CHILEAN JOURNAL of AGRICULTURAL RESEARCH

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RESEARCH ARTICLE

Estimation of protein content in wheat grains under different irrigation regimes via vegetation indices

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Received: 20 October 2024; Accepted: 28 January 2025, doi:10.4067/S0718-58392025000400481

ABSTRACT

Biotic and abiotic environmental factors during the production of bread wheat (*Triticum aestivum* L.) directly affect the yield and quality of the grain. Climatic factors are the most significant in the definition of yield, contributing to the greatest variability compared with genotype. This influence is also present in the quality characteristics that the bakery industry seeks, such as the protein content in grain and flour. These characteristics can be estimated during the production stage via vegetation indices. Therefore, the objective of this research was to identify the correlations between the vegetation indices, normalized differential vegetation index (NDVI) and green leaf index (GLI) with the total protein content, as well as with the yield components and characteristics of bakery and industrial quality, in flour wheat genotypes under different irrigation conditions. The NDVI was estimated each week during the grain filling period. To obtain the GLI, orthophotos were constructed from scheduled flights with an unmanned aerial vehicle (UAV) during the same period. Twenty-four genotypes of bread wheat were evaluated, of which 18 were commercial varieties and 6 were experimental lines, under four irrigation treatments: 0-55, 0-45-75, 0-45-75-100 and 0-35-75-85-105 d after sowing. The results revealed a significant positive correlation between the grain protein content and the NDVI and a highly significant correlation with the GLI. This correlation was more evident under contrasting irrigation conditions. Therefore, the GLI index obtained at the grain-filling stage can be used as a predictive tool to estimate the protein content in harvested grains.

Key words: Protein content, *Triticum aestivum*, vegetation indices, wheat.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the three main cereals produced worldwide, along with corn and rice, which makes it a key food crop (Wrigley and Nirmal, 2017). The FAO's preliminary forecast for world wheat production for 2024 is 797 million tons. However, the harvest of the new commercial cycle has been affected by various climatic conditions in some of the main producing countries, for which it is estimated that the production of cereal will experience a slight decrease of 7.5 Mt, reaching 782.0 Mt for the 2023-2024 cycle (USDA, 2024).

However, wheat is considered good baking quality because it has properties that allow it to produce desirable bread according to industry and/or consumer criteria (Wysocka et al., 2024). For this purpose, the bakery industry needs appropriate types of flour for different bakery products (Hughes et al., 2020). Therefore, producers must consider different factors to meet the demand for high-quality grains, such as suitable varieties, nutrient management, pest and disease management, irrigation, crop rotation, storage and postharvest handling. Various climatic environmental factors, such as temperature, solar radiation, and rainfall, impact the expression of the potential yield of crops and, therefore, determine the yield obtained (Rozbicki et al., 2015).

In a climate change scenario, the negative effects of wheat diseases, among other biotic and abiotic factors, are likely to increase (Santa-Rosa et al., 2016). There are antecedents that indicate that climatic components are the most significant in the definition of wheat yield, contributing 56% of the variability, whereas genotype contributes 23% (Fernández et al., 2019). These elements, in addition to influencing the phenological characteristics of the crop, have a direct effect on the quality of the grain, such as the protein content.

A field experiment was conducted at the ICAR-Indian Agricultural Research Institute, New Delhi, to study the interactive effects of irrigation and N fertilizer on the yield, grain protein content, and water and N use efficiency of wheat. The design of the experiment was split-plot with irrigation (I_0: rainfed, I_2: two irrigations, I_3: three irrigations, I_5: five irrigations) as the main plot and N (N_0: 0 kg N ha^{-1}, N_{30}: 30 kg N ha^{-1}, N_{60}: 60 kg N ha^{-1} and N_{120}: 120 kg N ha^{-1}) as the subplot treatment. The grain protein content was not significantly influenced by the irrigation level in either year. Averaged across years and N levels, I_0 resulted in a 6% higher grain protein content than did the other irrigation treatments (Pradhan et al., 2017). In one study, the effects of irrigation and N application on the grain yield, protein content and amino acid composition of winter wheat were evaluated. Field experiments were conducted in a split-plot design with three replicates in high-yielding land on the North China Plain. Three irrigation treatments were examined in the main plots: No irrigation, irrigation at jointing, and irrigation at jointing plus anthesis, while subplots were assigned to N treatment at four different rates: 0, 180, 240, and 300 kg N ha^{-1}, respectively. The results indicated that, compared with no irrigation, irrigation at jointing and at jointing plus anthesis improved the grain yield by an average of 12.79% and 18.65%, respectively, across the three cropping seasons. However, different irrigation treatments had nonsignificant effect on the grain protein content during any cropping season (Zhang et al., 2017). The opposite results were obtained in El Bajio, Mexico, where the effect of the number of irrigations on the industrial quality of wheat was evaluated. Ten commercial varieties were used, and irrigation was applied at 0-35, 0-35-70, 0-35-70-105 and 0-35-70-105-125 d (Cruz et al., 2017). The evaluated variables were hectoliter weight, grain hardness, protein content in flour, heading time, mass strength, tenacity-extensibility ratio and bread volume. With the three-irrigations treatment, which resulted in the highest protein content in the flour, the strength of the dough and bread volume increased, whereas the protein content decreased by four or five. In one experiment conducted in northwestern Mexico, three N levels (75, 150 or 250 kg ha^{-1}) and three levels of irrigation (3, 4 or 5 irrigations) were studied. Increasing the N rate decreased the yellow belly (YB) content, thousand kernel weight and hectoliter weight and increased the protein content and number of grains per spike. The number of irrigations did not affect the number of grains per spike. However, increasing the number of irrigations increased the YB content, thousand kernel weight and hectoliter weight; on the other hand, the protein content decreased (Rodríguez-Felix et al., 2014). These findings suggest that understanding the optimum regime of irrigation and N application is an important strategy for improving both grain yield and protein quality in winter wheat.

Wheat with moderate to high protein content (greater than 12%) is considered essential for bread production, whereas wheat with lower protein content is typically used for biscuits, noodles or animal feed (Ross, 2017; Jansone et al., 2024). The protein in the grain is the product of the accumulation of N during the vegetative stage until the filling of the grain (Sperotto et al., 2017; Argentel-Martínez et al., 2018). Proper management should be considered in grain production, since different types of stress affect the chlorophyll content and the metabolism of carbohydrates and proteins in plants and ultimately the quality of the grain (Argentel-Martínez et al., 2018).

One way to consider the quality of the grain during the production stage of the crop is through multispectral reflectance since the leaf surface of the plant strongly reflects the energy in the near-infrared range (dos Santos et al., 2023) and can be measured by vegetation indices. The reflectance is determined by the properties of the foliar tissues, and these anatomical characteristics of the plants affected by stressors, such as heat or drought, can cause variations in the values of the vegetation indices. The leaf epidermis, cuticle thickness, and arrangement of mesophyll cells influence the ability to reflect or absorb light. The presence of a thick cuticle or waxy layer can increase the reflectance in the visible and near-infrared ranges. On the other hand, water in leaf tissue is strongly absorbed in the mid-infrared region. Dehydration due to water stress reduces the ability of leaves to absorb these wavelengths, leading to an increase in reflectance. Chloroplasts, which are rich in chlorophyll, absorb strongly in the visible region. Thermal or water stress can degrade chlorophyll, resulting in a change in reflectance patterns via decreased absorption in the visible spectrum. A reduction in cell turgor can affect the internal structure, changing the reflectance in the NIR. Heat or drought stress reduces absorption in red light (due to lower chlorophyll content) and can modify reflectance in the NIR (due to structural changes), resulting in lower normalized differential vegetation index (NDVI) values (Karabourniotis et al., 2021; Melandri et al., 2021; Wang et al., 2025).

The reflectance measurements should be carried out during specific stages of the phenological development of the crops, such as grain filling, since in this period, the vegetation indices, for example, the NDVI, have better results when estimating yields (Magney et al., 2016). In terms of protein content, the grain-filling stage is fundamental and is directly related to yield since the increase in the availability of carbohydrates in this stage causes a negative relationship between grain yield and protein percentage (Maich et al., 2017; Fernández et al., 2019), and yield can be estimated via vegetation indices. Li et al. (2020) noted that using remote sensing data to predict grain quality and yield in crops such as wheat is becoming a more viable alternative to destructive laboratory testing methods. A close relationship between wheat grain protein content and vegetation indices such as the normalized difference vegetation index (NDVI) has been demonstrated (Tan et al., 2020). Wang et al. (2014) indicated that this relationship may depend on the phenological stage at which sampling is carried out; for example, the accuracy of grain protein content is improved when data collection is carried out during anthesis.

The indices used for prediction range from the use of the NDVI, which is the most common index, the leaf area index (LAI) (Ríos-Hernández, 2021), to the use of indices that measure the reflectance of the wavelengths corresponding to the primary colors of visible light, red, green and blue, which can provide good estimates, such as the green leaf index (GLI) (Chen et al., 2024).

Unmanned aerial vehicle (UAV) platforms equipped with multiple sensors capable of rapidly scanning entire fields have proven to be useful tools for collecting the nondestructive data on crop canopies necessary for the estimation of vegetation indices (Radoglou-Grammatikis et al., 2020; Xie and Yang, 2020; Jansone et al., 2024).

The objective of this research was to identify the correlations between vegetation indices, the normalized differential vegetation index (NDVI) and the green leaf index (GLI), and the total protein content, yield components and characteristics of bakery and industrial quality in flour wheat genotypes under different irrigation conditions.

MATERIALS AND METHODS

Experimental site

This research was carried out at the “El Bajío” Experimental Field (CEBAJ) of the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), located at 6.5 km Celaya-San Miguel de Allende Celaya, Guanajuato (20°32' N, 100°48' W; 1765 m a.s.l.) in the autumn-winter 2021-2022 and 2022-2023 production cycles. The CEBAJ region has registered precipitation values of 578 mm and an average annual temperature of 19.8 °C.

Handling the experiments

Twenty-four genotypes of flour wheat (*Triticum aestivum* L.) were evaluated, of which 18 were commercial varieties and 6 were experimental lines (Table 1). Four irrigation schedules were also evaluated: 0-55, 0-45-75, 0-45-75-100 and 0-35-75-85-105 d after sowing. The irrigation was performed by flooding via the sluice method. The irrigation sheets applied were 26 cm (14 + 12) for the two-irrigation treatment, 34 cm (14 + 10 + 10) for the three-irrigation treatment, 44 cm (14 + 10 +10 + 10) for the four-irrigation treatment and 54 cm (14 + 10 + 10 + 10 + 10) for the five-irrigation treatment. A split-plot experimental design with three replicates was used. In the large plot, the irrigation schedule was established, and in the small plot, the genotypes were established. The experimental plot consisted of two 3 m long furrows with a distance between them of 0.75 m, with an experimental plot of 4.5 m^2.

Table 1. Wheat genotypes used in the experiment.

No	Genotype	No	Genotype
1	Cortazar S94	13	Salamanca S75
2	Urbina S2007	14	V-17
3	Borlaug100 F2014	15	V-19
4	Maya S2007	16	V-21
5	Bárcenas S2002	17	Luminaria F2012
6	Ibis M2016	18	Cisne F2016
7	Bacorehuis F2015	19	Fort Mayo
8	Conatrigo F2015	20	V-27
9	Alondra F2014	21	V-28
10	Noreste F2019	22	Witness A
11	Hans F2019	23	V-35
12	Faisán s2016	24	Witness B

Estimation of the NDVI

To estimate the normalized differential vegetation (NDVI) index, four samplings were carried out with the GreenSeeker sensor (PTx Trimble, Westminster, Colorado, USA or AGCO Corporation, Duluth, Georgia, USA) on a weekly basis during the grain-filling period, from 21 March to 15 April 2022 for the first cycle and from 6 February to 8 March 2023 for the second cycle. The readings were taken in a period of time equivalent to 1 h before and 1 h after the solar zenith. The sampling of individual plots was carried out 60 cm from the crop canopy.

The NDVI was calculated via the following formula:

$$NDVI = (NIR - R)/(NIR + R) \qquad (1)$$

where NIR is the reflectance of near-infrared light and R is the reflectance of visible red light.

GLI estimation

To obtain the green leaf index (GLI), four scheduled flights were performed via an unmanned aerial vehicle (UAV) DJI Phantom 3 Pro unit (DJI, Shenzhen, China) on a weekly basis during the grain-filling period from 25 March to 14 April 2022 for the first cycle and from 6 February to 8 March 2023 for the second cycle to obtain orthophotos.

The images obtained with the UAV were analyzed via ImageJ software (Schneider et al., 2012) with the RGB measurement plugin to obtain basic statistics. The GLI index was subsequently obtained by averaging the reflectances of all the pixels contained in the image via the following formula:

$$GLI = ((G - R) + (G - B))/(2G + R + B) \qquad (2)$$

where G is the reflectance of visible green light, R is the reflectance of visible red light and B is the reflectance of visible blue light.

Measurement of protein content and quality characteristics

The protein content in the flour was measured with an infrared spectrophotometer (FOSS NIR Systems 5000-M Analyzer, Hillerød, Denmark) (method 39-10; AACC, 2005). A clean sample of 500 g was used for each genotype with three replicates, and the hectoliter weight (HLW) (kg hL^{-1}) of grain was determined on a volumetric scale (Seedburo Equipment Co., Chicago, Illinois, USA).

The grain hardness (GH) (%) was calculated from the pearling index in 20 g grains, which indicates the ease of partial removal of the outer layers via a standardized abrasion procedure. Values less than 47% are classified as soft endosperm grains. Using a Brabender mill (Quadrumat Senior, CW Brabender OHG, Duisburg, Germany) and sifting through a mesh 129 µm in diameter, refined flour was obtained. The strength (DS) and the tenacity/extensibility ratio (TE) of the dough were calculated from the alveogram, which was obtained from 60 g refined flour via the Chopin Alveograph (Tripette & Renaud, Asnières-sur-Seine, France) via method 54-30A of the AACC (2005).

The variables used for the statistical analysis were days to heading (DH), grain yield (GY), average of the NDVI (ANDVI), average of the GLI (AGLI), thousand-grain weight (TGW), harvest index (HI), biomass (BIO), grains per square meter (GM2), spikes per square meter (SM2), protein in grain (PG), hectoliter weight (HLW), grain hardness (GH), protein in flour (PF), dough strength (DS), toughness-extensibility relationship (TER) and yellow belly (YB).

Statistical analysis

The results obtained from the NDVI and GLI were correlated with the agronomic and quality data via Pearson's correlation with SAS version 9.4 software (SAS Institute, Cary, North Carolina, USA). For the principal component analysis (PCA), the FactoMIneR package was used (Lê et al., 2008), with the PCA function using the correlation matrix. To obtain the visualization based on ggplot2 (Wickham, 2016), the Factoextra package (Kassambara and Mundt, 2020) was used with the fviz_pca_biplot function; both packages were run on the free software R version 4.1.1 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS AND DISCUSSION

Table 2 shows that, in the two irrigations, protein in grain (PG) was negatively correlated with grain yield (GY), average of the green leaf index (GLI) (AGLI), grains per square meter (GM2), spikes per square meter (SM2), toughness-extensibility relationship (TER) and yellow belly (YB) and positively correlated with thousand-grain weight (TGW), harvest index (HI) and protein in flour (PF), which exemplifies the high correlation between the protein content in grain and the variables of industrial quality, vegetation indices and yield components. Similarly, this negative correlation between protein content and grain yield is explained by the interrelation between C and N metabolism in plants, since the increase in the availability of carbohydrates in the filling grain stage causes a negative relationship between grain yield and protein percentage (Fernández et al., 2019; Ganeva et al., 2024).

Table 2. Pearson's correlation between variables with $p \leq 0.05$ for the two irrigation schedules. DH: Days to heading; GY: grain yield; ANDVI: average number of shots of the normalized differential vegetation index (NDVI) index; AGLI: average number of shots of the green leaf index (GLI); TGW: weight of thousand grains; HI: harvest index; BIO: biomass; GM2: grains per square meter; SM2: spikes per square meter; PG: protein in grain; HLW: hectoliter weight; GH: grain hardness; PF: protein in flour; DS: dough strength; TER: toughness-extensibility relationship; YB: yellow belly.

	DH	GY	ANDVI	AGLI	TGW	HI	BIO	GM2	SM2	PG	HLW	GH	PF	DS	TER	YB
DH	1	0.40	0.63**	0.85**	-0.63	-0.45**	0.42**	0.55**	0.28	-0.69**	-0.23	-0.40*	-0.01	0.10	0.19	0.28
GY		1	0.67**	0.57**	-0.42*	-0.51**	0.95**	0.88**	0.74**	-0.59**	-0.21	-0.36	-0.24	0.17	0.31	0.65*
ANDVI			1	0.55**	-0.49**	-0.47*	0.71**	0.70**	0.56**	-0.46*	-0.27	-0.47*	-0.05	0.27	0.34	0.36
AGLI				1	-0.69**	-0.48*	0.53*	0.68**	0.35	-0.79**	-0.24	-0.55**	-0.20	0.28	0.40*	0.54**
TGW					1	0.49*	-0.44*	-0.72**	-0.42*	0.67**	0.05	0.66**	0.16	-0.43	-0.39	-0.28
HI						1	-0.57**	-0.49	-0.57**	0.45**	0.01	0.32	0.01	-0.03	-0.06	-0.41*
BIO							1	0.91**	0.78**	-0.49*	-0.19	-0.40	-0.07	0.22	0.25	0.56**
GM2								1	0.71**	-0.63**	-0.20	-0.58**	-0.12	0.36	0.38	0.53**
SM2									1	-0.41*	-0.04	-0.21	-0.13	0.02	0.13	0.48*
PG										1	0.12	0.51*	0.50**	-0.24	-0.50**	-0.60**
HLW											1	-0.11	-0.01	-0.03	-0.08	-0.12
GH												1	0.03	-0.6**	-0.60**	-0.28
PF													1	-0.05	-0.47*	-0.60**
DS														1	0.75*	0.13
TER															1	0.48*
YB																1

According to the three irrigations, the variable PG was only positively correlated with TGW and protein in flour (PF) and negatively correlated with YB (Table 3); in this sense, the negative correlation between the protein content in grain and the percentage of grains with a yellow belly has been described by Rodríguez-González et al. (2011), who also mentioned that the presence of yellow belly manifests itself mainly in unfavorable environments (Rodríguez-González et al., 2014).

Table 3. Pearson's correlation between variables with $p \leq 0.05$ for the three irrigation schedules. DH: Days to heading; GY: grain yield; ANDVI: average number of shots of the normalized differential vegetation index (NDVI) index; AGLI: average number of shots of the green leaf index (GLI); TGW: weight of thousand grains; HI: harvest index; BIO: biomass; GM2: grains per square meter; SM2: spikes per square meter; PG: protein in grain; HLW: hectoliter weight; GH: grain hardness; PF: protein in flour; DS: dough strength; TER: toughness-extensibility relationship; YB: yellow belly.

	DH	GY	ANDVI	AGLI	TGW	HI	BIO	GM2	SM2	PG	HLW	GH	PF	DS	TER	YB
DH	1	0.15	0.52**	0.81**	-0.62**	-0.88**	0.72**	0.46*	0.42*	-0.25	-0.08	-0.49*	0.12	0.44	0.35	0.02
GY		1	0.65**	0.51**	-0.29	-0.08	0.73**	0.79**	0.48*	-0.12	0.17	-0.32	-0.29	0.21	0.34	0.31
ANDVI			1	0.75**	-0.54**	-0.37	0.66**	0.71**	0.44*	-0.16	0.05	-0.52**	-0.04	0.30	0.41*	0.23
AGLI				1	-0.60**	-0.65**	0.82**	0.65**	0.45*	-0.38	0.069	-0.53**	-0.11	0.37	0.44*	0.34
TGW					1	0.61**	-0.62**	-0.78**	-0.45*	0.45*	-0.12	0.70**	0.14	-0.6**	-0.56**	-0.13
HI						1	-0.68**	-0.35	-0.26	0.28	0.03	0.49*	-0.19	-0.49*	-0.38	0.01
BIO							1	0.81**	0.56**	-0.31	0.12	-0.53**	-0.17	0.47*	0.46*	0.22
GM2								1	0.62**	-0.27	0.19	-0.61**	-0.25	0.49*	0.54**	0.21
SM2									1	0.09	-0.02	-0.32	0.07	0.33	0.31	-0.08
PG										1	0.06	0.31	0.65**	-0.18	-0.23	-0.61**
HLW											1	-0.14	-0.09	0.05	0.07	-0.13
GH												1	0.03	-0.7**	-0.82**	-0.22
PF													1	0.03	-0.11	-0.72**
DS														1	0.87**	0.10
TER															1	0.28

The vegetation indices average of the NDVI (ANDVI) and AGLI were positively correlated with the agronomic variables days to heading (DH) and GY in the four irrigation schedules, as shown in Table 4, which supports the predictive use of the vegetation indices during the heading stage to estimate performance. These characteristic conditions hinder the development of lines with high protein contents since the main characteristic that is sought during plant breeding is increased yield.

In the four irrigation schedules, a negative correlation between the protein content in the grain and the percentage of yellow belly and the grain yield was maintained, which was observed in three of the four irrigation schedules (Table 5). Similarly, a negative correlation was detected between the ANDVI index and the protein content in the grain, and a strong negative correlation was detected with the AGLI. This negative correlation between the vegetation indices and the protein content in the grain was detected in the two and five irrigation schedules. Irrigation regimes are contrasting irrigation regimes, which could indicate that this correlation occurs under extreme drought or humidity conditions.

Table 4. Pearson's correlation between variables with $p \leq 0.05$ for the four irrigation schedules. DH: Days to heading; GY: grain yield; ANDVI: average number of shots of the normalized differential vegetation index (NDVI) index; AGLI: average number of shots of the green leaf index (GLI); TGW: weight of thousand grains; HI: harvest index; BIO: biomass; GM2: grains per square meter; SM2: spikes per square meter; PG: protein in grain; HLW: hectoliter weight; GH: grain hardness; PF: protein in flour; DS: dough strength; TER: toughness-extensibility relationship; YB: yellow belly.

	DH	GY	ANDVI	AGLI	TGW	HI	BIO	GM2	SM2	PG	HLW	GH	PF	DS	TER	YB
DH	1	0.45**	0.55**	0.87**	-0.50*	-0.79**	0.68**	0.55**	0.50*	0.01	-0.11	-0.42*	0.12	0.48*	0.12	0.01
GY		1	0.42*	0.73**	-0.42*	-0.51**	0.91**	0.91**	0.64**	-0.56**	0.07	-0.54**	-0.47*	0.50*	0.26	0.56**
ANDVI			1	0.69**	-0.39	-0.51*	0.51**	0.47*	0.46*	-0.01	-0.02	-0.32	-0.03	0.31	0.02	0.15
AGLI				1	-0.51**	-0.80**	0.86**	0.76**	0.62**	-0.25	0.04	-0.53**	-0.16	0.47*	0.165	0.33
TGW					1	0.40**	-0.45*	-0.75**	-0.46*	0.27	-0.26	0.56**	0.30	-0.58**	-0.28	-0.19
HI						1	-0.81*	-0.55**	-0.40*	0.05	0.13	0.55**	0.02	-0.39	-0.22	-0.19
BIO							1	0.86**	0.63**	-0.40*	-0.03	-0.62**	-0.33	0.53**	0.25	0.47*
GM2								1	0.67**	-0.52**	0.16	-0.65**	-0.46	0.63**	0.32	0.50*
SM2									1	-0.13	0.04	-0.41*	-0.03	0.44*	-0.11	0.36
PG										1	-0.45*	0.36	0.71**	-0.19	-0.21	-0.71**
HLW											1	-0.36	-0.26	0.08	-0.003	0.29
GH												1	0.22	-0.76**	-0.50*	-0.22
PF													1	-0.04	-0.18	-0.78**
DS														1	0.54	-0.01
TER															1	-0.08
YB																1

Table 5. Pearson's correlation between variables with $p \leq 0.05$ for the five irrigation schedules. DH: Days to heading; GY: grain yield; ANDVI: average number of shots of the normalized differential vegetation index (NDVI); AGLI: average number of shots of the green leaf index (GLI) index; TGW: weight of thousand grains; HI: harvest index; BIO: biomass; GM2: grains per square meter; SM2: spikes per square meter; PG: protein in grain; HLW: hectoliter weight; GH: grain hardness; PF: protein in flour; DS: dough strength; TER: toughness-extensibility relationship; YB: yellow belly.

	DH	GY	ANDVI	AGLI	TGW	HI	BIO	GM2	SM2	PG	HLW	GH	PF	DS	TER	YB
DH	1	0.21	0.40	0.82**	-0.45**	-0.60**	0.31	0.17	-0.05	-0.56**	-0.39	0.24	0.41*	-0.14	0.37	0.01
GY		1	0.57**	0.55**	-0.03	0.12	0.31	0.25	0.27	-0.33	-0.04	-0.28	-0.23	-0.23	0.39	0.56**
ANDVI			1	0.63**	-0.15	-0.26	0.45*	0.33	0.41*	-0.47*	-0.16	-0.20	-0.31	-0.40	0.43*	0.15
AGLI				1	-0.32	-0.38	0.25	0.13	-0.02	-0.60**	-0.43*	-0.10	0.12	-0.12	0.54**	0.33
TGW					1	0.53**	-0.21	-0.45	0.06	0.37	0.46*	-0.03	-0.48**	-0.12	-0.04	-0.19
HI						1	-0.54**	-0.25	-0.14	0.27	0.26	-0.16	-0.29	0.20	0.15	-0.19
BIO							1	0.75**	0.71**	-0.36	-0.26	-0.03	0.11	-0.31	0.05	0.47*
GM2								1	0.49*	-0.30	-0.30	-0.07	0.19	-0.08	0.19	0.50*
SM2									1	-0.29	-0.07	-0.36	-0.01	-0.12	0.21	0.36
PG										1	0.39	0.27	-0.17	0.01	-0.37	-0.71**
HLW											1	0.38	-0.62**	-0.36	-0.40	0.29
GH												1	-0.0003	-0.39	-0.47*	-0.22
PF													1	0.47	0.02	-0.78**
DS														1	0.22	-0.01
TER															1	-0.08
YB																1

Figure 1 shows the values of the correlation matrix, which revealed that the first three principal components represented 73.1% of the total variation (PC1 49%, PC2 14.2% and PC3 9.9%).

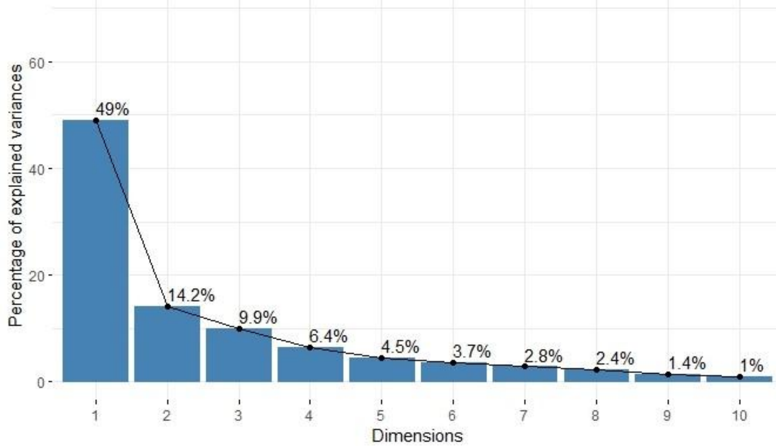

Figure 1. Percentage of the variance explained by the principal components.

The biplot in Figure 2 shows that the quality variables toughness-extensibility relationship (TER), dough strength (DS) and yellow belly (YB) had greater values in the four irrigation treatments, while in the five irrigation treatments, the highest values were in the weight of thousand grains (TGW), HI, grains per square meter (GM2), spikes per square meter (SM2) and biomass (BIO) variables. Similarly, the protein, PF and PG variables were positively correlated with each other and negatively correlated with the quality variables TER, DS and YB, whereas the average number of shots of the green leaf index (AGLI) and average number of shots of the normalized differential vegetation index (NDVI) (ANDVI) were positively correlated with each other and with grain yield (GY) and BIO, which coincides with the findings of Reznick et al. (2021) and Zajac et al. (2013), who described the correlation between the NDVI and BIO index, which can be explained by the intrinsic relationship that exists between yield and biomass, as suggested by Schierenbeck et al. (2015).

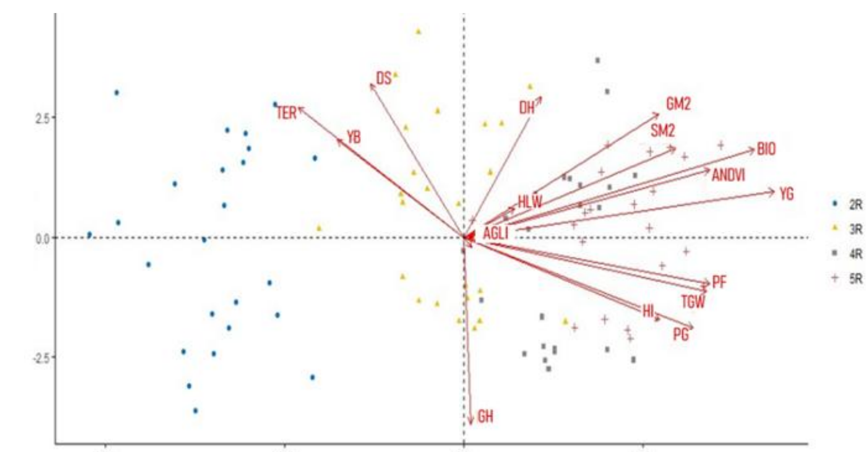

Figure 2. Biplot of the first two main components of the analysis of 24 genotypes evaluated in the four irrigation schedules. 2R: Two-irrigation schedule; 3R: three-irrigation schedule; 4R: four-irrigation schedule; 5R: five-irrigation schedule; DH: days to heading; GY: grain yield; ANDVI: average number of shots of the normalized differential vegetation index (NDVI); AGLI: average number of shots of the green leaf index (GLI); TGW: weight of thousand grains; HI: harvest index; BIO: biomass; GM2: grains per square meter; SM2: spikes per square meter; PG: protein in grain; HLW: hectoliter weight; GH: grain hardness; PF: protein in flour; DS: dough strength; TER: toughness-extensibility relationship; YB: yellow belly.

The results of multiple factor analysis (Table 6) revealed that the first two underlying factors (UFs) or CPs explained 88.03% of the total variance of the experiment, thus exceeding the range of minimum accumulated variance according to Field (2009), who suggested that the minimum values of accumulated variance should be in the range of 55%-65%. The factor with the largest eigenvalue has the greatest variance, which is successively reduced until the factors with small or negative eigenvalues that are generally omitted in the solutions are reached.

Table 6. Results of the multiple factor analysis.

PC	Eigenvalue	Difference	Proportion	Cumulative
1	240.457261	186.597313	0.7192	0.7192
2	53.859948	31.588877	0.1611	0.8803
3	22.271071	15.287027	0.0666	0.9469
4	6.984044	1.231392	0.0209	0.9678
5	5.752652	2.423618	0.0172	0.9850
6	3.329034	0.758335	0.0100	0.9949
7	2.570699	1.859299	0.0077	1.0026
8	0.711400	0.118381	0.0021	1.0047
9	0.593019	0.266268	0.0018	1.0065
10	0.326751	0.400790	0.0010	1.0075
11	-0.074039	0.100443	-0.0002	1.0073
12	-0.174483	0.191370	-0.0005	1.0068
13	-0.365853	0.087333	-0.0011	1.0057
14	-0.453185	0.203294	-0.0014	1.0043
15	-0.656480	0.125009	-0.0020	1.0023
16	-0.781489		-0.0023	1.0000

Significant differences were detected in the chi-square test (910.5, $p \leq 0.0001$) and the sufficiency of having used four UFs (190.3, $p \leq 0.0001$). The first is interpreted as interdependence between the variables evaluated, which justifies performing the AF (Table 7).

Table 7. Significance tests of factor analysis.

Test	DF	Chi-square	Pr >ChiSq
H0: There are no common factors	120	910.5746	< 0.0001
HA: At least there is one common factor			
H0: 4 Factors are sufficient	62	190.3368	< 0.0001
HA: More factors are needed			

In Figure 3, most of the variables evaluated are located in the first quadrant, with the exception of five variables (PG, HLW, PF, DS and TER); that is, there is no angle greater than 90° between most of the variables when the origin is taken as a reference, which gives rise to smaller angles between the vectors of each variable, and therefore, the positive correlations between them can be verified (Table 8) via Pearson correlation analysis (of the results through irrigation calendars). Four subgroups of variables were also formed, the first consisting only of the variable TER and the second consisting of the variables DS, PF, HLW and PG, with low associations between them, as observed in Table 8. The third group was formed with the variables SM2, GH and TGW because these variables are highly associated with each other (Table 8). The fourth group included seven variables, some of which were highly associated, such as GY and GM2, ANDVI and HI and AGLI with DH, all of which were positive and highly significant.

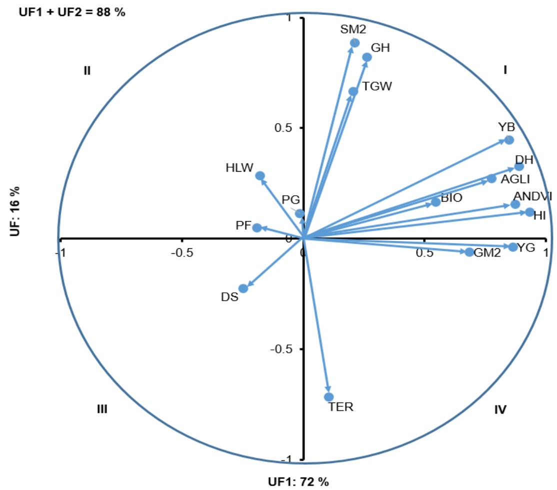

Figure 3. Multiple Factor Analysis across irrigation schedules for 15 variables, including agronomic and industrial quality variables. DH: Days to heading; GY: grain yield; ANDVI: average number of shots of the normalized differential vegetation index (NDVI); AGLI: average number of shots of the green leaf index (GLI); TGW: weight of thousand grains; HI: harvest index; BIO: biomass; GM2: grains per square meter; SM2: spikes per square meter; PG: protein in grain; HLW: hectoliter weight; GH: grain hardness; PF: protein in flour; DS: dough strength; TER: toughness-extensibility relationship; YB: yellow belly.

Table 8. Pearson's correlation between variables with $p \leq 0.05$ through irrigation schedules. DH: Days to heading; GY: grain yield; ANDVI: average number of shots of the normalized differential vegetation index (NDVI); AGLI: average number of shots of the green leaf index (GLI); TGW: weight of thousand grains; HI: harvest index; BIO: biomass; GM2: grains per square meter; SM2: spikes per square meter; PG: protein in grain; HLW: hectoliter weight; GH: grain hardness; PF: protein in flour; DS: dough strength; TER: toughness-extensibility relationship; YB: yellow belly.

9	DH	GY	ANDVI	AGLI	TGW	HI	BIO	GM2	SM2	PG	HLW	GH	PF	DS	TER	YB
DH	1.00	0.73**	0.90	0.66	0.49	0.91	0.72	0.58	0.49	0.11	-0.08	0.54	-0.17	-0.29	-0.13	0.87
		< 0.0001	< 0.0001	< 0.0001	0.00	< 0.0001	< 0.0001	< 0.0001	0.00	0.46	0.60	< 0.0001	0.25	0.05	0.39	< 0.0001
GY		1.00	0.76	0.73	0.18	0.77	0.40	0.66	0.11	-0.07	-0.10	0.24	-0.26	-0.29	0.02	0.72
			< 0.0001	< 0.0001	0.21	< 0.0001	0.01	< 0.0001	0.46	0.62	0.50	0.09	0.07	0.04	0.91	< 0.0001
ANDVI			1.00	0.58	0.22	0.90	0.65	0.51	0.31	0.13	-0.23	0.45	-0.10	-0.25	-0.03	0.79
				< 0.0001	0.14	< 0.0001	< 0.0001	0.00	0.03	0.37	0.11	0.00	0.51	0.09	0.86	< 0.0001
AGLI				1.00	0.34	0.65	0.04	0.57	0.41	-0.21	0.24	0.34	-0.44	-0.52	-0.13	0.90
					0.02	< 0.0001	0.79	< 0.0001	0.00	0.16	0.10	0.02	0.00	0.00	0.40	< 0.0001
TGW					1.00	0.17	0.36	0.11	0.65	0.14	0.39	0.54	-0.22	-0.22	-0.39	0.49
						0.24	0.01	0.45	< 0.0001	0.34	0.01	< 0.0001	0.14	0.12	0.01	0.00
HI						1.00	0.69	0.71	0.32	0.10	-0.21	0.40	-0.09	-0.24	-0.02	0.81
							< 0.0001	< 0.0001	0.03	0.50	0.16	0.00	0.55	0.10	0.90	< 0.0001
BIO							1.00	0.37	0.28	0.35	-0.34	0.37	0.20	0.15	-0.03	0.39
								0.01	0.05	0.01	0.02	0.01	0.17	0.31	0.86	0.01
GM2								1.00	0.14	0.03	0.15	0.15	-0.30	-0.36	0.01	0.55
									0.34	0.81	0.32	0.31	0.04	0.01	0.96	< 0.0001
SM2									1.00	0.21	0.26	0.80	-0.06	-0.34	-0.59	0.57
										0.16	0.08	< 0.0001	0.68	0.02	< 0.0001	< 0.0001
PG										1.00	-0.06	0.06	0.12	0.08	-0.03	-0.04
											0.67	0.67	0.40	0.59	0.84	0.77
HLW											1.00	0.16	-0.64	-0.62	-0.32	0.09
												0.27	< 0.0001	< 0.0001	0.02	0.54
GH												1.00	-0.02	-0.33	-0.70	0.58
													0.88	0.02	<.0001	< 0.0001
PF													1.00	0.76	0.00	-0.30
														< 0.0001	1.00	0.04
DS														1.00	0.23	-0.44
															0.11	0.00
TER															1.00	-0.24
																0.10
YB																1.00

Figure 4 shows the behavior of the protein content in the grain with respect to the vegetation index. Under the two-irrigation (Figure 3a) and five-irrigation (Figure 3d) calendars, high vegetation indices, NDVI and GLI, coincided with a relatively low protein content (crosses with relatively low contents (9.7% to < 10.3%)), and low vegetation indices coincided with relatively high protein content (triangles with relatively high protein contents in the grain (11.05% to < 11.36%)). However, in the three-irrigations (Figure 3b) and four-irrigations (Figure 3c) calendars, this behavior was not observed. This could indicate that this behavior occurs in contrasting irrigation environments.

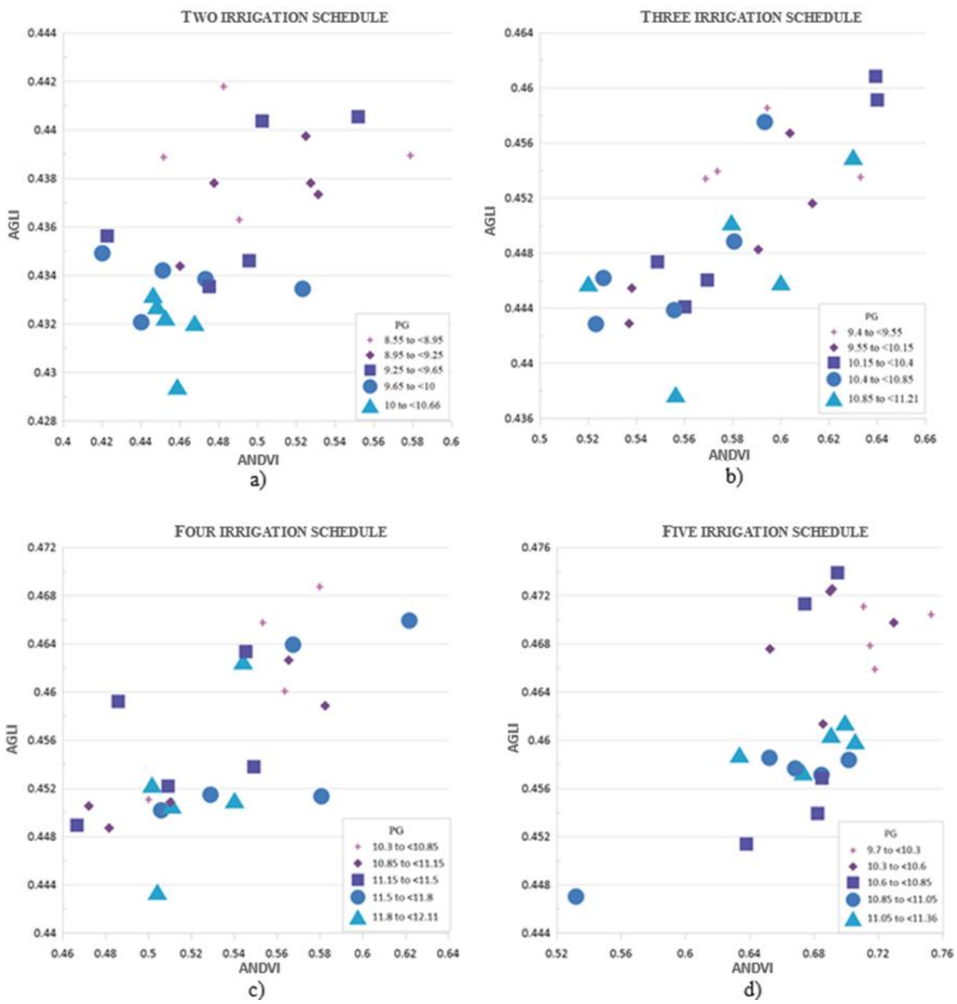

Figure 4. Bubble graph of the grain protein content (PG) in relation to the average normalized differential vegetation index (ANDVI) and the average green leaf index (AGLI). a) Two-irrigation schedule; b) three-irrigation schedule; c) four-irrigation schedule; c) five-irrigation schedule.

CONCLUSIONS

The significant correlation between wheat grain protein content and normalized difference vegetation index (NDVI) and green leaf index (GLI) is predominantly expressed in contrasting environments with respect to irrigation schedules. Therefore, vegetation indices NDVI and GLI obtained at stages prior to physiological maturity of grains can be used as a tool to predict yield and protein content of harvested grains.

Author contributions

Conceptualization: J.C.B-J., L.L-R., C.L.A-M. Methodology: L.L-R., E.S-M. Software: V.M-T., J.C.B-J. Validation: C.L.A-M., F.J.M-G. Formal analysis: J.C.B-J., V.M-T. Investigation: J.C.B-J., L.L-R., E.S-M., C.L.A-M. Resources: L.L-R., E.S-M. Data curation: J.C.B-J., V.M-T., F.J.M-G. Writing-original draft: J.C.B-J. Writing-review & editing: C.L.A-M. Visualization: E.S-M., V.M-T. Supervision: C.L.A-M. Project administration: L.L-R. E.S-M. Funding acquisition: L.L-R, E.S-M. All coauthors reviewed the final version and approved the manuscript before submission.

Acknowledgements

To the Secretariat of Science, Humanities, Technology and Innovation (SECIHTI), Mexico, for the scholarship awarded to J.C.B-J. for the scholarship granted for studies of Master of Science in Seed Production and Technology at TecNM/IT Roque.

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