ESTABLISHMENT OF PHENOTYPIC CORRELATIONS AND GENETIC DISTANCE IN A DIALLEL CROSS OF DURUM WHEAT

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Abstract

The aim of this research is to study phenotypic correlations and establishing the genetic distance between parents and their hybrids by cluster analysis. Experiments were conducted in three consecutive years in the experimental field of the Field Crops Institute - Chirpan, Bulgaria. The trials were performed in a randomized block design in three replications. Nine quantitative traits were observed, seven related to productivity and two related to grain quality. Significant differences between genotypes were established for all studied traits. The traits grains number per spike and grains weight per spike had a high positive correlation. This relationship was of great importance in determining the right strategy for leading a selection for productivity. At the first level the cluster analysis revealed two clusters genetically distant from each other. The bigger cluster was divided into two clusters with significant distance. Three clusters with a significant distance between them were observed. Parents and their hybrids fall into different clusters, indicating that hybrid combinations are genetically distant from their parents.

Key words: diallel cross, durum wheat, genetic distance, cluster analysis, correlations.

INTRODUCTION

Durum wheat (*Triticum durum* Desf.) is the most common type of tetraploid wheat. Its grain harvest is about 35 million tons of grain per year (Ranieri, 2015). Durum wheat has qualities that make it very special for on the world wheat market. It has a high vitreoussnes, strong gluten with high extensibility and low elasticity, high protein content and carotenoids and is used in pasta manufacturing. Durum wheat has the hardest grain of all types of wheat and is used to produce semolina (Dixon et al., 2009). It is a traditional crop that serves as a material for the production of pasta and is indispensable in this respect from common wheat.

The study of correlations between the individual characteristics of durum wheat plants and their practical use in the breeding of this crop is very important. The correlation analysis of traits makes it possible to establish the dependencies between them and especially those that determine productivity as the most important complex indicator and to look for opportunities for their change and obtaining stable and productive varieties (Dechev, 2004). Nayeem & Baig (2003) emphasize the grains' number per spike, test weight, and density. It has been reported that the number of grains per spike had a positive and significant correlation with the yield and an increase in this trait would lead to an increase in productivity (Dogan, 2009). Rachovska & Uhr (2010) concluded that a significant increase in yield would be achieved when the weight of grains per spike is increased. It is known that the weight of the grains per spike is directly related to the yield and as its component, it is important to establish the presence of correlations with other elements of the yield. This would help in selecting highly productive plants in the early stages of the breeding process. Mollasadeghi et al. (2011) found that the increase in productivity would be achieved if the traits grains number per spike, grains weight per spike, and the thousand-grain weight increased at the same time. Cifri (2012) reported that the elements related to yield were spike length, grains number per spike, and grains weight per spike. According to Popova & Neykov (2013), the largest positive relationship with the yield had the characteristics of grains number per spike and grains weight per spike. Slafer et al. (2014) confirmed the statement that the yield per unit area was influenced to the greatest extent by the number of grains per spike and thousand-grain weight. Nofouzi (2018) revealed that plant height, productivity tillering, spike length

and grains number per spike had a positive and significant correlation with yield.

The genetic distance of genotypes determines their potential for breeding progress when applying the method of hybridization, according to the available genetic distance of genotypes. The more genetically distant the parental forms, the greater potential for gene interaction in the form of dominance and epistasis leading to an increase in the potential for heterosis and transgression (Falconer, 1989). The method of hybridization is leading to the creation of genetically distant genotypes at the beginning of the breeding process. The success of the breeding, to create high-yielding varieties of wheat, largely depends on the correct set of parents for crossing. There are no rules on the basis of which combinations for obtaining promising lines can be foreseen in advance. By studying the quantitative traits, it is possible a greater understanding to combine the hereditary basis between genotypes in the hybridization process will be achieved. Increasing the number of genetically distant genotypes makes it possible to provide reliable sources of variation in different traits. By determining the genetic distance between genotypes, the correct selection of parental forms can be made in order to make significant progress in the yield potential in recombinant genotypes (Islam, 2004). The assessment of genetic distance between genotypes can be based on the phenotypic manifestation of quantitative and qualitative traits (Souza & Sorells 1991). Most often, genetic distance is measured as phenotypic distance (Kabir et al., 2009). It is assumed that if the genotypes are different phenotypically by more traits, they are also genetically distant. Eivazi et al. (2007) successfully applied cluster analysis to determine the genetic distance in plant breeding. The cluster analysis depicts a dendrogram that shows how genotypes are distant. Genetic distant groups, grouped based on cluster analysis in tetraploid wheat explain the differences between genotypes (Hailu et al., 2006). Cluster analysis is also useful for selecting parents to use in modeling the breeding program (Souza & Sorrells, 1991). Naroueirad et al. (2006) determined the genetic distance in local wheat varieties on the basis of several morphological traits. Talebi et al.

(2010) studied 24 durum wheat genotypes to determine their genetic distance, more important traits related to yield, and using cluster analysis identified three clusters. Hashjin et al. (2014) studied 116 genotypes from several different regions in order to establish the genetic distance between them.

The aim of this research was to study phenotypic correlations and identified the most important traits to improve durum wheat productivity. Establishing the genetic distance between parents and their hybrids by cluster analysis for complete and effective use of the results of diallel crosses.

MATERIALS AND METHODS

The research was conducted in the experimental field of the Field Crops Institute - Chirpan. The standard local technology for growing durum wheat adopted at the institute was used. The soil type is Eutric Vertisols (by FAO), characterized by medium organic matter (1.5-2.4%), with slightly acid to neutral soil reaction. The experiments were sown in the optimal period for durum wheat in Bulgaria on October 20-30. The genotypes heading time was on May 8-16. The plants were taken (harvested) on July 7-10 in full maturity. Meteorological conditions during the three-year period of the study were characterized by higher temperatures than the multi-annual norm. The first two harvest years of 2014 and 2015 were favourable in terms of soil moisture and rainfall higher than the average for many years. The third harvest year was characterized as the hottest and at the same time with 20% less precipitation. The genotypes were tested under field conditions by the randomized block design in three replications in the period 2014-2016. Each parent and F_1 was hand sown in two rows; each row was two-meter long; spaces between rows were twenty cm and five cm between plants. Modern durum wheat varieties were used as parents: Victoria (11), Deni (22), Superdur (33), Progres (44), Predel (55). The included varieties were crossed in a diallel design without reciprocal ones and their ten F_1 hybrid combinations were obtained: Victoria x Deni (12), Victoria x Superdur (13), Victoria x Progres (14), Victoria x Predel (15), Deni x Superdur (23), Deni x Progres (24), Deni x

Predel (25), Superdur x Progres (34), Superdur x Predel (35), Progres x Predel (45). The inclusion of F1 in the present study is due to the fact that the F1 generation is heterozygous but absolutely phenotypic homogeneous. It contains the genes of both parents and with the replications of the cross will always have the same genotype.

Nine quantitative traits were studied. Seven are related to productivity and two are related to grain quality. The following traits were studied: plant height, productivity tillering, spike length, spikelets number per spike, grains number per spike, grains weight per spike, thousand-grain weight, grain protein content, and wet gluten content. Productivity tillering by counting them (pcs.). Spike length by measurement of spike length in centimeters. Spikelets number per spike by counting (pcs.). Grains number per spike by counting (pcs.). Grains weight per spike by weighing the grains of the main spike in grams (g). Thousand-grain weight was calculated by the formula (grains weight per spike/grains number per spike)*1000 in grams (g). Grain protein content in percentage was estimated by measuring N according to the Kjeldahl method. The following formula was used: Protein, % = N (% DM) x 5.7 to convert the N content to protein content (BDS EN ISO 20483:2014). Wet gluten content in percentage was established by the standard mechanical method for their determination with perten glu-tomatic system (BDS EN ISO 21415-2:2008). In full maturity, from each replication, a total of twenty plants necessary for the biometric measurements and the technological analysis were randomly selected from plots every year.

The data from the three years were combined and their average value was calculated. On the results for the mean of parents and their hybrid combinations was conducted Duncan`s test for multiple comparing the means at the detected significant differences ($p < 0.05$) (Duncan, 1955). Correlation and variation analysis was performed by Lidanski (1988). From the multivariate methods was used hierarchical cluster analysis in order to group the genotypes by genetic similarity and distance by the method of Ward (1963), as the data was standardized to unify the scale. The data analysis software system Statistica 10 was used to calculate the data.

RESULTS AND DISCUSSIONS

Apart from the parents, the meanings of F_1 generations can also be considered as separate genotypes. They are heterozygous, but at the same time extremely homogeneous, due to the same genotype of each individual from the individual hybrid combination in this genera-tion. Duncan`s test for multiple comparing revealed significant differences between genotypes for all studied traits (Table 1). This was evidence of a significant diversity between the tested genotypes. The mean, maximum and minimum values indicate that the traits were within the normal range for durum wheat. The calculated coefficients of variation reveal that the trait of productivity tillering varied the most (CV = 10.7), followed by the trait grains weight per spike and the grains number per spike, and the trait with lowest CV was wet gluten content. The other traits occupied an intermediate position in terms of coefficients of variation. According to Alam et al. (2013) in durum wheat the grains number per spike had the highest coefficient of variation. Sabaghnia et al. (2014) reported that the traits of productivity tillering and grains number per spike were the most varied. Adhikari et al. (2018) report high coefficients of variation for the grains number per spike. A similar result for coefficients of variation for the trait of productivity tillering was reported by Tambe et al. (2013).

From a breeding point of view, correlations between the traits related to productivity are of particular importance. A correlation analysis was performed between the traits included in the study involving parents and F1 hybrids. The obtained values of correlation coefficients are presented in Table 2. From all 36 possible correlations, only 9 were statistically significant. The trait productivity tillering had only one significant positive correlation with the spike length (r = 0.61*). Nofouzi (2018) also reported the same correlation. Plant height had four significant positive correlation coefficients. One positive with a thousand grain weight (r = 0.72**) and three negative with spike length (r = -0.53*) with grain protein content (r = -0.87**) and wet gluten content (r = -0.63*).

Table 1. Duncan's multiple range test, mean values, standard deviation, min, max, CV and standart error of 15 genotypes for 9 quantitative traits

Traits / Genotypes	Plant height, cm	Product-ivity tillering, pcs	Spike length, cm	Spikelets number per spike, pcs	Grains number per spike, pcs	Grains weight per spike, g	Thousand grain weight, g	Grain protein content %	Wet gluten content %
Victoria	103.7[g]	7.4[ab]	6.3[a]	21.3[ab]	47.8[abc]	2.2[ab]	47.5[cd]	14.3[a]	29.6[a]
Deni	90.3[bcd]	8.3[abcd]	8.5[cefg]	22.9[ef]	43.4[a]	2.0[a]	46.5[bcd]	15.7[ab]	32.0[bc]
Superdur	79.6[a]	7.7[ab]	8.4[bcdef]	21.7[abcd]	50.2[abcd]	2.0[a]	39.3[a]	16.5[c]	32.0[ab]
Progres	98.5[cdef]	7.7[ab]	8.6[efg]	21.4[abc]	44.8[ab]	2.2[ab]	50.5[cd]	15.1[ab]	31.3[ab]
Predel	87.8[bc]	7.7[ab]	8.0[bcde]	22.1[bcde]	53.6[cde]	2.1[ab]	40.3[ab]	16.4[c]	32.0[bc]
Victoria x Deni	99.4[efg]	6.9[a]	7.9[bd]	23.6[f]	52.4[bcde]	2.4[ab]	47.7[cd]	15.6[ab]	30.9[ab]
Victoria x Superdur	98.0[efg]	8.1[abcd]	7.9[bce]	22.4[cde]	57.9[de]	2.6[bc]	47.4[cd]	15.7[ab]	32.3[bc]
Victoria x Progres	102.5[fg]	7.2[ab]	8.1[bcde]	22.7[def]	52.3[bcde]	2.4[abc]	49.6[cd]	15.2[ab]	31.0[ab]
Victoria x Predel	96.7[def]	7.6[ab]	8.0[b]	22.6[def]	56.8[de]	2.6[c]	47.0[cd]	15.8[bc]	31.2[ab]
Deni x Superdur	93.2[cde]	8.3[abcd]	9.1[gk]	22.9[ef]	56.9[de]	2.6[bc]	48.0[cd]	15.6[ab]	29.7[a]
Deni x Progres	92.7[cde]	9.7[cd]	9.2[k]	22.3[bcde]	49.8[abcd]	2.5[bc]	51.0[d]	15.7[abc]	31.4[ab]
Deni x Predel	89.2[bc]	9.0[bcd]	9.1[gk]	22.8[ef]	56.1[de]	2.6[bc]	46.0[bcd]	16.1[bc]	32.3[bc]
Superdur x Progres	87.6[bc]	8.0[abc]	8.5[cdefg]	20.6[a]	57.3[cde]	2.6[c]	46.0[bcd]	16.2[bc]	33.6[d]
Superdur x Predel	84.8[ab]	10.0[d]	8.5[bcdef]	21.4[abc]	58.2[e]	2.6[bc]	43.9[abc]	16.1[bc]	32.5[bc]
Progres x Predel	87.9[bc]	8.7[abcd]	8.9[fgk]	21.4[abc]	52.2[cde]	2.5[abc]	47.2[cd]	16.1[bc]	32.7[bc]
Mean	92.8	8.2	8.3	22.1	52.6	2.4	46.5	15.7	31.6
Min	79.	6.9	6.3	20.6	43.4	2.0	39.3	14.3	29.6
Max	103.7	10.0	9.2	23.6	58.2	2.6	51.0	16.5	33.6
CV	7.4	10.7	8.5	3.6	8.9	9.4	7.0	3.5	3.3
± m	1.7	0.22	0.18	0.20	1.22	0.05	0.84	0.14	0.27

Mean values ± standard deviation values (in each column), followed by the same letters (in index) are not significantly different at $p < 0.05$ according to Duncan's multiple range test (DMRT)

Table 2. Correlation analysis between 9 quatitative traits

Traits	1	2	3	4	5	6	7	8	9
1. Plant height	1	-0.51	-0.53*	0.31	-0.18	0.19	0.72**	-0.87**	-0.63*
2. Productivity tillering		1	0.61*	-0.19	0.24	0.36	0.02	0.37	0.37
3. Spike length			1	0.13	0.15	0.26	0.10	0.59*	0.41
4. Spikelets number per spike				1	0.03	0.04	0.20	-0.05	-0.39
5. Grains number per spike					1	0.76**	-0.20	0.42	0.26
6. Grains weight per spike						1	0.44	0.03	0.10
7. Thousand grain weight							1	-0.64*	-0.34
8. Grain protein content								1	0.73**
9. Wet gluten content									1

* - p<0.05, ** - p<0.01

From these correlation coefficients, it can be seen that tall genotypes had a larger grain, a shorter spike, and lower grain quality indicators. Authors Khan et al. (2013) and Rathwa et al. (2018) also find a significant positive correlation between plant height and the thousand grains' weight. Spikelets number per spike had no statistically significant correlation with another of the studied traits.

The grains number per spike correlated significantly only with the weight of grains per spike (r = 0.76**). The correlation was positive and showed that the increase in the number of grains per spike led to an increase in the productivity of the spike. Stefanova-Dobreva & Muhova (2020) reported a significant positive correlation between the number of grains per spike and grains weight per spike. A number of

studies showed that the weight of the grains per spike was the main breeding indicator determining the productivity of the genotype. Therefore, the number of grains per spike is extremely important for breeding. These two traits had no significant correlations with the other traits in the study. This means that the number of grains per spike was extremely important for productivity. Other authors also found a positive correlation between the grains number per spike and the weight of the grains per spike in durum wheat (Popova & Neykov, 2013; Rathwa et al., 2018). They concluded that increasing these two traits would lead to a significant increase in productivity. Therefore, when leading a selection in the next segregating generations, special attention must be paid to the grains numbers per spike. The grain protein content correlated negatively with the thousand-grain weight (r = -0.64*). The grain wet gluten content correlated very strongly and positively with grain protein content. Authors Taneva et al. (2019), Dragov et al. (2019), Stefanova-Dobreva & Muhova (2020a) also reported a significant and positive correlation between wet gluten content and grain protein content. Hannachi et al. (2013) and Gerema (2020) reported that grains number per spike and grain yield were in a positive significant correlation. On the other hand, grains weight per spike had a positive significant correlation with yield (Sourour et al., 2018). The established correlation coefficients could be used to optimize the selection in the segregating generations. They should be taken into account, especially in the breeding program for increasing the productivity of durum wheat. The most important traits were grains number per spike and grains weight per spike and selection should be led by these traits to increase productivity.

Cluster analysis was applied to measure the genetic distance of parents and their hybrids in this diallel cross. The data of the obtained results for parents and F1 hybrids on all studied traits were used. The values by traits for each of the studied years were included. This allowed a large group of small genes controlling the stability of traits to be included in the overall assessment and construction of the dendrogram. In this way, we got an enriched result with greater benefit for the

breeding. In order to unify the scale and more accurately assess the results obtained, the data were standardized (Siahbidi et al., 2013).

The dendrogram from the hierarchical cluster analysis is presented in Figure 1. The figure shows the separation of parents and their hybrids was in three main clusters with a sufficient degree of differences between them.
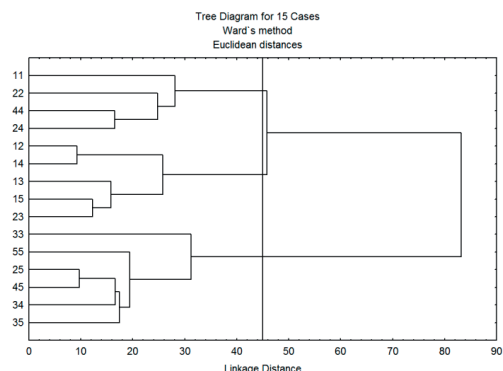

Figure 1. Dendrogram of 15 durum wheat genotypes from the diallel cross on 9 quantitative traits. Victoria (11), Deni (22), Superdur (33), Progres (44), Predel (55), Victoria x Deni (12), Victoria x Superdur (13), Victoria x Progres (14), Victoria x Predel (15), Deni x Superdur (23), Deni x Progres (24), Deni x Predel (25), Superdur x Progres (34), Superdur x Predel (35), Progres x Predel (45)

Cluster I included variety Victoria (11), variety Deni (22), variety Progres (44), and cross Deni x Progres (24). This cluster included three of the parents, as well as a cross between two of them, which indicates the presence of some genetic similarity between them. Cluster II included only crosses Victoria x Deni (12), Victoria x Progres (14), Victoria x Superdur (13), Victoria x Predel (15), and Deni x Superdur (23). This cluster included all crosses where the variety Victoria (11) participated as a parent. This indicates a strong influence of the variety Victoria (11) on the results of hybrids with it, which made them genetically closer to each other. Cluster III included variety Superdur (33), variety Predel (55) and crosses Deni x Predel (25), Progres x Predel (45), Superdur x Progres (34) and Superdur x Predel (35). Observation of this cluster shows that the variety Superdur (33) was different from the others in the cluster. This is easily explained given that the Austrian variety Superdur (33) was the only foreign variety involved in the

diallel cross. As expected, the Predel (55) variety falls into the same group as most of the hybrids in which it was a parent. Thus, a great similarity was observed between the variety Predel (55) and its F1 hybrids. A careful examination of the dendrogram shows some genetic similarity between cluster I and cluster II. On the other hand, it was observed a high degree of genetic distance between clusters I and II with cluster III. Therefore, it could be argued that the diallel cross led to the production of a relatively distant hybrid combination from their parents. In addition, some hybrids differed significantly from their parents. Diallel crossing made it possible to the creation of genetically distant hybrids in the complete combination of genetic material from a group of varieties.

Variety Predel is a standard for yield in Bulgaria. Hybrid combinations clustered with it were valuable in terms of breeding. In terms of breeding according to a complex assessment of all traits valuable were the hybrid combinations: Superdur x Predel, Superdur x Progres, Progres x Predel, Deni x Predel. They were genetically similar to the standard and in their segregating generations can be found generations that superiority it. Very valuable are the crosses showed heterosis on several traits: Deni x Predel by three traits and Superdur x Progres by two traits. These crosses can be used directly both for the production of transgression forms and for the development of hybrid breeding (Dragov, 2019). After continuing the individual selection in the hybrid combination Deni x Predel, it is possible to increase the yield and create a new durum wheat variety, as the Deni variety is genetically distant from the Predel variety. The genetic similarity and distance of the genotypes in the diallel cross from the dendrogram could be used in creating a strategy for conducting an effective breeding process in durum wheat.

The greater genetic distance between the parents was a prerequisite for heterosis manifestations in the segregated generations. Genetically closer genotypes need to be combined to achieve faster success. To achieve greater breeding progress, but in later segregating generations, it is advisable to cross genetically more distant parents. In an experiment using cluster analysis, Khodadadi et al. (2011) and Dimitrov et al. (2021) reached the same conclusion about the breeding strategy. Many researchers used cluster analysis to study agro-morphological similarities and distances between genotypes (Awan et al., 2014; Yadav et al., 2015). Cluster analysis based on quantitative traits gave an idea of the similarity between genotypes by calculating the euclidean distance (Awan et al., 2014). This could be used to improve durum wheat by selecting a variety for hybridization from different clusters (Ghafoor et al., 2012). To use transgressive breeding in wheat, the parents should be genetically distant (Ahmad et al., 2014; Zamanianfard et al., 2015). The greater the genetic distance between the parents, the more likely it was to observe heterosis and better-segregated generations (Mehari et al., 2015). In addition, the results showed that crosses involving parents from different clusters can lead to maximum heterosis and increase genetic distance (Hailegiorgis et al., 2011; Singh & Salgotra, 2014). The result of the study was a prerequisite for optimizing the breeding program and accelerating the breeding process in durum wheat.

CONCLUSIONS

Significant differences were found between genotypes on all studied traits. The traits grains number per spike and grains weight per spike had a high positive correlation. This relationship was of great importance in determining the right strategy for leading a selection for productivity. Diallel crossing made it possible to the creation of genetically distant hybrids in the complete combination of genetic material from a group of varieties. Parents and their hybrids fall into different clusters, indicating that hybrid combinations are genetically distant from their parents. The hybrid cross Deni x Predel is extremely valuable in terms of selection. Three clusters were observed with a significant distance between them. The genetic similarity and distance of the genotypes in the diallel cross from the dendrogram could be used in creating a strategy for conducting an effective breeding process in durum wheat. The hybrid cross Deni x Predel is extremely valuable in terms of breeding. The result of the study was a

prerequisite for optimizing the breeding program and accelerating the breeding process in durum wheat.

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