MAGYAR AGRÁR- ÉS ÉLETTUDOMÁNYI EGYETEM

Columella

Volume 10, Number 1, 2023

Columella
Journal of Agricultural and Environmental Sciences

Impact of N Supply on Some Leaf Characteristics of Maize Crop

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Abstract: Nitrogen (N) is an essential nutrient widely used in maize crop production. Application of a high N rate is commonly practiced by growers as a "guarantee" of optimal growth and yield. However, excessive nitrogen consumption can cause wastage, negatively impact plants, and adversely affects the environment. This paper reports on the impact of N supply on leaf characteristics in maize. Maize was grown in an experimental plot of the Department of Agronomy, The Hungarian University of Agriculture and Life Sciences, Hungary, during the spring and summer of 2021 (May-October). Four observation plots consisting of 10^2 m area size were evaluated for various N levels (0, 50, 100, and 150 kg ha^{-1} N a.i) with marked plants sampling in four replications. Data collection on leaf traits viz. leaf number plant^{-1} (B), leaf number plant^{-1} (S), temperature 0 °C (leaf surface), SPAD, leaf length (cm), leaf width (cm), and leaf area (cm^2) were measured one week after application in weekly sequences of N until the eighth week. The results showed that nitrogen fertilizer application increased the leaf number plant^{-1} (B), temperature, SPAD, and leaf width while contrasting with leaf number plant^{-1} (S). However, there was no difference in leaf length for all treatments studied. Although an increase occurred up to the use of 100 N. Whereas, 150 N treatment showed low performance and exhibited a negative correlation for all traits except temperature and number of leaves (S). The results suggest that treatment of 100 N produced the best results in most traits studied. Furthermore, detailed research study is needed to confirm the findings, as many other environmental factors influence maize plant growth.

Keywords: SPAD, leaf traits, LA, nitrogen, maize

Received 7 September 2022, Accepted 20 February 2023

Introduction

Maize (*Zea mays* L.), generally known as corn, is one of the major grain crops cultivated in many parts of the world. It's a good source of carbohydrates, vitamins, minerals, and phytochemicals (Shah et al., 2016). Maize production has expanded globally, surpassing other grain crops, making it the most valued staple food. The United States, China, Brazil, Argentina, and Ukraine were the top 5 maize-producing countries in the world, which produce about 8.02 hundred million tonnes (69.82%) of 1.15 thousand million tonnes, and the amount of maize production in Hungary amounted to 8.23 million tonnes in 2019 (Food and Agriculture Organization of the United Nations, n.d.)).

Globally, the latest challenges are human population growth and global climate change. It may need to manage by enhancing the use of Agro-technological aspects in agriculture. Therefore, more efficient resources, particularly N resources, must be utilized to ensure minimal wastage in crop production. In earlier studies, Urban et al. (2021) and Loch (2015) reported that maize is highly sensitive to nutrient deficiencies, especially in the early stages of growth. As a result, optimizing nutrition during the early vegetative period can boost yields. Nitrogen (N) is not only a required element but is also a limiting factor for the production yield of summer maize (*Zea mays* L.) grains. However, irrational N levels inhibit increased production and result in environmental pollution (Liu et al., 2018).

Loch (2015) underlines that the gradual increase in fertilizer use leads to further increases in average maize yield, except in drought years, an initial average of 6 tonnes ha^{-1} has been restored or exceeds conversely. Maize production can be associated with various reasons where the main reason is that maize is more sensitive to water shortages. According to Pepó (2017), the applications of Agro-technological components can decrease the negative consequences of climate change while also increasing crop production. Precision agriculture technologies can help to offset the effects of weather. This technology is increasingly widely used in agriculture industries of various crops production, including maize. Sensor sensing technology is one of the most vital tools in this methodology (Schepers et al., 1992; Simkó & Veres, 2019).

For crops, a chlorophyll meter Soil Plant Analyzer Development (SPAD-502) provides phenotyping readings and shows the chlorophyll a and chlorophyll b in the thylakoid membrane in leaf mesophyll chloroplast (Kandel, 2020). According to Huang et al. (2006); May (2000), chlorophyll is a green pigment present in the mesosomes of cyanobacteria and chloroplasts of algae and plants. It is a crucial component in the plant for the photosynthesis process, as it absorbs sunlight and uses the energy to synthesis carbohydrates from carbon dioxide and water. In addition, Ghimire et al. (2015) discovered that chlorophyll has direct roles in photosynthesis and hence closely relates to the photosynthesis capacity, development, and yield of crops.

Previous studies indicate that chlorophyll also influences the plant's leaf color, and chlorophyll concentration has a high correlation with the value measured with the Soil Plant Analysis Development (SPAD) meter. The SPAD meter can also estimate the concentration of Chlorophyll in leaves in a non-destructive manner (Amagai et al., 2022; Xiong et al., 2015).

Recently, chlorophyll meters widely used in agricultural systems to guide nitrogen (N) management by monitoring leaf N status. However, the effect of environmental factors and leaf characteristics on leaf N estimates is still unknown (Xiong et al., 2015). There is a non-linear relationship between leaf chlorophyll concentration and N rate, and chlorophyll SPAD value is applied to estimate the effect of N rate on leaf chlorophyll concentration. Kandel (2020); Richardson et al. (2002) reported that this tool is also used in estimating leaf N concentrations using SPAD data collection. The chlorophyll SPAD value quickly and readily assesses the N status of summer maize (Liu et al., 2018). Nitrogen (N) deficiency will have a direct impact on crop productivity. As a result, the elongation rate of maize stems, leaf area, and leaf or canopy net photosynthesis (Pn) are all reduced. Furthermore, it will also cause the hyperspectral reflection of the leaves to become very sensitive to N status, causing the plants to be shorter and have less dry matter. Therefore, early diagnosis of plant nitrogen deficit is essential to optimize the application of N fertilizers and crop yields (Zhao et al., 2003).

Another approach to improving N use efficiency (NUE) involves plant-based strategies that rely on monitoring the N status of crops by measuring chlorophyll content per leaf area (Xiong et al., 2015). The leaf area index (LAI) is a metric that determines the total leaf area for each unit of horizontal surface area. Leaf area index (LAI) is a metric that determines the total leaf area per unit of parallel surface area. LAI is directly involved in radiation capture, photosynthesis, energy exchange with the atmosphere, as well as influencing growth performance and yield. As a result, most agronomic investigations involving plant growth and yield analysis involve leaf area (LA) measurements (Berdjour et al., 2020).

When there is an influence of N supply, the physiological characteristics of the plant will be affected, and this can be determined at the field level. However, other environmental factors may have an impact on the outcome (Zhao et al., 2003). Therefore, to study the effects of various nitrogen supplies in maize associated with SPAD readings and leaf characteristics, we conducted maize experiments during the spring-summer 2021 growing season. The main objectives of this study were i) To record the photosynthetic activity of maize plants in various N applications, and ii) To determine the relationship between SPAD values, leaf area, and its characteristics in various N applications.

Materials and Methods

Experimental Site

The research was carried out at an experimental plot of the Department of Agronomy, The Hungarian University of Agriculture and Life Sciences, Hungary, during the spring and summer of 2021 (May-October). This plot is located at a hilly section of the country near-average climatic zone, 242 m above sea level (47°46′N, 19°21′E) on sandy loam, brown forest soil (Chromic Luvisol). In 2021, the average annual precipitation in Gödöllő was 531.0 mm (20.91 inches), while in Hungary, the estimated precipitation is between 400 to 500 millimeters (15.8 - 19.7 inches) per year. Generally, the west is slightly wetter than the east (Weather and climate, 2021). Maize hybrid seed variety namely MV 277 were sown using Wintersteiger Plotman maize planter machine with 75 thousand plant ha^{-1} planting density.

Treatment

Four observation plots consisting of a 10 m^2 area size were evaluated for various N levels (0, 50, 100, and 150 kg ha^{-1} N a.i) with marked plants sampling in four replications. Data collection on leaf traits viz. leaf number $plant^{-1}$ (B), leaf number $plant^{-1}$ (S), temperature °C (leaf surface), SPAD, leaf length (cm), leaf width (cm), and leaf area (cm^2) were measured one week after application in weekly sequences of N until the eighth week.

Measurement

Data collection on leaf parameters viz. leaf number $plant^{-1}$ (big (B)), leaf number $plant^{-1}$ (small (S)), temperature of leaf surface(0°C), SPAD, leaf length (cm), leaf width (cm), and leaf area (cm^2) were determined weekly. It started from 8 weeks to 16 weeks after planting (until all plants fully dried). The number of leaves of the selected plant was calculated by counting all the green leaves, while SPAD values and leaves temperature were measured on the same spot at the leaf surface. Besides, the Leaf chlorophyll index was measured by using a SPAD meter (SPAD 502 plus, Minolta, Japan). Meanwhile, the estimated leaf area (LA) has followed the procedure introduced by (Elings, 2000) by multiplying the leaf length by its widest width by alpha, where alpha is 0.743 ($L \times W \times \alpha$).

Statistical analysis

One way ANOVA between treatments was performed to compare the leaf number $plant^{-1}$ (B), leaf number $plant^{-1}$ (S), temperature (°C), SPAD, leaf length (cm), leaf width (cm), and leaf area (cm^2) at $P < 0.05$ level of significance. However, Post Hoc Multiple Comparisons using the Least Significant Difference (LSD) were used to compare the mean values of the various levels of nutrient treatments at $P < 0.05$. Analysis was conducted by using the IBM SPSS version 23.

Results and Discussion

The mean, maximum and minimum values, and standard deviations for leaf number plant^{-1} (B), leaf number plant^{-1} (S), temperature (°C), SPAD, leaf length (L), leaf width (W), and leaf area (LA) are shown in Table 1. The mean for leaf number plant^{-1} (B) was 9.87 with Std. Deviation (1.86), where the mean for leaf number plant^{-1} (S) was 1.69 with Std. Deviation (0.66). The leaf number plant^{-1} (B) showed the highest mean compared to the leaf number plant^{-1} (S), while the leaf number plant^{-1} (S) was the more consistent score. The mean for leaf temperature was 240 °C with Std. Deviation (3.85) and minimum and maximum temperature values were 15.30 °C and 34.80 °C, respectively. The SPAD value indicates that the mean value was 45.20 with Std. Deviation, minimum and maximum values were 9.94, 2.30, and 68.20, respectively. The results demonstrated that the mean values for leaf length (L) and leaf width (W) were correlated with leaf area (LA). Potdar and Pawar (1991) found a good correlation between leaf area and various combinations of leaf length (L) and leaf width (W) in the banana (*Musa acuminata* Colla). Also, Peksen (2007) has found a strong relationship between leaf area and a combination of lamina length (L) and lamina width (W) in *Vicia faba* L. by measuring leaf length and leaf width and calculating different combinations.

The ANOVA table (Table 2) illustrates there were highly significant differences in all characteristics measured viz. leaf number plant^{-1} (B), leaf number plant^{-1} (S), temperature (°C), SPAD value, leaf length (L), leaf width (W), and leaf area (LA) between the groups for different nutrient treatment (0, 50,100 and 150 kg ha^{-1} N). The different levels of N treatments had a significant impact on leaf number (big and small), F (3, 1602 and 3, 1504) = [17.892 and 54.196]; $p = 0.000$). Besides, temperature (°C) (F (3,1463) = [4.464], $p = 0.0040$), SPAD (F (3, 1463) = [35.157], p = 0.000), leaf length (L) (F (3,1461) = [5.063], $p = 0.002$), and width (W) (F (3,1462) = [6.283], $p = 0.000$), and leaf area (LA) (F (3,1461) = [8.221], $p = 0.000$).

Leaf number plant^{-1} (B)

Post hoc analysis (Figure 1(a)) using LSD ($P < 0.05$) indicated that there were no significant differences between the 0 N, 50 N, 100 N treatments in leaf number plant^{-1} (B) (M = 9.97, 10.13, 10.12 respectively). However, the 150 N treatment (M = 9.33) differed significantly among the other treatments. The results also showed that the N fertilizer content positively correlated with the plant leaf number $^{-1}$ of 0 N, 50 N, and 100 N but drastically reduced the leaf number plant^{-1} of 150 N treatment. However, many previous studies have proven that giving an extra N fertilizer can inhibit tree growth and leaf production in maize. In contrast to Vos et al. (2005), which stated that leaf appearance rate, leaf development period, and leaf number are not affected by nitrogen supply in maize crops. The average leaf number plant^{-1} (B) by week showed that the highest was in the 3rd weeks with 11 leaves (0 N and 100 N), followed by 10.75 and 10.5 (50 N and 150 N, respectively). The leaves number (B) started to decrease in week 6, and it happened after the plant was in the final stages of the reproductive phase (Figure 2 (a)). This situation occurs because the nutrients were directed toward grain development rather than leaf growth (Kandel, 2020).

Leaf number plant^{-1} (S)

The results of leaf number plant^{-1} (S) in various N treatments are shown in Figure 1(b).

 DOI: https://doi.org/10.18380/SZIE.COLUM.2023.10.1.15

Table 1: Mean, maximum, minimum and Std Deviation of leaf number plant^{-1} (B), leaf number plant^{-1} (S), temperature (°C), SPAD, leaf length (cm), leaf width (cm) and leaf area (cm^2) for various levels of nutrient treatments (0, 50, 100, and 150 kg ha^{-1} N).

	Leaf number plant^{-1} (B)	Leaf number plant^{-1} (A)	Temperature (°C)	SPAD	Leaf lenght (cm)	Leaf width (cm)	Leaf area (cm^2)
Mean	9.87	1.69	24.00	45.20	67.91	7.43	390.39
Std. Deviation	1.86	0.66	3.85	9.94	18.08	2.55	146.83
Minimum	2.00	1.00	15.30	2.30	5.00	0.40	1.50
Maximum	12.00	3.00	34.8	68.20	97.00	84.00	655.80

Table 2: Analysis of variance for leaf number plant^{-1} (B), leaf plant^{-1} (S), temperature (°C), SPAD, leaf length (L), leaf width (W) and leaf area (LA) for various levels of nutrient treatments (0, 50, 100, and 150 kg ha^{-1} N).

Parameter	Source of variation	Sum of Squares	df	Mean Square	*F*	Sig.
Leaf number plant^{-1} (B)	Between Groups	180.946	3	60.315	17.892	.000
	Within Groups	5400.394	1602	3.371		
	Total	5581.341	1605			
Leaf number plant^{-1} (B)	Between Groups	64.256	3	21.419	54.196	.000
	Within Groups	594.383	1504	.395		
	Total	658.639	1507			
Temperature (°C)	Between Groups	196.789	3	65.596	4.464	.004
	Within Groups	21497.851	1463	14.694		
	Total	21694.640	1466			
SPAD	Between Groups	9747.611	3	3249.204	35.157	.000
	Within Groups	135208.461	1463	92.419		
	Total	144956.072	1466			
Leaf length (cm)	Between Groups	4921.479	3	1640.493	5.063	.002
	Within Groups	473423.114	1461	324.040		
	Total	478344.593	1464			
Leaf width (cm)	Between Groups	121.468	3	40.489	6.283	.000
	Within Groups	9421.174	1462	6.444		
	Total	9542.641	1465			
LA (cm2)	Between Groups	523930.814	3	174643.605	8.221	.000
	Within Groups	31040000.000	1461	21244.002		
	Total	31560000.000	1464			

df: Degrees of freedom; Sig.: Significance; Significance level = $P < 0.05$.

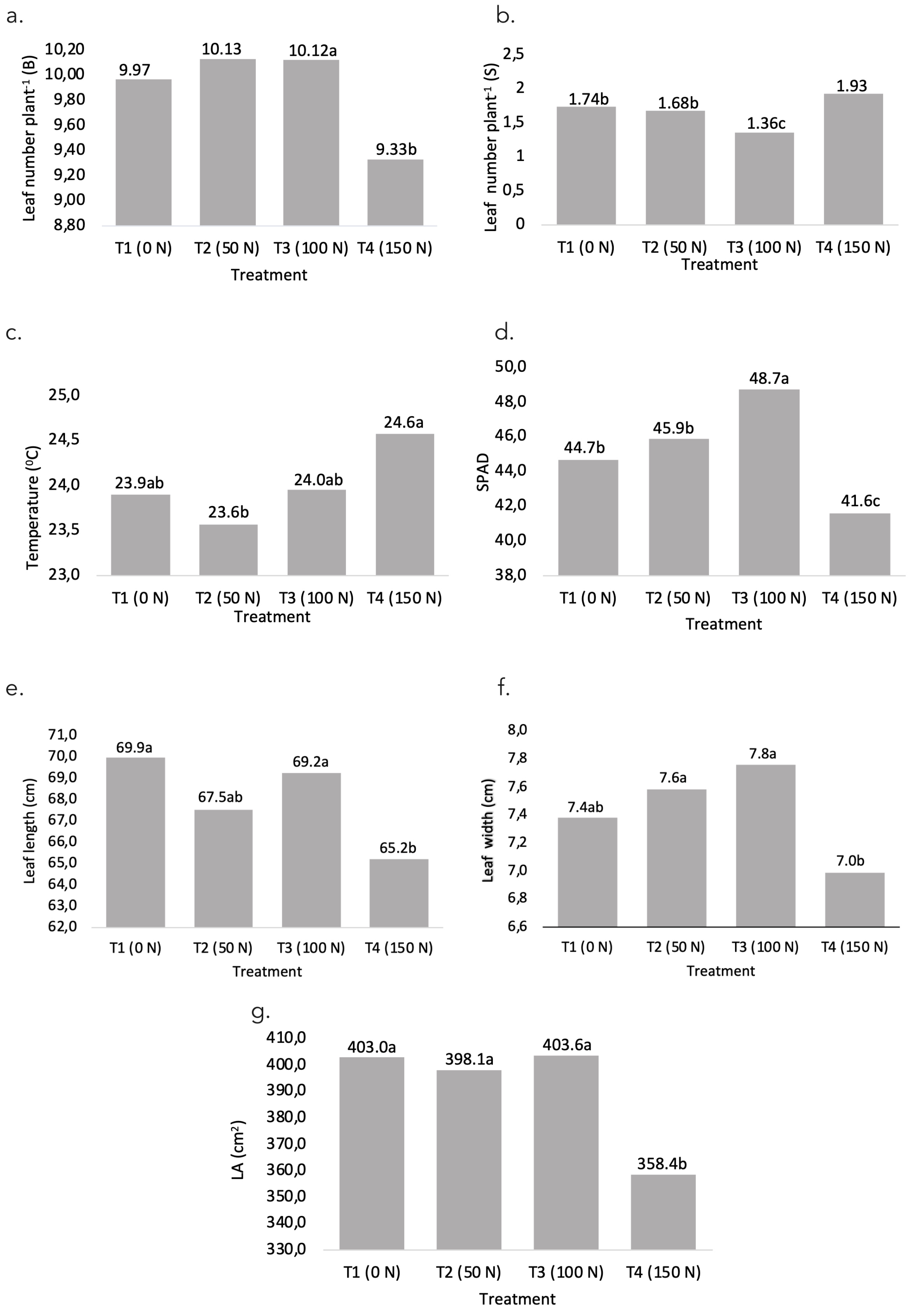

Figure 1: Mean values of (a) leaf number plant^{-1} (B), (b) leaf plant^{-1} (S), (c) temperature (°C), (d) SPAD, (e) leaf length (L), (f) leaf width (W) and (g) leaf area (LA) for various levels of nutrient treatments (0, 50, 100, and 150 kg ha^{-1} N)

 DOI: https://doi.org/10.18380/SZIE.COLUM.2023.10.1.15

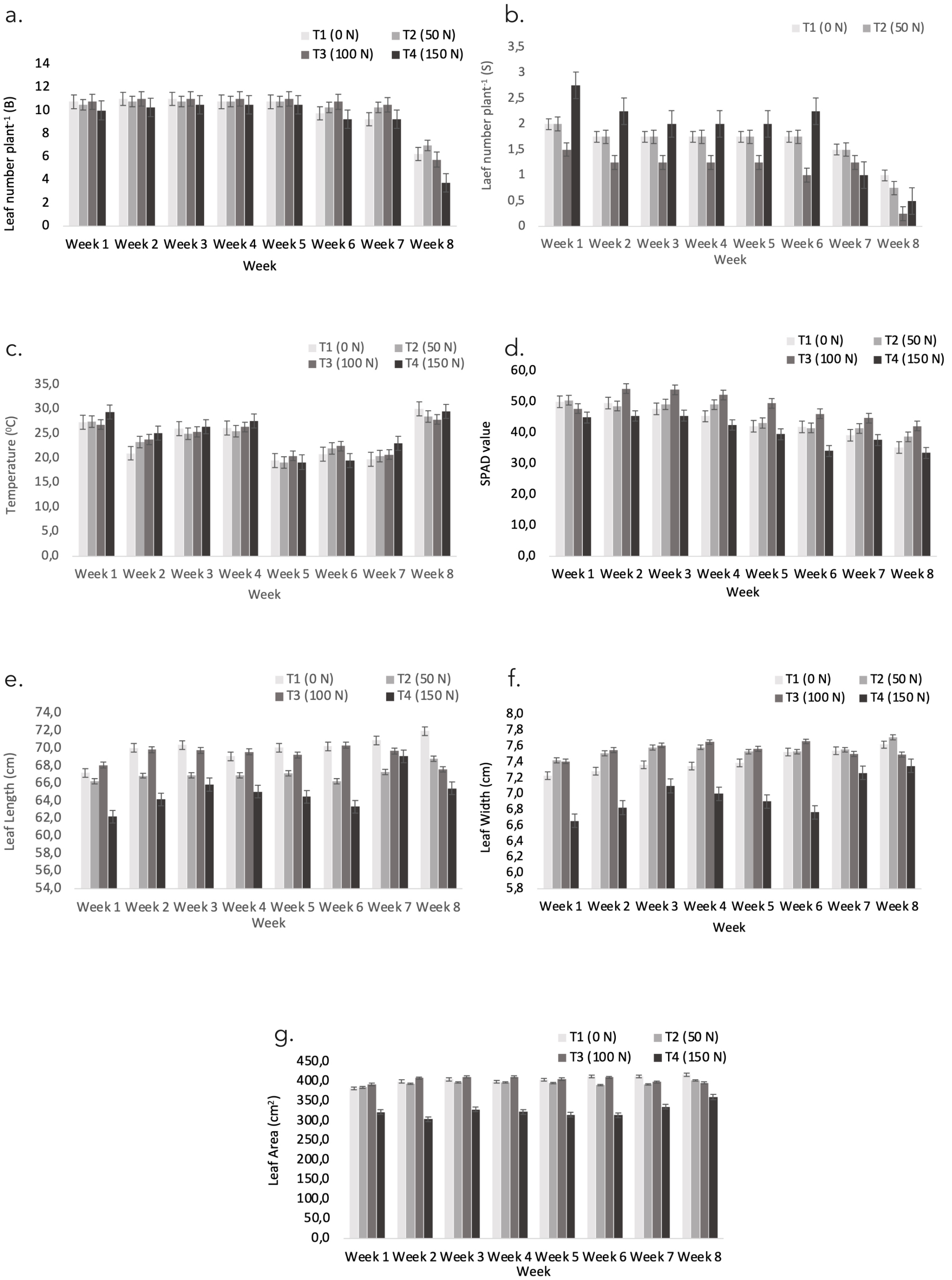

Figure 2: Average of (a) leaf number plant-1, (B), (b) leaf number plant^{-1} (S), (c) temperature (°C), (d) SPAD, (e) leaf length (L), (f) leaf width (W) and (g) leaf area (LA) for various levels of nutrient treatments (0, 50, 100, and 150 kg ha^{-1} N) by week.

The highest leaf number plant^{-1} (S) was produced by treatment of 150 N (1.93) and was significantly difference ($P < 0.05$) with 0 N, 50 N and 100 N (1.74, 1.68 and 1.36 respectively). Treatment of 150 N also consistently produced the highest average number plant^{-1} for every week up to the 6th week (Figure 2 (b)). This study implies that high nitrogen levels will increase the production number of small leaves. Similar findings were observed by Gungula et al. (2005)), who discovered that higher nitrogen rates produced more leaves and reduced leaf aging.

Temperature

Analysis showed no significant different between means ($P < 0.05$) of treatment 0 N, 50 N 100 N and 150 (23.9 °C, 23.6 °C, 24.0 °C, 24.6 °C respectively) (Figure 1(c)). However, the temperature of leaf in maize was highest in 8th week which is up to 30 °C for treatment 1 (0 N) and the lowest in 5th week which is down to 19.1 °C. In this experiment, treatment of 150 N had a relatively high temperature compared to others with 29.4 °C, 25.1 °C, 26.4 °C, 27.6 °C, 19.2 °C, 19.5 °C, 23.0 °C, 29.6 °C for 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, and 8th week respectively (Figure 2 (c)). Based on the performed evaluation and considering the results, this finding showed that the temperature of the leaves is related to the N application and ambient temperature. The temperature of the plant leaf had a direct influence on the biochemical reactions required for plant physiology. The temperature of the leaves rises as radiant energy is absorbed and drops as sensible and latent heat energy are transferred from the leaves to the air. As a result, environmental factors associated with this energy balance (light intensity, temperature, humidity, airflow velocity, and so on) influence leaf temperature, photosynthesis, and growth (Kitaya, 2019).

SPAD readings

The results in Figure 1(d) revealed that SPAD readings showed high significance in treatment 100 N and 150 N ($P < 0.05$). However, no difference in the treatment of 0 N and 50 N. The SPAD values responded to the application of N were quickly in 0 N, 50 N, and 100 N with 50.0, 50.5, and 47.8 respectively, and the lowest was 150 N (44.9). However, in the 2nd week, the SPAD values of 100 N were increased and produced the highest value and maintained until the 8th week (54.2, 53.9, 52.2, 49.5, 46.1, 44.8, and 42.1), while treatment of 150 N produced the lowest SPAD values viz. 44.9, 45.4, 45.4, 42.5, 39.5, 34.1, 37.6, and 33.5 in 1st week to 8th week respectively (Figure 2(d)). Similar findings revealed that the amount of chlorophyll content that can be extracted decreases in line with the increase in nitrogen levels (Simkó & Veres, 2019). This finding contrasted with Liu et al. (2018), where the value of SPAD chlorophyll increased significantly with the rate of N. However, the recommended treatment was 185 kg N ha^{-1}, which is an appropriate utilization rate for optimal grain yield, photosynthesis, and ultrastructure of chloroplasts. Schepers et al. (1992) also revealed similar findings in which the SPAD value obtained from the SPAD-502 device was directly correlated to the nitrogen status of the plant. Additionally, the SPAD meter is a widely used handheld device to measure leaf chlorophyll in a quick, accurate, and non -destructive manner. It is also a gadget to detect N deficiency in maize and guide N management in Agricultural systems. However, there is a major drawback that leaf greenery can vary between hybrids and is due to other plants and environmental factors (Piekielek et al., 1997; Xiong et al., 2015), including plant growth stage, type of hybrids, the timing of N fertilizer application, and N source (Schepers et al., 1992).

 DOI: https://doi.org/10.18380/SZIE.COLUM.2023.10.1.15

Leaf length

Leaf length of maize was not significantly different for all treatments measured ($P < 0.05$). However, the highest leaf length was in the treatment of 0 N, followed by 100 N, 50 N, and the lowest was 150 N (Figure 1(e)). The optimum leaf length value showed in the 7th week, and the leaf length value decreased sharply in the 8th week (Figure 2(e)). The results obtained show that the N rate does not affect the leaf length and, these results were correlated to the width and area of the leaves (Peksen, 2007).

Leaf width

The leaf width was not significantly different between N treatments ($P < 0.05$). The highest leaf width was in treatment 100 N, followed by 50 N, 0 N, and the lowest was 150 N (7.8 cm, 7.6 cm, 7.4 cm, and 7.0 cm, respectively). The highest leaf width showed in the 8th week and the lowest in the 1st week. In previous research stated that leaf width has a good correlation between leaf length (L) and leaf width (W), but the LA constant related to L and W varied in different cultivars (Potdar & Pawar, 1991).

Leaf Area (LA)

Post Hoc LSD results ($P < 0.5$) showed a significant difference between the treatment of 0 N, 50 N, and 100 N with 150 N. However, no significant difference between 0 N, 50 N, and 100 N treatments. Treatment of 100 N showed the highest leaf area starting in the 1st week until the 6th week. Besides, treatment of 150 N remained produced the lowest leaf area up to the 8th week (Fig. 2 (g)). The results showed that high N supplementation (150 N) resulted in less leaf area than the other treatments. However, previous related studies have found a positive relationship between N and leaf area. These findings supported by Berdjour et al. (2020); Valentinuz and Tollenaar (2006), who stated that higher N utilization rates influenced leaf area values produce positive effects on cell division and elongation, resulting in increased leaf length and rapid leaf development (Chiesa et al., 2000)). Furthermore, according to Chaudhry and Jamil (1998), higher nitrogen doses promote plant growth rather than yield. Nevertheless, the availability of N has a substantial impact on the development of leaf area only during vegetative growth (Muchow, 1988; Vos et al., 2005).

Conclusion

According to the findings of this study, nitrogen fertilizer application increased the leaf number plant^{-1} (B) up to 100 N and declined significantly at the 150 N. Contrasting results were obtained for the leaf number plant^{-1} (S), where the increase occurred at the treatment of 150 N. Meanwhile, the temperature at the leaf surface was positively associated with the increased in the application of the N rate for all treatments. In the SPAD values (leaf photosynthesis rate), the readings increased directly with the increase of N fertilizer application which decreased with treatment of 150 N, which may be related to the number of leaves in this study. Furthermore, the study's findings showed that leaf length (L) has no direct relationship with leaf width (W). Nonetheless, two of these parameters are directly related to leaf area (LA). The conclusion of this study indicates that N content affects the growth performance of maize crops, and plants from the treatment of 100 N produced the best results in the most parameters measured. Nevertheless, excessive nitrogen application harms plant growth. The environment, soil conditions, precipitation, temperature, pH, and plant variety can influence the plant's ability to absorb nitrogen from the soil.

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