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Investigation of soybean leaf area influenced by water supply

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Abstract— Soybean leaf area observations were carried out in two levels of water supply using two different varieties (*Sinara* and *Sigalia*) in Hungary. Half of the crops was grown using unlimited watering in evapotranspirometers, while the others received only 50% of their water requirements from the R1 (beginning bloom) stage (stressed crops). Out of four meteorological variables, the air temperature, the most easily accessible meteorological variable impacted the *LAI* (leaf area index) the most, irrespective of water supply. To obtain the variation in the vertical leaf area distribution, the LAI_{max} was selected and analyzed, when leaf area remained relatively invariable. Water deprivation in the reproductive phase significantly reduced the *LAI*, irrespective of the studied variety. The water stress condition significantly lowered the level-wise trifoliate area of *Sigalia* above the sixth leaf level but did not lower it in the case of *Sinara*. Increase of 1.5 in the number of leaf levels could be contributed to the higher *LAI* of crops with unlimited watering in comparison to water stressed ones. The area of the middle leaflet was significantly higher compared to the outer ones at the bottom and the top of the plant, but they were of about the equal size around the center of the plant height irrespective of the variety. According to our best knowledge, this morphological phenomenon has not been described yet.

Key-words: distribution of leaf area, soybean (*Glycine max* L.), evapotranspiration, water stress

1. Introduction

Leaf assimilatory surface size, photosynthesis, and crop biomass are the main crop indicators of final yield (*Kross et al.* 2015) including soybean. Most commonly, the ratio of the green leaf area in m^2 to the ground surface area (in m^2)

is expressed as leaf area index, *LAI*. Green leaf area is available for gas exchange processes (mainly CO_2 and water vapor) between the canopy and the atmosphere. *Vina et al.* (2011) specified these vegetation processes, including light and water interception (rainfall and fog), light attenuation through the crop stand, transpiration, photosynthesis, autotrophic respiration, and carbon and different nutrients' cycles. *Yang et al.* (2009) identified that the size of maximum *LAI* reflected the impacts of environmental conditions the most. *Malone et al.* (2002) concluded the soybean *LAI* reaches 3.5–4.0 in the growth stages from R2 (full bloom) to R5 (beginning seed) producing high seed yield. Similar values were also published by *Board et al.* (1997). In soybean evapotranspiration (*EVTR*), "threshold" *LAI* of 3.7 was communicated by *Campos et al.* (2017) at the University of Nebraska-Lincoln Research Centre, USA after which further growth in *LAI* does not increase canopy's evapotranspiration. This peak *LAI* was called as "saturation *LAI*" after *Bausch* (1993). In this study, the peak *LAI* was selected and analyzed from the growing period of soybean, where *LAI* remained relatively invariable (August). Selected time periods were also suitable for comparative water withdrawal investigations as growth stages between R4 (full pod) and R6 (full seed) are vulnerable to water deficiencies producing in significant yield loss (*Bagg et al.* 2009). *Hsiao* (1990) also highlighted *LAI* as affected by water stress as an important indicator of crop growth and final yield.

The green colored part of crop leaves is the photosynthetically active component which must be separated from the dried leaves.

Two main types of approaches were developed in the *LAI* estimation; direct (based on leaf collection) and indirect methods. In this second group, *LAI* is derived from one or more easily measurable leaf characteristics (*Jonckheere et al.* 2004). Indirect non-contact measurements are considered to be the most up-to-date, and non-destructive *LAI* observation group using remotely sensed canopy reflectance data that has been reviewed partially by *Verrelst et al.* (2015). Although it does not mean that other *LAI* estimation methods can not be of great significance under special circumstances.

The aim of this investigation was to find a simplified approach in soybean's leaf area estimation that allows easy experimental evaluation of the assimilatory (transpiration) surface size. Leaf area estimation (in the absence of planimeter) using empirical functions based on meteorological variables was also a vital part of the study target. The variation in the vertical distribution of the leaf area across two soybean varieties of three different water supply levels was also documented. To date, there are only limited number of studies that have attempted to take soybean leaflet area into account. Leaf area parameters are basic inputs for most of the crop models.

2. Materials and methods

Soybean (*Glycine max* L.) and meteorological observations were carried out at the Keszthely Agrometeorological Research Station (ARS) (latitude: 46°44′ N, longitude: 17° 14′ E, elevation: 124 m above sea level), over the vegetation period of 2017. A QLC-50 climate station fitted with a CM-3 pyranometer was operated at ARS. This standard station belongs to the Hungarian National Meteorological Network operated by the Hungarian Meteorological Service (OMSZ). On the recommendation of the seed supplier company of Karintia (2017), indeterminate *Sinara* (*Sin*), a water stress tolerant, and *Sigalia* (*Sig*), a variety bred for average weather conditions were included in the study. The length of the studied soybean's growing season ranged between 116 and 120 days. Soybean hand sowing occurred on May 9, 2017, and crops successfully emerged on May17, 2017. The planting distance between the crop rows was 0.24 m (planting population: 600,000 seed ha^{-1}). Before planting, 300 kg ha^{-1} nitrogen-phosphorus-potassium (NPK) fertilizer was applied at the time of sowing (N:P:K=1:1:1).

The phenological phases of soybean were used after *Fehr* and *Caviness* (1977).

Some of the treatments were grown in the growing pots of Thornthwaite-type compensation evapotranspirometers. Half of them got unlimited water supply in line with the nature of the equipment (*ET*). Stressed soybean canopies received only 50% of their water requirement from the reproductive phase (R1), producing detectable water stress conditions (*RO*). The stressed vessels' control system (magnetic swimmer and Reed-switch) were disconnected and its water supplier switch was connected to the non-stressed vessel's reed switch, so the stressed vessel's water supply was regulated by the non-stressed water compensation unit. Water deprivation of stressed crops was ensured by closing the water supplier tap every second day. This setup allowed to reduce water supply in about half of the reproductive stage. A data logger of HYGACQ V1.3 type was connected to log the amount of water use. The calculated hourly sums were memorized. The collected hourly data were downloaded to a computer, using the WHYGACQ program (*Anda* and *Soós*, 2014). As stressed crop's tap was closed every second day, pentad sums and evapotranspiration totals were calculated (water balance) and presented in the study.

Altogether, two water treatments and two varieties were included in the study with the following experimental design:

- *Sin ET* (water stress tolerant variety, unlimited water supply);
- *Sig ET* (variety of average weather conditions, unlimited water supply);
- *Sin RO* (water stress tolerant variety, crops exposed to moisture stress from generative phase, R1);
- *Sig RO* (variety of average weather conditions, crops exposed to moisture stress from generative phase, R1).

The leaflet area, L was determined as follows. Given the oval shape of the leaflets, the area was expected to be directionally proportional to the product of the length (a) and width (b). The parameter (a) was measured from the base of the leaf to the terminal part of the leaf blade. The maximum width of the leaflet (b) was taken at the widest lobes of the lamina perpendicular to the lamina midrib:

$$L = x \cdot ab. \tag{1}$$

In addition, destructive L measurement using segmentation was carried out from separate growing pots excluded from the water supply study. Each leaflet was placed on a flat surface of an evenly colored red cardboard with a scale at the side, directly under a vertically mounted camera (Canon EOS 7D digital camera with 17.9 megapixels. Histogram based threshold segmentation was applied in the image processing program (SGDIP 0.1; of our own) to count the area of the region of interest.

The x multiplier in Eq. (1) was determined with linear regression without intercept based on about 350 trifoliates.

Plant height of the crops was also registered for the same crops where L measurements were carried out.

The effect of the water supply on the trifoliate level was analyzed with a 3-way ANOVA method. The area of the trifoliate was the response variable, the water supply, the variety of the plants, and the trifoliate level were considered as exploratory variables. In the first step, all 2-way and 3-way interactions were included in the model. Iteratively, the non-significant interaction and main effect with the highest p-value was removed from the model. Tukey HSD posthoc test was used, if it is necessary.

The area of the middle leaflet compared to the outer ones within a trifoliate was examined. The proportion of the middle leaflet within the whole trifoliate was calculated for each trifoliate level of each plant. Given the two outer leaflet are of about equal size, the proportion being greater than 1/3 implies that the middle leaflet is larger than the outer ones. This proportion was analyzed with a 3-way ANOVA method on the same way as described at the analysis of the area of the trifoliate level.

Two-tailed one-sample t-test was applied to compare the proportion of the middle leaflet assumed to be 1/3 of trifoliate area, on each trifoliate level, separately. Pooled standard deviation was used in the t-test. To facilitate the presentation of the results, 95% confidence interval was calculated for each trifoliate level. All plants were considered as a single sample.

To illustrate the impact of meteorological elements (air temperature, T_a; water vapor pressure, e_a; wind speed, u; precipitation, P) on LAI, the Pearson's correlation analysis was applied. Multiple stepwise regression analysis was carried out to get the combined effect of different meteorological variables on

LAI. The Akaike information criterion (*AIC*) was applied to estimate different *LAI* projections (*Motulsky* and *Christopoulos,* 2004):

$$AIC = N \times \ln\left(\frac{SS}{N}\right) + 2K \quad , \tag{2}$$

where N is the number of data points, K is the number of parameters fit by the regression plus one, SS is the residual sum of squares value taken from the ANOVA-table of the regression analysis. The model with the smallest *AIC* value is most likely to be correct.

The tests were carried out with the SPSS Statistics software (IBM Corp., New York, USA) and R statistical software (R, 2017).

3. Results and discussion

3.1. Weather, crop development, and evapotranspiration (EVTR)

On a soybean seasonal average basis, the vegetation period in 2017 was 1.3 °C warmer ($p < 0.622$) than that of the long-term mean at Keszthely (1971–2000). Warmer months were particularly noticeable in summer (June-August), when the difference from the climate norms was 1.8–2.7 °C. The growing season's precipitation sum P was 37.0 mm lower ($p < 0.738$) than that of the long-term P total (384.4 mm). Monthly P sums in the growing season reduced with 17.4–37.4 mm in comparison to their climate norms (1971–2000). Although the distribution of monthly P sum was quite even during the soybean growing season in 2017. The only increased P in September was out of the soybean's vegetation period.

Durations of the soybean phenological stages were similar across all treatments (data not shown). The maximum difference in the length of the vegetation periods was only restricted to a few days (1–2 days).

Barely different seasonal daily mean *EVTR* rates of 5.88 and 5.63 mm day^{-1} was measured in *Sin ET* and *Sig ET*, respectively. Using unlimited watering, increase of 4.51% in daily average *EVTR* rate of *Sin ET* was not significant ($p < 0.224$), indicating no variation in *EVTR* rates between the two varieties with different water requirements. Irrespective of variety, 50% water withdrawal of the crop water requirement declined the seasonal daily mean *EVTR* rate by more than half (*Sin*: 74.68%, $p < 0.001$; *Sig*: 75.51%, $p < 0.001$). Surprisingly, there was no significant impact of variety on daily mean *EVTR* rates at both water supply levels (*ET*: 4.51%, $p < 0.224$; *RO*: 5.48%, $p < 0.165$). Even over water deprivation, the variety *Sin* bred for water shortage conditions statistically used the same amount of water as *Sig*.

Distribution in evapotranspiration pentad sums followed the usual pattern (*Fig. 1*); the peak 5-day evapotranspiration sums in the middle of the growing season were 51.6 (*Sin*) and 57.4 mm pentad^{-1} (*Sig*) for the *ET* in the middle of July, and 23.0 (*Sin*) and 24.7 mm pentad^{-1} (*Sig*) for the *RO* treatments, respectively, in the middle of August. Top water losses of water deprived treatments were late about one month in comparison to unlimited the water supply treatment. From the beginning of August, variety *Sin* generally had higher peak 5-day *EVTR* sums than the variety *Sig* in both water supplies. In August, the stressed soybean probably "addicted" to water shortage, and the evapotranspiration curves of *RO* got closer to the evapotranspiration curves of *ET*.

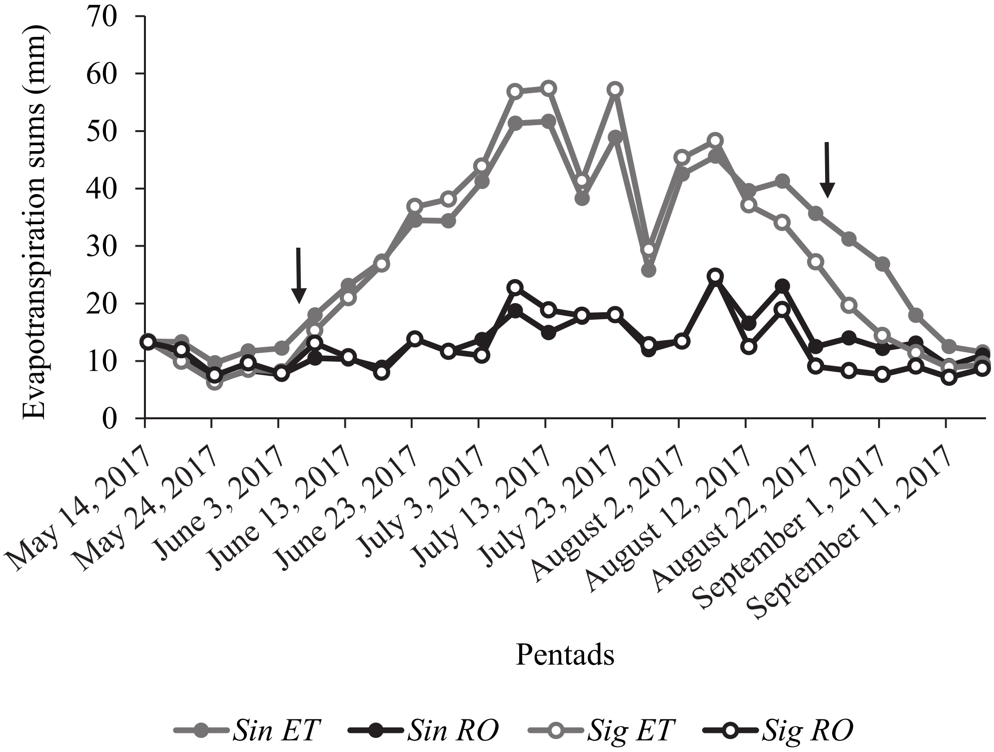

Fig. 1. Pentad sums of evapotranspiration in two soybean varieties (*Sinara* (*Sin*) and *Sigalia* (*Sig*)), using unlimited (*ET*) and water stressed (*RO*) canopies at Keszthely, during 2017. The arrows show the beginning and end of water deprivation.

The cumulative *EVTR* of 346.4 and 327.9 mm in *Sin RO* and *Sig RO*, respectively, stayed near the cumulative *P* of the growing period (2017: 347.4 mm) (*Fig. 2*). *EVTR* totals of both *ET* treatments (*Sin*: 759.3 mm; *Sig*: 725.8 mm) were about twice as high as the cumulative seasonal *P* at Keszthely, indicating that soil water coming from *P* probably would not be enough to satisfy soybean water needs. *Montoya et al.* (2017) reported similar *EVTR* totals ranging from about 400 to 800 mm for rainfed and 50–75% deficit irrigated soybeans (cultivar Don Mario) in Uruguay (31°22'S).

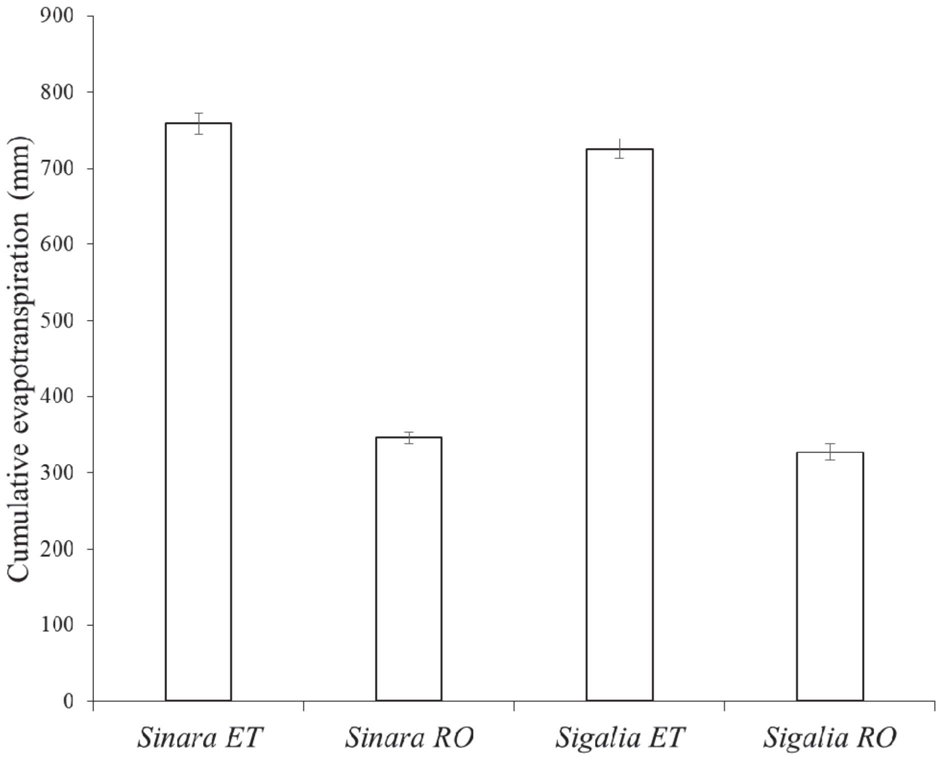

Fig. 2. Cumulative evapotranspiration of the two varieties *Sinara* (*Sin*) and *Sigalia* (*Sig*) using two levels of water supply; unlimited (*ET*) and water stressed (*RO*) canopies at Keszthely, during 2017.

3.2. Seasonal variation in LAI

Until photosynthesis shows an immediate response to environmental conditions, crop growth is the end result of photosynthesis, responds more slowly to environmental variation (*Lessmann et al.* 2001). This is why consecutive tracking of *LAI* is appropriate in crop growth monitoring.

LAI of soybean showed a period of increase that followed by a maximum value maintained for a shorter time period before decreasing. Weekly mean *LAI* increased from a minimum of 0.3 on June 6 to a maximum of 9.6 (both *ET*) on August 2 (*Fig. 3*). LAI_{max} of 6.5 in *Sin RO* was only slightly lower than that of LAI_{max}=7.0 measured by *Montoya et al.* (2017) in soybean (cultivar: Don Mario) at Salto, Uruguay (31°22' S). *Setiyono et al.* (2008) also detected somewhat lower LAI_{max} of 7.8 in irrigated soybean (variety P93M11) at Lincoln, NE (40°49' N). Natural senescence slightly declined the *LAI* to a value of 0.1 (*RO*). Seasonal mean *LAI* ranged from 5.3 (*Sin ET*) to 3.0 (*Sig RO*) over the growing season of 2017. Only, like a tendency, the seasonal mean *LAI* in *Sin* tended to be 1.9 (*ET*; $p < 0.349$) and 12.4% (*RO*; $p < 0.154$) higher than that of the average *LAI* of *Sig*. In turn, the seasonal mean *LAI* of both varieties were strongly impacted by water deprivation. Declines of 39.4 ($p < 0.001$) and 49.3% ($p < 0.001$) in water stressed *Sin* and *Sig* were observed, respectively, in comparison to *LAI* of crops with unlimited watering. *Kross et al.* (2015) explained lower soybean *LAI* under dryer conditions to have resulted from a greater investment in the root structure. Descending branch of weekly *LAI* curve was slightly steeper in the water stressed pots than that of in the *ET* treatments.

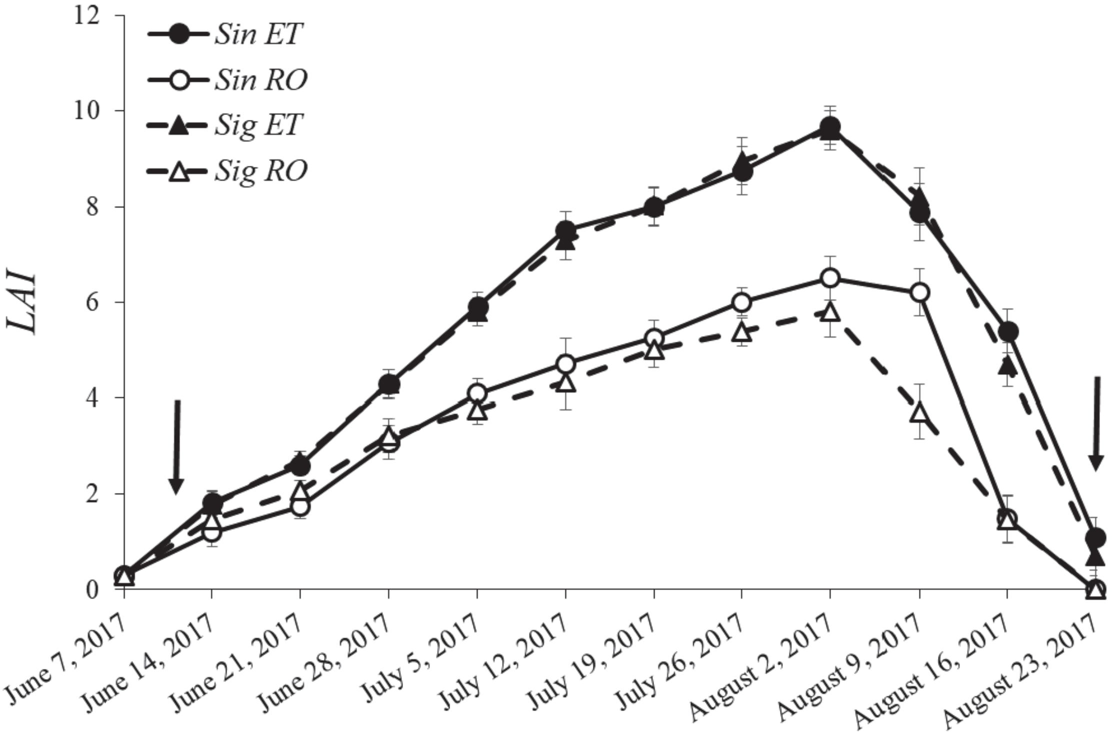

Fig. 3. Weekly variation in leaf area index (*LAI*) of varieties *Sinara* (*Sin*) and *Sigalia* (*Sig*) using two levels of water supply (*ET*: unlimited watering; *RO*: water stressed crops) in 2017. The arrows show the beginning and end of water deprivation.

3.3. Soybean's canopy structure

The *LAI_{max}* was selected for evaluation of canopy architecture, as during this growth period (R6), the final height of crops was reached and the *LAI* remained relatively invariable. *Yang et al.* (2009) concluded that *LAI_{max}* well reflects the impact of environmental conditions on the growth of crops.

The average number of leaf storeys were 15.0 ± 2.18 and 13.5 ± 1.75 in *ET* and *RO*, respectively. Due to the lower number of leaf levels, crop height in *ET* increased with 0.13 m (p <0.001) comparing to the height of *RO*. Unlimited water supply stimulated the plant growth through emerging more trifoliate layers contributing to increased *LAI*.

The multiplier x in Eq. (1). was determined with linear regression without intercept based on measured data of about 350 trifoliates:

$$L = 0.708\ ab. \tag{3}$$

Fig. 4 shows the calculated leaflet area values.

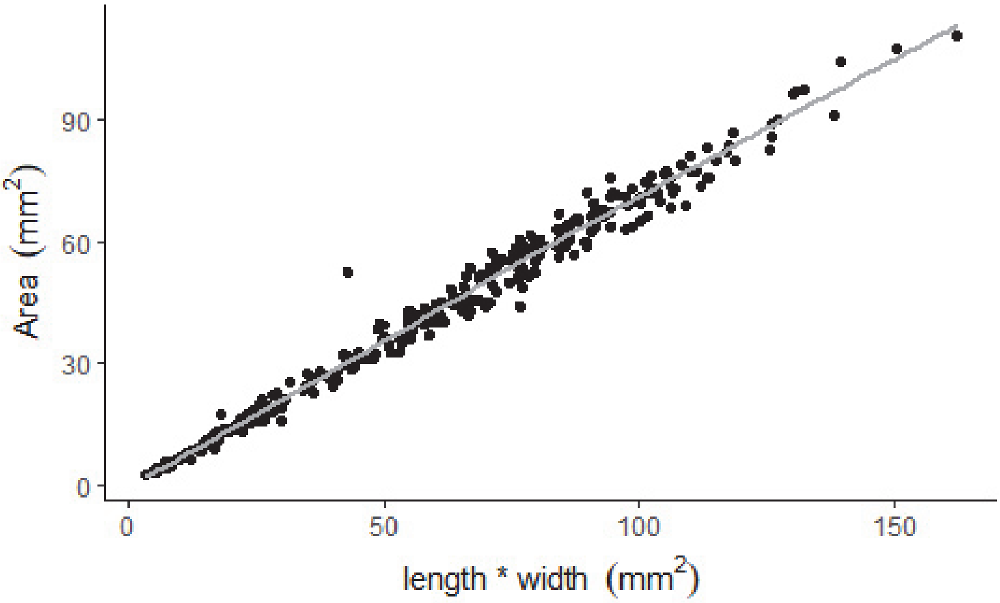

Fig. 4. Calculated leaflet area values based on Eq. (3).

The effect of the water supply was analyzed with a 3-way full factorial ANOVA. The 3-way interaction and two 2-way interactions were not significant, they were removed from the model. Finally, the main effect of the trifoliate level ($p = 3 \cdot 10^{-10}$), the water supply ($p = 0.0005$) and the water supply and variety interaction ($p = 0.034$) were found to be significant. The fact that interactions including trifoliate level were not significant shows that the effect of the water supply and the variety were much the same on each trifoliate level. To reveal the information hidden in the water supply – variety interaction, these two variables were combined into a single variable having four levels. The pairwise comparison was performed using the Tukey-HSD test. It pointed out that the trifoliate area was significantly lower in the case of *Sig* with water stress condition than in the other three groups (each $p < 0.05$). The difference among the other three groups was not significant (*Fig. 5*).

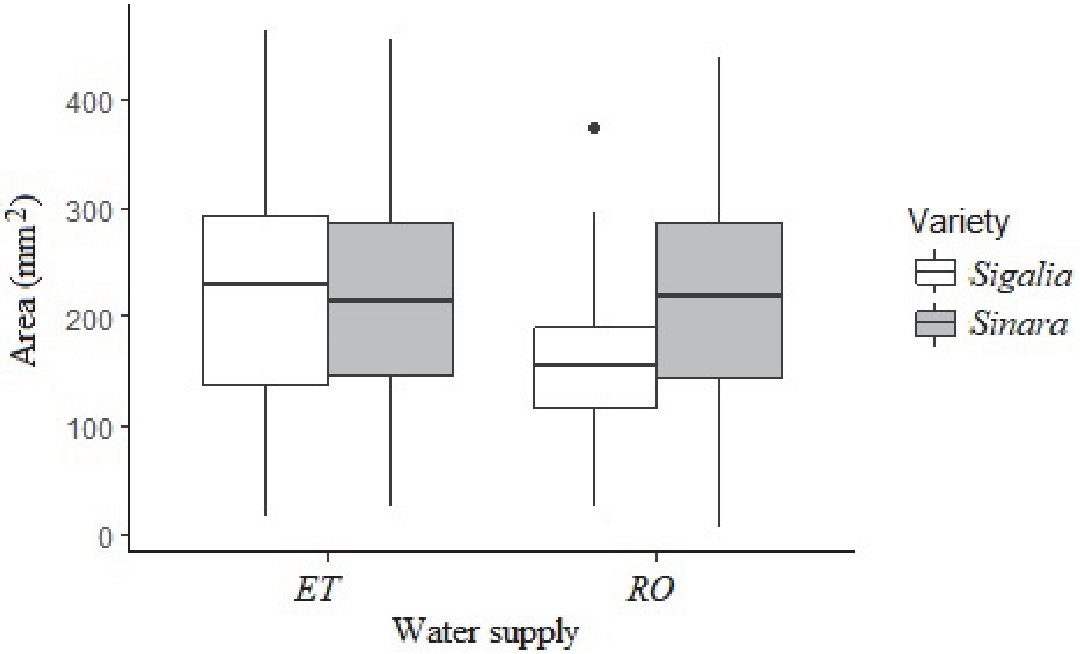

Fig. 5. Trifoliate area by water supply and variety of soybean. *ET*: unlimited watering, *RO*: water stress.

Analysis was carried out on the proportion of the middle leaflet within the whole trifoliate with 3-way ANOVA model. After removing the non-significant interactions and main effects, the final model contained only the intercept. Therefore, none of the factors had significant effect on the proportion of the middle leaflet. The mean of the proportion was compared to 1/3 using a one-sample *t*-test. The trifoliate levels above the 13th level were excluded from the analysis due to the low sample size. The results are shown in *Fig. 6*. The curve is bowl-shaped, the proportion of the middle leaflet is significantly higher than 1/3 on the lower four levels and from the 10th level with the exception of the 12th level. It shows that the area of the middle leaflet is approximately equal to the area of the outer ones around the center of plant height, but is greater at the bottom and the top of the plant height.

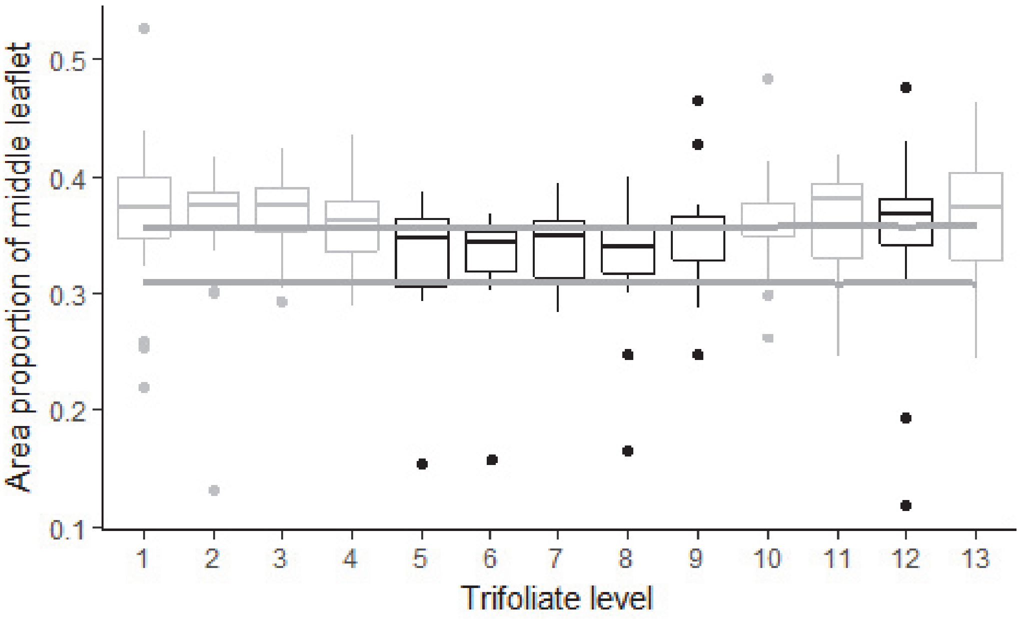

Fig. 6. Area of the middle leaflet compared to the whole area of the trifoliate. Thick grey line shows the 95% confidence interval. Boxplot is colored grey if the difference from the 1/3 is significant.

3.4. Impact of weather on the LAI

The influence of four meteorological variables (air temperature, T_a; vapor pressure, e_a; precipitation, P; and wind speed, u) on the weekly mean *LAI* of the two varieties using two levels of water supply was assessed by a correlation analysis (*Table 1*). Irrespective of the variety and water supply, the highest correlation coefficients (r) ranging from 0.59 (*Sig RO*) to 0.62 (both *ET* treatments) were computed between *LAI* and T_a. T_a alone explained at about 60–62% of the variability of *LAI*. Among four studied parameters (water stress, phenology, T_a, and light use), T_a significantly improved the vegetation production metrics, included *LAI* (*Nguy-Robertson et al.* 2015) in Nebraska, USA (41.165°N). Slightly lower positive correlations of 0.46 (*Sig RO*) – 0.56 (*Sin RO*) were observed between *LAI* and e_a. Weak negative correlations [(–0.07) – (–0.15)] between *LAI* and *P* were expected as crops were grown in evapotranspirometer under unlimited watering. Unexpectedly, negative correlation [(–0.21) – (–0.45)] between *LAI* and u was found referring to the favorable effect of calm weather conditions on soybean leaf growth. The correlation between *LAI* and u was only significant in *Sig RO*, see also *Table 1*. The probably reason might has been that the *LAI* values were the lowest in *Sig RO*, creating the most open canopy structure mostly exposed to the influence of wind. More closed soybean stands with higher *LAI* could hinder the impact of the wind.

Table 1. Correlation coefficients (r) for weekly measured leaf area index (LAI) and weekly weather variables (weekly means of air temperature, T_a; weekly means of water vapor pressure, e_a; weekly precipitation sums, P; weekly mean wind speed, u) included in the study. Number of observations was 12 for each correlation.

LAI	***Sin ET***	***Sin RO***	***Sig ET***	***Sig RO***
T_a **[°C]**	0.62^{**}	0.61^{**}	0.62^{**}	0.59^{**}
e_a **[kPa]**	0.51^{**}	0.56^{**}	0.52^{**}	0.46^{**}
P **[mm]**	–0.15	–0.07	–0.14	–0.08
u **[m/s]**	–0.21	–0.32	–0.24	$–0.45^{*}$

* Marginally significant correlation $|r|>0.1$, $p<0.01$

** Marginally significant correlation $|r|>0.1$, $p<0.001$

All of the meteorological variables mentioned above were included in the multiple stepwise regression analysis. In this statistical analysis the variables included stepwise in the resulted equations are dimensionless. As there was hardly enough difference in r between the two studied varieties (*ET*: $p < 0.809$; *RO*: $p < 0.115$), their data were treated together (*Table 2*) when identifying regression equation. On the basis of the Akaike information criterion (*AIC)* and the adjusted multiple correlation (R^2), T_a impacted the *LAI* the most, irrespective of water supply (in *ET*: $LAI = 0.996T_a - 16.796$, R^2=0.322 , *AIC*= 20.77; in *RO*: $LAI = 0.728T_a - 12.746$, R^2=0.309 , *AIC*= 13.25). It is a favorable response as T_a is the most easily accessible meteorological variable for all potential users including farmers. *Nielsen* (1990) called the attention for the importance of ambient T_a in governing soybean's physiological processes in USDA Central Great Plains Research Statin (40°9'N), as shown even for leaf growth in this study.

Table 2. Result of multiple stepwise regression analysis between meteorological variables and leaf area index, *LAI* in unlimited water supply, *ET* and using 50% water withdrawal, *RO*. Only one meteorological variable, the air temperature T_a remained in the regression equations. Equations included data of both varieties studied. *R* and *AIC* denoted coefficient of multiple correlation and Akaike Information Criterion, respectively.

	Adjusted R^2	***F*-value**	**Sig. of *F***	**Standard Error of coefficient**	**Regression equation**	***AIC***
ET	0.322	6.223	0.032	Const.=8.88 T_a=0.399	$LAI = 0.996T_a - 16.796$	20.77
RO	0.309	5.925	0.035	Const.=6.65 T_a=0.299	$LAI = 0.728T_a - 12.746$	13.25

3.5. Seed yield of the treatments

Based on a two-way ANOVA (*F*) test for seed yield, there were significant main effects of water supply (*F* (1, 16) = 87.396; *p* = 0.000) and variety (*F* (1, 16) = 8.082; *p* = 0.012) (The numbers in the bracket are the between-groups and the within-groups degrees of freedom separated by a comma. After the = are the *F* statistic and the significance level). The water x variety interaction on seed yield was not significant (*F* (1, 16) = 1.644; *p* = 0.218), indicating that the impact of the water supply was about the same on both varieties (*Fig. 7*).

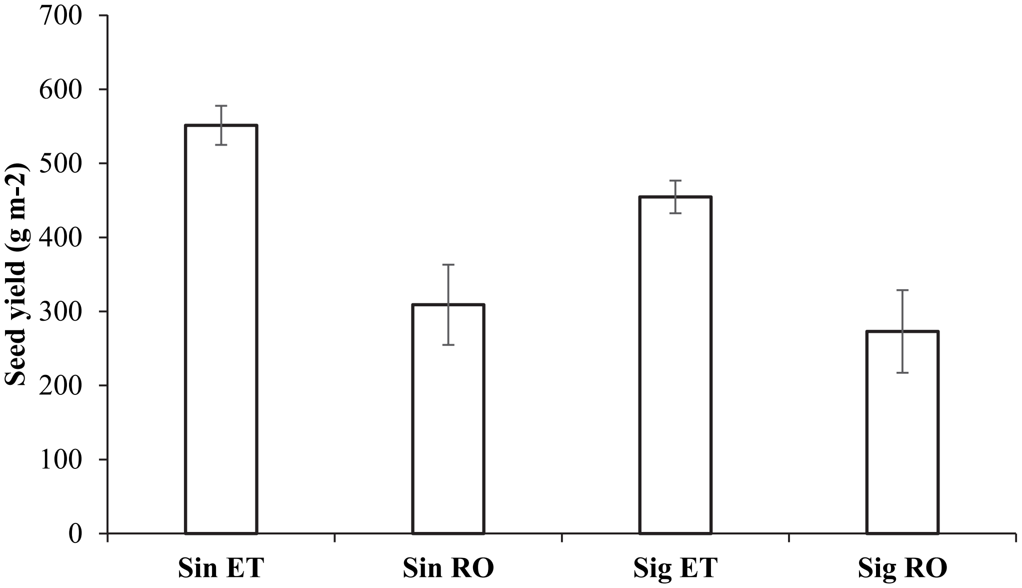

Fig. 7. Seed yield of soybean varieties *Sinara* (*Sin*) and *Sigalia* (*Sig*) in two watering levels (unlimited: *ET*; water stressed: *RO*).

4. Conclusion

Accounting different varieties and water supply levels, this study contributed to the better understanding of soybean canopy architecture. The new information – produced by the research – can widen the application related to the crop structure used in crop modeling.

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