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Gene action for grain protein content in durum wheat

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Abstract: The aim of this study was to determine the gene action and combining ability of durum wheat for grain protein content. During the three year period a diallel cross was carried out with five modern parents of durum wheat – 'Victoria', 'Deni', 'Superdur', 'Progres' and 'Predel'. Ten hybrid combinations and the parents were grown in the experimental field of the Field Crops Institute, Chirpan. The experiment was performed by the randomize block method design in three replications. It was found that in the inheritance of grain protein content dominance and overdominance in positive and negative directions were observed. Statistical processing of the results showed that both additive and non-additive genetic effects have influenced on inheritance. Non-additive gene effects (SCA) had a greater role in inheritance. This suggests that an effective selection for this trait could begin in later generations. The combining ability analysis has identified two good general combinators (Predel and Superdur varieties) that could be used as donors to increase the values of the trait protein content in grain. Several crosses showing positive and significant SCA effects have also been identified, suitable for achieving reliable transgressive genotypes.

Key words: gene action; combining ability; GCA effects; SCA effects; grain protein content; durum wheat

Delovanje genov za vsebnost beljakovin v zrnih trde (durum) pšenice

Izvleček: Namen raziskave je bil določiti vpliv delovanja genov in kombinacijske sposobnosti trde (durum) pšenice na vsebnost beljakovin v zrnih. V obdobju treh let so bila izvedena dialelna križanja s petimi sodobnimi starševskimi sortami trde pšenice-– Victoria, Deni, Superdur, Progres in Predel. Deset križancev in njihovi starši so bili gojeni na poskusnem polju Inštituta za poljščine v Čirpanu (Field Crops Institute, Chirpan). Poskus je bil izveden kot naključni bločni poskus s tremi ponovitvami. Ugotovljeno je bilo, da sta pri dedovanju vsebnosti beljakovin v zrnju udeležni dominanca in naddominanca v pozitivni in negativni smeri. Statistična obdelava podatkov je pokazala, da so na dedovanje vplivali aditivni in neaditivni genski vplivi. Neaditivni genski učinki so imeli pri dedovanju večjo vlogo. To nakazuje, da bi se učinkovita selekcija za to lastnost lahko začela že v prejšnjih generacijah. Z analizo kombinacijske sposobnosti sta bila prepoznana dva dobra splošna kombinatorja ('Predel' in 'Superdur'), ki bi ju lahko uporabili kot donatorja za povečanje vsebnosti beljakovin v zrnih. Številna križanja kažejo pozitivne in značilne neaditivne genske učinke (SCA), ki so primerni za doseganje zanesljivih transgresivnih genotipov.

Ključne besede: delovanje genov; kombinacijska sposobnost; aditivni genski učinki; neaditivni genski učinki; vsebnost beljakovin v zrnju; trda (durum) pšenica

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1 INTRODUCTION

Durum or pasta wheat is the only tetraploid species of commercial importance that is cultivated in a number of countries, and in some of them it is essential. Durum wheat is an important crop for the preparation of pasta, bulgur, couscous and other products (Ahmad, 2015). The protein content of the grain is of particular importance for the quality of durum wheat and its products. High values of this trait have a positive relationship with the yield of semolina from the grain and are desired by the milling industry. The use of plant proteins in human food is of great importance for healthy human nutrition. The protein content in the grain is a very important indicator of wheat related to its nutritional value and technological qualities of the products (Blanco et al., 2006). Protein content is highly influenced by the genotype and content of available nitrogen in the soil, as well as humidity and temperature during the growing season (Campbell et al., 1977; Fowler et al., 1990; Ames et al., 2003). According to Clarke et al. (1990) and Fowler et al. (1990) the highest protein content of the grain is due to the cultivation of varieties that can withstand higher rates of nitrogen fertilization. The improvement of the varieties must meet the requirements for the production of high quality products. This leads to an accelerated improvement of individual traits. Knowledge of the gene action of the trait is useful for the development of a high quality durum wheat variety. Diallel crosses are used to establish gene action and mechanisms for inheriting quantitative traits. It is a reliable method for selecting parents and providing a comprehensive assessment of their hybrid combinations. This analysis is suitable for studying quantitative traits in early generations. It requires relatively more work, but gives a very effective estimate. More detailed information can be obtained from diallel crosses with one generation F_1. The other important advantage is that the estimates obtained in F_1 can be confirmed by testing the second generation F_2. In diallel analysis, general combining ability (GCA) is associated with genes that have additive effects and describes the ability of parents to pass on their traits to hybrid generation. Specific combining ability (SCA) is the deviation from additive effects caused by dominance and epistasis. The establishment of gene action (additive and non-additive) is of great importance for determining the strategy for achieving more meaningful and rapid results in the development of new varieties. In a properly designed breeding program, knowledge of gene action is the key that will maximize the effectiveness of improvement breeding work.

A number of authors have studied gene action in inheritance of grain protein content in wheat. Lysa (2009), Akram et al. (2011), Yao et al. (2014), Pansuriya et al. (2014), Tiwari et al. (2015) have reported significant participation of both additive (GCA) and non-additive (SCA) gene effects in the inheritance of the studied trait. According to the ratio of GCA / SCA variance, additive gene effects ($6_g^2 > 6_s^2$) have played a greater role in inheritance. This suggests that this trait could be significantly more easily improved and it is possible for an effective selection of genotypes to be applied in earlier segregated generations. Other researchers (El-Habbad et al., 1996; Pansuriya et al., 2014; Tiwari et al., 2015) found preponderance of non-additive genetic effects, while Zahid et al. (2007) and Al-Naggar et al. (2015) noted the preponderance of additive gene effects. From the preponderance of the non-additive genetic effects reported by some authors, it could be assumed that dominance had a greater influence on this trait. Determining the degree of dominance would help to establish the possibility of purposeful work in the breeding program. Here, a link can be made with the use of specific results, aimed at reaching transgressive forms or creating heterosis varieties. According to Fonseca & Patterson (1968), Martin & Talbert (1995), Elfadl et al. (2006) dominance and overdominance correspond to the theoretical foundations of heterosis and suggest that it is the result of allelic and non-allelic interactions between the genetic material of the parents. Dominance in the inheritance of the trait was reported by Patel et al. (2018). The diversity of the results obtained is evidence of the great influence of the genotypes used and the environmental conditions in the inheritance of the trait. This suggests that modern domestic and foreign varieties of durum wheat should be included in the study and their genetic capabilities should be traced and determined. Plant breeding is the art and science of changing the heredity of plants and improving them for the benefit of human.

The aim of this research was to study the genetic difference between genotypes, as well as the type of gene action (additive and non-additive) in the inheritance of protein content in the grain. By determining the general and specific combining ability, we expected to identify the parents for successful combinations and to obtained promising genotypes in the earliest segregated generations. This will be of great benefit in optimizing the breeding process for durum wheat in terms of grain protein content.

2 MATERIAL AND METHODS

The study was conducted in the experimental field of the Field crops institute in Chirpan, Bulgaria in three consecutive years (2014-2016) using the standard, local cultivation technology. 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 in time-frame October 20-30. The genotypes heading time was in time-frame May 8-16. The plants were taken (harvested) in time-frame July 7-10 in full maturity. Meteorological conditions during the three-year period of the study were characterized by higher temperature 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.

A half diallel cross was performed with the including of five modern varieties of durum wheat: Victoria (BG), Deni (BG), Superdur (AT), Progres (BG), Predel (BG). The three-year period has allowed three generations of F_1 and two generations of F_2 to be grown. The parents, F_1 and F_2 hybrids were sown under field conditions by the block method design in three replications. Each parent or F_1 was sown in two rows, and each F_2 was sown in five rows; each row was 2 m long; spaces between rows were 20 cm and 5 cm between plants. In full maturity a total of 20 plants from F_1 and parents and 30 plants from F_2 were taken randomly from each replication every year. Part of the seeds were used for the sowing of F_2. With the remaining seeds, a technological analysis was performed for estimation of grain protein content. The grain protein concentration (GPC, %) was estimated by measuring of 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 ISO 20483:2014).

The results obtained were statistically processed by applying the method 2 model I of Griffing (1956) with software product of Mark D. Burow and James G. Coors 1994 (Burow & Coors, 1994). The same program was used for the analysis of variance. The general combining ability (GCA) of the parents and the specific combining ability (SCA) of the crosses were determined. The degrees of dominance in the individual hybrid combinations were calculated according to Ognyanova (1975). On the results for mean of parents and their hybrid combination was conducted Duncan`s test for multiple comparing the means at the detected significant differences ($p < 0.05$) (Duncan, 1955). Statistica 10 software program was used for the two analyzes performed above.

3 RESULTS AND DISCUSSION

The mean values for the trait grain protein content of the parents for the three years F_1 and both F_2 generations are presented in Table 1. It can be seen that there was a significant variation in the years of study, also and significant differences between mean values. The most favourable for grain protein content was 2014, while the most unfavourable was 2015. The parents had values from 13.75 % for the variety Victoria (F_1-2015) to 18.53 % for the variety Superdur (F_1-2014). The table also presents the average values of the hybrid combinations by years and generations. The highest value was found for the combination Victoria X Deni - 18.50 % (F_1-2014), and the lowest - 13.52 % for Victoria X Progres (F_2-2015). The same table includes the corresponding indices showing the ways and direction of inheritance. They show that there was a great diversity in inheritance. In the F_1 generation in 2014 positive overdominance (towards the better parent) prevailed. In 2015, there were more manifestations of intermediate inheritance, but there also were those with dominance and over-dominance to the weaker parent. In 2016 dominance and overdominance in both directions were observed. In the F_2 generation, several manifestations of overdominance were seen in a positive direction, but in most cases it was in a negative direction. In inheritance of the grain protein dominance and overdominance in both positive and negative directions were observed. Preponderance of dominance and overdominance for the trait grain protein content was established by other authors (Kumar & Maloo, 2011; Desale & Mehta, 2013; Patel et al., 2018).

Table 2 represents the analysis of variance for genotypes, general combining ability (GCA) and specific combining ability (SCA). Significant differences between genotypes were observed for all test cases. This makes it possible to conduct a diallel analysis for an in-depth study of the genetic causes controlling the trait grain protein content. The sums of the squares of the genotypes were in each case the largest and determine that the genotypes had the largest contribution to the overall variation of the studied trait. The values of the mean squares for genotypes, GCA and SCA were statistically significant for the three harvest years in both generations (table 2). Therefore, both additive gene effects (GCA) and non-additive gene effects (SCA) were involved in the inheritance of the trait. The results correspond to those obtained by a number of other authors (Barnard et al., 2002; Joshi et al., 2004; Lysa, 2009; Akram et al., 2011; Yao et al., 2014; Pansuriya et al., 2014; Tiwari et al., 2015; Patel et al., 2018).

Table 1: Mean values and indexes of inheritance for trait grain protein content (%).

Parents	Code	2014 y.	2015 y.	2016 y.	2015 y.	2016 y.
Victoria	11	14.74a	13.75ab	14.47a	13.75ab	14.47a
Deni	22	16.25abc	14.67bcd	16.27cde	14.67bcd	16.27cde
Superdur	33	18.53d	14.59abcd	16.43de	14.59bcd	16.43cde
Progres	44	15.35a	15.04d	15.09abc	15.04cd	15.09abc
Predel	55	17.70bcd	15.31d	16.21cde	15.31d	16.21cde
Hybrid combinations	Code	F_1-2014 y.	F_1-2015 y.	F_1-2016 y.	F_2-2015 y.	F_2-2016 y.
Victoria x Deni	12	$^{od+}$18.50d	$^{cd-}$13.89ab	$^{cd-}$14.55a	^{i}14.24abc	^{i}15.36abcd
Victoria x Superdur	13	^{i}16.57abcd	$^{cd-}$13.76ab	$^{od+}$16.78e	$^{od-}$13.65ab	$^{pd+}$16.11bcde
Victoria x Progres	14	$^{od+}$16.09ab	^{i}14.13abc	$^{od+}$15.39abcd	$^{od-}$13.52a	$^{od+}$15.54abcde
Victoria x Predel	15	$^{cd+}$17.43bcd	$^{cd-}$13.97ab	$^{cd+}$16.03bcde	$^{od-}$13.68ab	$^{cd-}$14.77ab
Deni x Superdur	23	^{i}17.56bcd	$^{od-}$13.76a	$^{od-}$15.52abcd	$^{od-}$14.48abcd	$^{od-}$15.49abcde
Deni x Progres	24	$^{od+}$17.28bcd	$^{cd+}$15.13d	$^{od-}$14.87ab	$^{od-}$14.18abc	$^{od-}$14.50a
Deni x Predel	25	$^{od+}$18.13cd	^{i}15.13d	$^{od-}$15.17abcd	$^{cd+}$15.18cd	$^{od-}$15.41abcde
Superdur x Progres	34	$^{pd+}$17.81bcd	$^{cd+}$15.18d	^{i}15.68abcde	$^{cd+}$14.99cd	$^{cd-}$15.14abc
Superdur x Predel	35	$^{cd-}$17.69bcd	^{i}14.96cd	$^{od-}$15.71abcde	$^{od-}$14.40abcd	$^{od+}$16.59de
Progress x Predel	45	$^{cd+}$17.95bcd	$^{od-}$14.56abcd	$^{od+}$16.03bcde	$^{od-}$14.19abc	$^{od+}$16.80e
M ± m		17.17±0.29	14.52±0.15	15.61±0.17	14.39±0.14	15.61±0.19

Mean values (in each column), followed by the same letters are not significantly different at $p < 0.05$ according to Duncan's multiple range test (DMRT). Indexes: i-intermediate, cd-complete dominance, pd-partial dominance, od-overdominance, minus-decrease, plus-increase

The ratio of GCA and SCA variances indicates a predominance of non-additive gene effects over additive ones in all cases except F_1-2015, where additives predominated. The same results, for the predominance of non-additive genetic effects, have been reported by a number of other authors (Perenzin et al., 1992; Singh et al., 2004; Joshi et al., 2004; Nazeer et al., 2011; Kumar, 2012; Khodadadi et al., 2012; Ahmad et al., 2017; Patel et al., 2018). On the other hand, the predominance of additive genetic effects in inheritance has been reported by other researchers (El-Habbad et al., 1996; Bnejdi & El-Gazzah, 2010; Sadeghi et al., 2012; Tiwari et al., 2015; Al-Naggar et al., 2015). The predominance of non-additive gene effects suggests that an effective selection in the breeding for grain protein content in durum wheat, in our set of parents, should be conducted in later segregated generations. This is associated with a reduced influence of non-additive genetic effects in later segregated generations.

Table 3 represents the values for parental GCA and hybrid crosses SCA. Predel variety manifested itself as a good general combiner for increasing the grain protein content. It had positive and significant GCA values for all test cases. This variety contained more genes with additive effects. Of interest was the variety Superdur, which in three of the five cases had significant and positive values of GCA. These genotypes could be successfully used in breeding of durum wheat to increase the values of the trait grain protein content. As a bad general combiner for the trait was Victoria variety showed significant negative values for all cases of research and lead to a decrease in the protein content in the hybrids obtained with it. The other parent varieties occupied an intermediate position. There was no observed clear outlined good combination of SCA effects in the hybrid crosses. In different years there was no hybrid combination with three or more significant effects in one direction. Of interest in this situation was the cross 'Victoria' X 'Superdur', which had two significant positive values for SCA. This combination was a cross between a parent with a negative GCA and a parent with a positive GCA. Other crosses with high SCA effects for two years were 'Progres' X 'Predel' and 'Superdur' X 'Progres'. Researchers Kumar & Maloo (2012) and Singh et al. (2012) reported that not all crosses with high SCA effects were obtained from the crosses of a 'Good'

Table 2: ANOVA by years for Genotypes, General combining ability (GCA), Specific combining ability (SCA) and relation to variances of GCA and SCA ($6_g^2 / 6_s^2$) for grain protein content

Year	Source of variation	Sum of squares	Mean squares	Significant (*,**,***)
F_1-2014	Genotype	53.769	3.841	***
	GCA	26.434	6.608	***
	SCA	27.336	2.734	***
	Error	29.506	1.054	
	$6_g^2 / 6_s^2$	0.32		
F_1-2015	Genotype	14.672	1.048	***
	GCA	10.023	2.506	***
	SCA	4.649	0.465	***
	Error	6.347	0.227	
	$6_g^2 / 6_s^2$	1.22		
F_1-2016	Genotype	20.008	1.429	***
	GCA	7.872	1.968	***
	SCA	12.13	1.214	***
	Error	11.713	0.418	
	$6_g^2 / 6_s^2$	0.11		
F_2-2015	Genotype	14.091	1.007	***
	GCA	8.012	2.003	***
	SCA	6.079	0.608	***
	Error	7.919	0.283	
	$6_g^2 / 6_s^2$	0.6		
F_2-2016	Genotype	23.806	1.700	***
	GCA	9.805	2.451	***
	SCA	14.001	1.400	***
	Error	15.219	0.544	
	$6_g^2 / 6_s^2$	0.17		

* - $p \leq 0.05$; ** - $p \leq 0.01$; *** - $p \leq 0.001$; n.s. – no significant

X 'Good' GCA combiner. Particularly, crosses with high SCA effects were obtained from crosses between 'Bad' X 'Bad' and 'Bad' X 'Good' general combiner. These researchers claimed that such manifestations were due to the involvement of dominance or epistatic gene effects.

Gami et al. (2011) and Tiwari et al. (2015) have determined that crosses with high SCA may be more likely to be sources of transgression. Transgressive lines on a given trait can be a source for creating high-nutrition varieties of durum wheat. According to them, assessments of gene action and variation explain the genetic potential of materials and contribute to breeding progress in durum wheat quality. The analysis of combining ability shows that non-additive genetic effects (dominance and epistasis) has played a major role in the inheritance of the trait grain protein content in durum wheat. Two good combiners have been identified to increase the grain protein content: 'Predel' and 'Superdur'. Furthermore as a promising combination was found the cross 'Victoria' X 'Superdur'. Varieties Predel and Superdur have been defined as good general combiners by other quantitative characteristics in our previous studies (Dragov & Dechev, 2015; Dragov, 2017; Dragov, 2020).

The study provided information on two of the most important moments in a successful breeding program - choosing parents for crossing and leading a purposeful selection on this trait. The choice of parents for crossbreeding is the basis for obtaining good results from a breeding program. Hybridization is a

Table 3: Values for general combining ability (GCA) of parents and specific combining ability (SCA) of crosses for grain protein content

Code	2014 y.	2015 y.	2016 y.	2015 y.	2016 y.
Parents / Error	±0.31	±0.14	±0.19	±0.16	±0.22
11 Victoria	-0.70*	-0.55*	-0.28*	-0.53*	-0.42*
22 Deni	0.13 n.s.	0.01 n.s.	-0.14 n.s.	0.15 n.s.	-0.05 n.s.
33 Superdur	0.52*	-0.04 n.s.	0.40*	0.05 n.s.	0.36*
44 Progres	-0.45*	0.27*	-0.21*	0.08 n.s.	-0.21 n.s.
55 Predel	0.50*	0.30*	0.24*	0.24*	0.33*
Hybrid combinations	F_1-2014 y.	F_1-2015 y.	F_1-2016 y.	F_2-2015 y.	F_2-2016 y.
Crosses / Error	±0.70	±0.32	±0.44	±0.36	±0.50
12 Victoria x Deni	1.9*	-0.09 n.s.	-0.62*	0.23 n.s.	0.22 n.s.
13 Victoria x Superdur	-0.41 n.s.	-0.16 n.s.	1.03*	-0.25 n.s.	0.55*
14 Victoria x Progres	0.08 n.s.	-0.11 n.s.	0.28 n.s.	-0.42*	0.56*
15 Victoria x Predel	0.46 n.s.	-0.30 n.s.	0.45*	-0.42*	-0.75*
23 Deni x Superdur	-0.26 n.s.	-0.73*	-0.35 n.s.	-0.11 n.s.	-0.42 n.s.
24 Deni x Progres	0.43 n.s.	0.31 n.s.	-0.37 n.s.	-0.44*	-0.84*
25 Deni x Predel	0.31 n.s.	0.28 n.s.	-0.53*	0.38*	-0.47 n.s.
34 Superdur x Progres	0.57 n.s.	0.41*	-0.12 n.s.	0.46*	-0.61*
35 Superdur x Predel	-0.51 n.s.	0.18 n.s.	-0.55*	-0.28 n.s.	0.28 n.s.
45 Progress x Predel	0.72*	-0.53*	0.39 n.s.	-0.53*	1.07*

* - $p \leq 0.05$; n.s. – no significant

basic method for increasing genetic diversity and obtaining valuable genotypes in segregating generations. When choosing parents, the following should be taken into account: genetic distance, adaptation potential and combining ability. Greater genetic variation and the possibility of transgressions were obtained from crosses with higher SCA effects. In turn, crosses with high SCA effects were obtained by crossing parents with high GCA effects with parents with medium or low GCA effects. This indicates that parents with high GCA effects should always be present in the hybridization scheme. In our set of parents in the diallel cross, two varieties: Superdur and Predel were established as the most valuable parents. The use of these varieties in the future hybridization program would show good results in segregated generations.

Successful selection in the early segregated generations F_2, F_3 is suitable for traits in which inheritance control is determined by additive gene effects. It should be noted that for the studied trait there was a significant participation of the additive genetic effects in our case. An effective selection in later segregated generations should be recommended for traits in which non-additive genetic effects predominate. Therefore, an effective selection on this trait should be applied in the later segregated generations, when the influence of the non-additive (dominance) decreases and the additivity increases. The significant influence of additive and non-additive gene effects found in our study suggests that the use of both types of gene effects is necessary to improve the trait. In the studied trait in F_1 in one year the inheritance was mainly controlled by additive gene effects while in the other two it was mainly by non-additive ones. The selection in different environmental conditions (years) would have a positive impact on breeding improvement work. This is due to the accumulation of different useful genes in different years.

4 CONCLUSIONS

In the inheritance of grain protein content there was complete dominance and overdominance, both in a positive and in a negative direction. Additive and non-additive genetic effects had a significant influence on the inheritance of this trait. Therefore, to maximize the grain protein content of durum wheat, a system that includes both types of gene effects at the same time should be used. The ratio of variances indicates that non-additive genetic effects prevailed over additive ones in most cases. This result shows that it is necessary for an effective selection to start in the later segregated generations where dominance decreases and additivity increases. The study identified two good combiners that increased the values of the trait: Predel and Superdur varieties. Crosses with these two genotypes suggest opportunities for promising hybrids. The hybrid combination 'Victoria' X 'Superdur' is also of selection interest according to the demonstrated significant values for SCA.

5 REFERENCES

Ahmad, E., Akhtar, M., Badoni, S., & Jaiswal, P. (2017). Combining ability studies for seed yield related attributes and quality parameters in bread wheat (*Triticum aestivum* L.). *Journal of Genetics, Genomics & Plant Breeding, 1*(1), 21-27. http://ejggpb.com/abstract.php?article_id=4

Ahmad, M. (2015). Genetical analysis of some agronomic traits in durum wheat. *International Journal of Environment, 4*(4), 336-341. http://www.curresweb.com/ije/ije/2015/336-341.pdf

Akram, Z., Ajmal, S., Khan, K. S., Quereshi, R., & Zubair, M. (2011). Combining ability estimates of some yield and quality related traits in spring wheat (*Triticum aestivum* L.). *Pakistan Journal of Botany, 43*(1), 221-231. http://www.pakbs.org/pjbot/PDFs/43(1)/PJB43(1)221.pdf

Al-Naggar, A., Shabana, R., El-Aleem, M., & El-Rashidy, Z. (2015). Diallel analysis of wheat grain protein content and yield in F_1 and F_2 generations under contrasting nitrogen conditions. *American Research Journal of Agriculture, 1*(4), 12-29. https://doi.org/10.21694/2378-9018.15002

Ames, N., Clarke, J., Dexter, J., Woods, S., Selles, F. & Marchylo, B. (2003). Effects of nitrogen fertilizer on protein quality and gluten strength parameters in durum wheat (*Triticum turgidum* L. var. *durum*) cultivars of variable gluten strength. *Cereal Chemistry, 80*, 203-211. https://doi.org/10.1094/CCHEM.2003.80.2.203

Barnard, A., Labuschagne, M., & Vanniekerk, H. (2002). Heritability estimates of bread wheat quality traits in the Western Cape province of South Africa. *Euphytica, 127*, 115–122. https://doi.org/10.1023/A:1019997427305

BDS ISO 20483:2014 . (2014). Cereals and pulses - Determination of the nitrogen content and calculation of the crude protein content - Kjeldahl method (ISO 20483:2013). ISO 20483:2013 specifies a method for the determination of the nitrogen content of cereals, pulses and derived products, according to the Kjeldahl method, and a method for calculating the crude protein content. The method does not distinguish between protein nitrogen and non-protein nitrogen. International Organization for Standardization (ISO Standard No. 20483). https://www.bds-bg.org/bg/project/show/bds:proj:87392

Blanco, A, Simeone, R., & Gadaleta, A. (2006). Detection of QTLs for grain protein content in durum wheat. *Theoretical and Applied Genetics, 112*, 1195-1204. https://doi.org/10.1007/s00122-006-0221-6

Bnejdi, F., & El-Gazzah, M. (2010). Epistasis and genotype-by-environment interaction of grain protein content in durum wheat. *Genetics and Molecular Biology, 33*(1), 125-130. https://doi.org/10.1590/S1415-47572010000100021

Burow, M., & Coors, J. (1994). DIALLEL: A microcomputer program for the simulation and analysis of diallel crosses. *Agronomy Journal, 86*, 154-158. https://doi.org/10.2134/agronj1994.00021962008600010028x

Campbell, C., Cameron, D., Nicholaichuk, W., & Davidson, H. (1977). Effect of fertilizer N and soil moisture on growth, N content, and moisture use by spring wheat. *Canadian Journal of Soil Science, 57*, 289-310. https://doi.org/10.4141/cjss77-035

Clarke, J., Campbell, C., Cutforth, H., DePauw, R., & Wilkleman, G. (1990). Nitrogen and phosphorus uptake, translocation, and utilization efficiency of wheat in relation to environment and cultivar yield and protein levels. *Canadian Journal of Plant Science, 70*, 965-977. https://doi.org/10.4141/cjps90-119

Desale, C., & Mehta, D. (2013). Heterosis and combining ability analysis for grain yield and quality traits in bread wheat (*Triticum aestivum* L.). *Electronic Journal of Plant Breeding, 4*(3), 1205-1213. http://www.indianjournals.com/ijor.aspx?target=ijor:ejpb&volume=4&issue=3&article=003

Dragov, R., & Dechev, D. (2015). Inheritance of plant height in durum wheat. *Agricultural University - Plovdiv, Scientific Works, 59*(2), 65-74. (Bg). http://nauchnitrudove.au-plovdiv.bg/8-inheritance-of-plant-height-in-durum-wheat/

Dragov, R. (2017). Genetic and breeding study of number of grains per spike in durum wheat. *(Rastenievadni nauki) Bulgarian Journal of Crop Science, 54*(2), 24-32. (Bg). https://cropscience-bg.org/page/bg/details.php?article_id=471

Dragov, R. (2020). Combining ability for the grain wet gluten content in durum wheat. *Bulgarian Journal of Agricultural Science, 26*(5), 998–1002. https://journal.agrojournal.org/page/en/details.php?article_id=3054

Duncan, D. (1955). Multiple Range and Multiple F-tests. *Biometrics II*, 1-42. https://doi.org/10.2307/3001478

Elfadl, E., Kling, C., & Melchinger A. (2006). Evaluation of heterosis in durum wheat (*Triticum durum* Desf.) "*Prosperity and Poverty in a Globalised World-Challenges for Agricultural Research*" *Tropentag,* October 11-13, 2006, Bonn, Germany. https://www.semanticscholar.org/paper/Evaluation-of-Heterosis-in-Durum-Wheat-(Triticum-Elfadl-Kling/bdd6affa1de55be2d0fbb5ab883f55fe135092cf

El-Habbad, L., Sarrafi, A., Fabre, J., & Aussenac, T. (1996). Genetic expression of some grain quality and yield components in exploid wheat (*Triticum aersticum* L.). *Cereal Research Communications, 24*(3), 323-239. from http://www.jstor.org/stable/23784219

Fonseca, S., & Patterson F. (1968). Hybrid vigor in seven parental diallel cross in common wheat (*Triticum aestivum* L.). *Crop Science, 8*, 85-88. https://doi.org/10.2135/cropsci1968.0011183X000800010025x

Fowler, D., Brydon, J., Darroch, B., Entz, M., & Johnston, A. (1990). Environment and genotype influence on grain protein concentration of wheat and rye. *Agronomy Journal, 82*, 664-666. https://doi.org/10.2134/agronj1990.00021962008200040002x

Gami, R., Tank, C., Chauhan, R., Patel, S., & Thakor, D. (2011). Heterosis for grain yield and quality components in durum wheat (*Triticum durum* Desf.). *Research on Crops, 12*(2), 496-498. https://www.researchgate.net/publication/294753725_Heterosis_for_grain_yield_and_quality_components_in_durum_wheat_Triticum_durum_Desf

Griffing, B. (1956). Concept of general and specific combining ability in relation to diallel crossing system. *Australian Journal of Biological Science, 9*(4), 463-493. https://doi.org/10.1071/bi9560463

Joshi S., Sharma, S., & Sain, R. (2004). Nonallelic interactions for yield and its components in hexaploid wheat (*Triticum aestivum* L.). *Indian Journal of Genetics and Plant Breeding, 64*(1), 63-64. http://www.indianjournals.com/ijor.aspx?target=ijor:ijgpb&volume=64&issue=1&article=018

Khodadadi, E., Aharizad, S., Sabzi, M., Shahbazi, H., & Khodadadi, E. (2012). Combining ability analysis of bread quality in wheat. *Annals of Biological Research, 3*(5), 2464-2468. https://www.researchgate.net/publication/228333030_Combining_ability_analysis_of_bread_quality_in_wheat

Kumar, R. (2012). *Genetic architecture of seed yield and quality parameters in bread wheat (Triticum aestivum L.) over environments*. Ph.D. (Agri.) thesis (unpublished) submitted to Anand Agricultural University, Anand. https://krishikosh.egranth.ac.in/handle/1/77239

Kumar, V., & Maloo, S. (2011). Heterosis and combining ability studies for yield components and grain protein content in bread wheat (*Triticum aestivum* L.). *Indian Journal of Genetics and Plant Breeding, 71*(4), 363-366. http://www.isgpb.org/documents/archive/10-sc-363-366.pdf

Kumar, V., & Maloo, S. (2012). Parental molecular diversity and its concurrence to heterosis in bread wheat (*Triticum aestivum* L.). *Indian Jorunal of Agricultural Sciences, 82*(3), 207-212. https://www.researchgate.net/publication/290349535_Parental_molecular_diversity_and_its_concurrence_to_heterosis_in_bread_wheat_Triticum_aestivum

Lysa, L. (2009). Identification of the genetic controlling system of the protein content in the grain of winter wheat. *Cytology and Genetics, 43*, 258-261. https://doi.org/10.3103/S0095452709040069

Martin, J., & Talbert, L. (1995). Hybrid performance in wheat as related to parental diversity. *Crop science, 35*, 104-108. https://doi.org/10.2135/cropsci1995.0011183X003500010019x

Nazeer, W., Farooq, J., Tauseef, M., Ahmed, S., Khan, M., Mahmood, K., Hussain, A., Iqbal, M., & Nasrullah, H. (2011). Diallel analysis to study the genetic makeup of spike and yield contributing traits in wheat (*Triticum aestivum* L.). *African Journal of Biotechnology, 10*(63), 13735-13743. https://doi.org/10.5897/AJB11.967

Ognyanova, A. (1975). Elements of biometrical genetics. Biometrical methods in agriculture, genetics and plant breeding. *Zemizdat-Sofia*, 216-304 (Bg).

Pansuriya, A., Dhaduk, L., Vanpariya, L., Savaliya, J., Patel, M., & Mehta, D. (2014). Combining ability over environment for grain yield and its components in bread wheat (*Triticum aestivum* L.). *AGRES – An International e-Journal, 3*(1), 39-46. http://arkgroup.arkgroup.co.in/upload/Article/1833-1-4.pdf

Patel, N., Dholariya, N., Delvadiya, I., & Akbari, S. (2018). Genetic analysis of grain yield, its components and quality characters in durum wheat (*Triticum durum* Desf.) over environmets. *International Journal of Pure & Applied Bioscience, 6*(2), 523-532. https://doi.org/10.18782/2320-7051.6196

Perenzin, M., Pogna, N., & Borghi, B. (1992). Combining ability for breadmaking quality in wheat. *Canadian Journal of Plant Science, 72*, 743-754. https://doi.org/10.4141/cjps92-090

Sadeghi, F., Dehghani, H., Najafian, G., & Aghaee, M. (2012). Estimation of genetic structure traits attributes breadmaking quality in bread wheat (*Triticum aestivum* L.) using GGEbiplot and diallel method. *Cereal Research, 2*(4), 263-277. https://www.sid.ir/en/Journal/ViewPaper.aspx?ID=376546

Singh, H., Sharma, S., & Sain, R. (2004). Heterosis Studies for yield and its components in bread wheat over environments. *Hereditas, 141*, 106-114. https://doi.org/10.1111/j.1601-5223.2004.01728.x

Singh, K., Sharma, S., Sharma, Y., & Tyagi, B. (2012). Combining ability for high temperature tolerance and yield contributing traits in bread wheat. *Journal of Cereal Research, 4*(1), 29-37. From http://epubs.icar.org.in/ejournal/index.php/JWR/article/view/35311

Statistica 10. StatSoft Inc. (2010).

Tiwari, R., Marker, S., & Ramteke, P. (2015). Gene action and combining ability analysis for quality traits macaroni wheat (*Triticum durum* Desf.). *Vegetos-An International Journal of Plant Research, 28*(1), 130-133. https://doi.org/10.5958/2229-4473.2015.00017.8

Yao J., Ma, H., Yang, X., Zhou, M., & Yang, D. (2014). Genetic analysis of the grain protein content in soft red winter wheat (*Triticum aestivum* L.). *Turkish Journal of Field Crops, 19*(2), 255-260. https://doi.org/10.17557/tjfc.18686

Zahid, A., Saif, A., Ali, A., & Muhammad, J. (2007). Genetic analysis of protein, lysine, gluten and flour yield in bread wheat (*Triticum aestivum* L.). *Pakistan Journal of Biological Sciences, 10*, 1990- 1995. https://doi.org/10.3923/pjbs.2007.1990.1995