

Effects of climatic factors on grain vitreousness stability and heritability in durum wheat

Gordana R. BRANKOVIĆ^{1*}, Dejan DODIG², Miroslav Z. ZORIĆ³, Gordana G. ŠURLAN-MOMIROVIĆ¹,
Vesna DRAGIČEVIĆ², Nenad ĐURIĆ⁴

¹Department of Genetics, Plant Breeding and Seed Science, Faculty of Agriculture, University of Belgrade, Belgrade, Serbia

²Maize Research Institute Zemun Polje, Belgrade, Serbia

³Institute of Field and Vegetable Crops, Maize Department, Novi Sad, Serbia

⁴Department for Cereal Crops, PKB Agroekonomik Institute, Belgrade, Serbia

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Abstract: Modern durum wheat breeding programs are becoming more oriented toward creation of not only high-yield cultivars but also high quality, with good stability for the trait of interest. Vitreousness of grain is associated with semolina granulation, color, and protein content; it is regarded as one of the most important characteristics in the grading industry, affecting the commercial value of the commodity. A set of 15 winter and facultative durum wheat genotypes was tested for grain vitreousness across 6 environments for 2 consecutive years. Three-way analysis of variance showed that genotype, location, and year contributed 4.1%, 20.6%, and 42.2% to the total sum of squares, respectively. Interaction terms, including genotype, contributed 6.1% to the total sum of squares, and location × year contributed 27.0%. Stability of grain vitreousness for the examined breeding lines and cultivars of durum wheat was shown by the site's regression. Broad-sense heritability of grain vitreousness was estimated to be 71%. Climatic variables were used for factorial regression modeling, and most of the interaction term for grain vitreousness was explained by mean temperatures in June (54.4%) and April (14.2%), and precipitation (14.4%) and sunshine hours (14.3%) in March. During the grain filling and grain ripening stages, the most influential climatic variables in explaining interaction were maximum temperature (43.4%), precipitation (30.9%), and sunshine hours (5.6%).

Key words: Durum wheat, climatic factors, heritability, stability, vitreousness

1. Introduction

Durum wheat (*Triticum durum* Desf.) is the only tetraploid wheat species of commercial importance in the food industry, due to its good grain quality. Traditionally, durum wheat is of narrower adaptation; it is cropped in dry and warm regions, where limited water supply and prolonged high temperatures during grain development are obstacles for common wheat cultivation (Rachon et al., 2009). According to FranceAgriMer (2011), the EU, Canada, US, and Turkey contributed 17 million tons to total annual world durum wheat production of 34.4 million tons in 2010. More recently, growing interest in durum wheat cultivation has been reported in other countries where the ecological conditions for its development are not optimal, like Germany, Hungary, Austria, Poland, and Serbia.

Durum wheat spring forms are widely grown, having good quality but low yield (Rachon et al., 2009). The most updated tendencies in durum wheat breeding include

winter forms, which are more productive, although possessing a lower content of protein and a poorer vitreousness of grain (Zalewski and Bojarczuk, 2004). The modern breeding strategy for genetic improvement of durum wheat germplasm, given the current circumstances of global climate change, is oriented towards creating high-yield and high quality cultivars resilient to stress and with good stability. Such efforts can lead to advances in agronomic production and in the food processing industry by increasing value and improving healthy properties of final products (Žilić et al., 2009).

The large quantities of yellow pigment, high vitreousness, test weight, and proteins, especially favorable gluten composition of good strength, are the durum cultivar qualities of primary interest to the food industry. The international grading of the durum wheat varieties is determined based on degree of grain vitreousness and hardness (Dexter et al., 1988; Dowel et al., 2000).

* Correspondence: gbrankovic@agrif.bg.ac.rs

Different concepts of stability have been established (Lin et al., 1986; Becker and Leon, 1988). Quality traits of a genotype, like quantitative ones, show similar patterns of reaction to environment (Rharrabti et al., 2003), and are regarded as stable if the genotype \times environment interaction (GEI) is low. The change of genotype ranks in diverse environments (crossover GEI) represents an impediment in the identification of the superior and stable varieties (Epinat-Le Signor et al., 2001).

The nature and reasons for GEI can be explained if information on individual environmental covariates is available (Kang et al., 2005). It can be a good direction not only in its understanding but also exploitation. The crop potential for production and quality is initiated during the vegetative growth phases (Jamieson and Wilson, 1993). Shooting, grain filling, and grain ripening are the most important development phases determining the final quality of the grains, and weather conditions impact product characteristics during these stages (Paredes-Lopez et al., 1985; Marta et al., 2011). Annicchiarico (2002) emphasized that collecting climatic information at the test locations and measuring the morpho-physiological traits of genotypes can be useful in characterizing the subregions, extending the results to new locations, adaptation modeling, identifying adaptive traits, and assessing their potential as indirect selection criteria for breeding.

The objectives of this study were to examine the variability and stability of durum wheat genotypes for grain vitreousness, to determine components of variance and heritability, and to assess the effects of climatic factors on GEI for grain vitreousness by using factorial regression.

2. Materials and methods

2.1. Plant materials and experimental design

The genetic material used in this research was represented by 15 durum wheat genotypes (breeding lines and cultivars) of the winter and facultative varieties, obtained from the GeneBank of Maize Research Institute Zemun Polje, Belgrade, Serbia (Table 1). Durum wheat genotypes were tested at 3 locations (Zemun Polje (ZP) (44°52'N; 20°19'E), Rimski Šančevi (RS) (45°19'51'N; 19°50'59'E), and Padinska Skela (PS) (44°57'N 20°26'E)) in Serbia during 2 consecutive seasons (2010–2011 and 2011–2012). Climatic variables during the March–June vegetation period were measured at the field locations and were provided by the PKB Agroekonomik Institute (Padinska Skela) and the Hydrometeorological Service of Serbia. Average values for maximum temperature (mxt), minimum temperature (mnt), mean temperature (mt), relative humidity (rh), sunshine hours (sh), and precipitation (pr) for March (1), April (2), May (3), and June (4) are given in Table 2. Flowering time of all genotypes changed from mid- to the last week of May.

The experimental design was a randomized complete block design (RCBD) with 4 replications. Each plot consisted of 5 rows of 1-m length with plants spaced 20-cm apart. The middle 3 rows were used for analysis and the calculated plot size was 0.6 m² (3 \times 0.2 m \times 1 m). The autumn plowing was done to a 30-cm soil depth. Fertilization included the application of mineral fertilizers (NPK 15:15:15, MAP) before seeding according to the recommendation based on the analysis of soil chemical properties and available content of phosphorus, potassium, and mineral nitrogen reserves. Seeds were treated with the fungicides Dividend 0.30 FS (Syngenta) (active ingredient: difenoconazole, 30 g/L) in the 2010–2011 season and Raxil 0.60 FS (Bayer) (active ingredient: tebuconazole, 60 g/L) in the 2011–2012 season. Sowing was done mechanically at the RS location and by hand at the PS and ZP locations. In the spring, fertilization included urea, KAN, and AN (34% N). Integral protection against pests and weeds was accomplished by the appropriate use of adequate pesticides and its efficacy was monitored and crop damage was avoided.

Grain vitreousness expressed in percentages were measured according to the method given in Kaludjerski and Filipović (1998) with a farinator, a device that allows 50 wheat kernels to be held firmly while a blade cuts them transversely. Vitreous grains are translucent and transparent when cut, while starchy grains are white and opaque, due to the existence of air pockets in the endosperm that diffract and diffuse the light (Hoseney, 1986). The percentage of vitreous kernels is determined by examining the cross-section of the kernels and represents the mean of the 50 \times 2 following the formula:

$$\text{Grain vitreousness (\%)} = A + \frac{3}{4} B + \frac{1}{2} C + \frac{1}{4} D,$$

A-number of fully vitreous grains

B-number of vitreous grains with more than 75% of grain cross-section being vitreous

C-number of vitreous grains with 50% to 75% grain cross-section being vitreous

D-number of vitreous grains with 25% to 50% grain cross-section being vitreous.

2.2. Statistical analysis

For the analysis of grain vitreousness data, 3-way ANOVA was used based on RCBD.

The empirical mean response (Y_{ijkl}) of the i th genotype in the j th location, k th year and l th replication is expressed as:

$$Y_{ijkl} = \mu + \gamma_{jl} + g_i + l_j + y_k + (gl)_{ij} + (gy)_{ik} + (ly)_{jk} + (gly)_{ijk} + \varepsilon_{ijkl},$$

where μ is the grand mean, γ_{jl} is the effect of the l th replication in the j th location, g_i is the effect of the i th genotype, l_j is the effect of the j th location, y_k is the effect

Table 1. Names (codes), origin, type, and pedigree information of tested genotypes of durum wheat (*Triticum durum* Desf.).

Code	Genotype	Origin	Type	Pedigree
D1	37EDUYT No. 7922	Mexico (CIMMYT)	facultative**	ALTAR84/STINT//SILVER_45/3/POHO_1/4/ GREEN_14//YAV_10/AUK
D2	37EDUYT No. 7896	Mexico (CIMMYT)	facultative**	AINZEN_1/3/SRN_3/AJAI_15//DON87/4/MINIMUS/ COMB DUCK_2//CHAM_3
D3	37EDUYT No. 7817	Mexico (CIMMYT)	facultative**	SNITAN/3/STOT//ALTAR84/ALD
D4	Varano	Italy	winter*	CAPEITI-8/CRESO//CRESO/3/VALFORTE(VALF)/ TRINAKRIA
D5	37EDUYT No. 7821	Mexico (CIMMYT)	facultative**	AINZEN-1//PLATA_6/GREEN_17
D6	37EDUYT No. 7880	Mexico (CIMMYT)	facultative**	ALTAR 84/STINT//SILVER_45/3/LLARETA INIA/4/
D7	10/I.	Serbia	winter**	WINDUR (Germany)//RODUR (Romania)
D8	SOD 55	Slovakia	winter*	KORALL ODESSKIJ (Soviet Union)//GK PANNONDUR (Hungary)
D9	37EDUYT No. 7803	Mexico (CIMMYT)	facultative**	RASCON_37/2*TARRO_2/4/ROK/FGO//STIL/3/ BISU_1/5/MALMUK_1/SERRATOR_1
D10	DSP-MD-01 No. 66	Syria (ICARDA)	facultative**	848.10.6/Otb2//Gdr1
D11	34/I	Serbia	winter**	SOD 55 (Slovakia)//KORIFLA (ICARDA)
D12	37EDUYT No. 7820	Mexico (CIMMYT)	facultative**	AINZEN-1/3/MINIMUS_6/PLATA_16//IMMER
D13	37EDUYT No. 7857	Mexico (CIMMYT)	facultative**	CBC 514 CHILE/SOMAT_4/3/HUI/YAV79//DON87
D14	37EDUYT No. 7849	Mexico (CIMMYT)	facultative**	CBC 505 CHILE/LLARETA INIA/3/D86135/ACO89// PORRON_4
D15	120/I	Serbia	winter**	WINDUR (Germany)//KAVADARKA (Macedonia)

* cultivar, ** breeding line; CIMMYT-International Maize and Wheat Improvement Center; ICARDA- International Center for Agricultural Research in the Dry Areas; 37EDUYT – 37th Elite Durum Unreplicated Yield Trial; DSP-MD-01 – Durum Segregating Populations – Mediterranean Dryland (season 2000–2001)

of the k th year, $(gl)_{ij}$ is the interaction of the i th genotype with the j th location, $(gy)_{ik}$ is the interaction of the i th genotype with the k th year, $(ly)_{jk}$ is the interaction of the j th location with the k th year, $(gly)_{ijk}$ is the interaction of the i th genotype with the j th location and with k th year, and ε_{ijkl} is the average error.

Variance components were estimated based on the combined 2-way ANOVA according to Falconer (1981) as follows:

$$V_g = \frac{MS_g - MS_{ge}}{re}$$

$$V_{ge} = \frac{MS_{ge} - MS_{er}}{r}$$

$$V_{er} = MS_{er}$$

$$V_p = V_g + \frac{V_{ge}}{e} + \frac{V_{er}}{re}$$

where V_g , V_{ge} , V_{er} , and V_p are the variances due to genotypes, GEI, experimental error, and phenotypes, respectively. MS_g , MS_{ge} , and MS_{er} are the mean squares of

Table 2. Averages of climatic variables by months measured at the locations used in 2010–2011 and 2011–2012.

Month	Location					
	2010–2011			2011–2012		
	RS	PS	ZP	RS	PS	ZP
average maximum temperature (°C)						
March	11.0	11.7	11.9	15.6	15.6	15.4
April	18.9	18.9	19.1	18.9	19.7	19.3
May	22.6	23.3	22.6	22.6	23.9	22.7
June	<u>26.8</u>	<u>27.5</u>	<u>27.3</u>	<u>29.3</u>	<u>30.6</u>	<u>29.9</u>
average minimum temperature (°C)						
March	1.2	0.3	2.2	1.2	-0.5	1.7
April	7.6	4.5	7.6	7.2	4.0	7.7
May	10.9	7.1	11.3	11.7	7.9	11.5
June	<u>15.0</u>	<u>11.6</u>	<u>15.4</u>	<u>15.8</u>	<u>11.1</u>	<u>16.4</u>
average mean temperature (°C)						
March	5.7	5.7	8.0	8.1	7.7	8.9
April	13.2	12.1	14.4	13.0	12.4	13.5
May	16.8	15.4	17.5	17.4	16.0	17.0
June	<u>20.9</u>	<u>19.9</u>	<u>22.2</u>	<u>22.9</u>	<u>21.7</u>	<u>24.3</u>
average relative humidity (%)						
March	<u>77.6</u>	79.4	<u>70.2</u>	55.4	60.9	55.3
April	62.6	67.8	58.5	68.5	72.8	65.2
May	72.7	<u>80.1</u>	68.5	<u>70.4</u>	<u>79.8</u>	<u>70.6</u>
June	69.2	77.7	63.3	61.7	72.1	56.4
precipitation sum (mm)						
March	26.2	21.6	18.6	4.1	1.6	2.5
April	22.8	25.8	14.1	<u>82.8</u>	63.0	73.3
May	<u>63.0</u>	<u>90.0</u>	<u>94.8</u>	52.2	<u>72.0</u>	<u>81.8</u>
June	36.9	41.4	23.0	27.5	15.0	16.1
sunshine hours sum (h)						
March	159.6	162.0	103.9	241.4	253.9	234.9
April	205.9	222.1	191.2	204.2	209.2	145.3
May	269.5	255.9	244.5	253.4	230.7	199.5
June	<u>284.5</u>	<u>280.3</u>	<u>257.6</u>	<u>359.0</u>	<u>344.3</u>	<u>313.5</u>

RS: Rimski Šančevi; PS: Padinska Skela; ZP: Zemun Polje; maximum values for each climatic variable at the locations used are underlined.

genotypes, GEI, and pooled error, with e being the number of environments and r the number of replications. Broad-sense heritability is expressed as:

$$h^2 = \frac{V_g}{V_p} \times 100$$

The factorial regression (Denis, 1988; van Eeuwijk et al., 1996) modeling assumes inclusion of environmental information to describe the interaction term. In our study we applied multiple factorial regressions of climatic variables to explain interaction term for grain vitreousness of durum wheat genotypes tested.

In order to evaluate genotypes for their performance and stability regarding grain vitreousness, both genotype (G) and GEI effects must be considered simultaneously. Thus the sites regression (SREG) model was used (Crossa and Cornelius, 1997) to obtain GGE biplots. The average-environment coordination (AEC) view of the GGE biplot was used to estimate the mean performance and stability

of tested durum wheat genotypes. The GGE biplot for this purpose was created using the genotype-metric preserving singular value procedure (SVP = 1), which is more suitable for genotype evaluation. Data analysis was done within the R computing environment (<http://www.R-project.org>).

3. Results

3.1. Variability of grain vitreousness for durum wheat genotypes

Data regarding grain vitreousness average values are given in Table 3. The average value of grain vitreousness across all genotypes and all environments was 83.1%. The ZP location in 2010–2011 showed the lowest average grain vitreousness across all genotypes (46.4%), and the absolute lowest value of 28.2% for genotype D15 was observed. The location that showed the highest average value of grain vitreousness (96.5%) across all genotypes was ZP in 2011–2012. Among the genotypes, the highest value for grain vitreousness across all environments was recorded in D13 (88.5%) while D15 had the lowest grain vitreousness (72.6%). The genotype performance for grain vitreousness

Table 3. Average grain vitreousness (%) for durum wheat genotypes (D1–D15) by locations and years of experimentation.

Genotype	2010–2011				2011–2012				Mean for 2-year period
	RS	ZP	PS	Mean	RS	ZP	PS	Mean	
D1	85.5	44.1	86.9	72.2	97.4	95.9	92.8	95.3	83.8 bac
D2	82.6	40.4	88.5	70.5	93.0	96.6	93.9	94.5	82.5 bdc
D3	81.4	47.2	92.0	73.5	97.3	96.5	95.2	96.4	84.9 bac
D4	82.5	37.4	90.5	70.1	97.9	99.7	94.9	97.5	83.8 bac
D5	82.2	51.1	88.6	74.0	97.0	99.2	94.1	96.8	85.4 bac
D6	83.4	55.0	85.9	74.8	94.7	95.9	87.9	92.8	83.8 bac
D7	81.5	45.9	85.0	70.8	90.5	92.1	95.2	92.61	81.7 dc
D8	73.6	28.2	84.8	62.2	93.4	95.1	90.9	93.1	77.7 d
D9	88.9	56.6	89.8	78.4	96.1	97.5	93.0	95.6	87.0 ba
D10	80.50	44.4	90.8	71.9	98.0	97.2	94.7	96.7	84.3 bac
D11	79.5	55.8	88.1	74.5	97.2	97.1	97.1	97.2	85.8 bac
D12	78.2	43.8	83.9	68.6	95.2	97.2	91.1	94.5	81.6 dc
D13	84.9	69.2	89.8	81.3	97.2	97.1	92.6	95.7	88.5 a
D14	82.5	48.2	85.5	72.1	96.2	95.5	91.7	94.5	83.3 bc
D15	49.5	28.2	81.0	52.9	90.2	95.4	91.0	92.2	72.6 e
Mean	79.8 d	46.4 e	87.4 c	71.2	95.4 ba	96.5 a	93.1 b	95.0	83.1

The means in columns and rows labeled with the same letter are statistically nonsignificant at the 0.05 probability level. Tukey's (HSD) test was used. Details for genotypes and locations are given in Tables 1 and 2.

in different locations and years is presented in the genotype versus grain vitreousness biplot (Figure 1). As shown, genotypes achieved higher values for grain vitreousness in all 3 locations in the second year of experimentation, but the magnitudes of differences among genotypes were lower compared to the first season. The mean differences for grain vitreousness among all 3 locations in the first year were significant ($P < 0.05$) as shown by Tukey's (HSD) test, but only between the ZP and PS locations in the second year (Table 3). The differences among means across genotypes were also significant ($P < 0.05$) (Table 3).

3.2. ANOVA, components of variance, and heritability

The results of 3-way ANOVA for grain vitreousness obtained from 2 years and 3 locations are given in Table 4. All observed sources of grain vitreousness variation, genotype, year, location, and their interactions proved to be significant ($P < 0.001$). The most influential effect was year, which contributed 42.2% to the total sum of squares, followed by location (20.6%) and genotype (4.1%). Among interactions, location \times year was the highest (27.0%), followed by genotype \times year (2.4%), genotype \times location (2.0%), and genotype \times location \times year (1.7%).



Figure 1. Genotype versus grain vitreousness for durum wheat genotypes (D1–D15) by locations in 2010–2011 and 2011–2012. PS: Padinska Skela; RS: Rimski Šančevi; ZP: Zemun Polje. Details for genotype are given in Table 1.

Table 4. Three-way ANOVA for grain vitreousness.

Source of variations	df	SS	SS (%)	MS [†]
Genotype (G)	14	4994	4.1	356.7***
Location (L)	2	24,865	20.6	12,431.6***
Year (Y)	1	51,096	42.2	51,092.7***
G \times L	28	2417	2.0	86.3***
G \times Y	14	2899	2.4	207.1***
L \times Y	2	32,631	27.0	16,316.9***
G \times Y \times L	28	2059	1.7	73.5***
Error	267	6951	-	26.0

*** $P < 0.001$, [†] tested against error mean square.

Estimates of components of variance, broad-sense heritability, and coefficients of genotypic and phenotypic variation for grain vitreousness are presented in Table 5. As genotype interactions with year and location were rather low, the above calculations were based on 2-way combined ANOVA (data not shown), where year and location factors were combined as environment. The variance components due to genotype and genotype \times environment were 10.5 and 19.9, respectively. Coefficients of genotypic and phenotypic variation were 3.9 and 4.6, respectively. The estimated broad-sense heritability of 71% for grain vitreousness was moderate.

3.3. Stability of durum wheat genotypes for grain vitreousness

The average-environment coordination (AEC) view of the site regression biplot was used to estimate mean performance and stability of tested durum wheat genotypes for grain vitreousness (Figure 2). The GGE

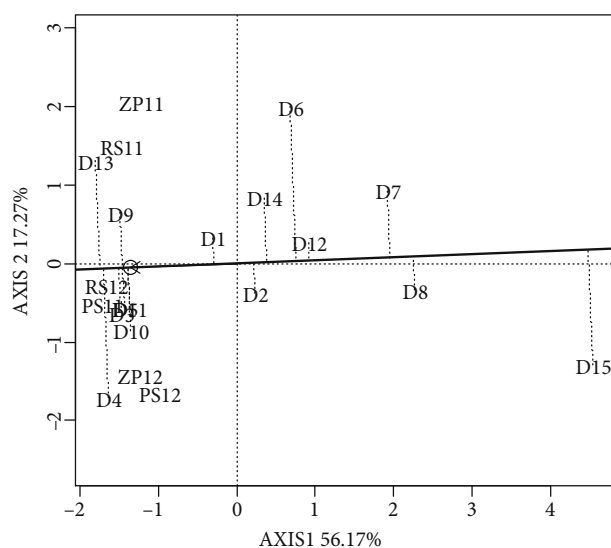


Figure 2. The average-environment coordination (AEC) view of the GGE biplot to show the mean performance and stability of grain vitreousness for durum wheat genotypes over tested locations. The details for genotypes and locations are given in Tables 1 Table 2.

biplot accounted for 73.4% of the total G + GE variation for the grain vitreousness of durum wheat genotypes under study. The length of the average-environment vector was sufficient to select genotypes based on mean grain vitreousness. Genotypes with above-average mean grain vitreousness were D1, D10, D11, D3, D5, D9, D13, D4, and D11. The below-average mean grain vitreousness was observed in the following genotypes: D15, D8, D7, D12, D6, D14, and D2. Genotype D15 had the lowest mean grain vitreousness across environments and unsatisfactory stability. The best stability was observed in genotype D12, but it had a below-average mean grain vitreousness value. On the other hand, the most unstable genotypes for the grain vitreousness quality were D6, D4, D15, and D13, and among them D4 and D13 showed the best grain vitreousness.

3.4. Effect of climatic factors on grain vitreousness

The factorial regression procedure included choice of climatic variables in the combined analysis. It led to information regarding level of the interaction explained. Models with all variables included and models by months of durum wheat vegetative cycles were generated (Table 6). A heat map of the Pearson correlation coefficients of climatic variables pairs measured at the 3 locations and averaged across 2010–2011 and 2011–2012 years is given in Figure 3.

By including all available variables in the above-mentioned analysis for the multiple environment combined analysis, a model (mt4, pr1, sh1, mt2) ensued, explaining 97.2% of the genotype \times environment sum of squares and leaving a small residual (2.8%) (Table 6). Models that considered climatic variables by months of durum wheat genotypes vegetative cycle in combined analysis showed that maximum temperature was the most important variable in June (43.4%), minimum temperature in May (26.4%), relative humidity in April (54.0%), and sunshine hours in March (53.6%) (Table 6).

Table 5. Estimates of components of variance, broad-sense heritability, and coefficients of genotypic and phenotypic variation for grain vitreousness.

σ_g^2	σ_{ge}^2	σ_e^2	σ_p^2	h^2 (%)	CV_g (%)	CV_p (%)
10.5	19.9	4.4	14.9	71	3.9	4.6

σ_g^2 : genetic variance; σ_{ge}^2 : variance of the genotype \times environment interaction; σ_e^2 : environmental variance; σ_p^2 : phenotypic variance; h^2 : broad-sense heritability; CV_g : coefficient of genetic variation, CV_p : coefficient of phenotypic variation.

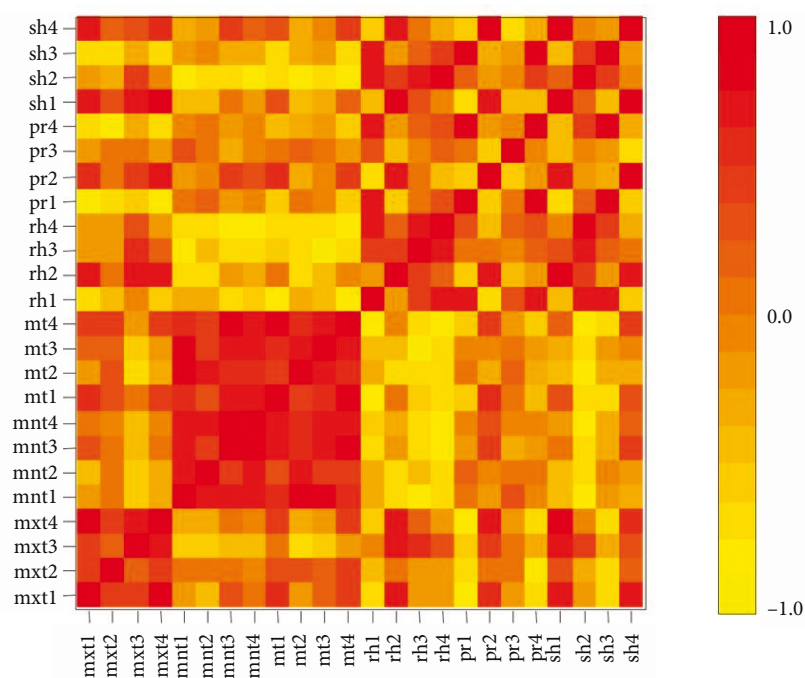


Figure 3. A heat map of the average Pearson correlation coefficients of climatic variables pairs measured at the 3 locations during 2010–2011 and 2011–2012. The legend on the right depicts the correlation color scale. The details for climatic variables are given in Table 2.

Table 6. Multiple factorial regressions of climatic variables explaining interaction term for grain vitreousness of durum wheat genotypes.

Model	Environmental variables included in the final model [§]	Residual
All variables	mt4 (54.4); pr1 (14.4); sh1 (14.3); mt2 (14.2)	2.8
	Variables by months of vegetation period	
March	sh (53.6); pr (22.9); rh (9.3); mnt (6.2)	8.1
April	rh (54.0); sh (12.2); mt (10.6); mnt (10.1)	13.1
May	mnt (26.4); sh3 (23.5); rh (21.5); pr3 (15.1)	13.5
June	mxt (43.4); pr (30.9); sh (5.6)	20.1

[§] Variable significance is tested against error mean square $P < 0.01$; mxt: average maximum temperature; mnt: average minimum temperature; mt: average mean temperature; pr: precipitation sum; rh: average relative humidity; sh: sunshine hours sum. All reported values are given as a percentage of the explained variance of interaction by the term.

4. Discussion

Grain vitreousness values for durum wheat genotypes grown at the locations in Serbia were in the range of 72.6%–88.5%, and similar to the values reported for other regions, such as northeastern Turkey (Khalaf et al., 2005;

Korkut et al., 2007; Bilgin et al., 2010; Taghouti et al., 2010). In some irrigated areas a slightly lower range for durum vitreousness (61.5%–86.7%) was reported, whereas in dry land this trait was expressed in the range of 77.4%–99.0% (Baum et al., 1995).

The predominant source of variation for grain vitreousness in our study proved to be year and year \times location interaction. This is not surprising, as several previous studies in wheat and other crops showed that differences among consecutive years are larger than differences among test locations within southeastern Europe (Rizza et al., 2004; Sudaric et al., 2006; Dodig et al., 2008). Although our locations are not geographically distant, there were probably still differences in microclimate that could further explain our findings. Despite being far lower than the influence of environmental factors, the influence of genotype and its interaction with environment (year and location combination) in our study was highly significant and thus not to be neglected. The interactions of genotype \times year and genotype \times location in our study can be explained by differences in the magnitude of individual responses in each genotype in each particular year and location, while no significant crossover interactions were observed.

Dodig et al. (2003) determined the genotype effect to be 6% and GEI to be 14% for grain vitreousness of 3 durum genotypes grown under normal and drought conditions. A GEI greater than genotype in contribution to grain vitreousness in durum wheat was also found by Korkut et al. (2007) and Baum et al. (1995). Korkut et al. (2007) examined 44 durum wheat genotypes for grain vitreousness over 6 environments for 2 seasons in Turkey and showed that genotype, genotype \times year, genotype \times location, and genotype \times year \times location explained 10.3%, 5.3%, 11.9%, and 11.9% of the total sum of squares, respectively. According to Baum et al. (1995), under dryland conditions, G, environment (E), and GEI contributed 11.4%, 70.0%, and 16.9% to the total sum of squares for vitreousness variation, and 14.1%, 58.1%, and 23.0% under Mediterranean irrigated conditions, respectively.

Rharrabti et al. (2003) conducted 10 field trials of 10 durum wheat genotypes during 2 seasons in the north and south of Spain, evaluating many traits, including grain vitreousness. Their finding was that G, GEI, and E participated with 4.9%, 25.5%, and 52.4% of the total sum of squares, respectively. Our values showed that G/G \times E relation of grain vitreousness variation was 0.68, which was 3.58 times higher than the previously mentioned investigation, and 2.96 times higher than in Taghouti et al. (2010), inferring a smaller contribution of G \times E to the grain vitreousness variation. However, the influence of year on grain vitreousness in our study was prevalent, including 42.2% of the total sum of squares, which justified the use of factorial regression in climatic variables modeling.

The relation of genetic variance (σ_g^2) and variance of the GEI (σ_{ge}^2) for grain vitreousness in our study was relatively low (0.53) compared to the value of 0.85 reported by Bilgin

et al. (2010). This relation might be an impediment for breeding generally adaptable genotypes of durum wheat for improved grain vitreousness. Ideally, this relation should be higher than 1. Heritability helps in predicting the genetic potential of breeding germplasm and in identifying superior combinations in hybridizations and suitable methods of selection (Ahmad et al., 2013). Higher heritability for the trait of interest leads to greater genetic improvement (Allard, 1960). In our study, the broad-sense heritability estimate for grain vitreousness was moderately high (71%), higher than the values of 62.4% and 39.1% reported by the Bilgin et al. (2009) and Bilgin et al. (2010), respectively. Our heritability value indicated moderate genotypic effects on this trait, repeatability, and the ability to make genetic gains. Other authors reported mainly lower values of heritability for vitreousness. For example, Taghouti et al. (2010) estimated the broad-sense heritability for 12 durum wheat cultivars over multiple environments to be only 3%. The broad-sense heritability estimate in irrigated conditions was lower (59%) than in dryland conditions (79%) for the durum wheat genotypes developed at the International Maize and Wheat Improvement Center (CIMMYT) and the International Center for Agricultural Research in the Dry Areas (ICARDA), according to Baum et al. (1995). The large difference in the values of heritability under dryland and irrigated conditions suggested that increased moisture had a negative impact on grain vitreousness.

According to Yan et al. (2000), ideal genotypes should have large projections of their markers towards the AEC abscissa to show high performance, and small projections onto the AEC ordinate to show high stability of performance.

The use of covariates, which can be any environmental factor such as humidity, precipitation, temperature, or environmental index, allows researchers to investigate possible causes of GEI (Magari and Kang, 1993). Multiple factorial regressions were used to search for informative sets of environmental covariables. According to Voltas et al. (2005), environmental modeling of GEI by factorial regression can be regarded as a predictive strategy for recommendation purposes, and outcomes of factorial regression analyses could be integrated into geographical databases, in which annual environmental data are recorded over a relevant temporal scale. Climatic factors were very useful in accounting for the GEI of grain vitreousness in our study. The model, which included mean temperatures in June (54.4%), mean temperatures in April (14.2%), and precipitation (14.4%) and sunshine hours (14.3%) in March, left a residual of only 2.8%.

In June, when grain filling and grain ripening occur, the most important climatic factor that explained GEI for grain vitreousness was the average maximum temperature

(43.4%) (Table 6). The average maximum temperature in June was inversely correlated with the sum of precipitation in June (Figure 3). Our results are in accordance with previous findings that the agronomic conditions (water and nitrogen availability), climatic factors (temperature and light intensity) during grain filling, and the drying intensity at maturity influence grain vitreousness (Parish and Halse, 1968; Bechtel et al., 2009; Edwards, 2010, Nair et al., 2010). In all tested locations, mean temperatures and precipitations in June were inversely related, respectively, in the second compared to the first season. This resulted in higher vitreousness in the second season of about 20% (95.0 vs. 75.2%). It was documented that the hot and dry seasons cause yield instability, but lead to the better expression of quality parameters such as vitreousness (Borghini et al., 1997). The increase in the mean daily relative air humidity and the sum of rainfall during the first 10 days of June, and the insufficient mean daily temperatures during subsequent wheat developmental stages had a negative effect on vitreousness according to Stoeva (2012).

The factorial regression approach dissected GEI for grain vitreousness, and also showed the impact of climatic variables in earlier stages of wheat growth such as tillering (March) and stem elongation (April). GEI for grain vitreousness can be explained by mean precipitations and sunshine hours in March and mean temperatures in April (each explains about 14%). Although it may be unexpected that grain quality traits depend on climatic variables in the period when grains are not even formed, these findings are not unknown in the literature. In Australia, it was found that the cumulative precipitation during the vegetative period (from May to September) was negatively associated with grain protein content (Correll et al., 1994). Rainfall and soil water availability dilute early nitrogen reserves by vegetative proliferation, and leaching and soil nitrogen loss may augment soil moisture reserves, favoring carbohydrate assimilation and translocation more than that of nitrogen, in turn leading to a reduction in protein content (Smith and Gooding, 1999; Marta et al., 2011). Several authors found a positive medium to high correlation between grain vitreousness and protein

in durum wheat (Budak and Budak, 1998; Bilgin et al., 2010; Taghouti et al., 2010). Oweis et al. (1999) showed that under rain-fed conditions, N raised values for the vitreousness to as high as 92% from 57% under unfertilized conditions, whereas with irrigation the maximum was 75%. This infers climate importance in the sense that if the growing season is predominated by cool wet conditions in the spring, crop biomass and yields will be high, but the N content and wheat quality will be low (Salinger et al., 1995). The vitrification hypothesis proposes that accumulated nonreducing sugars and highly hydrophilic proteins enter a glassy state during dehydration, immobilizing membranes and macromolecules in the cytoplasm, preventing them from denaturizing and coagulating (Sakurai et al., 2008). Hosney (1986) pointed out that as the cytoplasm in the endosperm dries, it shrinks, and the protein matrix and starch granules either adhere tightly (vitreous grains) or rupture, leaving air spaces (opaque kernels).

In conclusion, grain vitreousness was affected more by climatic conditions of consecutive years than other variables. Although they were far lower, the contributions of G and GEI were still significant and thus worth studying. It is important to identify and understand the pattern of GEI to be able to use it constructively in selecting genotypes. Modeling by months showed that the most influential climatic variables in explaining interaction for grain vitreousness were maximum temperature (43.4%), precipitation (30.9%), and sunshine hours (5.6%) during grain filling and ripening. The heritability estimate was moderately high and should not encourage us to expect good breeding perspectives. Thus, including more locations and years is needed to understand the nature of grain vitreousness.

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