds were pre-inoculated or not.

The morpho-physiology of both '*Pannonia Kincse*' and '*Boglár*' genotypes was measurably affected by P fertilization, with more significant effects on stomatal conductance and plant height traits. In addition, pod number per plant and, consequently, the final seed yield were noticeably affected by the application of P fertilizer, however, the high rate (90P) did not significantly increase these traits compared to the lower rate (45P). P application significantly increased the oil concentration in the produced seeds, with more significant effect under drought stress conditions, whereas it did not affect the protein concentration trait.

Treating drought-stressed soybean plants with 1 mM of H₂O₂ could alleviate the negative influence of drought and enhance both the morpho-physiology and the yield of both '*Pannonia Kincse*' and '*Boglár*' genotypes; its effect was more noticeable on the leaf area index (LAI) as H₂O₂-sprayed plants had higher (LAI) values than both drought-stressed and fully-irrigated counterparts. The effect size was also noticeable on the relative water content (RWC) of both genotypes.

It can be reported that SPAD is not recommended as a reliable trait when evaluating the effect of drought stress on soybean, at least on the studied genotypes, in the study area, as it showed different trends through stages of different genotypes. On the contrary, LAI trait presented a steady trend through stages and genotypes under both drought stress and irrigated conditions, which might suggest this trait to be the most reliable physiological trait to count on in evaluating soybean's performance in the study area.

In the chamber room experiments, the results showed that significant differences among PEG concentrations, between genotypes and their interaction were recorded. For both "*ES Mentor*" and "*Pedro*" genotypes, germination ratio (GR) and root elongation (RE) decreased as the PEG concentration increased. Both germination energy (GE) and ultimate germination (UG) decreased, whereas mean period of ultimate germination (MPUG) and percentage inhibition increased with increasing water stress. '*ES Mentor*'

could maintain higher (GR) than '*Pedro*' under all PEG concentrations except 15%, whereas (RE) was lower under all concentrations.

'*ES Mentor*' could achieve higher germination ratio under different water deficiency levels; however, germinated seeds of '*Pedro*' could tolerate relative water stress better, as the roots could elongate deeper searching for available water. The seed size had a noticeable effect on germination ratio.

For both genotypes, increasing PEG concentration was accompanied by decreasing SPAD values at all stages. Concerning chlorophyll content, Chl_a , Chl_b and Chl_{x+c} decreased as PEG concentration increased at all stages of '*ES Mentor*'; the reduction was insignificant at vegetative stages (V_2 and V_4 stages) and significant at reproductive stages (R_2 and R_4), whereas for '*Pedro*' 2.5% PEG treatment had the best Chl_a and Chl_{x+c} contents at V_2 stage. However at the following stages, control treatment could maintain the best values, and the increase in PEG concentration was accompanied by a decrease in both contents. Chl_b , on the other hand, was significantly higher for 2.5% PEG treatment than control at both vegetative stages, whereas in the reproductive stages it insignificantly decreased with increasing PEG concentration.

Maximum photochemical efficiency of PSII (F_v/F_m) of both genotypes followed one trend throughout the studied stages; it decreased with increasing PEG concentration. Increasing PEG concentration was accompanied by a non-significant decrease in the actual photochemical efficiency of PSII ($\Phi PSII$) of '*ES Mentor*' in all stages, whereas for '*Pedro*' 2.5% PEG treatment resulted in better $\Phi PSII$ compared to control treatment at both vegetative stages, however, control was the highest at later stages and $\Phi PSII$ decreased with increasing PEG concentration. Significant differences were recorded for both genotypes in response of stomatal conductance to PEG application.

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11. PUBLICATION LIST



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List of publications related to the dissertation

Hungarian scientific articles in Hungarian journals (1)

1. Ábrahám, É. B., **Basal, O.**: Különböző éréscsoportú szójafajták tesztelése a Hajdúságban.
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- Foreign language conference proceedings (5)
20. **Basal, O., Szabó, A.:** Morphology and seed yield of drought-stressed soybean as affected by H₂O₂ spraying.
In: Proceedings of ISER 226th International Conference, [s.n.], Helsinki, 1-3, 2019.
21. **Basal, O., Szabó, A.:** The Effects of Phosphorus Application and Rate on Soybean Physiology and Growth under Drought Stress.
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Foreign language abstracts (3)

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09 October, 2020



12. DECLARATION

I prepared this dissertation within the framework of Kálmán Kerpely doctoral school of the University of Debrecen, in order to obtain a doctoral (PhD) degree from the University of Debrecen.

Debrecen, 20

..... ..

the signature of the candidate

DECLARATION

I certify that Oqba Basal; a doctoral candidate between 2016-2020, and within the framework of the above-mentioned doctoral school, has carried out his work under my guidance / direction. The independent contribution of the candidate to the results included in the dissertation, the dissertation is the independent work of the candidate. I suggest / recommend the acceptance of the dissertation.

Debrecen, 20

..... ..

signature of the supervisor (s)

13. ANNEXES

Bokréta	Bólyi 612	ES Pallador	ES Mentor	Pannonia Kincse	Coraline	Ananda	DS
Bokréta	Bólyi 612	ES Pallador	ES Mentor	Pannonia Kincse	Coraline	Ananda	FI

Annex 1. The experimental design of the soybean gynotype experiment.

			Replication	Irrigation Regime
35N	0N	105N	IV.	NI
105N	0N	35N	III.	
35N	105N	0N	II.	
0N	35N	105N	I.	
35N	0N	105N	IV.	HI
105N	0N	35N	III.	
35N	105N	0N	II.	
0N	35N	105N	I.	
35N	0N	105N	IV.	FI
105N	0N	35N	III.	
35N	105N	0N	II.	
0N	35N	105N	I.	

Annex 2. The experimental design of the experiment on the effect of drought stress and nitrogen fertilization on '*Pannonia Kincse*' genotype.

						Replication	Irrigation Regime
35N	0N	105N	35N	0N	105N	IV.	NI
105N	0N	35N	105N	0N	35N	III.	
35N	105N	0N	35N	105N	0N	II.	
0N	35N	105N	0N	35N	105N	I.	
35N	0N	105N	35N	0N	105N	IV.	HI
105N	0N	35N	105N	0N	35N	III.	
35N	105N	0N	35N	105N	0N	II.	
0N	35N	105N	0N	35N	105N	I.	
35N	0N	105N	35N	0N	105N	IV.	
105N	0N	35N	105N	0N	35N	III.	FI
35N	105N	0N	35N	105N	0N	II.	
0N	35N	105N	0N	35N	105N	I.	
Inoculated (+)			Non-inoculated (-)				

Annex 3. The experimental design of the experiment on the effect of drought stress and nitrogen application on '*Boglár*' genotype.

90P	0P	45P	90P	0P	45P	III.	non-irrigated
45P	90P	0P	45P	90P	0P		
0P	45P	90P	0P	45P	90P		
90P	0P	45P	90P	0P	45P	III.	fully-irrigated
45P	90P	0P	45P	90P	0P		
0P	45P	90P	0P	45P	90P		
Pannonia Kincse			Boglár			I.	

Annex 4. The experimental design of the experiment on the effect of drought stress and phosphorus application on 2 soybean genotypes.

DW	FI	HP	DW	FI	HP	III.	
HP	DW	FI	HP	DW	FI		
FI	HP	DW	FI	HP	DW		
Pannonia Kincse			Boglár			I.	

Annex 5. The experimental design of the experiment on the effects of drought stress and exogenous application of hydrogen peroxide (H₂O₂) on 2 soybean genotypes.