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Diversity, correlating traits and phenotypic distance among modern durum wheat genotypes

Krasimira Taneva, Rangel Dragov*, Maria Videva and Violeta Bozhanova

Agricultural Academy, Field Crops Institute, 6200, Chirpan, Bulgaria
**Corresponding author:* dragov1@abv.bg

Abstract

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The studies of phenotypic/genetic diversity, correlating traits between various agronomic and quality traits in specific environmental conditions and phenotypic distance among genotypes, that are included in hybridization programs, are essential for building an appropriate breeding strategy for durum wheat improvement. In this regard 90 modern durum wheat genotypes of diverse origins were grown under field conditions for three years, and 14 traits related to yield and grain quality were monitored. The greatest variation and correspondingly the highest coefficient of variation was found for the traits: SDS-sedimentation value (CV- 41.6%), grain yield (CV-14.3%) and kernels weight per spike (KWS) – (CV-11.9 %). According to the analysis of variance (ANOVA) genotype, years of cultivation and interaction between them had a reliable effect on the variation of the all studied traits. The expression of three grain quality traits: SDS-sedimentation value, vitreousness and yellow index and six yield related traits: thousand kernel weight, test weight, number of spikelets per spike, number of kernels per spike and spike length depends in our conditions to the greatest extent on the genotype. The environment/year of cultivation has a determining influence on the phenotypic variation of the traits: grain yield, plant height, grain protein content and grain wet gluten. Both positive and negative correlations were established between agronomic and quality-related indicators in our study. A positive correlation was found between yield and grain protein content ($r = 0.28$) contrary to the commonly accepted negative correlation between these traits. Positive correlations were also found between grain protein content and plant height ($r = 0.48$); wet gluten content in grain and plant height ($r = 0.50$). Positive correlations were found for grain vitreousness with the following traits: plant height ($r = 0.52$), test weight ($r = 0.39$), yield ($r = 0.43$), number of kernels per spike ($r = 0.30$), kernels weight per spike ($r = 0.39$). These positive correlations can help to increase the yield and quality of the genotypes, and therefore, it is recommended to breed for these traits under our conditions. The strong, positive, and significant correlations established in our study between number of kernels per spike and kernels weight per spike ($r = 0.79$), and between number of spikelets per spike with number of kernels per spike ($r = 0.64$), suggest that these traits can be considered as important factors in the selection of genotypes with higher grain yield. The largest variation and, respectively, the highest coefficient of variation was reported for SDS-sedimentation value (CV-41.6%).

Keywords: quality; yield; variation analysis; analysis of variance; correlation analysis; cluster analysis

Introducton

A major priority in durum wheat breeding is the creation of high-yielding varieties with excellent grain quality. The strong gluten matrix and the high content of yellow pigments are two of the most important components of the technological quality of this crop (Vida et al., 2022). Whereas the former is important to determine the culinary quality of pasta, the latter is an major marketing characteristic. Small differences in the variation of genes controlling major quality traits in modern durum wheat cultivars, are a significant limitation in their breeding improvement (Blanco and De

Giovanni, 1995). This makes it necessary to include a large number of genotypes with different ecological-geographical origins in breeding programs. Studying the existing genetic diversity and incorporating it into hybridization programs, is important to further yield and quality improvement.

The presence of negative correlations among some yield-related agronomic traits and quality-related traits impede the breeding work (Rharrabti et al., 2000; Bilgin et al., 2010). Subira et al. (2014) established that the yield increase in the 20th century was largely, due to the decrease in protein content of the newly created varieties. Similar results were also reported by a number of other researchers (Motzo et al., 2004; De Vita et al., 2007; Dotlacil et al., 2010; Nazco et al., 2012). On the other hand, the selection of high-yielding genotypes for individual traits is also difficult, due to different interactions and compensatory mechanisms between the individual yield components, depending on the genotype and environment interactions (Slafer et al., 2014). The environmental and genotypic impact on quality-related indicators has been studied repeatedly by different groups of researchers in various locations and conditions, with different genotypes (Peterson et al., 1992; Rharrabti et al., 2003; Sakin et al., 2011; Subira et al., 2014). It was established that the environmental conditions (locations and years of cultivation) had a significant statistical impact on protein content, SDS – sedimentation value, gluten index, yellow pigments content. Kiliç, and Yaˇgbasanlar (2010) investigated the effect of drought on yield components, and some traits related to the grain quality of durum wheat varieties. They reported that the SDS-sedimentation value, which is related to gluten strength, was 20.2% higher in the grain of the drought-exposed plots than in the irrigated controls. It was also found that protein content and vitreousness depend to a greater extent on the environmental conditions, whereas yellow pigment content and SDS-sedimentation value – on genotype (Nachit et al. 1995; Kiliç, and Yaˇgbasanlar, 2003). Among the environmental factors, high temperatures and humidity at grain filling (Jenner, 1991; Blumenthal et al., 1993), rainfall distribution (Campbell et al., 1981) and nitrogen fertilization (Rao et al., 1993) had the greatest impact on quality. According to Guzmán et al. (2016), heat stress reduces the physical parameters of grain – test weight and thousand kernel weight, as well as Minolta b* values, but increased protein and zinc content. Variation in the concentration of yellow pigments is determined by genotype, but is also influenced by the environmental conditions. Rharrabti et al. (2003) associated a higher mean temperature with an increase of yellow pigment content, and drought – with an increase in SDS-sedimentation volume values, and therefore, an improvement in gluten strength. The same authors reported an inverse correlation between protein content and SDS-value, concluding that the relationships between quality characteristics were influenced to some extent by the climatic conditions during grain filling. According to Graham and Rosser (2000), moisture deficit during the different stages of grain filling or high temperatures affects the pigment concentration and composition, and is the cause for variation due to genotype and environment interaction. According to the same authors, the concentration of pigments correlates weakly positively with the mean temperatures, and weakly negatively with precipitation in this stage. Ficco et al. (2014) concluded that genotypes are difficult to select based on grain carotenoid content, as it is a quantitative trait that is encoded by multiple QTLs on all chromosomes.

By determining the genetic distance and by suitable selection of parental forms, significant progress can be made in the yield potential of recombinant genotypes (Islam, 2004). For breeders to take advantage of transgressive segregation, genetic divergence between parents is required (Ahmad et al., 2014; Zamanianfard et al., 2015). Estimation of genetic distance between genotypes can be based on the phenotypic expression of quantitative and qualitative traits (Souza and Sorells, 1991). Genetic distance is most commonly measured as phenotypic distance (Gashaw et al., 2007; Debnath et al., 2008; Kabir et al., 2009). It is suggested that if genotypes are phenotypically different in many traits, they are also genetically distant in their genomes. Cluster analysis is a suitable method to determine the distance of genotypes, as well as the degree of genetic affinity, and the distance of genotypes from each other (Mohammadi and Prassana, 2003; Eivazi et al., 2007; Kumar et al., 2014; Mishra et al., 2015). Cluster analysis results may show partial differences between genotypes. Furthermore, separation by cluster analysis is best for estimating the genetic distance between them (Khodadadi et al., 2011).

Before being included in a hybridization program, genotypes known to carry valuable grain quality indicators should also be evaluated for yield and basic agronomic traits.

The aim of this investigation was to determine the diversity variation; the impact of genotype, environment and genotype-environment interaction on the expression of the important traits; the associations between the yield and quality-related traits, and the phenotypic distance between the studied durum wheat genotypes.

Material and Methods

The study included 90 modern durum wheat genotypes – varieties and breeding lines from all over the world. All the genotypes were grown under field conditions for three years 2020 – 2022. A randomized block method was used in four replications with an experimental plot size of 15 m^2. The accepted technology for growing durum wheat was used.

The following traits were monitored: grain yield (Y)– t/ha, plant height (H)– cm, productive tillering (PT) – number, spike length (SL) – cm, number of spikelets per spike (NSS) – number, number of kernels per spike (NKS) – number, kernels weigth per spike (KWS) – g, grain protein content (GPC) – percentage, wet gluten content in grain (GWG) – percentage, SDS-sedimentation volume (SDS) – cm^3, vitreousness (VIT) – %, test weight (TW) – kg/hl, thousand kernel weight (TKW) – g, Minolta yellow index b* (MK).

The grain protein content was determined by the Kjeldahl method (N x 5.7) according to BNS EN ISO 20483: 2014, and wet gluten – according to BNS EN ISO 21415-2: 2008. The gluten strength was evaluated by measuring the sedimentation volume of wholemeal flour in a lactic acid – sodium dodecyl sulfate (SDS) solution at a standard sedimentation time (ICC 151: 1990). Vitreousness was determined according to BNS EN 15585: 2008. Thousand kernel weight was determined according to BNS EN ISO 520: 2010. Test weight was determined according to BNS EN ISO 7971-1: 2009. The values of yellow colour b* were measured according to CIE L*a*b* cubic colour space, which is considered the most operational and informative one. The measurement was made per grain using a Minolta CR-410 chroma meter. The higher the b* value, the greater the amount of carotenoids. The chroma meter was calibrated with a standard calibration plate.

The obtained results were processed statistically, by variation and correlation analysis, two-factor analysis of variance (ANOVA) and cluster analysis. All statistical analyses were performed using the Statistica 13.0 program (TIBCO, Software, 2018).

Results and Discussion

All three years are characterized as warmer than the multi-year norm. The first year is the hottest. It should be noted that for the January, temperatures in the three years of the study, no negative mean day-night temperatures were recorded. October is significantly warmer than normal. November is characterized by an average day-night temperature around the norm, and only during the 2020 harvest is it lower. The months of December, January, and February in all three years have an average daily temperature above the average norm. In the month of March, in the first year the temperature is higher than the norm; in the second year it is around the norm, and in the third year it is lower than the norm for a multi-year period. In the month of April, the average day-night temperature in the first two years is lower than the norm, and in the third year it is higher. The month of May is characterized by temperatures around the norm. The temperature in June in the first two years is around the norm, and in the third year is above the norm. Rainfall is extremely unevenly distributed during the growing season of durum wheat. The second and third years are above the norm for a multi-year period. The next year is drier, and they deviate from the norm. In 2020, the precipitation is relatively evenly distributed in the individual months.

The presence of genetic variability in breeding materials for important traits is a prerequisite for success of plant breeding programs. The coefficient of variation (CV), which is estimated by analysis of variance, is commonly used to

Table 1. Meteorological characteristics for the three years of testing.

	Average daily air temperature, °C				Montly amount of precipitation, mm			
Years / Months	2019 – 2020	2020 – 2021	2021 – 2022	Norm 1928 – 2022	2019 – 2020	2020 – 2021	2021 – 2022	Norm 1928 – 2022
October	15.0	15.2	11.3	12.7	48.2	67.3	150.5	38.6
November	11.2	6.6	7.9	7	82.4	7.4	14.2	47.3
December	3.6	5.8	3.9	1.4	21.6	70.4	108.8	54.0
January	1.4	3.2	1.8	-0.2	1.5	108.6	21.4	44.3
February	5.3	4.5	4.2	1.7	55.5	25.8	40.1	37.7
March	8.3	5.2	4.2	5.7	67.4	39.1	22.4	37.0
April	10.5	10.3	12.2	11.8	62.2	84.0	36.0	45.3
May	16.6	16.9	17.3	16.9	50.3	34.9	29.4	64.1
June	20.5	20.6	22.0	20.7	62.6	42.8	80.5	65.4
July	24.7	25.6	25.1	23.1	12.0	49.0	7.7	54.1
Sum	117.1	113.9	109.9	100.8	463.7	529.3	511	487.8

Source: Authors' own elaboration

determine the variability present in a population.

An variation analysis of agronomic and grain quality traits was performed on the mean values of the three harvest years 2020–2022 (Table 2). The greatest variation and correspondingly the highest coefficient of variation (CV- 41.6%) was found for SDS-sedimentation value, and ranged from 16.7 to 57.8 cm^3. Mean for the three years of research, yield varied in the range of 3.35–6.85 t/ha (CV-14.3%), kernels weigth per spike (KWS) – within 1.5–2.7 g (CV-11.9 %), and number of kernels per spike (NKS) – within 36.3–54.8 (CV-9.3%). Productive tillering (PT) ranged within 2.73–4.1 tillers (CV-8.8%), plant height (H) – within 71.1–110.3 cm (CV-7.8%), and spike length (SL) – within 5.82–8.7 cm (CV-7.5%). Vitreousness (VIT) ranged within 67.9–95.6% (CV-6.5%), thousand kernel weight (TKW) – within 36.6–50.2 g (CV-6.2%), and number of spikelets per spike (NSS) – within 18.3–23.9 (CV-5.7%). Grain protein content (GPC) varied from 12.8 to 16.3% (CV-4.1%), and wet gluten (GWG) – from 27.1 to 34.2% (CV-4.1%). A low level of variation characterized yellow pigment content in grain (b*), with variation in the range of 15.6–17.8 Minolta b* and CV-2.9%. The lowest coefficient of variation and correspondingly the least variation was found for test weight (CV-2.8%), with a range of variation from 68.8 to 78.0 kg/hl. The rest of the examined traits were also characterized by a relatively low level of variation.

The phenotypic coefficient of variation (CV) values were classified (Sivasubramanian and Menon, 1973) as low (<10%), moderate (10–20%) and high (> 20%). According to this classification, in the studied sample of 90 genotypes, there is a great diversity for quality trait SDS-sedimentation value and moderate for yield and kernels weigth per spike. In general, a moderate and high coefficient of variability indicates that there are a sufficient opportunities for selection of promising elite genotypes from a certain population for the relevant trait. Low values indicate the need for creation of variability, either by hybridization or mutation, followed by selection (Tiwari et al., 2011).

The results obtained from our research reveal the existence of sufficient variability among studied genotypes on two important traits: gluten strength (SDS-sedimentation value) and grain yield, that could be exploited in our future breeding program for durum wheat improvement.

The phenotypic expression of many agronomic and quality indicators is highly dependent on environmental conditions or on the interaction between genotype and environment, which makes selection difficult. The study of factors influencing the expression of important traits under specific soil and climatic conditions can facilitate the breeding process. The genotype-environment interaction is dependent on the genetic nature of the trait and the genetic diversity of the used genotypes. The speed of the selection process for a given quantitative trait depends on the presence and size of the genotype-environment interaction.

Therefore, in our study, the contribution of genotype, environmental conditions, and the interaction between them to the total phenotypic variation for each of the traits studied in a sample of 90 genotypes grown in three consecutive years

Table 2. Descriptive statistics of the studied traits

Trait	Mean	Standart error	Minimal	Maximal	CV%
Y	5.34	0.08	3.35	6.85	14.3
H	90.2	0.74	71.1	110.3	7.8
PT	3.4	0.03	2.73	4.1	8.8
SL	7.3	0.06	5.82	8.7	7.5
NSS	21.4	0.13	18.3	23.9	5.7
NKS	43.7	0.43	36.3	54.8	9.3
KWS	1.9	0.02	1.50	2.7	11.9
TKW	43.0	0.28	36.6	50.2	6.2
GPC	14.8	0.06	12.8	16.3	4.1
SDS	26.8	1.17	16.7	57.8	41.6
MK	16.6	0.05	15.6	17.8	2.9
GWG	31.2	0.13	27.1	34.2	4.1
VIT	86.8	0.59	67.9	95.6	6.5
TW	74.7	0.22	68.8	78.0	2.8

Yield (Y), Plant height (H), Productivity tillering capacity (PT), Spike length (SL), Number of spikelets per spike (NSS), Number of kernels per spike (NKS), Kernels weigth per spike (KWS), Thousand kernel weigth (TKW), Grain protein content (GPC), Sedimentation volume (SDS), Minolta kernels (MK), Grain wet gluten (GWG), Vitreousness (VIT), Test weigth (TW)

Source: Authors' own elaboration

was determined. Analysis of variance (ANOVA) is commonly applied to determine the presence and magnitude of a genotype-environment interaction using multiyear data.

According to the analysis of variance (Table 3) genotype, years of cultivation and interaction between them had a reliable effect on the variation of the studied traits. The variation of SDS-sedimentation vlue (96.58%), thousand kernel weight (61.15%), test weight (58.70%), number of spikelets per spike (53.07%), vitreousness (50.33%), kernels weigth per spike (49.44%), number of kernels per spike (47.96%), Minolta yellow index b* (46.36%) and spike length (39.25%) was mainly due to genotype.

In agreement with our results, Sourour et al. (2018) reported a significant influence of genotype on the variation of thousand kernel weight and gluten strength, and a significant impact on the genotype-environment interaction for all studied traits. According to Yagdi and Sozen (2009), the variation of test weight is influenced to the greatest extent by gen-

Table 3. Analysis of variance of the traits included in the study

Yield (Y)						
Source	SS	df	MS	F critical	significance	η^2%
Genotype	472.16	89	5.31	29.0	***	29.73
Year	724.63	2	362.3	1983.7	***	45.63
GxY	292.70	178	1.6	9.0	***	18.43
Error	98.63	540	0.18			
Plant height (H)						
Source	SS	df	MS	F critical	significance	η^2%
Genotype	40068	89	450	2719	***	42.77
Year	41962	2	20981	126702	***	44.80
GxY	11554	178	65	392	***	12.33
Error	89	540	0,16			
Productivity tillering capacity (PT)						
Source	SS	df	MS	F critical	significance	η^2%
Genotype	238.962	89	2.685	1.597	***	14.49
Year	65.878	2	32.939	19.588	***	4.00
GxY	435.824	178	2.448	1.456	***	26.43
Error	908.040	540	1.682			
Spike length (SL)						
Source	SS	df	MS	F critical	significance	η^2%
Genotype	244.20	89	2.74	176	***	39.25
Year	235.03	2	117.52	7531	***	37.78
GxY	134.46	178	0.76	48	***	21.61
Error	8.43	540	0.02			
Number of spikelets per spike (NSS)						
Source	SS	df	MS	F critical	significance	η^2%
Genotype	1177.6	89	13.2	1021	***	53.07
Year	229.1	2	114.6	8838	***	10.32
GxY	805.2	178	4.5	349	***	36.29
Error	7.0	540	0.01			
Number of kernels per spike (NKS)						
Source	SS	df	MS	F critical	significance	η^2%
Genotype	13374	89	150	130	***	47.96
Year	1805	2	902	781	***	6.47
GxY	12084	178	68	59	***	43.33
Error	624	540	1.15			
Kernels weigth per spike (KWS)						
Source	SS	df	MS	F critical	significance	η^2%

Table 3. Continued

Genotype	44.065	89	0.495	182	***	49.44
Year	4.887	2	2.443	899	***	5.48
GxY	38.712	178	0.217	80	***	43.43
Error	1.467	540	0.003			
Thousand kernel weigth (TKW)						
Source	SS	df	MS	F critical	significance	η^2%
Genotype	5727	89	64	1966	***	61.15
Year	1544	2	772	23586	***	16.49
GxY	2077	178	12	357	***	22.18
Error	18	540	0,03			
Grain protein content (GPC)						
Source	SS	df	MS	F critical	significance	η^2%
Genotype	302.4	89	3.4	348	***	24.95
Year	725.1	2	362.6	37128	***	59.83
GxY	179.1	178	1.0	103	***	14.79
Error	5.3	540	0.009			
Sedimentation volume (SDS)						
Source	SS	df	MS	F critical	significance	η^2%
Genotype	99852.2	89	1121.9	1290.9	***	96.58
Year	497.9	2	249.0	286.5	***	0.48
GxY	2568.1	178	14.4	16.6	***	2.48
Error	469.3	540	0.9			
Minolta kernels (MK)						
Source	SS	df	MS	F critical	significance	η^2%
Genotype	185.2	89	2.1	57	***	46.36
Year	106.7	2	53.3	1470	***	26.71
GxY	88.0	178	0.5	14	***	22.03
Error	19.6	540	0.03			
Grain wet gluten (GWG)						
Source	SS	df	MS	F critical	significance	η^2%
Genotype	1325.2	89	14.9	606	***	24.81
Year	3187.0	2	1593.5	64869	***	59.67
GxY	815.6	178	4.6	187	***	15.27
Error	13.3	540	0.02			
Vitreousness (VIT)						
Source	SS	df	MS	F critical	significance	η^2%
Genotype	25427	89	286	16436	***	50.33
Year	12700	2	6350	365320	***	25.14
GxY	12386	178	70	4003	***	25.52
Error	9	540	0.01			
Test weigth (TW)						
Source	SS	df	MS	F critical	significance	η^2%
Genotype	3587	89	40	1683	***	58.70
Year	1340	2	670	27980	***	21.93
GxY	1171	178	7	275	***	19.16
Error	13	540	0.02			

*** – $p \leq 0,001$

Source: Authors’ own elaboration

otype. The variation in the concentration of yellow pigments was determined by genotype and environmental conditions. Braaten et al. (1962) and Rharrabti et al. (2003) reported a significant influence of genotype and small influence of environment on the variation of yellow pigment content. Despite the greater importance of genotype, a significant impact of the genotype-environment interaction was established on the variation of kernels weigth per spike (43.43), number of kernels per spike (43.33), number of spikelets per spike (36.29%), and a significant influence of environment on spike length (37.78%). The high coefficient of variation and the results of the analysis of variance for SDS-sedimentation value, which showed a minor influence of the environmental conditions (0.48%), and the genotype-environment interaction (2.48%) on the variation of this trait, demonstrated that the studied sample was characterized by high genetic diversity by this indicator. The greatest environmental impact was found in the variation of grain protein content (59.83%), wet gluten content in grain (59.67%), yield (45.63%) and plant height (44.80%). According to Blanco and De Giovanni (1995), breeders' efforts to increase grain protein content achieved little success, because it is a complex heritable trait, whose expression is highly dependent on environmental conditions. It is well known that the selection of genotypes with high protein content is impeded by the existing negative correlation with yield and the significant influence of environmental conditions on the variation of this trait (Blanco et al. 2006; Würschum et al. 2016). The genotype effect was significant on plant height (42.77%), yield (29.73%), protein content (24.95%) and wet gluten content (24.81%). The productive tillering indicator was influenced to the greatest extent by the genotype-environment interaction (26.43%), with a significant impact of genotype (14.49%). The presence of a reliable genotype-environment interaction on yield (18.43%) hampered the effective genotype-by-phenotype selection for this important trait. The interaction between genotype and year can be explained by the differences in the magnitude of the individual response of each genotype to each individual year of cultivation. This necessitates the need for long-term studies on productivity, or establishment of phenotypic stability of yield from different genotypes by statistical methods (Nsarellah et al., 2011; Mohamed et al., 2013).

As a summary of the conducted analysis of variance, it can be highlighted that the expression of three grain quality traits – SDS-sedimentation value, vitreousness, and yellow index – and six yield-related traits – thousand kernel weight, test weight, number of spikelets per spike, number of kernels per spike, and spike length – depends, in our conditions, to the greatest extent on the genotype. Nevertheless, reliable genotype-by-phenotype selection could be conducted only for the trait gluten strength (SDS-sedimentation value), due to the established minor influence of the environmental conditions (0.48%), and the genotype-environment interaction (2.48%) on the variation of this trait. The environment/year of cultivation has a determining influence on the phenotypic variation of the traits: grain yield, plant height, grain protein content and grain wet gluten. This necessitates the testing of the breeding materials to be carried out in several consecutive years/different environments, for a more accurate selection of appropriate genotypes for these characteristics.

To select suitable genotypes in breeding programs, it is extremely important to have a good knowledge of the correlations between the traits characterizing yield and quality. Correlations, although reliable only within the studied genotypes and conditions, point to relationships that could be used to increase the effectiveness of breeding programs (Backer et al., 1971). The presence of a high correlation coefficient between two traits suggests the existence of a strong hereditary relationship between them, and probability of a close genetic basis (Gaines, 1991). To detect relationships between agronomic and grain quality traits, a correlation analysis was conducted based on the mean data from the three years. The significant correlations established at the phenotypic level mean over the three years of the study, are reflected in Table 4.

Weak positive but statistically significant correlations were found for grain protein content (GPC) with yield (r = 0.28), kernel weight per spike (r = 0.28), and a relatively high one with plant height (r = 0.48). A higher positive correlation was found between GPC and vitreousness (r = 0.63), and the commonly accepted correlation with wet gluten content (r = 1.00). A weaker negative correlation was found between GPC and SDS-sedimentation value (r = -0.38), and a stronger one for yellow index b* (r = -0.68). Plant height was positively correlated with yield (r = 0.55), vitreousness (r = 0.52), wet gluten content (r = 0.50), test weight (r = 0.45), thousand kernel weight (r = 0.44), and negatively with SDS-sedimentation value (r = -0.50) and yellow index b* (r = -0.41). A relatively high positive correlation was found for thousand kernel weight with test weight (r = 0.49), and weaker positive with kernels weigth per spike (r = 0.35), and yield (r = 0.27), as well as a weak negative correlation with the SDS-sedimentation value (r = -0.37). Under our conditions, a positive correlation was established for test weight with yield (r = 0.48), kernels weigth per spike (r = 0.39), vitreousness (r = 0.39), wet gluten content (r = 0.21), and negative associative correlation with SDS-sedimentation value (r = -0.51). Yield correlated positively with vitreousness (r = 0.43), wet gluten content (r = 0.28), kernels weigth per spike (r = 0.25), and negatively with SDS-sedimentation value (r = -0 .59) and

Table 4. Phenotypic correlations between quantitative traits related to productivity and grain quality

	GPC	H	TKW	TW	Y	PT	SL	NSS	NKS	KWS	SDS	MK	GWG	VIT
GPC	1.00	0.48*	0.14	0.21	0.28*	0.07	-0.03	0.19	0.20	0.28*	-0.31*	-0.64*	1.00*	0.63*
H		1.00	0.44*	0.45*	0.55*	0.15	-0.02	-0.08	-0.11	0.20	-0.50*	-0.41*	0.50*	0.52*
TKW			1.00	0.49*	0.27*	0.19	0.05	-0.16	-0.09	0.35*	-0.37*	-0.01	0.15	0.13
TW				1.00	0.48*	0.17	-0.09	-0.10	0.12	0.39*	-0.51*	-0.12	0.21*	0.39*
Y					1.00	0.20	-0.23	-0.08	0.04	0.25*	-0.59*	-0.22*	0.28*	0.43*
PT						1.00	-0.08	0.04	0.05	0.06	-0.30*	0.04	0.08	0.13
SL							1.00	0.46*	0.17	0.17	0.14	0.03	-0.05	-0.03
NSS								1,00	0.64*	0.48*	-0.09	-0.14	0.18	0.08
NKS									1.00	0.79*	-0.21*	-0.15	0.21*	0.30*
KWS										1,00	-0.33*	-0.18	0.29*	0.39*
SDS											1.00	0.33*	-0.34*	-0.38*
MK												1.00	-0.66*	-0.68*
GWG													1.00	0.65*
VIT														1.00

* – $p \leq 0,05$;

Source: Authors' own elaboration

yellow index b* (r = -0.22). A negative correlation was found between productive tillering and SDS-sedimentation value (r = -0.30), and a positive one between spike length and number of spikelets per spike (r = 0.46). Number of spikelets per spike correlated strongly positively with number of kernels per spike (r = 0.64) and more weakly with kernels weigth per spike (r = 0.48). Number of kernels per spike was strongly positively correlated with kernels weigth per spike (r = 0.79) and weakly correlated with wet gluten content (r = 0.21) and vitreousness (r = 0.30). The same trait was found in a weak negative correlation with SDS-sedimentation value (r = -0.21). Kernels weigth per spike correlated weakly positively with vitreousness (r = 0.39) and wet gluten content (r = 0.29), and weakly negatively with SDS-sedimentation value (r = -0.33). SDS-sedimentation value was found in a positive associative relationship with yellow index b* (r = 0.33), and in a negative one with vitreousness (r = -0.38) and wet gluten content (r = -0.34). A strong negative correlation was found for yellow index b* with vitreousness (r = -0.68) and wet gluten content (r = -0.66).

The correlation between yield and thousand kernel weight has been noted so far by many authors (Li et al., 2006; Akram et al., 2008; Nofouzi et al., 2008; Khan et al., 2013; Nikkhahkouchaksaraei et al., 2017; Laddomada et al., 2021). Therefore, thousand kernel weigth is considered to be one of the principal yield components, which is used to select high-yielding genotypes in breeding programs (Topal et al., 2004).

A number of authors reported a negative correlation of protein content with yield, and a significant influence of the environmental conditions on the variation of this trait (Blanco et al. 2006; Würschum et al. 2016). However, Grahmann et al. (2014) also reported on similar to the positive correlation we found, which deviates from the often reported negative correlation between these two traits. The impact of high temperatures during grain filing is well-known. The occurrence of very high temperatures (in the range of 35–40 °C) during grain filling significantly affects dry matter and protein accumulation in different parts of the plant (Corbellini et al. 1997; Zingale et al., 2023). Colecchia et al. (2015) reported higher protein concentration in humid conditions, whereas other authors reported higher protein levels in dry years (Di Fonzo et al., 2001; De Vita et al., 2007). Primary data from our research shows that harvest years 2019 and 2021 were more favorable for the accumulation of higher amounts of protein in the grain for all genotypes. The analysis of weather conditions during the three harvest years indicates that these results may be related to the larger amount of precipitation in June of those two years, compared to the relatively lower amount in the same month of 2020. At the same time, in the harvest years 2019 and 2021 during the ripening the amount of July, precipitation has been much lower compared to the long-term average amount and to that of 2020 harvest year. Thus, obtained results reveal that moister conditions during the grain-filling phase could have a positive influence on the grain protein content. Nevertheless, further more detailed research is needed to clarify the relationship of protein content with the weather conditions, environmental and genetic factors, that are responsible for the positive correlation we found between yield and protein content among studied genotypes. The results of such investigation could contributed to future breeding progress for the simultaneously improvement of yield and protein content in durum wheat under changing climatic conditions in our region. Acording to Tanin et al. (2022), complications of achieving this breeding goal are determined by several important factors: GY and GPC are highly influenced by the environment, which is also confirmed in the present study; the typical negative correlation between both traits and low to moderate level of heritability for Yield and CPC, also found in our previous research (Taneva et al., 2019)

In agreement with our results, Kendal (2019) reported a positive correlation of yield with wet gluten content and vitreousness, and a negative correlation with yellow pigment content and SDS-sedimentation value. The same author also found a positive correlation between spike length and number of spikelets per spike, thousand kernel weight with test weight, plant height with thousand kernel weight, test weight, wet gluten content and a strong positive correlation between number of spikelets per spike and number of kernels per spike. A negative correlation was also reported between the number of kernels per spike and SDS-sedimentation value, height and yellow pigment content, thousand kernel weight with SDS-sedimentation value and yellow pigment content. Similar to our results, a positive correlation between the plant height and yield was reported by Usadad et al. (2022). Nikkhahkouchaksaraei et al. (2017) and Sourour et al. (2018) reported a negative correlation between these two traits. A number of authors also reported a strong positive correlation between number of kernels per spike and kernels weigth per spike (Abinasa et al., 2011; Singh, 2016; Usadad et al., 2022). The strong positive and significant correlation found in our study between these two yield-related traits, suggests that these traits can be considered as two important factors in the selection of genotypes with higher grain yield.

According to a number of authors, vitreous grains are associated with high protein content (Dexter et al., 1988,1989; Samson et al., 2005; Sieber et al., 2015). Other authors reported conflicting data on the correlation between vitreousness and grain protein content (Longin et al., 2013; Pinheiro et al., 2013). Oktem and Oktem (2019) reported a strong positive correlation between the studied traits. Trad et al. (2022) found a strong correlation in the negative direction between these two traits, whereas Fu et al. (2018) found a non-significant correlation between the level of hard vitreous grains and protein content. Research on the relationship between high protein but low HVK (hard vitreous kernels), and low protein but high HVK, is still limited. There is also limited information on the relationship between grain vitreousness, yellow pigment content and pasta colour (Fu et al., 2018).

The positive correlations that we established for plant height with grain protein and wet gluten content were similar to the findings of Dagnaw et al. (2022). Therefore, it is difficult to select genotypes as donors for hybridization based on plant height, in order to obtain high-yielding genotypes with the desired levels of protein and wet gluten content in the grain. The introduction of the dwarfing gene through modern breeding approaches can improve grain yield along with the desired levels of protein and wet gluten content in the grain (Thuillet et al., 2005).

The negative correlation that we found between gluten strength (SDS) and plant height was also reported for other genotypes and growing conditions (Clarke et al., 2010). According to Clarke et al. (2010), this correlation is due to linked genes or to pleiotropy. The Rht1 allele, which is the likely source of gibberellic acid insensitivity in semi-dwarf wheat genotypes, is known to be located on chromosome 4B (Blanco et al., 1996; Ellis et al., 2002). At the same time, Elouafi et al. (2000) reported a QTL on 4B affecting SDS-sedimentation value.

The negative correlations established in our study between gluten strength (SDS) and some of the yield structural elements: number of kernels per spike and kernels weigth per spike, are in agreement with the negative correlations between these traits, reported by other authors (Kayyal et al., 1995; Yagdi and Sozen, 2009).

There is a conflicting information regarding the relationship between grain protein amount and gluten strength. Some authors reported a slightly positive correlation between protein and SDS-volume (Carrillo et al., 1990; Kovacs et al., 1995; Clarke et al., 2010; Oktem and Oktem, 2019; Singh et al., 2022), and another group of authors reported no correlation (Autran et al. 1986; Pena, 2000), or a negative correlation (Zhang et al. 2008).

The most significant correlations established in our study, both between yield components and between some yield components and quality indicators, can facilitate breeding work not only for creating high-yielding genotypes but also for the simultaneous genetic improvement of grain yield and quality traits, such as vitreousness (VIT) and wet gluten content in grain, in durum wheat under our pedo-climatic conditions. Despite our finding of a positive correlation between yield and protein content, further research is needed to clarify the relationship of protein accumulation with weather conditions, environmental factors, and genetic factors, in order to achieve simultaneous breeding improvement of both traits. The significant negative correlations found between SDS and yield/yield-related traits will make it difficult to select high-yielding genotypes with strong gluten.

Establishing the phenotypic distances between genotypes before including them in a hybridization program, is essential for building an appropriate breeding strategy for durum wheat improvement.

The genetic diversity analysis performed by cluster analysis is shown in Figure 1. A dendrogram was generated to examine the relationships among 90 durum wheat genotypes of diverse origins based on Euclidean distances. The cluster analysis was conducted on the basis of fourteen quantitative traits related to productivity and quality.

Genotypes fall into three major clusters. Cluster I includes mostly spring-type genotypes. Cluster II includes cultivars and advanced breeding lines, created at FCI-Chirpan. Cluster II is divided into two sub-clusters. Cluster III includes the majority of foreign varieties, as well as advanced breeding lines and cultivars created at FCI-Chirpan. The use of cluster analysis in breeding programs is an important step to determine the breeding strategy. Considering the genotypes distance, it is possible to build a strategy for building a hybridization program. To achieve faster but smaller progress, it is necessary to cross genotypes falling within the same cluster. On the other hand, a slower but greater progress requires cross-breeding of genotypes from different clusters. According to the large number of genotypes and the many traits included in the study, the following specific proposals for hybridization can be made. Cluster II includes the most valuable varieties and genotypes from the breeding program. They should be used mainly as parents to get better and desired results. Given their small genetic distance, hybrids between them will decay in less time, and the results will be smaller. The Predel variety is a yield and quality standard for Bulgaria, and hybridization between it and other genotypes from cluster II will lead to success in obtaining more productive new genotypes, with good grain quality. Regarding earlier maturing genotypes, they can be crossed with genotypes from cluster I. Cluster III contains the largest number of foreign varieties. Hybridization between the Predel, Heliks, Reyadur and Saya genotypes from cluster II will result in a smaller increase in genetic diversity, but faster results. It is recommended that these varieties be crossed with cluster I genotypes to obtain earlier genotypes and increase genetic diversity. Crosses between the individual clusters will lead to a significant increase in genetic diversity in the breeding program and obtaining new valuable genotypes.

Conclusions

As a result of the conducted research, important conclusions have been drawn related to: determining of phenotypic diversity; the influence of genetic and environmental

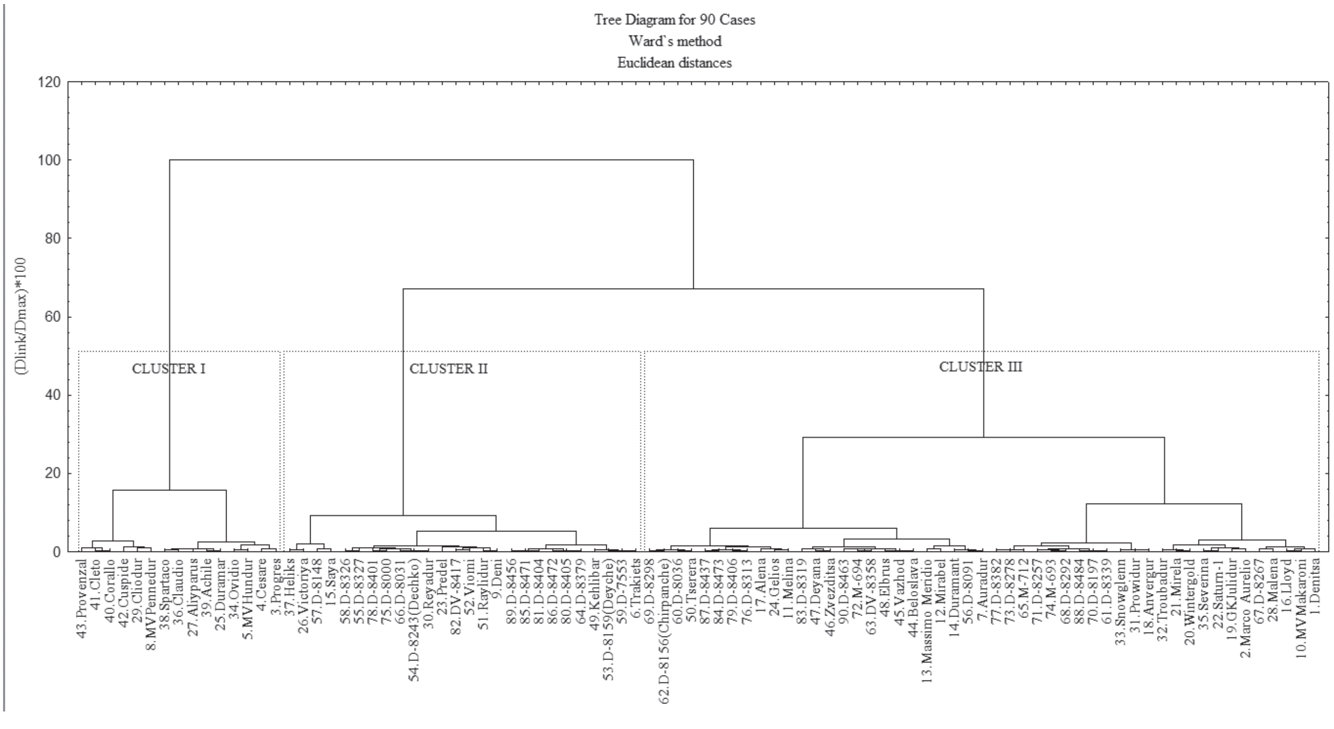

Fig. 1. Dendrogram of cluster analysis of ninety genotypes for fourteen quantitative traits
Source: Authors' own elaboration

factors and their interaction on the phenotypic expression of the traits related to yield and grain quality; the associations between them, as well as the phenotypic distance between durum wheat genotypes with diverce origine. These conclusions will serve to build an appropriate breeding strategy for durum wheat improvement under the changing climatic conditions of our region.

The greatest variation and correspondingly the highest coefficient of variation was reported for SDS-sedimentation value (CV-41.6%), and the lowest coefficient of variation and correspondingly the least variation was found for test weight (CV-2.8%). The results obtained reveal the existence of sufficient variability among studied genotypes on two important traits: gluten strength (SDS-sedimentation value) and grain yield, that could be exploited in our future breeding program.

According to the analysis of variance, the genotype, years of cultivation and interaction between them have a reliable influence on the variation of the studied traits. The expression of three grain quality traits: SDS-sedimentation vlue, vitreousness and yellow index and six yiled related traits: thousand kernel weight, test weight, number of spikelets per spike, number of kernels per spike and spike length depends in our conditions to the greatest extent on the genotype. Nevertheless, reliable genotype-by-phenotype selection could be conducted only for the trait gluten strength (SDS-sedimentation value), due to the established minor influence of the environmental conditions (0.48%) and the genotype-environment interaction (2.48%) on the variation of this trait. The environment/year of cultivation has a determining influence on the phenotypic variation of the traits: grain yield, plant height, grain protein content and grain wet gluten. This necessitates the testing of the breeding materials to be carried out in several consecutive years/different environments for a more accurate selection of appropriate genotypes for these characteristics. A significant impact of the genotype-environment interaction was established on the variation of the yield related traits: kernels weigth per spike (43.43), number of kernels per spike (43.33), number of spikelets per spike (36.29%). This necessitates the need for long-term studies on productivity or establishment of phenotypic stability of yield from different genotypes by statistical methods.

The most significant correlations established in our study between yield components and between some yield components and quality indicators, can facilitate the breeding work not only for creating high-yielding genotypes, but also for simultaneously genetic improvement of grain yield and quality traits as vitreousness (VIT), wet gluten content in grain in durum wheat at our pedo-climatic conditions. Despite our finding of a positive correlation between yield and protein content, further research is needed to clarify the relationship of protein accumulation with the weather conditions, environmental and genetic factors, to achieve a simultaneous breeding improvement of both traits.

The significant negative correlations found between SDS and yield/yield related traits will make it difficult to select high-yielding genotypes with strong gluten. Therefore, under our conditions, the selection for improving both importan grain quality traits can lead to a decrease in grain yield.

Genetic distance established in this study gives an accurate idea of the remoteness of genotypes. The varieties Predel, Saya, Heliks and Reyadur should be used in the breeding program to obtain genotypes with higher productivity and better quality.

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