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Genetics and Breeding

Correlations between quantitative traits of winter common wheat-breeding tool for increasing grain yield

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Abstract. *Information on the relationships between quantitative traits affecting yields is extremely important for winter wheat. For it, the annual genotype*environment interaction is palpable and often masks the influence of individual traits on grain yield. The aim of the study is to determine the traits through the selection of which the grain yield could be significantly increased in the future. The data from three field multifactorial experiments were used (FERT, PGR, ABC), in which a significant influence of various factors (year, point, density, fertilization) on the size and variation of all studied traits was established. In the database thus formed the observed strong variance in the values of the traits is a great prerequisite for the established correlations to be accepted with a high degree of reliability. The mutual influence in the formation of each of the traits is a good basis for their grouping, according to the type of their effect on yield. 1) The characteristics, number of grains per m² (NGm), grain weight per spike (WGS) and number of productive tillers per m² (NPT) have a significantly positive effect on grain yield, 2) the weight per 1000 grains (TGW) and number of grains in spike (NGS) are traits that have a direct effect, but it is unstable in manifestation and 3) the traits, as height of stem (HOS), total plant biomass (TBM), and harvest index (HI) do not show a direct effect on grain yields. A significant increase in yield in the breeding of winter wheat can be achieved by increasing the number of grains per unit area (NGm). This is possible while maintaining the achieved level of number of grains in spike (NGS) with a parallel increase of tillering productive ability (NPT). The increase of this trait by selection should be taken into account when reducing the grain size (TGW). This will increase the chance of increasing the number of grains in the spike (NGS), will reduce the weight of the grain per spike (WGS), which in turn will be a prerequisite for optimizing the stability of lodging.*

Keywords: *Triticum aestivum* L., grain yield, components of productivity, correlations, path-analysis

Introduction

Increasing the productive potential of any crop is inextricably linked to creating and maintaining a certain balance between the components of productivity (Slafer et al., 2014; Lichthardt et al., 2020). This "balance" between the different traits on which grain is built is different. In the case of winter wheat, this balance largely depends on the climatic (natural) conditions for its cultivation. It is known that, under favourable environmental conditions, the correlations between the traits have some values, and under stress - others (Tsenov et al., 2015; Mihova et al., 2017; Mondal et al., 2020). The opinion of many researchers is that it is the growing conditions that cause a change in the correlations between the traits that directly or indirectly affect grain yield (Slafer et al., 2014; Gubatov et al., 2016; Xiong et al., 2020). In relation to a possible strategy for breeding of grain productivity or quality against the background of the growing influence of environmental conditions: a) correlations between the traits in conditions without appreciable stress (or irrigation) (Lichthardt et al., 2020; Mondal et al., 2020), b) correlations under conditions of constant stress (thermal or soil water deficit) (Mondal et al., 2020; Soares et al., 2021) and c) correlations in which mentioned conditions are changing on a seasonal or annual basis (Öztürk and Korkut, 2017; Quintero et al., 2018; Kanwal et al., 2019; Yang et al., 2019). In our country the environments are so dynamic that they could hardly be framed. However, there are some trends for lasting (principled) correlations between traits that should be considered in wheat breeding (Herrera et al., 2020; Tsenov et al., 2020a). In recent decades, with the advancement of computer programs, the scale of the influence of the environment on yield and its elements has been established (Chairi et al., 2020; Ramirez-Villegas et al., 2020; Xiong et al., 2020). The genotype by environment interaction is a complex factor that affects the traits and changes the balance between them, which ultimately affects the size of grain yield in specific environmental conditions (year, location, fertilization, etc.), (Sadras and Slafer, 2012; Gubatov et al., 2016; Xiong et al., 2020). The direction and nature of the environment by variety interactions is the main reason for the drastic change in the correlation

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between two traits such as direction and value (Quintero et al., 2018; Kanwal et al., 2019; Rajičić et al., 2020). The greater the differences in conditions during the study period, the more difficult it is to establish lasting and reliable interdependencies between traits (Mondal et al., 2020; Tsenov et al., 2020a). This has been known for a long time, which is why researchers often "speculate" by calculating correlations between traits by years, at locations with similar conditions, or in contrasting different variants of various factors (variety, irrigation, fertilizing, foliar treatment, etc.), (Ivanova and Tsenov, 2011; Rajičić et al., 2020). Conclusions are drawn that are sometimes quite far from the objective correlations between the traits (Zvizdojević and Vukotić, 2015). Variation of any nature caused by a given factor(s) masks the correlations and is a prerequisite for incorrect conclusions. All environmental factors that cause the dispersion of each trait ultimately make it difficult to build a correct breeding concept for stable relationships between traits in order to increase grain yield. Global climate change, which is already constantly observed in cereal crops such as wheat, is the reason why scientists are introducing simulation models for breeding according to climatic features and their possible change (Chairi et al., 2020; Ramirez-Villegas et al., 2020; Xiong et al., 2020). From the point of view of practical selection, simulation models give only general approximate information about the tendency of breeding strategy to change over time (Lichthardt et al., 2020; Le Gouis et al., 2020; Senapati and Semenov, 2020). This requires the application of certain statistical approaches or models to establish the basic relationships between the traits. The already established lasting trends of climate change in the world (Ayalew and Worku, 2019; Le Gouis et al., 2020) and in Bulgaria (Kazandjiev et al., 2011; Spiridonov and Valcheva, 2017) require a rethinking of our knowledge on the relationship between the quantitative characters of yield and quality of wheat (Chamurliyski et al., 2016; Raykov et al., 2017).

In the conditions of the temperate continental climate of the Balkan Peninsula, several studies have been published on the topic of cereals (Mihova et al., 2017; Iancu et al., 2019; Öztürk et al., 2019). The correlations between the elements of productivity in barley, durum wheat, triticale, oats and wheat have been studied repeatedly (Mandea et al., 2019; Öztürk et al., 2019). Much of the information about them is difficult to summarize because they apply only to specific conditions of breeding or agronomic experiments (Ivanova and Tsenov, 2011; Nankova et al., 2014). In Bulgaria, there is up-to-date information on the correlations for barley (Gocheva et al., 2017), for durum wheat (Popova and Neykov, 2013), for triticale (Stoyanov, 2019; Derejko et al., 2020), for oats (Dyulgerova and Savova, 2020) and for wheat (Desheva, 2016; Tsenov et al., 2019; Angelova et al., 2020). In winter wheat, the prevailing opinion is that the grain yield is a result of the size of the number of grains in spike (Desheva, 2016; Raykov et al., 2017; Tsenov et al., 2020a) and the weight of the grain in spike, in which the share of grain size (TGW) is lower than the number of grains in spike (Ivanova and Tsenov, 2012; Tsenov et al., 2015).

In recent years, there has been an increase in research looking for correlations between traits under a reliable genotype*environment interaction (Quintero et al., 2018; Yang et al., 2019). For example, Xiong et al. (2020), found that climate change in recent decades has increased the genotype*environment interaction in wheat by 49%. This prompted the idea of looking for correlations between traits subject to direct selection in wheat in data from multifactorial field trials conducted with other intentions (Tsenov et al., 2020b).

The main target is to establish reliable correlations between the components of productivity in wheat grown in the conditions of appreciable genotype*environment interaction, which causes the maximum possible variation of the traits. In this way, a balance should be established between the main traits - elements of productivity, the change of which in different directions significantly affects the grain yield.

Material and methods

The analysis of the correlations between the characteristics related to productivity was made on data collected from different field experiments (Table 1). The databases reflect the impact of several main factors, each of which causes variation in the values of each studied trait. The first database (FERT) consists of a study of 18 varieties over a two-year period (2017-2018), in which the main factors are crop density (450, 550, 650 and 750 germinated seeds/m^2) and several levels of nitrogen nutrition (400, 500, 600 and 700 kg/ha ammonium nitrate). The second database (PGR) includes the use of a growth regulator to shorten stem height and several seed densities (500, 600 and 700 germinated seeds/m^2) for 10 varieties that are part of the first 18th. The third group of data (ABC) includes 40 varieties (which include 18^{ths} from the first database) studied in three locations of Bulgaria (Dobrich, Rousse and Yambol), where the climate is significantly different for the magnitude of grain yield. The variation of each trait is large enough to attempt to find any relationships between them (Table 2). The change in the values of the traits (CV), as a result of their interaction with the described factors, is in the range of 10.1% to 25.5% for the different traits.

Table 1. Multifactor Databases used for statistical analyses

Code	Number of variants	Main factors				
		Year	Location	Variety	Density	Other
FERT	324	2	1	18	2	4
PGR	456	2	1	10	4	2
ABC	360	2	3	40		
Total	1140	2	4	68		

Table 2. Descriptive Statistics of traits in Multi Environment Trails

No	Trait	Number	Range	Min	Max	Mean	Std. Dev	CV, %
1	GY	1140	7.60	3.85	11.45	8.10	1.135	14.0
2	NPT	1140	693	332	1025	671	113.505	16.9
3	TGW	1140	30.8	27.2	58.0	44.9	4.603	10.3
4	WGS	1140	1.39	.73	2.12	1.23	0.205	16.7
5	NGS	1140	30.8	16.4	47.2	27.6	4.709	17.1
6	NGm	1140	19965	8744	28709	18201	2972.182	16.3
7	HOS	1140	54.0	57.0	111.0	89.2	10.559	11.8
8	TBM	684	7.73	1.83	9.56	4.47	1.137	25.5
9	HI	684	.36	.29	.65	0.43	0.062	14.4
10	LOD	456	100	0	100	23.5	36.067	10.1

Legend: GY- grain yield, t/ha, NPT- number of productive tillers, TGW- 1000 grain weight, WGS- weight of grain per spike, NGS- number of grains per spike, NGm- number of grains per m^2, HOS- height of stem, TBM- total biomass, HI- harvest index and LOD-lodging tolerance.

Investigated traits related to grain yield

Several quantitative traits were studied: grain yield (GY), number of productive tillers (NPT), number of grains per spike (NGS), 1000 grain weight (TGW), weight of grain per spike (WGS), number of grains per m^2 (NGm), stem height (HOS), total plant biomass including grain (TBM), harvest index (HI) and degree of lodging in % (LOD). The field measurements are on three replications of each individual character, without the indices (WGS, NGm). The latter are calculated from the values of the three main traits: NPT, TGW, NGS and are accepted as quantitative traits, along with the others. Data for height of stem (HOS) and total biomass, are from the first (FERT) and third (ABC) databases, and those for lodging tolerance (LOD) are from the second one (PGR).

Statistical analyses

The correlations between each of the traits and grain yield, as a result of their manifestation, were analysed using all possible basic statistical approaches to this, including path-analysis (Akintunde, 2012). In order to confirm the magnitude of the correlations with grain yield, the trait data were further evaluated by multi-regression analysis (MPA) and by principal component analysis (PCA). The statistical packages IBM SPSS 23, Statgraphics XVIII and Statistica 10 were used. Before being analysed, the numerical values of each trait were arranged and standardized according to the model Xs = (x-M) / sd) in MS Excel.

Results

Grain yield (GY) was positively affected by each of the traits: NGm (r=0.814), WGS (r=0.382) and TGW (r=0.376) and (NPT, r=0.229) (Table 3). In addition, NPT showed a strong indirect positive effect (r=0.550) on yield, although its indirect effects through other traits were low. Negative in value are the correlations between the TGW and the number of grains per spike (NGS), (r= -0.24), as well as that between the number of grains per m^2 (NGm) and their size (TGW), (r= -0.45). The value of the NGm is determined almost equally strongly by the two traits that form this index - NPT (r=0.62) and NGS (r=0.66). It is the only one that has reliable correlations with six of the eight traits studied. In addition to the positive effects of its constituent productivity traits, the height of the stem has a positive effect on it (HOS, r=0.35). The correlations of the NGm index with the grain size (TGW, r= -0.45), the grain weight per spike (WGS, r= -0.29) and the total biomass of the plant (TBM, r= -0.24), are negative. The sum of the indirect effects in the positive or negative direction of the trait with the other traits as a result is negative - r= -0.27 and ranks second in effect on yield after NPT.

Table 3. Path-correlation analysis of quantitative traits and grain yield

Trait	Direct effect	Indirect effects									Total indirect effect
	GY	NPT	TGW	WGS	NGS	NGm	HOS	TBM	HI	LOD	
NPT	**0.229**		0.11	0.04	0.07	0.05	0.17	0.03	0.10	-0.02	**0.55**
TGW	**0.376**	0.06		-0.04	**-0.24**	**-0.21**	0.19	0.09	0.13	0.03	-0.05
WGS	**0.382**	0.12	-0.04		**0.10**	-0.13	-0.19	0.08	0.06	0.04	-0.08
NGS	**-0.138**	-0.03	0.09	-0.04		-0.11	-0.02	0.02	0.01	0.02	-0.02
NGm	**0.814**	**0.62**	**-0.45**	**-0.29**	**0.66**		**0.35**	**-0.24**	-0.11	-0.18	**-0.27**
HOS	-0.032	0.00	-0.02	0.02	0.00	-0.01		0.00	-0.01	0.00	-0.02
TBM	0.020	0.01	0.00	0.00	0.00	-0.01	0.00		0.00	0.00	0.00
HI	-0.003	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00
LOD	0.015	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		-0,01

Legend: Values in **bold** are different from 0 with a significance level alpha=0.05, GY- grain yield, t/ha, NPT- number of productive tillers, TGW- 1000 grain weight, WGS- weight of grain per spike, NGS-number of grains per spike, NGm- number of grains per m^2, HOS- height of stem, TBM- total biomass, HI- harvest index and LOD-lodging tolerance.

Calculated by path-analysis, the correlations do not give a real picture of what the relationships between the important characters for breeding - elements of productivity are. For example, the relationship between the traits NGm and TGW, once is r= -0.45 and the second time is r= -0.21, or between the traits TGW and NGS (r=0.09, r= -0.24). Which of these values should we accept as true? For this reason, the correlations of the three main approaches to this were calculated (Figure 1). The data are presented in the figure because the values of the correlation coefficients calculated by each method (Pearson, Spearman and Kendall) are similar in traits and can be easily compared.

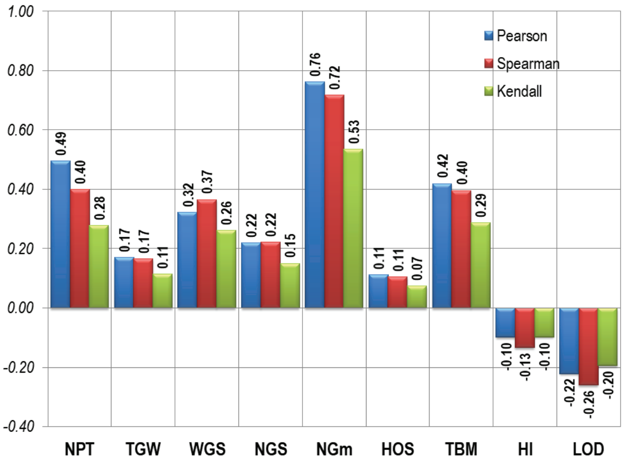

Figure 1. Correlations between GY and all traits by Pearson, Spearman and Kendall models
Legend: NPT- number of productive tillers, TGW- 1000 grain weight, WGS- weight of grain per spike, NGS- number of grains per spike, NGm- number of grains per m^2, HOS- height of stem, TBM- total biomass, HI- harvest index and LOD- lodging tolerance.

The data in the figure differ significantly from those in Table 3. To some extent, they correspond to the information on the relationships between traits, collected in previous studies (Tsenov et al., 2013, 2014, 2020a). The traits number of productive tillers per m^2 (NPT), total plant biomass (TBM) and the number of grains per m^2 (NGm) index play a decisive positive impact on the yield, without a doubt. On the other hand, stem height (HOS), harvest index (HI) and grain size (TGW) do not play a significant effect on grain yield, and lodging (LOD) naturally reduces grain yield to varying degrees.

Grain yield depends to the maximum extent on the balance that the variety can provide between the three main characteristics – productive tillering (NPT), grain size (TGW) and number of grains per spike (NGS). For this reason, their mutual influence as a manifestation in a given season is important for the size of the specific yield. With increasing productive tillering (NPT) trait, the other two grain size (TGW) and number of grains per spike (NGS), decreased (Table 4). The increase of grain size is accompanied by a decrease in the number of grains per spike, which is completely natural.

Table 4. Correlations between quantitative traits by Pearson model, (r-correlation coefficients) below and (R^2) above the diagonal

Traits	NPT	TGW	WGS	NGS	NGm	HOS	TBM	HI	LOD
NPT		0.01	**0.41**	0.31	0.25	0.06	0.11	0.01	0.00
TGW	**-0.10**		0.07	0.12	0.25	0.05	0.03	0.01	0.00
WGS	**-0,64**	**0.26**		**0.65**	0.01	0.03	0.00	0.03	0.01
NGS	**-0.55**	**-0.35**	**0.81**		0.18	0.09	0.02	0.05	0.02
NGm	**0.50**	**-0.50**	**0.11**	**0.43**		0.00	0.06	0.02	0.05
HOS	**0.25**	**0.21**	**-0.18**	**-0.30**	-0.04		0.01	0.00	0.01
TBM	**0.34**	**0.17**	-0.04	**-0.14**	**0.25**	**0.10**		0.08	0.00
HI	**0.08**	**0.11**	**-0.17**	**-0.23**	**-0.14**	0.01	**-0.28**		0.00
LOD	**-0.07**	0.02	**-0.12**	**-0.14**	**-0.22**	**-0.11**	0.00	0.00	

Legend: Values in **bold** are different from 0 with a significance level alpha= 0.05, NPT- number of productive tillers, TGW- 1000 grain weight, WGS- weight of grain per spike, NGS- number of grains per spike, NGm- number of grains per m^2, HOS- height of stem, TBM- total biomass, HI- harvest index and LOD- lodging tolerance.

Particularly negative is the relationship between the traits NGm and TGW (r= -0.50), which is an indication that in a team the two traits should be changed so that this negative correlation is reduced, which is a certain guarantee for a gradual increase in grain yield. In addition, the index grain weight per spike (WGS) is more strongly influenced by the number of grains per spike (NGS, r=0.81) than by the grain size (TGW, r=0.26). The high correlation between two quantities does not necessarily imply the existence of a causal relationship between them. However, correlation analysis is a tool for studying coefficients (correlations) between variables, in our case traits. Pearson correlation is generally a linear relationship between them. From a biological point of view, it is very difficult to count that a positive correlation between two traits (WGS-NGS, r=0.81) that causes a change in a third trait GY will be positive for it. There are complex relationships between traits that change with each other and this change affects the change in the resulting trait - grain yield. For this reason, in order to establish real causal relationships between the elements of productivity, the researchers used the regression model of data analysis to evaluate (Table 5). In this study, it was done through the SPSS program 23.

Table 5. Multi regression analysis by SPSS 23

Traits	Standardized coefficients	t	p-value	Correlations		Collinearity statistics	
				Partial	Semi-Part	Tolerance	VIF
1	2	3	4	6	7	8	9
(Constant)	-4.507	-12.59	.0000				
NPT	**.229**	5.26	.0000	**.215**	.055	.037	26.83
TGW	**.376**	12.53	.0000	**.466**	**.170**	.033	30.44
WGS	**.382**	12.99	.0000	**.422**	**.116**	.012	**81.00**
NGS	-.138	-9.04	.0000	-.160	-.040	.010	**103.66**
NGm	**.814**	41.01	.0000	**.643**	**.208**	.039	25.90
HOS	-.032	-1.58	.1136	-.113	-.028	.817	1.22
TBM	.020	3.72	.0002	.069	.017	.594	1.68
HI	-.003	-1.36	.1741	-.010	-.002	.778	1,29

Legend: Values in **bold** are different from 0 with a significance level alpha= 0.05, NPT- number of productive tillers, TGW- 1000 grain weight, WGS- weight of grain per spike, NGS- number of grains per spike, NGm- number of grains per m^2, HOS- height of stem, TBM- total biomass and HI- harvest index.

The correlations presented in column 2 of Table 5 reflect the direct effect of a single trait on grain yield. They are exactly the same as in Table 3 of the path analysis. What are the correlations indicated in Table 5 as Partial and Semi-Part? A partial correlation is that between an independent variable and a dependent variable, taking into account the influence of other variables on both the independent and dependent variables. The values of this type of correlations largely coincide with those in column 2, although the influence of each of the other traits on each of them is taken into account, too. The correlation between two variables (independent and dependent) is semi-partial after taking into account one or more of the other variables. It does not take into account the influence of confusing variables on the dependent variable. The semi-partial correlation provides information about that part of the unique variance that explains the independent variable (GY) as a proportion of the total variance in the dependent variable and not just the variance not accounted for by the monitored variables. After the "removal of all noise" from the variation of the traits, the picture of the influence of each of them on the yield in "pure" form is quite different. The traits that have an effect on the yield under the background of the change of the others are the grain size (TGW), the number of grains per unit area (NGm) and the grain weight per spike (WGS). Here, however, we encounter a statistical problem related to collinearity.

The term collinearity (multicollinearity) is a phenomenon in which a variable trait used as a determinant in multiple regressions can be linearly predicted by other traits with a significant degree of accuracy. Multicollinearity refers to a situation in which two or more explanatory variables in a multiple regression model are strongly linearly related. We have perfect multicollinearity if, for example, as in the equation above, the correlation between two independent variables is equal to +1 or -1. In the presence of multicollinearity, the assessment of the impact of a trait on the dependent character (GY) will be less accurate than if the independent traits are not related to each other. In a sense, collinear traits contain the same information about the dependent trait.

Multicollinearity is measured by tolerance (=1-R^2) or by inflation coefficient of variation VIF, (=1/tolerance). At a tolerance of less than 0.20 or 0.10 and/or a VIF of 5 or 10 and above indicates a problem with multicollinearity. Statisticians advise to use the model as it is, despite the multicollinearity. The presence of multicollinearity does not affect the efficiency of extrapolating the established model to new data, provided that the changing traits follow the same model of multicollinearity in a new data analysis. The results obtained here are completely similar to those in a previous study (Tsenov et al., 2020a). When a strong interaction of the traits with the environmental conditions is established, the grain size (TGW), the number of grains per unit area (NGm) and the grain weight per spike (WGS) are essential factors for grain yield.

The presence of collinearity in the database could be overcome to some extent by applying principal component analysis (PCA). It shows the magnitude and directions of variation of each of the traits (Figure 2) and presents them as "independent" vectors, the location of which is judged by the correlations between them. Four are the established reliable components of variation, according to the values of the parameter Eigenvalue >1. This is an indication of a linear and significant nonlinear genotype*environment interaction, which causes a strong variance in each of the traits studied.

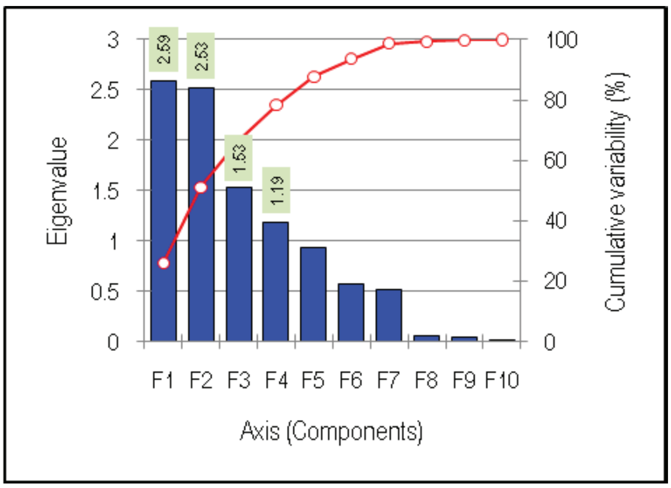

Figure 2. Screen plot of Eigenvalues in Principal Component Analysis

The division of each of the characters into components (Table 6) shows the shares of its variation (%) in each of the four components. Grain yield and most of the traits have a “stratification” of the dispersion by components. In general, the more stratified the variance of a trait, the more difficult it is to establish correlations with another trait with similar variation behaviour. This largely explains the difference between the values of the correlations between the traits calculated by the different methods of analysis. The values of the components for grain yield (GY), number of grains per spike (NGS), number of grains per m^2 (NGm), and weight of grain per spike (WGS) gradually decreased from F_1 to F_3 in a similar way (Table 6). In the case of productive tillering (NPT) the strongest variation is in (F_2=31.8), and in the case of grain size (TGW=55.28) the share of F_3 is the highest. The characteristics of HOS, TBM, HI and LOD, which practically do not show a relationship with the yield, have the largest share of F_4 and very low values of the first three components of variation.

Table 6. Principal Component Analysis: Contribution of the variables (%)

Trait	F1	F2	F3	F4
GY	14.234	13.858	7.473	0.401
NPT	2.755	31.801	1.082	0.417
TGW	2.304	0.549	55.283	0.238
WGS	22.326	7.678	11.605	2.438
NGS	30.002	5.377	1.806	1.328
NGm	17.976	14.259	6.976	0.196
HOS	2.385	8.342	6.134	11.594
TBM	1.686	9.717	9.233	23.269
HI	5.248	0.394	0.379	29.774
LOD	1.084	8.025	0.029	30.345
Sum of Fn	100.000	100.000	100.000	100.000

Legend: GY- grain yield, t/ha, NPT- number of productive tillers, TGW- 1000 grain weight, WGS- weight of grain per spike, NGS- number of grains per spike, NGm- number of grains per m^2, HOS- height of stem, TBM- total biomass, HI- harvest index and LOD- lodging tolerance.

In this complex picture of variation, however, what are the traits that affect grain yield? The answer to this essential question is presented in Figure 3. As a result of the application of PCA, each trait is presented in the dispersion space as a vector. The one that makes an acute angle with that of the grain yield has a positive effect on it and vice versa. The right angle is an indication of a complete lack of correlation (r=0), and an obtuse angle between the vectors means a negative correlation. The data on the graph represent ¾ (75.7%) of the total variance of the changes in the traits, which is a sufficient reason to draw correct conclusions about the correlations between them. The number of grains per spike (NGS) is the one that is essential for the yield. It also affects it directly through its participation in (WGS) and (NGm) indices. The other two main components of productivity, grain size (TGW) and productive tillering (NPT), do not have a direct effect on yield, but only indirectly through the two indices mentioned. The harvest index (HI), which a number of authors still cite as the most determining yield (Verma et al., 2019; Baye et al., 2020), in this study shows low and negative correlation. The traits lodging tolerance and stem height do not significantly affect the yield.

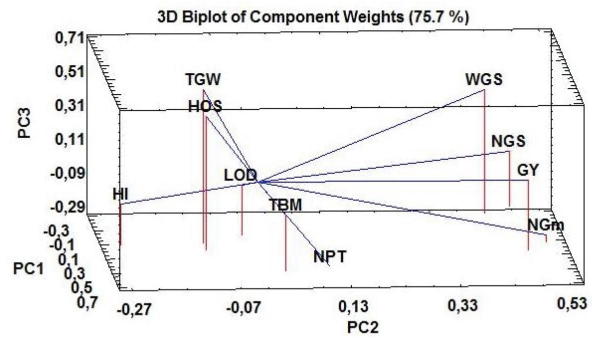

Figure 3. 3D biplot of all trait vectors by PCA
Legend: GY- grain yield, t/ha, NPT- number of productive tillers, NGS- number of grains per spike, TGW- 1000 grain weight, WGS- weight of grain per spike, NGm- number of grains per m^2, HOS- height of stem, TBM- total biomass, HI- harvest index and LOD- lodging tolerance.

The magnitude of the correlations between the studied traits is different according to the approach for their evaluation. They change in value and sometimes in direction (Table 7). For example, the correlation between grain yield (GY) and number of grains per spike (NGS), according to path-analysis is (r= -0.138), according to Pearson is (r=0.227), and according to Partial is (r=0.643). True, the values reflect different assessment approaches, but they are still single pairs of traits.

Table 7. Summary information on the values of the correlations between grain yield and all the traits, according to the different methods for their determination

Trait	Type of correlations						Rank impact
GY	Path	Person	Spearman	Kendall	Partial	Semi-Part	
NGm	**0.814**	**0.766**	**0.728**	**0.539**	**0.215**	0.055	
WGS	**0.382**	**0.323**	**0.376**	**0.266**	**0.466**	**0.170**	1
NPT	0.229	**0.492**	**0.402**	**0.285**	**0.422**	**0.116**	
TGW	0.376	0.172	0.177	0.118	-0.160	-0.040	2
NGS	-0.138	0.227	0.226	0.158	**0.643**	**0.208**	
HOS	-0.032	0.111	0.112	0.079	-0.113	-0.028	
TBM	0.020	0.428	0.407	0.295	0.069	0.017	3
HI	-0.003	-0.101	-0.132	-0.107	-0.010	-0.002	
LOD	0.015	-0.222	-0.262	-0.207			

Legend: Values in **bold** are different from 0 with a significance level alpha= 0.05, GY- grain yield, t/ha, NGm- number of grains per m^2, WGS- weight of grain per spike, NPT- number of productive tillers, TGW- 1000 grain weight, NGS- number of grains per spike, HOS- height of stem, TBM- total biomass, HI- harvest index and LOD-lodging tolerance.

Without taking into account each of the possible correlation analyses, drawing objectively accurate conclusions is unthinkable. If only one of the mentioned models is accepted, the probability of errors is high. Comparing the data from the different models, the traits in relation to their influence on the yield can be divided into three groups: 1) traits that have a significant positive effect (NGm, WGS, NPT); 2) traits that have an effect but it is unstable (TKW, NGS, TBM) and 3) traits that do not affect grain yield, at all (HOS, HI).

Each trait is influenced to some extent by the others. Are the characters of the first two groups negatively affected by some of the others? The partial correlations between them, presented in Figure 4, provide sufficient information about this. Significantly negative are the correlations between: NGm and TGW, WGS; WGS and NPT, NGm; NPT and WGS, NGS; TGW and NGS, NGm; NGS and NPT, TGW. Increasing the values of each of them would reduce the values of the cited traits, which disturbs the balance between them. The increase of (NGm) by selection will cause a decrease in grain size (TGW) and grain weight per spike (WGS), which in turn is a consequence of the first trait. If the grain weight per spike (WGS) is to be increased, this would lead to a decrease in the productive tillering (NPT) and, accordingly, the resulting number of grains per m^2 (NGm). Increasing by selection of (NPT) trait will lower the number of grains per spike (NGS) and through it the weight of the grain per spike (WGS). Apparently the three main components of productivity (NPT, TGW and NGS) are in a state of very precise balance, which ultimately forms the yield. That is why this balance is maintained, but how? In both of the yield indices (NGm and WGS) total is the number of grains per spike (NGS). Therefore, in order to increase the yield, this trait can be increased, but this must be done by reducing the grain size (TGW), which is strongly negatively associated with it (r= -0.499). According to the review of Passioura's (2020) research, the number of grains per spike is the only "universal" trait that can effectively increase yields by selection, regardless of environmental conditions. It is no coincidence that for several years this feature has been identified as extremely important in clarifying the reasons for the increase in yield (Dolferus and Richard, 2011; Slafer et al., 2014; Elía et al., 2016). It forms higher levels of traits (NGm and WGS), which in turn are direct factors in increasing grain yield.

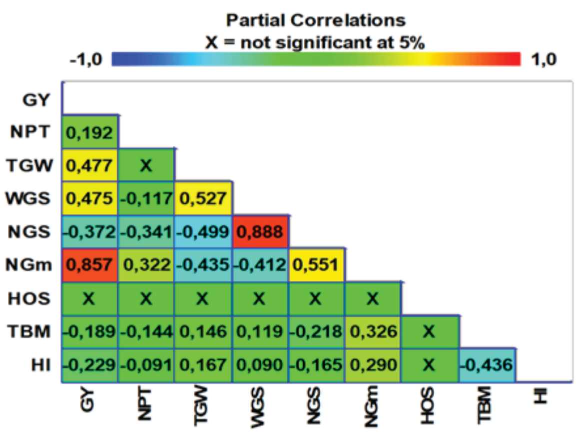

Figure 4. Patrial correlations between all the characters analysed by Statgraphics XVI software

Legend: GY- grain yield, t/ha, NPT- number of productive tillers, TGW- 1000 grain weight, WGS- weight of grain per spike, NGS- number of grains per spike, NGm- number of grains per m^2, HOS- height of stem, TBM- total biomass, HI- harvest index and LOD- lodging tolerance.

However, in order to achieve a balance between them, it is necessary for theTGW to be "fixed" within values of 33-38 g. This is valid for the conditions of the Balkan Peninsula (Tsenov et al., 2021).

Discussion

Grain yield in wheat is an effective trait. Its magnitude in a particular environment is subject to direct and indirect effects of a number of quantitative traits (Quintero et al., 2018). From the breeding point of view, it is extremely important to know in detail the basic relationships between traits that can be changed by selection, annually (Quintero et al., 2018; Al-Ashkar et al., 2020; Tsenov et al., 2020b) The data obtained eloquently outline the trends of relationships between the quantitative traits selected. It is interesting to note that harvest index (HI) has nothing to do with grain yield. Mitchell and Sheehy (2018) report that its gradual increase in the UK will increase grain yield up to 20 t/ha. The same opinion is shared by Mondal et al. (2020), especially in conditions of thermal stress or soil drought.

These results are completely different from the general opinion that the harvest index (HI) and the total plant biomass (TBM) are the traits that have a significant share in increasing grain productivity (Baye et al., 2020; Mondal et al., 2020; Pour-Aboughadareh et al., 2020), in the conditions of thermal stress or soil water deficit.

The variation of these traits is complex and non-linear, which probably affects the result that there are no correlations with grain yield or, as with the TBM, the correlations are contradictory in values and the conclusions about them are approximate (Table 7). The harvest index is even negatively related to the characteristics of grain weight per spike (WGS) and number of grains per spike (NGS), which is disturbing from the point of view of selection practice (Table 4). Total biomass positively affects the traits number of productive tillering (NPT) and number of grains per unit area (NGm), which is good news. According to large-scale studies by CIMMYT, Mexico (Mondal et al., 2020), the TBM trait is one of the reserves for future overcoming of the plateau in wheat productivity. In this regard, the data in the study are favourable for breeding in our country because they are consistent with the information of Xie et al. (2016), who show that the magnitude of biomass before harvest is related to the change in each of the traits that form grain yield.

The stem height trait does not have a significant correlation with any of the studied traits, therefore it can be changed without affecting the others. In a study of 31 advanced winter wheat breeding lines, Uhr et al., (2020) found no correlation between (GY) and (HOS), but the latter has a significant correlation with TGW. This suggests that by reducing TGW the stem height (HOS) can be easily reduced. This is a signal for further optimization of it at a level that provides higher tolerance of lodging. The productive tillering (NPT) should also be increased to a degree that compensates for the decrease in height of stem (HOS), thus maintaining the achieved total biomass (TBM) level, which is important for increasing (NGm, r=0.326*), as well as in conditions of stress.

The number of grains per spike (NGS) is a trait that can increase grain yield, both in favourable (Mitchell and Sheehy, 2018; Lichthardt et al., 2020) and under stress conditions (Dolferus and Richard, 2011; Passioura, 2020). In turn, it determines the level of the number of grains per m^2 (NGm), which in practice forms the level of grain yield in the climate of Bulgaria. It is highly dependent on productive tillering (NPT), which in turn has a negative correlation with the number of grains per spike (NGS). The latter has long been the key in the breeding of wheat in our country to increase its productive potential (Gubatov et al., 2016; Desheva, 2016; Tsenov et al., 2020a).

Conclusion

A significant increase in yield in the breeding of winter wheat can be achieved by increasing the number of grains per unit area (NGm). This is possible while maintaining the achieved level of number of grains per spike at level (NGS=30-35) and gradually increasing the number of productive tillers per m^2 (NPT). This could be obtained by observing the balance that exists between TGW and number of grains per spike (NGS), in order to minimize the negative effects between them. Therefore, the increase in productive tillering capacity (NPT) must be at the expense of a reduction in grain size (TGW). This will increase the chance of increasing the number of grains per spike (NGS), will reduce the weight of the grain per spike (WGW), which in turn will be a prerequisite for optimizing the tolerance of lodging. Total plant biomass (TBM) is an important character when wheat is grown under thermal stress after anthesis. Its correlations with grain yield are very contradictory in terms of values, according to the statistical model for analysis. The trait has a positive effect on the traits: number of productive tillers per m^2 (NPT), number of grains per m^2 (NGm) and TGW, as well. This is a sufficient reason for the (TBM) trait to be taken into account in the selection, together with the other traits. Stem height (HOS) does not have a significant negative effect on yield or any of its components. Therefore, it could be reduced to limits that would increase the harvest index and lodging tolerance, without reducing the total plant biomass. The last few traits will directly help to further increase grain yield.

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