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Chlorophyll content and leaf area correlated with corn (*Zea mays*) yield components in F_1 hybrids

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ABSTRACT

Chlorophyll content and leaf area are important biochemical and biophysical regulators of water, energy and carbon exchange during photosynthesis. The present study was carried out during 2020 (autumn) and 2021 (spring) at the agronomy experimental station, College of Agricultural Engineering Science, Salahaddin University, Erbil, Iraq to identify the relationship of chlorophyll and leaf area with yield and yield-related characteristics among 8 introduced corn (*Zea mays* L.) F_1 hybrids. Results demonstrated that the 8 hybrids were significantly different from each other for the number of kernels/row, number of leaves/ear, chlorophyll content and leaf area. Hybrid-by-year interaction was significant for ear yield, ear length, ear diameter, number of rows/ear, number of kernels/row and number of leaves/ear. No relationships phenotypically and genetically were found among chlorophyll with yield and yield-related traits in both the years. However, phenotypic and genotypic correlations were significant between leaf area and yield contributing traits in autumn 2020. Short vectors or obtuse angles by biplot analysis showed the same direction for correlation analysis. In conclusion, more information in future plant breeding programmes at phenotypic and DNA levels are required to represent the relationship between chlorophyll content with yield and yield-related traits. However, different correlations between leaf areas in the two growing years might be owing to the fluctuated local environmental factors during the plant growth period and/or no adaptation of new hybrids to the local environments.

Keywords: Corn, Chlorophyll content, Correlation, F_1 hybrids, Leaf area, Regression analysis

Several socio-economic and political events have affected agricultural production in Iraq in the last century. Based on the data presented by the Knoema website (2021), the area and yield of corn (*Zea mays* L.) in Iraq during the period 1960–2021 highly fluctuated, because of plant restrictions, unstable conditions, and environmental factors. The environmental conditions, planting practices, and factors affecting corn yield all vary due to the wide variety of corn planting areas (Van Loon *et al*. 2019). Therefore, it is critical to look into the factors that influence corn yield (Li *et al*. 2019). Leaf area and chlorophyll content are two parameters indicating corn's growth and are correlated with yield. One-sided green leaf area per unit of horizontal ground area is referred to as leaf area. It is one of the most frequently used biometric parameters for corn used to predict biomass, net dry matter productivity, and potential photosynthetic activity efficiency (Lykhovyd *et al*. 2019). Numerous factors have an impact on leaf area, including the size of the leaf area, the chlorophyll content of the leaves, ecological factors, plant density (Eszter 2015), a lack of water and nutrients (Bencze 2019), the number of growing degree days, the intensity of the light, developed maize hybrids (Dwyer *et al*. 1986), and corn defoliation (Lambert *et al*. 2014). In addition, the chlorophyll content of leaves is one of the most important groups of light-absorbing pigments in the photosynthesis process (Zhang *et al*. 2019). Total chlorophyll content (chlorophyll a and b) on a leaf area basis is defined as the leaf chlorophyll content (Houborg *et al*. 2015). According to Yang *et al*. (2022), the amount of chlorophyll in a plant's leaves can indicate the population's overall photosynthetic productivity. Chlorophyll levels in leaves are strongly correlated with primary production (Schull *et al*. 2015) and nitrogen levels in leaves (Ramoelo *et al*. 2015), the type of plant (Gitelson *et al*. 2006), disease, nutritional deficiency, and environmental stresses (Datt 1999), as well as plant phenology (Zhang *et al*. 2019).

Chlorophyll content and leaf area are correlated to the grain components (Sheikh Sharoush 2013), and grain filling period in corn (Al-Hadidi 2007). Identifying the characters closely related and contributing to yield becomes highly

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essential. Therefore, the present study was planned to evaluate yield and yield contributing traits of the genotypes and to determine the simple correlation, genetic correlation, regression analysis, and biplot analysis between chlorophyll content and leaf area with yield components.

MATERIALS AND METHODS

Present study was carried out during July to November 2020 (autumn) and March to June 2021 (spring) at the agronomy experimenta1 station, College of Agricultural Engineering Science, Salahaddin University, Erbil (8 km southwest, 36.101.16"N and 44.009.25"E and 415 m amsl). The region's climate is classified as a semi-arid area. The geographical properties of the experimental environments for the two years are provided in Table 1. The soil was silty clay loam having *p*H 7.5.

Hybrid collection and experimental design: Eight introduced corn F_1 hybrids, viz. SyBatanga (SYB), Reserve (RES), Nklucius (NKL), Syinore (SYI), SyauTex (SYA), DKC6589 (DKS), DEKALB6664 (DEK) and DKC5401 (DKF) from various sources and different origins were collected and evaluated in randomized complete block design (RCBD) with three replications.

Cultural practices: The uniform cultural practices were applied to all experimental plots. The experimental plots were ploughed twice to a depth of 15 cm to 30 cm, followed by soil restoration before planting. The hybrid seeds were manually planted in plots with four 3-m long rows at a planting density of 0.70 m × 0.20 m. Two seeds were manually sown per point. Ten days after planting, the plants were thinned to just one plant per point. At the rate of 120 kg/ha N, 120 kg/ha P_2O_5, and 120 kg/ha K_2O, fertilizers were applied using the compound fertilizer (NPK15:15:15) at 7 days after planting, and Urea (46% N) in equal splits at 15 and 35 days after planting. Weeds were manually controlled. Dripped irrigation was subjected throughout the plant growth period.

Table 1 Temperature, relative moisture and rainfall of the study site during two years (2020–21)

Year	Month	Air temperature °C		Relative moisture (%)	Rainfall (mm)
		Minimum	Maximum		
2020	July	28.9	43.4	13.3	np
	August	26.6	40.6	15.5	np
	September	26.90	38.90	16.1	np
	October	20.00	32.50	17.9	2.30
	November	13.70	21.50	38.00	37.00
2021	February	6.80	14.80	56.8	27.50
	March	8.70	20.00	42.5	26.10
	April	10.60	28.90	26.6	0.20
	May	24.30	35.30	17.3	0.20
	June	23.9	39.4	13.3	np

np, No precipitations.

Data collection: Data were taken from each plot, and 10 plants in the middle two rows were used for data collection and sampling. In both years, the data were taken for ear yield (EY), ear length (EL), ear diameter (ED), number of rows per ear (NRE), number of kernels per rows (NKR), number of leaves per ear (NLE), stem diameter (SD), leaf number per plant (LNP), chlorophyll content (Chl), and leaf area (LA).

Leaf area was measured for 10 samples by taking the longest and widest leaf in the middle of each plant, as suggested by Montgomery (1911)

$$\text{Leaf area} = \text{Leaf length} \times \text{Leaf width} \times 0.75$$

The chlorophyll content of the leaves was measured using an atLEAF Chl meter (FT Green LLC, Wilmington, DE) (Zhu *et al*. 2012).

Statistical analysis: The data obtained for different characters were analyzed with a range of statistical and multivariate methods. Combined analysis of variance (ANOVA) was carried out using the SAS software version 9.4 (SAS Institute Inc. 2014). Subsequently, data were standardized using NTSYS-pc program version 2.1 (Rohlf 2002), using the equation given as:

$$s = (x_{ij} - \bar{x})/\sigma$$

where s, Standardized vale; x_{ij}, Observation from i^{th} genotype in j^{th} block; $\bar{x}$, Mean value of the trait measure; σ, Standard deviation of the trait measure.

Simple correlation (Proc Corr) and genetic correlations (Proc mixed covtest asycov) were executed among the hybrids using the same SAS software version 9.4. Furthermore, regression was measured using Excel (Microsoft Office LTSC Professional Plus). In addition, the XLSTAT program was used to show biplot analysis each year to determine the influence of leaf area and chlorophyll content on yield components among the hybrids.

RESULTS AND DISCUSSION

Yield is very difficult to improve by selecting the genotypes for yield per se, because of it being a complex character (Wright 1921). Despite collecting the hybrids from different sources and origins, combined analysis of variance (ANOVA) of the two years (Table 2) detected that the differences between the evaluated hybrids were not significant, which proved that the hybrids used in this study had not divergence for yield and most yield-related traits. While, hybrids were significant for NKR, NLE, Chl, and LA, indicating that the hybrids are different just for these traits. However, the significant effects of years for yield-related traits indicate high differential hybrids responses across the different years. The variation in temperature across the different years was considered a major underlying causal factor for the hybrid year interaction for yield and yield related traits. Year relative magnitude was much higher than the hybrid effect for EL, ED, NRE, NKR, and LNP, suggesting that the performance of each hybrid is influenced more by year factors for these traits. Conversely,

Table 2 Mean squares from combined analysis of variance for 10 traits across two years, measured on 8 corn hybrids

S. O. V	d.f.	Mean square									
		EY	EL	ED	NRE	NKR	NLE	SD	LNP	Chl	LA
Year	1	1365.33	46.31**	188.25**	16.56*	378.73**	0.75	0.75	11.30*	2.31	1728.84
Rep (Year)	4	29173.45	11.18*	3.25	0.33	15.10	0.63	38.08*	0.85	71.77*	9310.89*
Hybrid	7	48421.46	4.64	12.71	1.69	52.10*	4.22*	7.54	2.58	131.20**	32282.81**
Hybrid × Year	7	169933.19*	6.00*	22.26*	4.64*	73.15*	3.19*	2.84	1.47	11.09	527.88
Error	28	31108.93	2.28	7.92	1.58	19.53	1.40	8.78	1.38	23.57	1463.30
CV		18.97	8.06	6.90	7.98	14.75	13.40	16.48	9.43	9.83	7.68

* and ** are significantly different at 0.05 and 0.01.

EY, Ear yield; EL, Ear length; ED, Ear diameter; NRE, Number of rows per ear; NKR, Number of kernels per rows; NLE, Number of leaves per ear; SD, Stem diameter; LNP, Leaf number per plant; Chl, Chlorophyll content; LA, Leaf area.

the mean values of the hybrid effect were higher than year effects for EY, NLE, SD, Chl, and LA, indicating that the performance of hybrids is affected by the type of hybrids for these traits. Previous studies found significant variations in agronomic characteristics among corn hybrids tested in various environments (Mustafa 2021, Yue *et al*. 2022).

Results on phenotypic and genotypic correlations and regression analysis between Chl and LA with yield and yield-related traits are presented in Table 3 for both years. Chl was not significant phenotypically with yield and yield components for both years. Similarly, in a study conducted on the evaluation of yield and Chl in corn hybrids during 2013, 2014 and 2015 by Eszter (2015), no correlation has been found between Chl and yield during these three years. In the present study, Chl was also genetically not significant in both growing years. This implies that the genetic effects on Chl are independent of the other traits. Regression analysis showed that Chl is not an effective trait for yield and other traits for both years. As a result, the charge of this trait was unable to obtain a yield on hybrids that was sufficient to reach maximum yield on different treatments. Stepwise regression was used by researchers to compare other traits in corn hybrid cultivars to grain yield, which was the dependent variable (Zinali *et al*. 2004).

There was a significant phenotypic correlation between LA and other traits in the autumn of 2020. Regression analysis also showed significant effects on all of the traits under study. This depicted that leaf area significantly affected yield and yield components for this year. In contrast, for the 2021 growing spring season, there was no significant correlation or regression between LA and other traits except for NRE, which was negatively significant. This big difference between the years refers to two factors. First, it is due to the influence of temperature during the year 2021, specifically at night, which was less than 10°C till early April 2021. This obligates us to plant the hybrids out of the recommended period for corn planting in Iraq, which was early to middle March. The lowest optimum temperature at night for corn planting should be more than 10°C (Al-Kharbouli 2020). Secondly, the type of hybrids also affects the Chl and LA as represented by ANOVA (Table 2). Eszter (2015) reported a weak to medium correlation between LA and yield in corn hybrids in three years (2013, 2014 and 2015). Bencze (2019) also showed a moderate positive correlation between LA and Chl with average yields. In terms of the magnitudes of genetic correlation, there was a high genetic correlation between LA and other characters in the autumn of 2020. Johnson (1974) showed a genetic correlation between LA in corn with grain yield. However, no genetic correlation was discovered for most of the characters in the spring of 2021, except for ED and NRE, which shows a negative genetic correlation. A significant negative genetic correlation indicates that by reducing LA, ED, and NRE tend to increase.

According to the biplot analysis for the Autumn of 2020 (Fig. 1), the total variation explained was 79.92% (66.33 and 13.59% for PC1 and PC2, respectively). While the biplot analysis for the spring of 2021 (Fig. 2), explained 60.90% total variation (38.29 and 22.61% for PC1 and PC2, respectively). According to Yan (2001), in the biplot, the approximate correlation coefficient is the cosine of the angle between any two or more trait vectors. Acute angles, which are less than 90°, indicate a positive correlation, obtuse angles, which are more than 90°, indicate a negative correlation, and right angles, which are equal to 90°, indicate no correlation. The trait might not be correlated with other traits if the vector is small. In the present study, a short vector, obtuse angles (more than 90°), and right angles (equal to 90°) between Chl and yield and other traits in both years indicated that there was no significant correlation between them (Fig. 1 and 2). A positive and significant correlation existed between LA and yield and yield components in the autumn of 2020 for hybrids DEK, SYA, SYI, DKS, and RES. This indicates that the greater the leaf area the higher the yield components for the mentioned hybrids. These results are supported by a phenotypic correlation and regression among the traits in Table 3. While based on the biplot analysis in the growing spring of 2021, there was no correlation between LA and Chl almost with all yield-related traits because there was no angle between them, except for ear yield which had an obtuse angle >90° (Fig. 1), these results also supported by

Table 3 Pearson correlation, genetic correlation and regression analysis between chlorophyll content and leaf area with yield and yield-related traits in 2020 and 2021

Trait	Autumn 2020								
	$Corr_P$	$Corr_G$	Regression equation	R^2	Trait	$Corr_P$	$Corr_G$	Regression equation	R^2
EY Chl	0.156	0.274	$Y = 0.156x + 8\times10^{-16}$	0.024	EY LA	0.834*	1.000*	$Y = 0.834x + 1\times10^{-15}$	0.695*
EL Chl	0.168	0.326	$Y = 0.167x - 1\times10^{-15}$	0.028	EL LA	0.723*	0.991*	$Y = 0.723x - 1\times10^{-15}$	0.522*
ED Chl	0.129	0.178	$Y = 0.129x - 5\times10^{-16}$	0.016	ED LA	0.752*	0.980*	$Y = 0.752x - 3\times10^{-16}$	0.566*
NRE Chl	0.325	0.542	$Y = 0.325x - 3\times10^{-16}$	0.106	NRE LA	0.799*	1.000*	$Y = 0.798x + 7\times10^{-16}$	0.638*
NKR Chl	0.166	0.164	$Y = 0.166x - 6\times10^{-16}$	0.028	NKR LA	0.869*	0.995*	$Y = 0.869x - 4\times10^{-16}$	0.755*
NLE Chl	-0.159	-0.152	$Y = -0.159x + 2\times10^{-15}$	0.025	NLE LA	0.844*	0.915*	$Y = 0.844x + 2\times10^{-15}$	0.712*
SD Chl	-0.188	-0.218	$Y = -0.188x + 1\times10^{-15}$	0.035	SD LA	0.832*	1.000*	$Y = 0.832x + 1\times10^{-15}$	0.693*
LNP Chl	0.417	0.530	$Y = 0.416x - 8\times10^{-16}$	0.174	LNP LA	0.775*	0.943*	$Y = 0.755x - 4\times10^{-16}$	0.600*
Trait	Spring 2021								
	$Corr_P$	$Corr_G$	Regression equation	R^2	Trait	$Corr_P$	$Corr_G$	Regression equation	R^2
EY Chl	-0.188	-0.279	$Y = -0.188x - 5\times10^{-16}$	0.035	EY LA	-0.179	-0.230	$Y = -0.179x - 4\times10^{-16}$	0.032
EL Chl	0.346	0.106	$Y = 0.346x - 2\times10^{-15}$	0.120	EL LA	-0.473	-0.268	$Y = -0.473x + 3\times10^{-15}$	0.223
ED Chl	-0.339	-0.723	$Y = -0.339x + 2\times10^{-15}$	0.115	ED LA	-0.527	-0.761*	$Y = -0.527x + 2\times10^{-15}$	0.278
NRE Chl	-0.109	-0.224	$Y = -0.109x - 1\times10^{-15}$	0.012	NRE LA	-0.898*	-0.614*	$Y = -0.898x - 6\times10^{-16}$	0.806*
NKR Chl	-0.593	-0.251	$Y = -0.593x + 4\times10^{-16}$	0.351	NKR LA	-0.001	0.258	$Y = -0.001x + 3\times10^{-16}$	1×10^{-6}
NLE Chl	0.363	0.267	$Y = 0.363x + 2\times10^{-15}$	0.132	NLE LA	0.391	0.405	$Y = 0.391x + 2\times10^{-15}$	0.153
SD Chl	0.557	0.401	$Y = 0.557x + 4\times10^{-16}$	0.311	SD LA	0.374	0.268	$Y = 0.374x + 1\times10^{-16}$	0.140
LNP Chl	0.645	0.048	$Y = 0.654x - 7\times10^{-16}$	0.428	LNP LA	-0.075	-0.002	$Y = -0.074x - 6\times10^{-16}$	0.006

*numbers are significantly different.

EY, Ear yield; EL, Ear length; ED, Ear diameter; NRE, Number of rows per ear; NKR, Number of kernels per rows; NLE, Number of leaves per ear; SD, Stem diameter; LNP, Leaf number per plant; Chl, Chlorophyll content; LA, Leaf area; $Corr_{P,}$ Phenotypic correlation; $Corr_{G,}$ Genotypic correlation; R, Regression analysis

phenotypic correlation. No correlation in the spring of 2021 might be due to the influence of the temperature throughout the plant growth period and the type of hybrids.

Chlorophyll content and leaf area are two important traits that are related directly to the photosynthesis process. In the present study, besides the presence of high variability among the 8 corn hybrids, there was no relationship between chlorophyll content and other traits in both the years. However, a highly significant relationship was found between leaf area and all of the traits studied in the growing autumn of 2020. This suggested that increasing leaf area tends to increase the other traits. But this was not found in the growing spring of 2021. The results supported by biplot graphs in both years, were highly consistent with

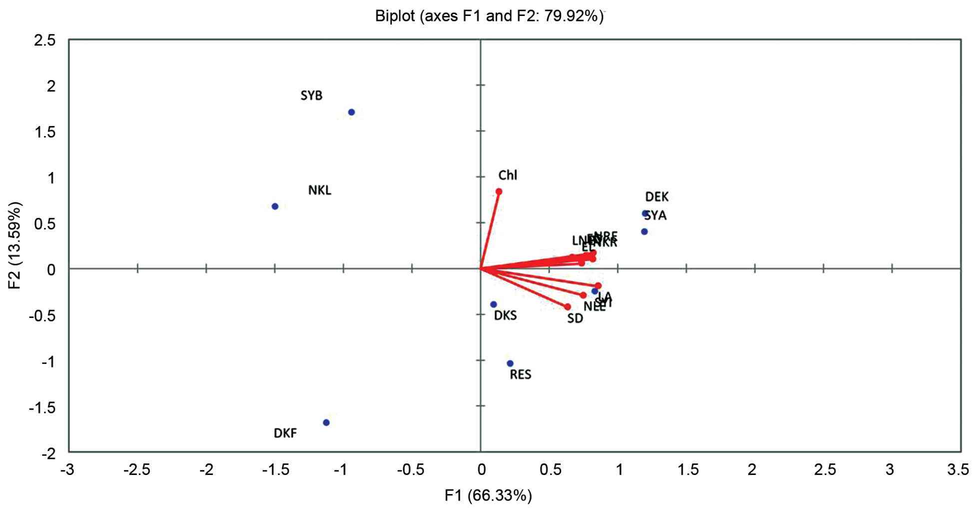

Fig. 1 Biplot based on 2020 data of 8 corn hybrids showing relationships among traits and hybrids.

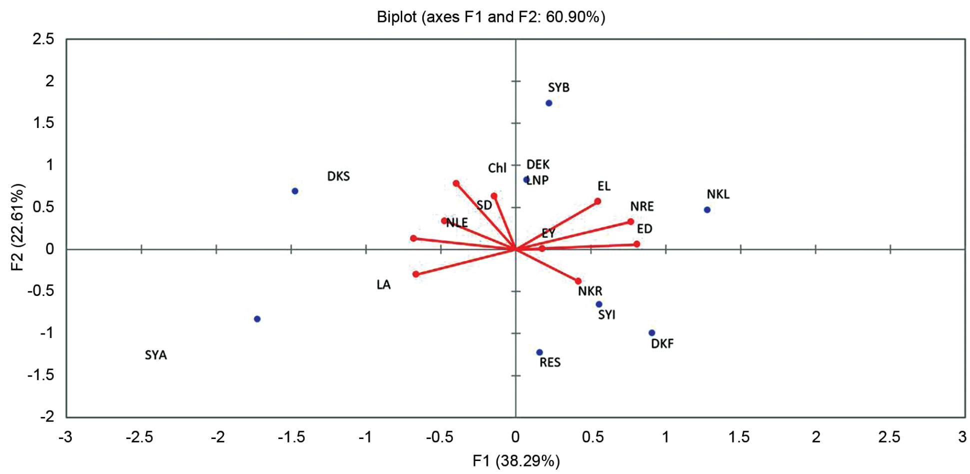

Fig. 2 Biplot based on 2021 data of 8 corn hybrids showing relationships among traits and hybrids.

correlations and regression analysis. The reasons behind these differences between the two years might be due to environmental factors during the plant growth period and/or no adaptation of new hybrids to the environments.

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