

Article

Morphological Characterization of 1322 Winter Wheat (*Triticum aestivum* L.) Varieties from EU Referent Collection

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Abstract: The wheat grain yields increased in EU from 4.98 t ha⁻¹ to 5.45 t ha⁻¹ in the periods from 2006 to 2014 to from 2015 to 2023. It is hypothesized that changes in specific morphological traits over the years resulted in grain yield increase due to the utilization of new wheat varieties in production. To highlight the current status and changes over time, we evaluated a comprehensive panel of 1322 wheat varieties that included testing of morphological traits of varieties recognized from period from 2006 till 2023. Positive relation of registration year with traits such as seed color, glaucosity of neck of culm, plant height, ear length, scurs and awns length, ear color, and shape of the beak of the lower glume was obtained. The most significant changes over time resulted in a darker color of the seed, decreased area of hairiness of the convex surface of the apical rachis segment, enhanced glaucosity of the neck of the culm and decreased frequency of the plants with recurved flag leaves. It was shown that traits such as the frequency of plants with recurved flag leaves, time of emergence, glaucosity of flag leaves, existence of scurs and awns, and area of the hairiness of the convex surface of the apical rachis segment had significant decreases over time. This research demonstrated the importance of twelve morphological traits in the varietal improvement of grain yield over the time from 2006 to 2023.

Keywords: grain yield; regression; seed color; year of registration

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1. Introduction

Wheat (*Triticum aestivum* L.) is one of the world's most important food crops, and a primary food source for an estimated 35% of the world's population [1]. Wheat alone supplies a fifth of global food calories and protein [2]. Although global wheat production is currently over 700 million tons [3], the demand for wheat production is projected to increase by 60% by 2050. Moreover, it is expected that wheat production will decline due to decreases in land suitability in low latitude areas and to climate changes (high temperatures, heat waves, and droughts) [4], while the world population will increase. Furthermore, it is not negligible that over time, new pests and diseases and new races of existing diseases have emerged [5]. Therefore, there is a timely, dire need to evolve new wheat varieties with traits that could tolerate different stresses, but also produce higher grain yield. Traits such as crop architecture, phenological date, and spike- and grain-related morphological characteristics are involved in grain yield formation [6]. Due to variations in climate-, biotic-, and abiotic stresses, there are demands for the adaptation of wheat varieties with different phenotypic traits.

The interaction of complex networks of genes with each other and the environment underlies wheat adaptation and influences many phenotypic traits of wheat [7]. According to Hyles et al. [8], wheat adaptation could be achieved through variation in phenology (seasonal timing of the lifecycle) and related traits (e.g., those affecting plant architecture). Nevertheless, the phenological expression of wheat plants is impacted by the environment and genetics, thus allowing wheat genotypes to achieve optimum productivity in

the environment in which they were primarily created [2]. In the same research it was also reported that environmental effects (E) explained approximately 72.2% of the total variation, whereas genotype (G) and GE interactions explained 6.9 and 18.3%, respectively. The assessment of genetic diversity is carried out via different marker techniques, such as morphological, biochemical, and molecular markers [9]. The morphological characteristics of wheat differ according to the genetic composition of the variety [10]. Moreover, the evaluations of phenotypic diversity or morphological traits are important in the differentiation of wheat varieties [11]. Further, wheat genotypes created at breeding programs undergo varietal testing for morphological characteristics known as distinctness, uniformity, and stability (DUS) before their release into production, and are granted plant variety protection (PVP), the most common system of intellectual property protection. PVP is also known as the breeder's right [12], and was introduced in 1961 by the International Union for the Protection of New Varieties of Plants [13]. The DUS testing is carried out in a trial field or greenhouse following internationally agreed protocols and UPOV guidelines. However, in many EU countries, according to CPVO, 27 morphological traits are characterized to distinguish DUS protocol [14].

The Croatian Agency for Agriculture and Food in Osijek maintains a collection of *Triticum* spp., including more than 1000 genotypes of *T. aestivum*, and provides DUS testing. Such a collection represents a wide source of genetic variability for morphological traits, potentially useful in modern breeding programs. The information on the relationship between traits and year of variety registration can be of great use to breeders, as it could indicate the traits on which selection should be directed in order to increase grain yield. It has been reported that indirect selection, targeting the morphological traits that contribute to grain yield, can be more efficient than direct selection for higher grain yield [15]. The aim of the present study was to evaluate which morphological traits, in various ways, contributed to the improvement of grain yield from 2006 to 2023.

2. Materials and Methods

A total of 1322 winter wheat (*Triticum aestivum* L.) varieties were grown in official DUS trials at the station of the Croatian Agency for Agriculture and Food in Osijek at 45°32' N, 18°44' E longitude and altitude of 94 m above mean sea level. The experimental field plot consisted of 10 rows, 5 m long, with a 12.5 cm inter-row spacing.

All trials were adjusted to CPVO protocol [14] for DUS testing, which guarantees the optimized conditions for morphological scoring of varieties.

All varieties were grown during two growing seasons (2020/21–2021/22) to evaluate their morphological characteristics. Fertilizer application and other agronomical management practices were performed, as per recommendations for the test location. During the growing season, insecticides and herbicides were applied as needed for weed and aphid control, as well as fungicides. At physiological maturity, the harvest was carried out manually taking randomly 100 ears with stems in both seasons during the first half of July.

2.1. Investigated DUS Traits

Assessment of DUS traits followed the method for observation of the characteristics indicated by the Table 1 [14].

Seed: color (A), seed coloration with phenol (B), and coleoptile: anthocyanin coloration (C) (Table 1) were DUS traits evaluated on the submitted seed. Eleven traits (labelled D–M and XYZ in Table 1) were measured in a field plot during growing season. Thirteen traits (labelled as M–XY in Table 1) were evaluated on samples after harvest from the field-grown plots.

Table 1. Morphological characteristics used to evaluate the 1322 winter wheat varieties.

Label	DUS Characteristic	States of Expression
A	Seed: color	1 white, 2 reddish, 3 purple, 4 bluish
B	Seed: coloration with phenol	1 absent or very light, 3 light, 5 medium, 7 dark, 9 very dark
C	Coleoptile: anthocyanin coloration	1 absent or very weak, 3 weak, 5 medium, 7 strong, 9 very strong
D	Plant: growth habit	1 erect, 3 semi-erect, 5 intermediate, 7 semi-prostrate, 9 prostrate
E	Plants: frequency of plants with recurved flag leaves	1 absent or very low, 3 low, 5, medium, 7 high, 9 very high
F	Flag leaf: anthocyanin coloration of auricles	1 absent or very weak, 2 medium, 3 strong
G	Time of ear emergence (first spikelet visible on 50% of ears)	1 very early, 3 early, 5 medium, 7 late, 9 very late
H	Flag leaf: glaucosity of sheath	1 absent or very weak, 3 weak, 5 medium, 7 strong, 9 very strong
I	Flag leaf: glaucosity of blade	1 absent or very weak, 3 weak, 5 medium, 7 strong, 9 very strong
J	Ear: glaucosity	1 absent or very weak, 3 weak, 5 medium, 7 strong, 9 very strong
K	Culm: glaucosity of neck	1 absent or very weak, 3 weak, 5 medium, 7 strong, 9 very strong
L	Lower glume: hairiness of external surface	1 absent, 9 present
M	Plant: length (stem, ears, awns, and scurs)	1 very short, 3 short, 5 medium, 7 long, 9 very long
N	Straw: pith in cross section	1 thin, 2 medium, 3 thick or filled
O	Ear: density	1 very lax, 3 lax, 5 medium, 7 dense, 9 very dense
P	Ear: length (excluding awns and scurs)	1 very short, 3 short, 5 medium, 7 long, 9 very long
R	Ear: scurs and awns	1 both absent, 2 scurs present, 3 awns present
S	Ear: length of scurs or awns	1 very short, 3 short, 5 medium, 7 long, 9 very long
T	Ear: color	1 white, 2 colored (red)
U	Ear: shape in profile	1 tapering, 2 parallel sided, 3 slightly clavate, 4 strongly clavate, 5 fusiform
V	Apical rachis segment: area of hairiness of the convex surface	1 absent or very small, 3 small, 5 medium, 7 large, 9 very large
Z	Lower glume: shoulder width	1 very narrow, 3 narrow, 5 medium, 7 broad, 9 very broad
X	Lower glume: shoulder shape	1 strongly sloping, 3 slightly sloping, 5 horizontal, 7 slightly elevated, 9 strongly elevated
Y	Lower glume: length of beak	1 very short, 3 short, 5 medium, 7 long, 9 very long
XX	Lower glume: shape of beak	1 straight, 3 slightly curved, 5 moderately curved, 7 strongly curved, 9 geniculate
YY	Lower glume: area of hairiness on internal surface	1 very small, 3 medium, 5 very large
XY	Seasonal type	1 winter type, 2 alternative type, 3 spring type

2.2. Statistical Analysis

Descriptive statistics, a Pareto chart (general regression model), regression analysis (multiple regression), and principal component analysis were performed by Statistica software (version 14). The Pareto chart as a frequency histogram showed the amount of the effect of each factor on the response in decreasing order, and a line going across the columns indicated how large an effect has to be (i.e., how long a column must be) to be statistically significant ($p < 0.05$).

3. Results

The data for grain yield were obtained from The World Bank data [16]. It was evident that grain yield gradually increased from the period from 2006 to 2014 (4.98 t ha^{-1}) till the period from 2015 to 2023 (5.45 t ha^{-1}).

Mean, minimum, and maximum values and standard deviations for 27 continuously variable descriptors are reported in the Supplementary Table S1 and Figure 1. Only ear color and seasonal type were dimorphic traits, while all other traits showed polymorphism. Three traits viz., hairiness of external surface of lower glume, pith in cross section of straw and coleoptile color were trimorphic. Seed color and anthocyanin coloration of auricles of flag leaves recorded four states of expression.

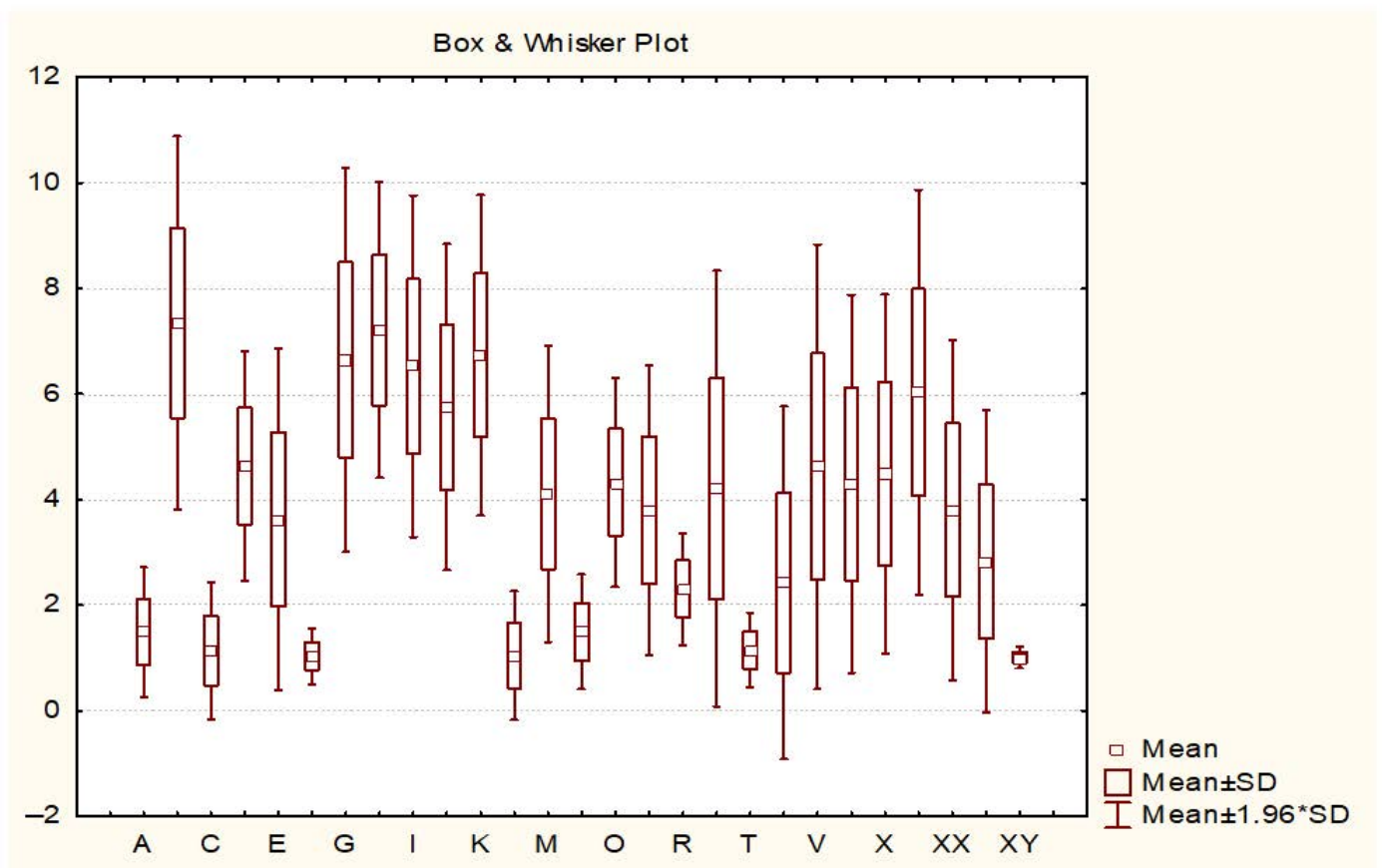


Figure 1. Box-plots for the 27 DUS testing traits (A–XY) in 1322 wheat varieties. For the letter in the x-axis, refer to Table 1.

3.1. Regression Analysis of Investigated DUS Traits

The Pareto chart showed the amount of effect of each factor on the response in decreasing order (Figure 2). From 27 investigated traits, 12 were significantly changed over the years. Those traits were in descending order: seed color, area of hairiness of convex surface of apical rachis segment, glaucosity of neck of culm, frequency of plants with recurved flag leaves, scurs and awns, ear length, glaucosity of flag leaves, plant length, time of emergence, ear color, shape of beak of lower glume, and length of scurs and awns.

The regression analysis among variables is reported in Table 2. Nine relations between observed traits and year of registration of varieties were highly significant ($p < 0.01$) while three traits that influenced year of registration were significant at $p < 0.05$. The results showed that any increase in seed color, glaucosity of neck of culm, plant length, ear length, scurs and awns length, ear color, and shape of beak of lower glume will result in proportionate increase as the years go by.

On the opposite, as years go by, proportionate decrease in values of traits such as frequency of plants with recurved flag leaves, time of emergence, glaucosity of flag leaves, scurs and awns, and area of hairiness of convex surface of apical rachis segment is obtained.

3.2. Principal Component Analysis of Twelve Investigated DUS Traits

The first eleven factors, obtained from original data, accounted for 100% of total variation (Figure 3). Principal component analysis showed that the first four PCs (PC1 to PC4) had eigenvalues > 1.0 and cumulatively accounted for 59.17% of the total variation.

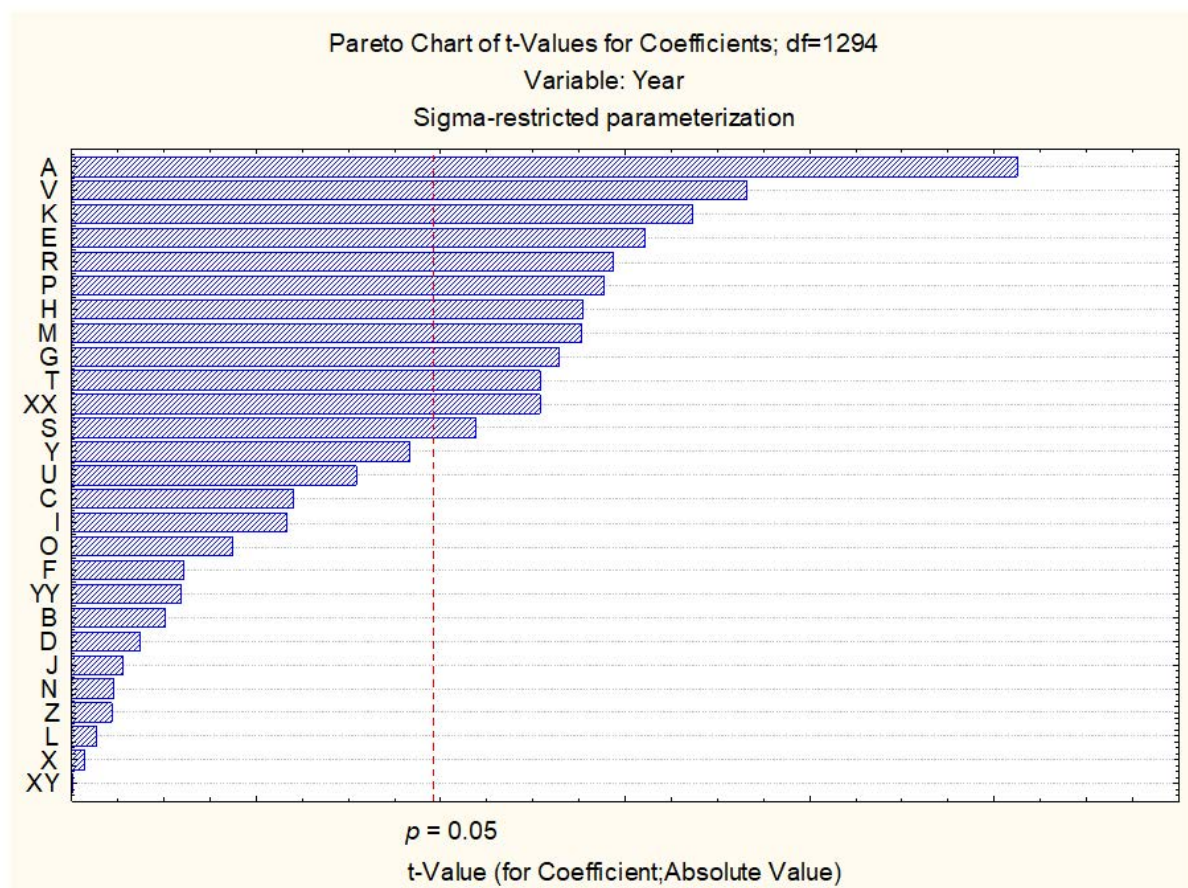


Figure 2. Pareto chart for identifying the significant changes of investigated traits over years. Explanation of letters (A–XY) can be found in Table 1 (Section 2).

Table 2. Regression analysis of 27 traits against the registration year of the wheat varieties.

$R = 0.37474083$ $R^2 = 0.14043069$ Adjusted $R^2 = 0.12249532$ $F(27,1294) = 7.8298$ p				
	Unstandardized Beta (B)	Standard Error of B	t(1294)	p-Level
Seed: color	0.279	0.055	5.126	0.000 **
Seed: coloration with phenol	0.009	0.018	0.504	0.614
Coleoptile: anthocyanin coloration	−0.056	0.046	−1.202	0.229
Plant: growth habit	0.012	0.031	0.368	0.713
Plants: frequency of plants with recurved flag leaves	−0.064	0.021	−3.107	0.002 **
Flag leaf: anthocyanin coloration of auricles	0.069	0.114	0.604	0.546
Time of ear emergence (first spikelet visible on 50% of ears)	−0.059	0.022	−2.644	0.008 **
Flag leaf: glaucosity of sheath	−0.099	0.036	−2.767	0.006 **
Flag leaf: glaucosity of blade	0.033	0.028	1.166	0.244
Ear: glaucosity	0.008	0.028	0.277	0.782
Culm: glaucosity of neck	0.117	0.035	3.368	0.001 **
Lower glume: hairiness of external surface	−0.007	0.050	−0.135	0.893
Plant: length (stem, ears, awns, and scurs)	0.066	0.024	2.767	0.006 **
Straw: pith in cross section	0.013	0.058	0.229	0.819
Ear: density	0.028	0.032	0.873	0.383
Ear: length (excluding awns and scurs)	0.071	0.025	2.886	0.004 **
Ear: scurs and awns	−0.387	0.132	−2.937	0.003 **

Table 2. Cont.

$R = 0.37474083$ $R^2 = 0.14043069$ Adjusted $R^2 = 0.12249532$ $F(27,1294) = 7.8298$ p				
	Unstandardized Beta (B)	Standard Error of B	t(1294)	p-Level
Ear: length of scurs or awns	0.072	0.033	2.189	0.029 *
Ear: color	0.232	0.091	2.540	0.011 *
Ear: shape in profile	0.030	0.019	1.542	0.123
Apical rachis segment: area of hairiness of convex surface	−0.054	0.015	−3.662	0.000 **
Lower glume: shoulder width	0.004	0.021	0.218	0.827
Lower glume: shoulder shape	−0.001	0.020	−0.073	0.942
Lower glume: length of beak	0.050	0.027	1.833	0.067
Lower glume: shape of beak	0.053	0.021	2.539	0.011 *
Lower glume: area of hairiness on internal surface	0.014	0.024	0.596	0.551
Seasonal type	0.001	0.290	0.003	0.998

*, **—significance at $p < 0.05$ and 0.01 , respectively.

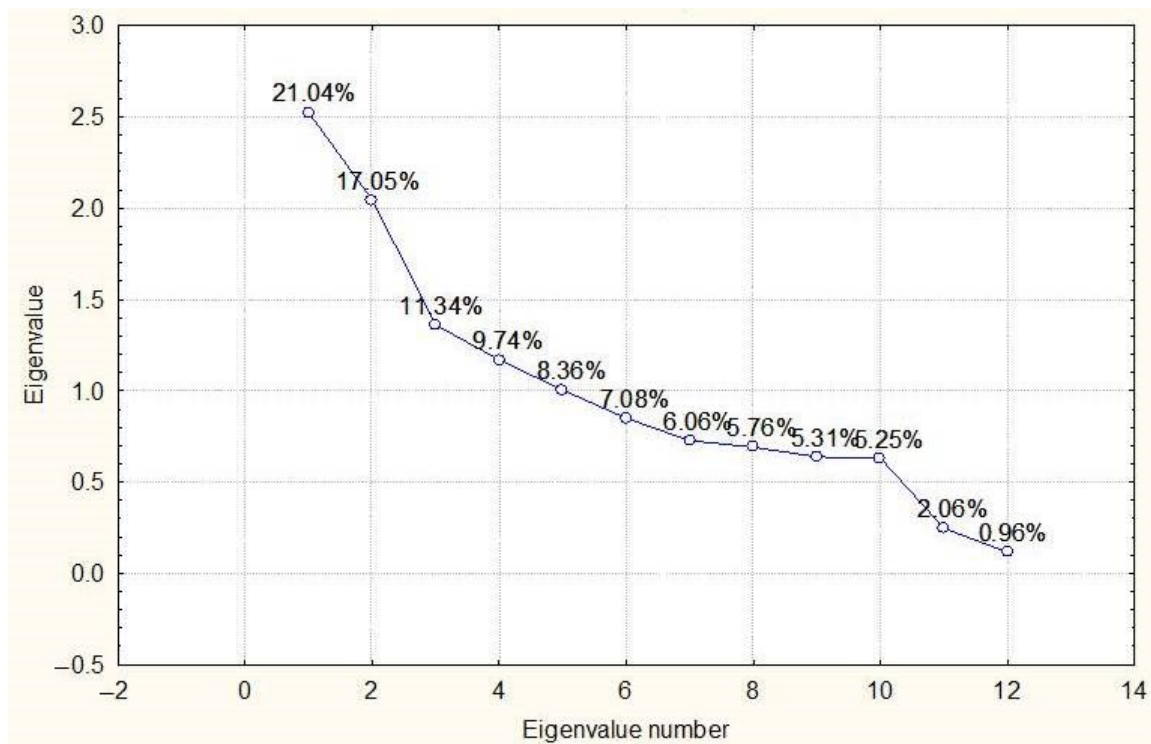


Figure 3. Eigenvalues of correlation matrix of twelve principal components.

The first PC axes accounted for 21.04% of the total variation, whereas the second PC explained 17.05% (Figure 4). The high level of variation in all four PC axes showed a high level of variation for these twelve characters. Characters with the largest absolute value closer to unity within the first principal component influences the clustering more than those with lower absolute value closer to zero. Accordingly, the major contributing traits for diversity in first principal component (PC1) were scurs and awns and their length (Supplementary Table S2). Presence of positive and negative correlation trends between the components and the variables are interpreted by positive and negative loading. Similarly, for second principal component (PC2), scurs and awns and the glaucosity of the neck of the culm were major contributors for the diversity. The major contributing character for the diversity in the third principal component 3 (PC3) was seed color, while area of convex surface of apical rachis segment in principal component four (PC4). Traits with high coefficients in the PC1 to PC2 should be considered as more significant because these axes explain almost 40% of the whole variation.

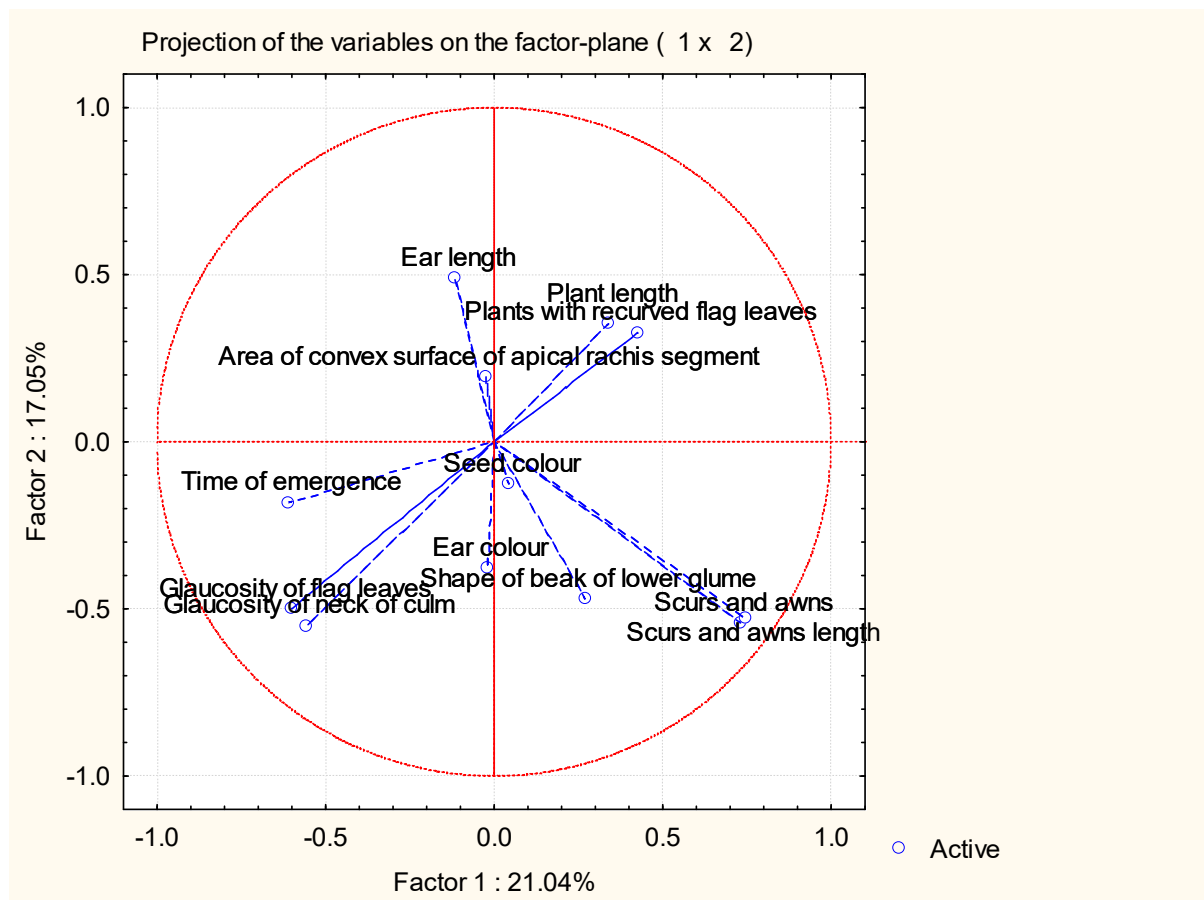


Figure 4. Principal component analysis of twelve DUS traits and registration year as supplementary variable.

4. Discussion

A range of DUS descriptors was elaborated for 1322 diverse winter wheat accessions maintained at the Croatian Agency for Agriculture and Food, and these have uses for both the characterization of germplasm and its evaluation for use by farmers and breeders. Each morphological trait among the 27 investigated was evaluated over two years and a wide range of diversity was observed. Diversity in the germplasm is essential to meet different purposes of the crop, such as the enhancement of different agronomical properties, better adaptability, improved grain and flour quality, and pest and disease resistance [17]. It is important to assess diversity in wheat germplasm to understand the influence of environmental fluctuations and to exploit genetic resources in breeding programs. Similar patterns of DUS descriptors were observed for varieties in an investigation by Malik et al. [18]. Morphological characteristics were used for descriptive purposes, and are traditionally used to distinguish plant varieties. The findings of Wang et al. [19] indicated that with the current breeding objectives, the selection of some noneconomic characteristics of wheat varieties, such as awn color, stem color, and glume color, seemed to be able to enrich the genetic diversity of varieties.

4.1. Morphological Characters of Different Plant Tissues or Whole Plant

In the current research, most of the morphological characteristics used to evaluate the 1322 winter varieties were rich in variation, indicating the high genetic diversity among them in morphological traits. This also means that 1322 winter wheat materials have high genetic diversity in morphological traits. This high variability of different varieties might be as a result of different origin, selections made by breeders according to aims, and environmental conditions [20]. However, the anthocyanin coloration of coleoptile,

anthocyanin coloration of auricles of flag leaf, hairiness of external surface of the lower glume, and seasonal type had fewer variations. On the other hand, Wang et al. [19] observed little difference in awn color, stem color, and glume color among the 195 wheat varieties. It could be explained that these characteristics were irrelevant to the current-breeding objectives.

4.1.1. Seed Characteristics

Based on subjective visual evaluation, seed colors of wheat varieties were divided into four classes: white seeds, reddish seeds, purple seeds, and bluish seeds. According to mean value the most represented were varieties with white and reddish seeds. It is known that the red seed coat color in wheat is a highly heritable trait, where catechin tannin is a precursor of a reddish-brown pigment in the seed coat [21]. All colored wheat seeds (red, purple, and blue) contain anthocyanins, which have a high antioxidant capacity and may help in the prevention of different chronic diseases, because anthocyanin coloration enhances nutritional quality of seed [22]. Li et al. [22] also reported that, however, wheat varieties with colored seed usually have lower stress resistance or tolerance and lower grain yield. However, seed color may not provide a reliable indication of grain quality and end-use quality value in the milling and baking industries [23]. In the current research, we might assume based on the seed color that wheat collection consisted mostly of varieties that were bred for high grain yield. Further, seeds were objectively evaluated by a phenol test: a rapid variety identification technique for seed coloration. The majority of wheat varieties had seeds of dark to very dark color. This was expected, as the phenol color reaction is governed by one or two genes in common wheat [24].

4.1.2. Flag Leaf and the Whole Plants Characteristics

The anthocyanin coloration of coleoptile and of the auricles of flag leaves were, in most varieties, absent or very weak. Those could be the traits that should be enhanced by the wheat breeding program, as anthocyanins are flavonoid pigments important for plant adaptation under biotic and abiotic stress conditions. For example, the purple color of coleoptile, culm, and anthers was related to a resistance to bunt [25]. Based on the mean value of plant growth habits, the varieties mostly belonged to the intermediate type. Normally, erect plant types are preferred as they increase light capture and thus, through photosynthesis, efficiently synthesize food for the growth and development of the plant [26]. Based on variation in the frequency of plants with recurved flag leaves, the varieties mostly had a low frequency of recurved flag leaves. This trait is not desirable in plants, as recurved flag leaves might disrupt the actual distribution of photosynthetic tissues [27]. The time of ear emergence of wheat varieties was a variable where mean value showed late maturity to very late maturity in wheat varieties. It is known that plant height and heading date (in the current research evaluated as time of emergence) are adaptive traits, which means that the optimum plant height and heading date depend on the target environment [2]. The most frequent were varieties with short to medium plant length. However, complete understanding of different morpho-physiological characters, such as plant height, is important in obtaining high grain yield. Khalid et al. [28] reported that reduction in plant height is generally associated with an increase in lodging resistance, drought tolerance and grain yield. It was observed that the character of the straw pith in cross section in the present investigation in most of the varieties expressed thin to medium size. Straw pith containing less lignin with a thin-walled parenchyma can cause drainage problems during maturity [29]. Therefore, it is evident that thicker or filled straw pith in cross section are more desirable.

4.1.3. Glaucosity of Different Wheat Tissues

Based on the glaucosity of different plant organs, most of the varieties showed a tendency for a stronger expression of glaucosity in different tissues. However, there was variability of glaucosity in 1322 wheat varieties. Wang et al. [19] showed that varieties

differed with respect to the glaucosity of different plant parts such as the flag leaf sheath, flag leaf blade, ear, and peduncle. It was shown that glaucousness is related to an increased tolerance to environmental stress such as drought [30]. However, wax composition varies with developmental stage, between organs, and with genetic and environmental conditions [31]. From the current research, it could be seen that among the organs, ear glaucosity was less pronounced in most varieties, compared to the glaucosity of the sheath and blade of the flag leaf and the neck of the culm.

4.1.4. Ear Morphological Characteristics

The ear has a photosynthetic capacity that is at least comparable to that of the flag leaf and shows a far greater increase in net photosynthesis than the flag leaves under elevated CO₂, and therefore, the ear contributes more to grain formation [32]. Further, the glumes, which are the main photosynthetic organs of the ear, are believed to be a significant source of assimilates for seed filling. This could be especially important at the late stages of grain filling due to the higher photosynthetic capability of glumes. Thus, morphological traits of glumes can also be of particular interest for wheat breeders. The shoulder width of the lower glume, shoulder shape of the lower glume, length of the beak of the lower glume, and shape of the beak of the lower glume are traits that can be used to classify varieties between the time of anthesis and full maturity. For standardization purposes, all observations are made on the lower glume, which can be identified by its lower point of attachment to the rachis. It is evident that longer glumes may contribute to longer seeds due to the formation of extended floral organs called lemma and palea, which form an envelope around the seed [33]. Moreover, glumes could provide a greater photosynthetic resource to the developing seed. The hairiness of the external surface of the lower glume was mostly absent, while the hairiness on the internal surface was mostly medium. Moreover, glume hairiness is an important morphological trait with high heritability and is related to the resistance to biotic and abiotic stresses [34]. The relation of glume hairiness with several important genes/loci, such as resistance to *Blumeria graminis* (DC) Speer f. sp. *tritici* Marchal and spikelet size and number, was previously observed [35]. Therefore, in wheat selection, more attention needs to be given to the hairiness of the external and internal surface of the lower glume.

The ear density is one of the yield-related traits [36] usually influencing the number of grains per ear and the thousand kernel weight. The ear is denser as the closer is the distance between the spikelets. However, in the current research, most varieties had medium ear density. Studies have shown that traits such as ear length, number of spikelets per ear, number of grains per ear, and volume weight are positively correlated with wheat grain yield [19]. Thus, besides ear density, the length of the ear is also related to grain yield, which, in the current research, was mostly short to medium.

The awns may have a positive effect on grain filling by increasing the photosynthetic area and activity, but the final impact on grain yield will depend on the prevailing environmental conditions [37]. The awns may have negative effects by increasing ear temperature. Most varieties had short scurs and short awns that did not result in an increase in ear temperature. Further, most varieties showed ears (glumes) of a white color. It is interesting that Fujii et al. [38] reported that breeders using a 'glume color' marker controlled by *Rg1* locus could select wheat elite lines with red-colored glumes to strengthen dough properties. They reported a link between glume color and *Glu-B3* alleles on 1BS. Further, the most frequent were varieties with a tapering ear shape in profile that was in accordance with the research of Othmani et al. [39], who reported on the most abundant white tapering ears in bread-wheat varieties. The hairiness of the convex surface of the apical rachis segment indicated a high morphological diversity, but more than half the varieties showed a small to medium score. The large score of this trait was found much more in landraces [40].

4.2. Morphological Traits That Changed over the Period from 2006 to 2023

Among the 27 measured morphological characteristics, regression analysis showed the positive relation of the year of the registration of wheat varieties and seven traits.

The first trait that showed the highest change was seed color. Unlike the common seed of white wheat, the color of reddish, purple, and bluish seed of wheat is mainly related to anthocyanins. It can be seen that wheat breeding programs worked on the selection of varieties with reddish seed that has a larger nutritional value than regular white wheat. Regarding anthocyanin content, previous studies have reported that the darker the color of wheat, the higher the anthocyanin content, showing a trend of deep/dark purple grain > blue grain > purple grain > red grain > amber grain wheat [22]. The seed color was closely clustered at PCA to ear color, that was also increased in more varieties over time. That means that newer varieties had a darker color of glumes, compared to older ones, but also with an increase in the shape of the beak of the lower glume.

An increase in plant height was observed over time, with the newer varieties being taller than the older ones. However, it has been reported that plant height has a significant correlation with wheat yield, and reducing plant height can improve harvest index and enhance lodging resistance [19]. The unrestricted reduction in plant height can also limit the improvement in biological yield, so both plant height overabundance and overweigh can cause a reduction in yield. Thus, in the current research due to more robustness, wheat breeding programs made a shift to tallness.

Climate changes over the past 20–30 years significantly affected the date of ear emergence and consequently grain yield [41]. A consistent trend of earlier heading (time of emergence) can be observed in varieties that were registered in recent years. Therefore, differentiation in the time of emergence was observed in the investigated varieties, although they probably have identical genetic control factors according to the requirements for vernalization and photosensitivity. Similarly, Tsenov et al. [42] reported on Bulgarian winter wheat varieties that also differed in grain-filling period. Liu et al. [31] reported that the glaucosity of different organs is responsible for drought tolerance due to reduction in the leaf water potential, which is essential to ensure plants have a relatively high photosynthesis rate. Therefore, the glaucousness intensity on the flag leaf, stem, ear, and other organs can be used as an indicator for drought-resistant and stress-resistant wheat varieties. The results showed that the glaucosity of the neck of the culm showed a gradually increasing trend in varieties registered over time, which suited the needs for the drought- and stress-resistance of wheat. Moreover, this trait was the major contributor of diversity according to PCA. It was expected that wheat breeders select drought tolerant varieties, as drought stress is becoming more evident as a result of global warming [43]. It can be observed that varieties that were registered in later years have an increased glaucosity of the neck of the culm. However, the same varieties showed reduction in traits such as the glaucosity of flag leaves, hairiness of the convex surface of the apical rachis segment, recurved flag leaves, and the occurrence of scars and awns.

The length of the ear gradually increased over the investigated time, where the highest distribution was observed in short-to-medium lengths of the ear. This trait has importance, as it was reported that traits such as ear length, number of spikelets per ear, number of grains per ear, and volume weight were positively correlated with wheat grain yield [19].

4.3. The Most Related Morphological Traits over the Years

The morphological traits were fully expressed and clearly distinguished from each other, which were suitable for subsequent relation analysis. It is known that in wheat breeding, selection for multiple traits is required, including grain-yield and grain-quality traits, as well as other agronomically important or physiologically adaptive traits such as developmental stage, plant architecture, and disease resistance [44]. At the PCA, seed color showed the most significant positive relation with year of registration. High positive correlations were observed between the glaucosity of flag leaves and the glaucosity of the neck of the culm, scurs, and awns and their length, plant length, and plants with recurved

flag leaves, indicating that these traits could reflect important characteristics of the wheat varieties as multiple traits.

Moreover, a positive relation was observed between the glaucosity of flag leaves and the neck of the culm with the time of ear emergence. This means that varieties with a stronger glaucosity of flag leaves and glaucosity of the neck of the culm will be later in maturity. A positive correlation between the time of ear emergence and grain yield was observed, although the change depended on environmental factors [42]. The glaucosity of the different tissues of wheat can have an important role in its resistance to biotic and abiotic stresses that will influence grain yield. For example, analysis of wheat wax deletion mutants without a glaucous appearance in the leaves was considered to be a tool to get many response genes [45]. In the same research, mutant-waxy had a slow loss rate, maintaining a higher leaf-water content. The positive relation of the shape of the beak of the lower glume and the scurs and awns, and their length, was also observed. Glumes, awns, and scurs have photosynthetic potential during the grain filling stage, contributing to grain yield, especially under stress conditions [46]. Therefore, obtainment of a positive correlation between those traits was expected.

5. Conclusions

Significant relations between twelve morphological traits and the registration year of wheat varieties were observed, which demonstrated the importance of those traits in varietal improvement. According to Pareto chart the highest changes were observed for seed color, followed by area of hairiness of convex surface of apical rachis segment, glaucosity of the neck of the culm, and the frequency of plants with recurved flag leaves. Therefore, it can be concluded that wheat morphological traits changed over time from 2006 to 2023, with the newer wheat varieties being taller with darker seed and more colored ears, enhanced ear length, enhanced scur and awn length, increased glaucosity of neck of culm, and a more curved shape of the beak of the lower glume, compared to older varieties. Older varieties had more plants with recurved flag leaves and a larger area of hairiness of the convex surface of the apical rachis segment, and were later in maturity. Moreover, in the future, involvement of genomic markers in the DUS system should be planned to potentially speed up the process testing.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agriculture14040551/s1>, Table S1: Mean, minimum and maximum values and standard deviations for 27 continuously variable descriptors for 1322 winter wheat varieties; Table S2: Factor-variable correlations (factor loadings), based on correlations.

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