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Influence of photosynthetic apparatus on the productivity of high-oleic sunflower depending on climatic conditions in the left-bank forest-steppe of Ukraine

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Abstract

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The influence of chlorophyll content and leaf surface area on crop productivity is acknowledged but information regarding these impacts on sunflower productivity is scanty. Also, the correlation between sunflower leaf surface area and chlorophyll content is largely unknown. Moreover, it is unclear the extent to which the leaf surface area and chlorophyll content influence sunflower productivity. Environmental conditions can also limit sunflower development and productivity. Hence, a two year (2016-2017) field experiment was conducted with the high-oleic sunflower hybrid PR64H32 under the environmental condition of the Left-bank forest-steppe of Ukraine. Analysis of weather conditions, particularly Hydrothermal coefficient (HTC) established that the vegetative period of 2016 had a normal moisture (HTC =1.00), while the year 2017 was dry (HTC = 0.45). Phenological observations revealed that generally, the vegetation period in 2016 was 120–122 days whereas that of 2017 was 115-117 days. In 2016, there was a significantly ($P < 0.05$) higher plant height, stem diameter, number of leaves, number of seeds per head and seed weight per head than in 2017. In contrast, both absolute chlorophyll concentration “a” and “b” and relative chlorophyll content (SPAD-502 plus readings) were higher in 2017 but there was no significant difference compared to 2016. Insufficient rainfall in 2017 caused a shortage in yield (seed weight per head) by 23.4%. Determination of seed quality with Spinlock Magnetic Resonance Solutions resulted in an oil content of 49.5% in 2016 but a slightly higher value (50.4%) in 2017. On the contrary, oleic acid content reduced from 81.6% in 2016 to 76.4% in 2017. Regression analysis uncovered an inverse proportional relationship between sunflower leaf surface area and the chlorophyll content (correlation coefficient, $r = 0.67$). Further analysis provided compelling evidence that, the leaf surface area impacted significantly on sunflower productivity (seed weight per head) than the chlorophyll content – a major interest in sunflower production.

Keywords: photosynthetic apparatus; forest-steppe; high-oleic; sunflower; leaf surface

Introduction

Sunflower (*Helianthus annuus* L.) is among the four most important oilseed plants globally (along with palm, soy, and rape seed) and branded as one of the two most essential oil crops in Europe, together with rapeseed (Jocić et al., 2015). Ukraine has maintained its position as leading producer of sunflower seeds in the world for the past decade. It is recently reported in 2018 that, Ukraine currently (2017−2018) ranks first in sunflower production worldwide with a 28.2% share of the total world sunflower output of 46.11 million metric tons (United States Department of Agriculture – USDA, 2018).

High oleic sunflower oil has the highest oleic acid content (over 90%) compared to all vegetable oils present in the global market (Jocić, et al., 2015), and it has greater oil resistance to auto-oxidation, which prevents the build-up of toxic products during oil processing, storage, and direct uses (Kaya et al., 2015). Concerning diet, higher oleic acid (70%) and lower linoleic acids (20%) are desired. Hence, breeding for oil quality in sunflower has chiefly focused on modifying the relative amount of fatty acids by raising oleic acid to have stable and healthy oil and enhancing stearic acid for a stable and healthy fat (Zambelli et al., 2015).

It is recently indicated that, the major goal in sunflower breeding is to create hybrids with high genetic potential for seed yield beyond 5 t/ha and oil content in seed greater than 50% that provide high oil yield per hectare of over 2.5 t/ha (Jocić et al., 2015). Also, Škoric (2012) revealed that just 10–20% of the yield potential of sunflower plants originates from genetic capacity and that, phenotypic difference is largely determined by genetic factors such as leaves and roots and environmental conditions including soil fertility and climate. Additionally, sunflower seed yield is reportedly a complex trait with polygenetic base that is greatly influenced by the environment (Skoric et al., 2007).

An imperative component in plant production is the development of the assimilation surface, particularly the leaf surface area and the chlorophyll content in them. The leaf surface area could determine the photosynthetic capacity and consequently the productivity of plants since sunlight for photosynthesis is absorbed by the leaf surface. Indeed, leaf area growth determines light interception and is a key parameter in determining plant productivity (Gifford et al., 1984; Koester et al., 2014). A larger leaf surface area permits more plant pigments to absorb light energy, facilitating a more productive photosynthesis.

Chlorophylls are the most vital photosynthetic pigments in plants (Silla et al., 2010). Leaf chlorophyll concentration is also a significant parameter that is often measured as an indicator of chloroplast development, photosynthetic capacity, leaf nitrogen content, or general plant health (Ling et al., 2011; Neto et al., 2017). In the laboratory, it is generally determined photometrically after extraction of the pigments using an organic solvent, such as acetone or dimethyl formamide (Arnon., 1949; Porra et al., 1989). Though this technique is reliable, it is time-consuming, destructive (the leaf material must be cut out from the plant, and is lost), labor-demanding, costly, and requires the use of toxic or flammable chemicals (Ling et al., 2011; Neto et al., 2017). The amount of chlorophyll in leaves is usually expressed in terms of either concentration (μg Chl/g tissue) or content (μg Chl/cm^2 tissue) and can differ significantly in value among different plant kingdoms and growth stages (Taiz et al., 2007).

In general, non-destructive methods to estimate chlorophyll content of vegetation are of great significance to agricultural management operations, especially in the area of precision farming (Gitelson et al., 2003). The scientific interest is verified by Kaufman et al. (2010), indicating that chlorophyll content is included in the parameters most frequently investigated in agricultural hyperspectral studies. Those investigations strongly depended on rapid, non-destructive and accurate in situ reference measurements. Two commercially accessible meters are broadly used (Konica Minolta, model SPAD-502 (Konica Minolta Sensing, Inc., Sakai, Osaka, Japan) and Opti-Sciences, model CCM-200 (Opti-Sciences, Inc., Hudson, NH, USA). An upgraded version of SPAD-502 meter (SPAD-502 plus) is also available (Spectrum Technologies, 2011). If optical techniques for measuring leaf chlorophyll content are applied, index values (e.g. SPAD-Value) are normally employed to specify the relative leaf chlorophyll content, but not absolute chlorophyll content or concentration (Richardson et al., 2002).

The influence of chlorophyll content and leaf surface area on crop productivity is acknowledged but information regarding these impacts on sunflower productivity is scanty. Also, the correlation between sunflower leaf surface area and chlorophyll content is largely unknown. Moreover, it is unclear the extent to which the leaf surface area and chlorophyll content influence sunflower productivity.

The present study therefore investigates the influence of chlorophyll content and leaf surface area on productivity of sunflower under the climatic condition of the Left-bank Forest-steppe of Ukraine. We also determined the relationship between leaf surface area and relative chlorophyll content (SPAD readings). We further verified that sunflower leaf surface area had a greater impact in forming seed weight (productivity) than chlorophyll content.

Materials and Methods

Experimental site and design

A two-year (2016 and 2017) field research was performed in Poltava region in the Forest-steppe of Ukraine. The experimental site was located 10 km SW from Poltava (Latitude: 49.6; Longitude: 34.9; altitude 113 m) on black soil, characteristic for coarse-medium loam. Seeds of high oleic sunflower hybrid (PR64H32) were sown on May, 12 and May, 20 and correspondingly harvested on September, 14 and September, 27 in 2016 and 2017. The experiment was conducted in Randomized Complete Block Design (RCBD) with three replications. The seeds were sown at a plant density of 60 000 plants/ha with 4 rows in each plot. An inter row gap of 70 cm was kept. Background fertilizer was applied to the soil during sowing at the rate of $N_{30}P_{30}K_{30}$.. All other cultural practices including weed, pest and disease control were done.

Data collection

Phenological observations on the growth and development of sunflower crops were conducted in accordance with the methodology of the state variety for testing of agricultural crops (Volkhodava, 2001).

Data on the following parameters were collected and/or determined from 30 typical tagged plants at reproductive phase before flowering (R-1 to R-4) (Schneiter & Miller, 1981): number of leaves; width of 7th leaf and length of 7th leaf before leaf surface area was determined using the method described by Osipova & Litun (1988). An estimate of the chlorophyll content was as well determined with SPAD-502 plus chlorophyll meter (Spectrum Technologies, 2011). Briefly, measurements were taken in triplicate from the seventh (7th) leaf of tagged plants and then averaged. The same samples were immediately moved to the laboratory on ice where the chlorophyll "a" and "b" concentrations were determined by the classical technique using the ULAB-102 Spectrophotometer. The pigment contents were calculated as mg/g fresh weight.

Harvesting was done through manual approach at maturity by harvesting two inner rows in each plot. Number of seeds and seed weight were then determined according to SSTS-4138-2002 (State Standard of Ukraine, 2003). Accounting, measurement, and related observations were undertaken in accordance with methods of field experience by Dospekhov (1985). The oil and oleic acid contents of the seeds were determined by employing the device Spinlock Magnetic Resonance Solutions.

Data analysis

Data were subjected to statistical ANOVA (Analysis of variance) followed by Duncan multiple range test at 5% level of probability using the Statistica 8 software (StatSoft. Inc.).

Results and Discussion

Weather conditions

Analysis of weather conditions, particularly Hydrothermal coefficient (HTC) as described (Selyaninov, 1937), showed that the vegetative period of 2016 had a normal moisture (HTC = 1.00), whereas the year 2017 was dry (HTC = 0.45). HTC were computed by the formula:

$$HTC = \sum K / \sum T \times 10,$$

where $\sum K$ is the amount of rainfall (mm), for a period with an average daily air temperature above 10°C; $\sum T$ is the sum of temperatures (°C), for the period with average daily air temperature beyond 10°C. Our determination of HTC was necessary for subsequent analyses of the influence of weather (environmental) conditions on sunflower plant phenology, morphology, physiology and productivity.

Data on rainfall, air temperature, solar radiation and relative humidity for the growing period in the research area was obtained from Poltava Regional Center for Hydrometeorology of Ukraine. The growing season in 2016 was characterized by excessive rainfall in certain months. The humidification level was characterized by excessive rain in May (109.4 mm), June (71.4 mm) and August (62.7 mm). Respectively, the figures were higher than the average long-term (perennial) data by 61.5 mm, 8.2 mm and 24.4 mm. There was insufficient rainfall in July (40.3 mm) and this was lower by 20.4 mm. However, the amount of precipitation in September (7.0 mm) and that of the perennial average were the same (Figure 1a).

For the year 2017, the vegetation period had insufficient rainfall compared to 2016. In May, June, July and August, the rainfall recorded were respectively, 39.4 mm, 41.2 mm, 29.9 mm and 7.2 mm. When compared with the long-term average, the rainfall respectively decreased by 8.5 mm, 22 mm, 30.8 mm and 31.1 mm. Thus, both July (30.8 mm) and August (31.1 mm) recorded the two least rainfall in comparison with the perennial average rainfall. In contrast, the amount of rainfall in September (16.6 mm) exceeded the long-term average rainfall by 9.6 mm (Figure 1a). Total rainfall for the growing season of 2016 was higher (290.8 mm) than that of 2017 (134.3 mm) with the long-term recording the least (217.1 mm).

There were no considerable difference in temperature between the two years during the vegetative season except for August and September, where there were considerably higher temperatures in 2017. The following average temperatures were recorded in the months of the vegetative period of 2016: May (15.1°C); June (19.9°C); July (22.6°C); August (22.6°C); September (15.2°C).The temperature for each month of the growing season exceeded the average perennial temperature, except for May, which generated a lower (negative) temperature. Specifically, the following temperature deviations were respectively obtained: May (-0.7°C); June (0.5°C); July (0.8°C), August (1.7°C); September (0.3°C) (Figure1b).

The following average temperatures were recorded in the months of the vegetative period of 2017: May (14.8°C); June (20.1°C); July (21.5°C); August (24.0°C); September (18.0°C). The temperatures in May and July were respec-

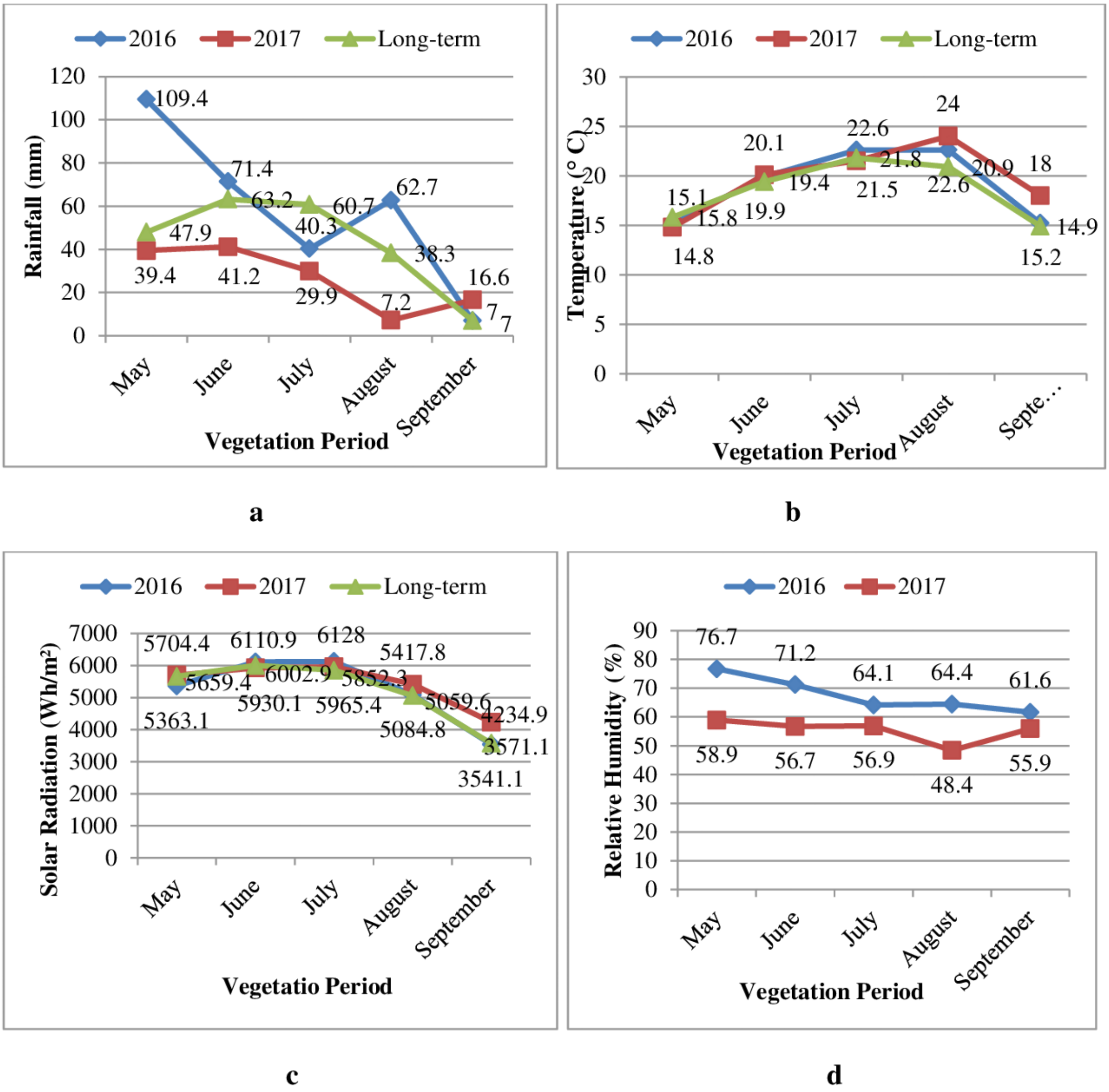


Fig 1. Weather conditions for the vegetative period of 2016 and 2017: *a* – Rainfall, *b* – Temperature, *c* – Solar Radiation, *d* – Relative Humidity

tively lesser than the perennial average by 1°C and 0.3°C. All other months of the vegetation season had a higher temperature than the long-term average. In particular, the highest increases were in both August and September by 3.1°C, while there was an increase of 0.7°C in June (Figure 1b). Total temperature for the growing season of 2017 was higher (98.4°C) than that of 2016 (95.4°C) with the long-term recording the least (92.8°C).

Regarding solar radiation, between the two years, the highest was recorded in July (6128 Wh/m^2) in 2016 and the least was in September (3541.1 Wh/m^2) of the same year (Figure 1c). Total solar radiation for the vegetative period of

2016 was slightly lower (26 227.9 Wh/m^2) than for the year 2017 (27 252.6 Wh/m^2), while the long-term recorded the least (26 145.3 Wh/m^2).

Relative humidity for the vegetative period was higher for every month in 2016 compared to 2017 (Figure 1d). Thus total relative humidity for the year 2016 was higher (338%) than for 2017 (276.8%).The highest recorded were in May; 76.7% in 2016 and 58.9% (2017) while the lowest were in September (61.6 %) in 2016 and August (48.4 %) in 2017.

Phenology

Traits which have the greatest dominant effects on environment modification of plant for maximizing production are phenological associated traits (Golparvar et al., 2013). In order to detect the influence of climatic conditions of the year on the development of plants, phenological observations were made of the progress of the main stages of plant development, and the dates of their onset were recorded based on the BBCH scale (Meier, 2001; Spaar, 1999). Weather conditions at the time of seed germination in 2016 led to the emergence of seedlings (BBCH 10) within 9–10 days. Flowering began (BBCH 61) between 72–74 days and lasted 8–10 days. In general, the period of vegetation was 120–122 days.

However, it was observed that, there was more intensive development of plants in the dry year of 2017. So, the seedlings emerged between 7–9 days. The inflorescence (star) stage (BBCH 51) began at 56–57 days. The first flowering plants (BBCH 61) were detected between 60–62 days. Flowering lasted 7-8 days. The period of vegetation was 115–117 days.

Morphological parameters

The main morphological parameters of the sunflower crop are plant height, stem diameter, number of leaves and their area. The results of the analysis of variance (ANOVA) found significant difference between these indicators depending on the conditions of the year (Table 1). Thus, the average plant height in the year 2016 was 188.3 cm, which is 27.3 cm higher than that of 2017. Based on ANOVA results, it was revealed that, plants grown under the conditions of 2016 were characterized by significantly higher values (Table 1).

The difference between the stem diameter, the number of leaves and the leaf surface area is slightly less evident. In 2016, plants formed an average stem diameter of 2.94 cm, while the average stem diameter in 2017 was 2.22 cm. It should be noted that, there was significantly more variation in the number of leaves. In the arid year of 2017, this parameter was smaller and varied from 18 to 23 pieces (pcs).

The main indicator that affects plant productivity is the leaf surface area. The ANOVA results revealed that, significantly larger assimilation surface was formed in the hybrid PR64H32 in the 2016 condition (Table 1). Thus, this parameter in 2016 was 0.68 m^2 (square meters) but in 2017, this indicator was only at the level of 0.49 m^2, which is significantly less (Duncan test = 0.06). This can be explained by the fact that the dry conditions in 2017 (shortage of rainfall and high temperature conditions) were limiting factor for realizing the biological potential of the hybrid, in particular leaf development. Similar to our findings, it is earlier reported that, during vegetative development, drought decreased the main stem height, stem diameter, number of nodes or leaves, and leaf area (Onemli & Gucer, 2010).

Chlorophyll "a" and "b" concentrations and SPAD readings

At the same time, it is noteworthy that the chlorophyll content was by contrast higher in 2017 for both absolute chlorophyll "a" and "b" concentrations and relative chlorophyll content (SPAD readings), but for both, there was no significant difference between the two years (Table 2). Specifically, the concentration of chlorophyll "a" and "b" in 2016 and 2017 were respectively 1.59 and 1.67 mg/g fresh weight. A similar trend was observed in determining the content of pigments using SPAD-502 plus. Thus, the average SPAD value in 2016 was 40.93, while a higher value of 46.21 was obtained in 2017. Water is an essential constituent of plants for supporting leaf structure and shape, photosynthesis, and thermal regulation (Ullah et al., 2013). The water stress (arid) condition in the year 2017 could have caused a decreased in absolute chlorophyll concentration, SPAD values as well as leaf surface area compared to normal moisture condition in 2016. The results of the present study support earlier work by Kiani et al. (2008) who confirmed a reduc-

Table 1. Morphological parameters of sunflower plants of the hybrid PR64H32 depending on meteorological conditions (2016–2017 years)

Year	Plant height, cm	Stem Diameter, cm	Number of leaves, pcs	Leaf surface area, m^2
2016	188.3a	2.94a	23.0a	0.68a
2017	161.0b	2.22b	20.1b	0.49b
Duncan test at 5%	2.78	0.17	0.82	0.06

Values followed by different letters are significantly different at 5% level of probability

Table 2. Effect of photosynthetic apparatus on productivity of sunflower hybrid PR64H32 depending on meteorological conditions (2016–2017 years)

Year	Chlorophyll «a» and «b» concentration, mg/g fresh weight	Chlorophyll content, SPAD value	Number of seeds per head, pcs	Seed weight per head, g
2016	1.59a	40.93a	1296.6a	84.9a
2017	1.67a	46.21a	1015.1b	65.0b
Duncan test at 5%	0.12	7.8	159.8	12.1

Values followed by the same letter are not significantly different at 5% level of probability

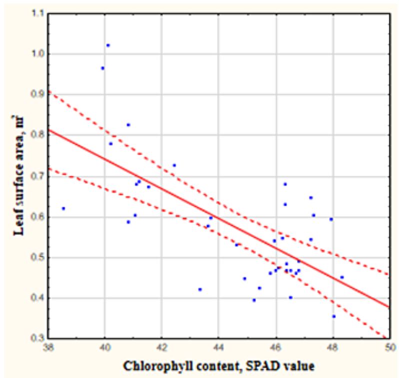
tion in plant chlorophyll status to a significant level at greater water deficits in sunflower plants. Again, our claim corroborated recent studies by Neto et al. (2017) which concluded that, water stress reduced the water and chlorophyll contents of sunflower (Sunbright Supreme variety). These authors also explained that, the progressive dehydration of sunflower plants stimulated an oxidative stress, which caused chlorophyll degradation and deficiency of this pigment synthesis in leaves. Additionally, the photosynthetic activity was notably altered by the biochemical damages of enzymes.

Productivity (seed number and seed weight)

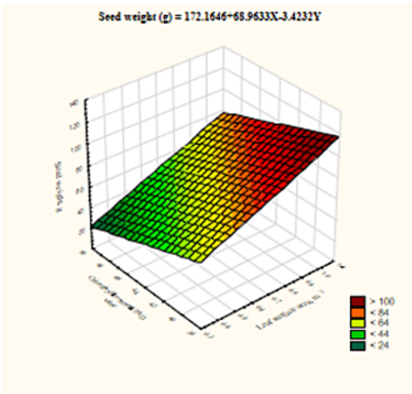
The main elements of the structure of the sunflower harvest are the number and seed weight formed per head on one plant. The results of the analysis of variance revealed a significant difference in each of the two indicators depending on the conditions of the year (Table 2). Significantly higher indicators (number of seeds and weight of seeds) were characterized by plants grown under normal moisture conditions of 2016. Thus, on the same plant, an average of 1296.6 seeds was produced with a total seed weight of 84.9 g. However, the average number of seeds formed per plant in 2017 was lower (1015.1 pcs) and had a lesser seed weight (65.0 g) as well. Deficit rainfall possibly slowed water exchange, as a consequence of the intensity of the formation of organic matter, seed weight accumulated slowly behind seed weight formed in 2016. Pourmohammad et al. (2013) reported that, sunflower yield correlated positively with plant height, number of seeds and 1000-seed weight and head diameter. These authors suggested that, based on heritability and correlation between traits, under drought stressed conditions in breeding programs, number of seeds, plant height and head diameter can be dependable criteria for selection of tolerant genotypes with higher yields. Besides, seed number is known to have the largest direct influence on seed yield (Skoric, 1974). Previously, Bannari et al. (2007) also mentioned that photosynthetic pigments are strongly linked to the physiological condition of the plant and its productivity. Therefore, in the arid year of 2017 in the present study, the negative effect of deficiency of photosynthetic pigments (lower chlorophyll content) on sunflower productivity (number of seeds and seed weight) cannot be over ruled.

Relationship between leaf surface area and chlorophyll content and their impact on sunflower productivity

Interestingly, the results of our regression analysis in relation to the leaf surface area and chlorophyll content of the same leaves of the plants, revealed an inverse proportional relationship between these two parameters. Consequently, for an increase in the assimilation surface, the chlorophyll content decreased. The correlation coefficient (r) was 0.67 (Figure 2a).



a)



b)

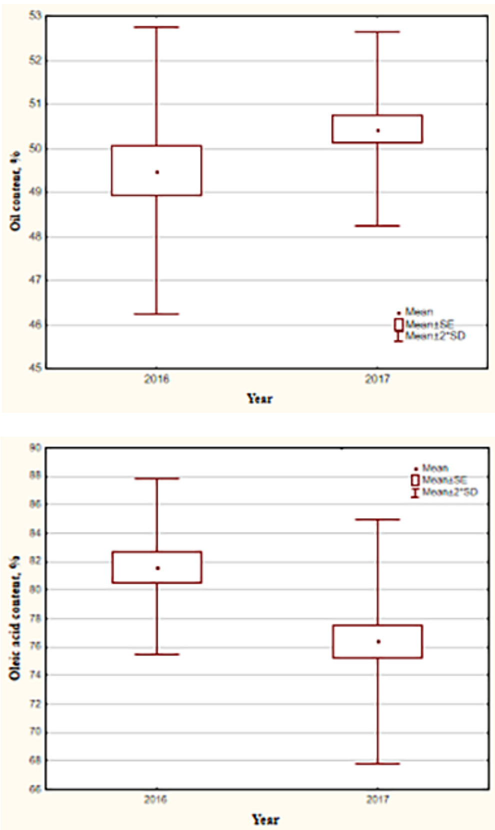
Fig. 2. Regression analysis of parameters of sunflower plants: *a* – leaf surface area and chlorophyll content; *b* – productivity of sunflower plants depending on the leaf surface area and chlorophyll content

Clearly, the impact of the main elements of the photosynthetic apparatus on sunflower productivity is shown (Figure 2b). So, for this sunflower plant (PR64H32), the leaf surface area (X-axis) clearly had a significant influence on the formation of seed weight (Z-axis) than its chlorophyll content in leaves (Y-axis).

Oil and oleic acid content

The main indicators for the quality of high-oleic sunflower seeds are the contents of oil and oleic acid. Consequently, in 2016, seeds with oil content of 49.5% and oleic acid content of 81.6% were produced. However, a slight increase in oil content (50.4%) was noticed in the dry year of 2017, yet the oleic acid content reduced to 76.4% (Figure 3a and 3b). Recently, Jocić et al. (2015) pointed out that, the majority of modern sunflower hybrids have 45-50% oil in seed. Besides, in sunflower, it is noted that grain (seed) oil concentration is chiefly determined by the total photosynthetically active radiation intercepted per plant during the grain filling period (Andrade & Ferreiro, 1996; Dosio et al., 2000; Izquierdo et al., 2008). The vegetative period for the arid year of 2017 in our study had a slightly higher total solar radiation (27 252.6 Wh/m^2) than 2016 (26 145.3Wh/m^2). Moreover, the grain filling period in the present study likely occurred mainly between August and September with solar radiations higher in 2017 than 2016 for these periods. This could partly explain the slightly higher oil content obtained in 2017.

Temperature is apparently a key environmental factor that influences fatty acid composition of the seed. In addition, it is mentioned that high temperature boosts the oleic acid content of standard sunflower cultivars (Low Oleic) (Ferfuia et al., 2012), but contradictory results are reported about temperature effects on oleic acid content of high oleic acid cultivars: no influence (Lacombe & Berville, 2000; Lagravére et al., 2000, Pourmohammad et al., 2013) or increase of oleic acid content with temperature (Triboi et al., 2000; Izquierdo & Aguirrezábal, 2008; Pourmohammad et al., 2013). Our study further conflicts these earlier studies in that, we recorded higher oleic acid content (81.6%) in the year (2016) that had normal moisture and lower temperature compared to 2017 which was dry with higher temperature. However, it is known that different genetic background and modifier genes could cause differences between high oleic inbred lines (Lacombe et al., 2004). Previously, Ferfuia et al. (2012) suggested that, factors such as water and nitrogen may modify the fatty acid composition of high oleic sunflower genotypes. Our determination that the moisture condition for the year 2016 was normal (high water) compared to the dry (less water) condition for 2017 could partly help explain our oleic acid results that found high oleic acid content in 2016 than in 2017 (Figure 3b).



a)

b)

Fig. 3. Analysis of variance of quality parameters of sunflower seeds depending on the conditions of the year: *a* – oil content, %; *b* – oleic acid content, %

Conclusion

This study shows that sunflower hybrid PR64H32 had more favorable conditions for the growth and development of plants with higher yield and quality created by normal moisture condition for the year 2016 than the dry condition in 2017. The deficit rainfall in 2017 led to a shortage in yield (seed weight per head) by 23.4%. Analysis of components of the photosynthetic apparatus discovered an inverse proportional relationship between the leaf surface area and the chlorophyll content in leaves of sunflower plants. We further showed a more significant effect of the leaf surface area than the chlorophyll content on the productivity (seed weight per head) of sunflower plants – a major interest in sunflower production.

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