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Understanding the impact of cultivar characteristics and environmental conditions on grain protein content and yield in wheat

Abstract. In the face of changing climatic conditions, there is a growing need to better understand the mechanisms influencing wheat yield and grain quality, particularly grain protein content (GPC). While genotype-by-environment interactions (GEI) have been widely studied, few investigations have focused on how specific environmental and varietal traits contribute to these interactions.

In this study, we applied the classification and regression tree (CART) method and a linear mixed model (LMM) to analyze field trial data across different wheat cultivars and varying environmental conditions. The analysis included factors such as soil nutrient content, rainfall distribution during the growing season, and varietal characteristics including plant height and growth duration. Our results revealed that GPC was primarily determined by rainfall during the grain-filling phase and the level of available nitrogen in the soil, while grain yield (GY) was strongly influenced by total rainfall during stem elongation and certain morphological traits. The variable "falling number" was included in the initial analysis but was excluded by the model due to its lack of predictive significance.

This study provides detailed insights into which environmental and varietal traits are most influential in shaping GEI effects on GPC and GY. The use of CART modelling enabled the identification of key predictors affecting cultivar responses under diverse growing conditions. These findings can support breeding and agronomic decision-making by offering predictive tools to select cultivars with improved stability in yield and grain quality under variable climatic conditions.

Keywords: winter wheat, grain yield, protein content, CART analysis

INTRODUCTION

Common wheat (*Triticum aestivum* ssp. *vulgare*) is a widely cultivated grain, used mainly to produce flour, which is used to make bread, cakes, cookies and other bakery products. It is an important source of food for populations around the world, rich in carbohydrates, plant protein, fiber, B vitamins and minerals such as iron, magnesium and zinc [FAO 2019]. However, despite its importance, common wheat faces various threats, including disease, pests, climate change and economic pressures.

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In response to these challenges, breeders and scientists are making efforts to improve wheat through breeding and genetic improvement. As part of these efforts, some scientists worldwide are working to refine breeding programs and evaluation methods to better understand the genetic basis of wheat traits and enable more precise recommendations to farmers [Mladenov et al. 2001]. Improved breeding programs can produce more productive and resistant wheat varieties that can more effectively meet food demand under changing climatic and economic conditions [Mladenov et al. 2001, Bustos-Korts et al. 2019].

To do this effectively, breeders need to understand how yield and protein content in grain are influenced, especially given the changing climatic conditions that increasingly affect crop outcomes. However, for this to be achieved, the genotype-environment (GE) interaction phenomenon needs to be well understood. Although there is a substantial amount of research on genotype-environment (GE) interactions, few studies have focused on explaining the observed genotype-environment interactions (GEI) through environmental characteristics and selected traits of varieties. Understanding the explanation of the observed GE interaction would significantly facilitate breeders' work and increase its effectiveness in these uncertain times.

During breeding work and official variety evaluations, a wide range of data on their properties is collected. This information can potentially be used to identify the causes of specific variety reactions to different environmental conditions by explaining the GE interactions in this way.

In this context, a particularly promising analytical tool could be the classification and regression trees (CART) algorithm [Breiman et al. 2017, Iwańska et al. 2018]. The CART algorithm allows for the construction of decision models that can identify patterns and relationships between various variables. In the context of studying GEI interactions, the use of CART enables the analysis of how different environmental factors and a variety of traits influence yield and protein content. This approach can provide a more precise and comprehensive understanding of the dynamics of these processes.

Analysis using the CART method can indicate which environmental features have the greatest impact on yield and protein content in various varieties and allows for the detection of interactions between the genetic traits of varieties and environmental conditions, which are difficult to identify using traditional statistical methods. Additionally, we believe that models based on CART can be used to predict how specific varieties will respond to future climate changes and environmental conditions [Zheng et al. 2009, 2010, Breiman et al. 2017].

Research on common wheat is of great importance in many fields, including agriculture, nutrition and environmental protection [Shewry and Hey 2015, Savary 2019]. The objective of this study is to examine the impact of soil-environment factors such as MgO and K_2O content, soil pH, average annual temperature, and rainfall on the quality parameters and grain yield of common wheat. This research aims to utilize modern analytical methods, such as CART analysis, to obtain more precise results that can have practical applications in agriculture. By better understanding these relationships, the study will contribute to advancements in wheat crop improvement, which is essential to address future challenges and ensure global food security. The greatest influence on the protein content of the grain was exerted by Zeleny's sedimentation value (SV) and resistance to fungal diseases, while the grain yield (GY) was influenced by the thousand-grain weight (TGW), disease resistance, and average annual temperature. These results indicate the need for an integrated approach to the assessment of varietal characteristics and environmental conditions in the wheat breeding process.

MATERIAL AND METHODS

Observations for yield and protein content were carried out in a field experiment, were obtained from 12 locations where multi-environmental winter wheat trials were conducted from 2015/2016 to 2019/2020 growing seasons by the Polish Research Center for Cultivar Testing (COBORU). The results of observations from the 12 study trial locations, based on selected soil-environmental characteristics, are presented in table 1. A total of 55 varieties were tested. The varieties selected for the experiments were divided into technological groups based on the Polish quality system E (highest), A (good quality) and B (bread-making). According to the Polish quality system of the 55 common wheat varieties tested, 28 (49%) belonged to class A, 26 (49%) belonged to class B, and one (2%) belonged to class E. The study involved the application of a single level of crop management: the high level of crop management (HIM). Under HIM, a higher nitrogen rate (40 kg N ha^{-1} at BBCH 59) was applied compared to the optimal rate for soil conditions at each site. In addition, foliar fertilizers were used (MgO 250 g ha^{-1}, Cu 50 g ha^{-1}, Mn 150 g ha^{-1}, Zn 80 g ha^{-1}), as well as two fungicides at BBCH 31–32 (carbendazim, dose 625 g ha^{-1}) and BBCH 49–60 (fenpropidin, dose 550 g ha^{-1}), and a growth regulator (trinexapac-ethyl, dose 125 g ha^{-1}) at BBCH 31. A high level of crop management was adopted to demonstrate the optimal yield potential of common wheat. Each experimental field was divided according to a split-block design with two replications, where crop management was the main factor and varieties were a side factor. The area of a single plot was 15 m^2. Year and location combinations were treated as separate environments. Agronomic traits evaluated included grain yield (GY) and grain protein content (GPC) in percent. Grain samples were milled in the laboratory using a Brabender Quadrumat Senior mill. Protein content (N × 5.7) was determined using the Kjeldahl method (Foss Tecator, Denmark) based on the ICC 105/2 method [ICC 1994].

Table 1. Characteristics of agroecological parameters in 12 trial locations

Locations	Longitude and latitude	Range of annual mean temperatures (°C)^A	Range of annual mean rainfall (mm)^A	Mean yield (t ha^{-1})^A	Arable land suitability groups class^B	Soil class^C	P_2O_5 content in soil (mg 100 g^{-1})^A	K_2O content in soil (mg 100 g^{-1})^A	MgO content in soil (mg 100 g^{-1})^A	Soil pH^A
Bezek	51.11; 23.15	8–10	560–580	9.22	2	IIIa–IVb	15.0	13	4.0	5.6
Białogard	54.0; 15.59	6–8	600–750	8.05	4	IIIb	15.0	12	3.0	5.8
Glubczyce	50.194; 1782	8.5–9	600–700	12.02	1	II–IVa	30.3	21	13.8	6.5
Jelenia Góra	50.856; 15.70	8–10	650–700	10.02	2	IVa	26.7	27	4.0	6.1
Kaweczyn	52.168; 20.34	8–9.5	600–700	9.74	4	IIIb	20.0	15	9.0	6.5
Kościelna Wieś	51.48; 18.01	7–9	550–650	10.72	2	IIIa	17.0	18	8.0	6.2
Krzyżewo	53.025; 22.75	7–8	650–700	9.12	4	IIIb	31.0	18	11.0	6.4
Pawłowice	50.454; 22.75	6–8	550–750	12.64	2	IIIb	9.2	19	7.6	6.0
Sulejów	51.351; 19.867	8–10	550–650	9.38	2	II–IVa	11.0	25	10.0	6.6
Węgrzce	50.119; 19.082	7–9	550–700	8.72	2	IIIb	14.0	20	4.0	5.8
Lisewo	54.195; 18.52	8.5–9	600–700	8.57	2	II	19.0	21	7.0	6.6
Ruska Wieś	53.79; 22.20	6–8	550–650	11.13	2	IIIb	20.0	12	4.0	6.8

^{A} across growing seasons; ^{B} 1 – best, 14 – worst; ^{C} I – best, VI – worst

Table 2 presents the evaluation of the basic characteristics, encompassing the assessment of the fundamental grain quality traits and frost resistance as well as the average resistance to fungal diseases. These data were used to elucidate the effect of genotype or environment on yield and protein content using the CART method. This characteristic evaluation was conducted during the variety registration trials as part of the value for cultivation and use (VCU) assessments.

Table 2. Characteristics of winter wheat cultivars based on registration value for cultivation and use (VCU) assessments

Cultivars	Quality group	TGW	GPC	SV	GY	FN	Frost resistance	Fungal disease resistancy
Ambicja	A	vh	m	vh	h	vh	3.5	7.8
Apostel	A	h	m	vh	h	vh	3.5	7.7
Bataja	A	vh	m	vh	Vh	h	4	7.6
Błyskawica	A	h	l	h	h	m	4	7.7
Comandor	A	l	m	h	vh	vh	4.5	7.7
Euforia	A	m	m	h	vh	vh	6	8
Formacja	A	h	m	vh	h	vh	4.5	7.9
Impresja	A	h	m	h	m	vh	5	7.8
Kariatyda	A	h	m	h	m	h	4.5	8.4
Kometa	A	m	m	h	h	h	4	7.8
KWS Firebird	A	l	l	vh	vh	h	3	7.6
KWS Spencer	A	m	m	vh	vh	vh	3.5	8.1
KWS Universum	A	m	m	vh	vh	h	4.5	7.8
LG Keramik	A	m	m	vh	m	h	4	7.1
Lindbergh	A	m	m	h	h	h	4	6.7
Lokata	A	m	h	h	h	h	3.5	7.7
Nordkap	A	h	h	h	vh	vh	5	7
Opoka	A	h	h	vh	vh	vh	4.5	7.8
Patras	A	h	h	h	good	h	4	7.5
Plejada	A	m	l	h	vh	h	3.5	7.9
Reduta	A	l	m	vh	m	vh	5	7.9
RGT Kilimanjaro	A	m	m	h	vh	vh	4.5	7.7
RGT Metronom	A	m	h	vh	vh	vh	4.5	8
SY Cellist	A	m	m	vh	vh	vh	2.5	8
SY Dubaj	A	vh	h	vh	vh	h	5	8.1
SY Yukon	A	m	m	vh	vh	vh	4	7.9
Venecja	A	m	m	vh	h	h	5	7.8
Admont	B	l	l	h	vh	h	4.5	7.9
Argument	B	h	l	vh	h	h	4	7.7
Artist	B	h	m	vh	vh	vh	3.5	7.5
Bonanza	B	h	m	h	h	vh	4.5	7.9
Bosporus	B	l	l	h	vh	h	4	7.7
Hybery F1	B	m	m	h	h	h	3.5	7.9
KWS Donovan	B	m	l	vh	vh	h	3	8
KWS Talium	B	l	l	h	h	h	3	7.8
LG Jutta	B	m	l	h	vh	vh	5.5	7.9

Cultivars	Quality group	TGW	GPC	SV	GY	FN	Frost resistance	Fungal disease resistancy
Medalistka	B	m	m	h	h	h	5.5	7.6
MHR Promienna	B	l	l	vh	h	h	3.5	7.3
Opcja	B	m	m	h	h	h	3	7.7
Owacja	B	m	m	h	vh	vh	3	7.6
RGT Bilanz	B	m	h	h	h	h	4.5	7.9
RGT Provision	B	m	l	h	h	m	4	8
RGT Ritter	B	vh	l	h	h	h	2.5	7.7
RGT Specialist	B	l	l	vh	vh	vh	4	7.7
Rivero	B	l	m	h	h	h	3.5	7.8
Sfera	B	l	m	h	h	h	4	7.6
SU Mangold	B	m	l	h	vh	vh	4.5	8
SU Petronia	B	h	l	h	vh	m	4	7.6
SU Tarroca	B	vh	l	m	vh	m	2.5	7.6
SU Viedma	B	h	h	vh	h	vh	3.5	7.8
SY Orofino	B	h	l	h	vh	m	4	7.6
Symetria	B	l	l	h	vh	vh	4	7.6
Titanus	B	h	l	vh	h	h	3	8.1
Tytanika	B	h	h	h	h	Vh	5	7.7
Moschus	E	h	h	vh	h	vh	4	7.8

Quality group based on the Polish quality scheme: E – superior cultivar, A – good quality cultivar, B – bread cultivar, C – non-baking cultivar. Values of studied variables: very high – vh, high – h, medium – m, low – l, where
TGW – thousand-grain weight (g): low 35–37; medium 38–40; high 41–43; very high > 44;
GPC – grain protein content (%): low < 9; medium 9–10; high 11–13, very high > 13;
SV – Zeleny sedimentation value (ml); low 40–42; medium 43–45; high 46–48; very high > 48;
GY – grain yield (dt ha^{-1}): low 56–58; medium 59–61; high 62–63; very high > 63;
FN – Hagberg falling number (s); low 290–300; medium 301–310; high 311–320; very high > 321.

The grain yield and grain protein contents were analyses a single-stage approach for a linear mixed model (LMM 1):

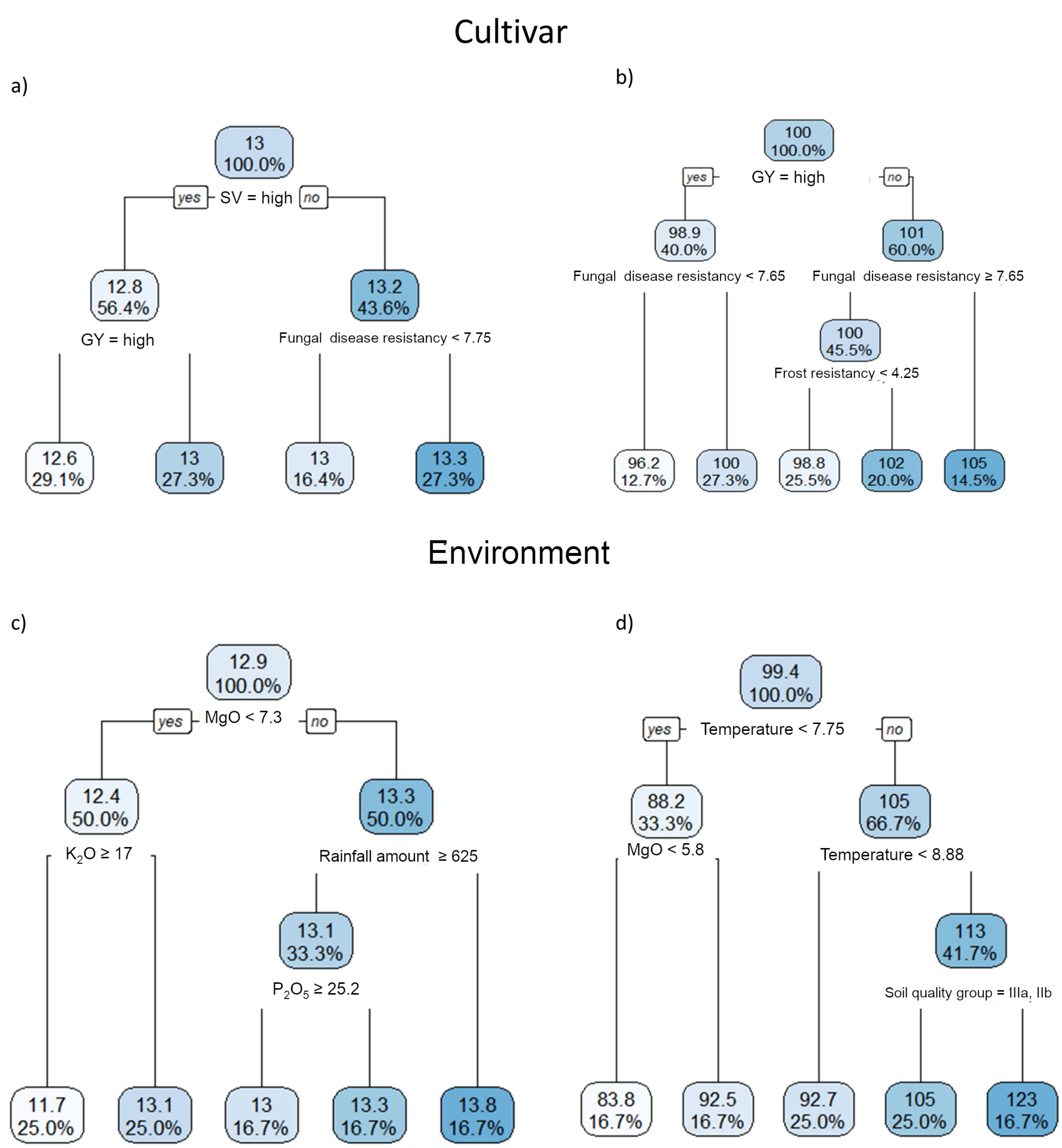
$$y_{ijklhn} = \mu + g_k + l_j + a_i + ga_{ki} + g_{lkj} + l_{aji} + g_{lakji} + r_{jih} + b_{jihn} + e_{ijklhn}$$

In the above equation y_{ijklhn} represents the grain yield. The following terms represent fixed and random effects in the model. The fixed effects include μ (overall mean), g_k (random effect of k^{th} cultivar), l_j (fixed effect of j^{th} location). a_i is the random effect of i^{th} growing season. The random interaction effects are represented by ga_{ki} (random interaction effect of k^{th} cultivar and i^{th} growing season), gl_{kj} (random interaction effect of k^{th} cultivar and j^{th} location), la_{ji} (random interaction effect of i^{th} growing season and j^{th} location), gla_{kji} (random interaction effect of k^{th} cultivar, j^{th} location and i^{th} growing season). r_{ji} account for the random effect of h^{th} replication nested in j^{th} location at i^{th} growing season. b_{jihn} is the random effect of n^{th} block nested in h^{th} replication at j^{th} location and i^{th} growing season. Lastly, e_{ijklhn} represents the random error associated with the yield observation (y_{ijklhn}).

The adjusted means for grain yield and grain protein content for varieties and the adjusted means for environments obtained from LMM model (LLM 1) were used in classification and regression trees (CART) methods. This allowed for assessing the impact of trial locations (characteristics from table 1) and for cultivar (characteristics from table 2) on yield and protein values yield and protein content in the study experiment. The sample size for CART analysis was 55 for cultivars and 60 observations for environments (combination of trail locations and growing seasons). The division into splits was based on the minimisation of the Gini impurity measure. To evaluate the CART model, cross-validation was performed using the k-fold method. This method was applied with 5 groups and 50 iterations. The use of cross-validation in this way allowed the determination of two parameters describing the fitting of the model estimation – accuracy and Cohen's kappa coefficient.

For the statistical analysis, we used the R 4.2.1 software package, the CART was obtained used rpart package.

Figure 1. Results of CART analysis for grain protein content (a, c) and grain yield (b, d) in cultivars and environments



GY – grain yield, TGW – thousand-grain weight (g), GPC – grain protein content (%), SV – Zeleny sedimentation value (ml), FN – Hagberg falling number

RESULTS

A CART analysis was conducted to examine causal relationships between wheat grain variables. Figure 1a shows the results of the CART analysis of GPC (grain protein content) based on variety-related variables. This model was characterized by the following values of the fit parameters – accuracy 0.91 ±0.3, Cohen's kappa coefficient 0.85 ±0.02.

The varietal trait that contributes most significantly to the reduction of variability in grain protein content (GPC) is SV (Zeleny sedimentation value). The "high" SV class corresponds to a lower GPC (12.8%) compared to other SV classes such as "very high", "medium", and "low" where the average GPC is 13.2%. For the "high" SV class, GPC is additionally influenced by grain yield (GY). When GY is classified as "high" the GPC is 0.4% lower. For other SV classes, the next variable affecting GPC is resistance to fungal diseases. If resistance is lower than 7.75, GPC decreases by 0.3%. Conversely, increased resistance to fungal diseases leads to higher GPC in common wheat varieties.

Figure 1c presents GPC results based on environmental characteristics, with value of accuracy 0.95 ±0.4 and Cohen's kappa coefficient 0.89 ±0.02. Locations with an average MgO dose below 7.3 kg ha^{-1} show a lower protein content (12.4%). Subsequently, lower GPC values are differentiated based on the K_2O level. For lower K_2O doses, protein content was 1.4% higher, reaching 13.1%. On the other hand, when the MgO content exceeds 7.3 kg ha^{-1}, higher GPC values are further divided based on the amount of precipitation – as rainfall increases, the grain protein content decreases.

The CART analysis results for grain yield (GY) based on varietal traits are presented in figure 1b. The fitting parameters had values: accuracy 0.91 ±0.3; Cohen's kappa coefficient 0.84 ±0.04. The most important variable contributing to data variance reduction is TGW (thousand grain weight). For "high" and "very high" TGW classes, the yield is lower. The data are then split based on the variety's resistance to fungal diseases.

Figure 1d shows the relationship between environmental conditions and GY. This model was characterized by the following values of the fit parameters – accuracy 0.87 ±0.5, Cohen's kappa coefficient 0.81 ±0.03. When the average annual temperature is below 7.75°C, grain yield is lower. In locations with higher MgO doses (above 5.8 kg ha^{-1}) and better soil quality, GY is higher.

DISCUSSION

Classification and regression trees (CART) analysis is widely utilized in various fields of scientific research and data analysis, including plant breeding, agronomy, and agricultural engineering [Zhang et al. 2017, Iwańska et al 2018]. In similar studies, CART analysis is primarily employed to identify significant influencing factors, model complex relationships, classify, and predict outcomes [Breiman et al. 2017, Johansson et al. 2020].

In our study, an increase in grain yield (GY) led to a decrease in grain protein content (GPC). Similar findings were reported by Oury and Godin [2007]. In our study, significant effects Zeleny sedimentation value (SV) and no significant Hagberg falling number (FN) of total on yield were observed. Additionally, the CART analysis provided valuable insights into the causal relationships between variables. The analysis demonstrated the significance of SV in reducing the variability in protein content, where high SV values were associated with lower GPC compared to other SV classes. Furthermore, the interaction between GY and SV affected GPC, while resistance to fungal diseases influenced GPC in specific SV classes. Similar studies, such as Zhang et al. [2017], utilized CART analysis to identify key genetic and environmental traits affecting baking properties. This study also examined other aspects related to wheat breeding and bread baking quality, including analysis of differences between wheat cultivars. The authors noted the need to compare various wheat cultivars in terms of their ability to produce high-quality bread, assessing differences in genetic traits such as protein content, gluten strength, and starch content, which affect bread baking quality. Assessment of environmental impact: Since environmental conditions can significantly impact wheat development and quality, this was highlighted as crucial in many related studies [Hatfield et al. 2011, Zhang et al. 2017, Derejko et al. 2021].

Given that such findings confirm the necessity of continuing research, we believe incorporating the latest data and scientific achievements related to the study topic can make our research more current and useful. Similar to this study, we acknowledge the value of CART analysis for such investigations.

In our study, the characterization of locations revealed that MgO dose and rainfall amount affected GPC. Lower MgO doses and higher rainfall were associated with lower protein content in grains. In the study by McIntyre et al. [2010], the authors investigated environmental factors affecting wheat grain protein content. CART analysis was applied to identify the key factors influencing this quality trait. The authors recognize the value of CART analysis in such studies, as it enables a thorough understanding of the complex relationships between variables and the identification of the most important factors influencing grain quality.

The CART analysis results for GY based on varietal traits highlighted the importance of thousand-grain weight (TGW) in reducing data variance. We believe that, besides studying environmental effects, it is also crucial to investigate varietal traits. Authors in studies [Oury et al. 2007, Sanchez-Garcia et al. 2015] share this perspective. Resistance to fungal diseases was also found to be significant for determining GY. Moreover, the relationship between environmental conditions and GY showed that lower average annual temperatures were associated with lower yields. Locations with higher MgO doses and better soil quality exhibited higher grain yields.

Our study is significant for several reasons. Firstly, it confirms previous findings on the impact of genetic and environmental traits on wheat quality and yield [Iwańska et al. 2017, Derejko et al. 2021, Wójcik-Gront et al. 2022]. Secondly, the use of CART analysis allowed for a deeper understanding of the complex relationships between the studied variables, which can contribute to more precise recommendations for wheat breeders. Furthermore, our results highlight the necessity of further research on the impact of environmental conditions on wheat quality and yield, which is crucial in the context of changing climatic and economic conditions.

Future research should focus on integrating the latest scientific achievements and data regarding wheat breeding and assessing the long-term effects of climate change on yields and wheat quality. Additionally, it is essential to continue efforts in identifying and breeding wheat cultivars resistant to diseases and varying environmental conditions, which can contribute to enhancing global food security.

CONCLUSIONS

Integrating genetic and environmental variables in CART and path analyses enables breeders to make informed decisions and select cultivars that meet specific objectives. Further investigations incorporating a broader range of variables will enhance our knowledge of the complex dynamics governing grain quality and yield in this important crop. They will contribute to more accurate cultivar recommendations and the development of resilient cultivars for various environmental conditions.

These conclusions demonstrate the progress made in wheat breeding and the need for continued integrated approaches to further improve the yield and quality of future wheat cultivars.

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