Original Contribution

STUDY OF GENETIC PARAMETERS AND ASSOCIATION BETWEEN CHARACTERS IN EINKORN GENOTYPES

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ABSTRACT

The study is carried out in the experimental field of Institute of Plant Genetic Resources “Konstantin Malkov”-Sadovo, Bulgaria during 2013-2015 growing seasons. Twenty two einkorn wheat accessions were evaluated to estimate of variability, heritability, genetic advance and associations among characters. The values of mean square for all twelve characters showed highly significant differences between einkorn genotypes. The phenotypic coefficients of variation were higher than genotypic coefficient of variation (GCV). High heritability accompanied with moderate expected genetic advances was recorded for number of spikelets per spike. High values for the genetic advance expressed as a percentage of the mean and moderate values for the heritability in broad sense were observed in the traits total number of tillers per plant and number of productive tillers per plant. The number of productive tillers per plant, grain weight per spike and spike length without awns had the strongest direct effect on grain yield per plant. The total number of tillers per plant had the highest negative direct effect on the grain yield per plant. Stepwise multiple linear regression analysis revealed that three traits included grain weight per spike, number of productive tillers per plant and total number of tillers per plant with $R^2 = 85.30\%$, had justified the best prediction model.

Key words: einkorn, variability, genetic advance, heritability, correlation, pat analysis, stepwise regression

INTRODUCTION

Einkorn (*Triticum monococcum* L.) is an old cereal known to have been grown 9 000 years ago, and its use extended to the Balkans and Central Europe in the neolithic period (1). It is very useful and promising plant breeding material for its resistance to disease (rusts, powdery mildew) (1-8). The crop is suitable for low input systems - organic farming - because of its better adaptability in marginal areas (in comparison with other crops) (1, 9). Einkorn is also valued for unique quality of grain and simple genome, which can provide an easy way to identify toxic fraction causing celiac disease. Grain contains 16-28 % of protein, 3-6 times more carotenoids than polyploid wheats and no anti-nutritious compounds (10-16). The attention toward this ancient species has also been renewed by the increasing demand for traditional products, the request for species suitable to be grown in marginal areas and the need to preserve genetic

diversity and to develop new genotypes between interspecific crosses (9).

Study of statistical parameters as mean, variance, heritability, genetic advance, etc., is not only to evaluate the genetic stability and performance of any particular genotype but it is also a measure to determine the effectiveness of selection for a particular trait in that genotype. Wider range of variability makes it convenient to select a particular trait with ease and efficacy (17). Variability studies are limited in einkorn wheat. It is fact that the higher the variability among the genotypes betters the chances for further improvement in the crop (18). Heritability estimates provide an indication of the expected response to selection in a population. Information on the nature and magnitude of heritability in a population is one of the prerequisites for successful breeding programs in selecting genotypes with desirable characters (19). High heritability indicates less environmental influence in the observed variation (20). High heritability (broad sense) associated with high genetic advance reveals strong contribution of additive genetic variance for expression of the traits and offers the most effective condition for selection (21, 22).

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Phenotypic and genotypic coefficients of variations, heritability and genetic advance have been used to assess the magnitude of variance in wheat breeding material (23). Correlation and path coefficient analysis are used as an important tool to bring information about appropriate cause and effects relationship between yield and some yield components (24, 25). Therefore the present study was designed to estimate the degree of genetic variability, heritability, genetic advance and associations among characters in 22 einkorn wheat genotypes.

MATERIAL AND METHODS

The study is carried out in the experimental field of Institute of Plant Genetic Resources “Konstantin Malkov”-Sadovo, Bulgaria during 2013-2015 growing seasons. Twenty two einkorn wheat accessions were examined. The accessions were sown in harvest plots each of 1 m² in three replications, in the randomized block design. Each plot consisted of five rows of 1.0 m length with 20 cm and 5 cm spacing between and within rows, respectively. All the agronomical packages and practices were applied to raise healthy crop. At the time of maturity, ten competitive plants of each genotype from each replication were randomly selected. Data were registered for length of second leaf (cm), width of second leaf (cm), total number of tillers per plant, number of productive tillers per plant, plant height (cm), spike length with awn (cm), spike length without awn (cm), number of spikelets per spike, number of grains per spike, grain weight per spike (g), thousand grain weight and grain yield per plant (g).

The mean data from all eight characters were used to analysis of variance according to Lidansky (26). Ecological, genotypic and phenotypic variances, genotypic and phenotypic coefficient of variability, broad sense heritability were calculated according to Singh and Chaudhary (27). Genetic advance in

terms of percentage of means was assessed by Brim et al. (28). Phenotypic correlations were calculated by using phenotypic variances and covariance. Correlation coefficients were calculated as followed by Lidansky (26) whereas path coefficient analysis was conducted to Dewey & Lu (29) using phenotypic correlations to assess direct and indirect influences of different yield components on grain yield per plant. Stepwise regression analysis was applied for determination of the best model, which accounted for variation exists in grain yield as dependent variable. Statistical analyses were performed using the statistical program SPSS 13.0.

RESULTS AND DISCUSSION

Analysis of variance, heritability in broad sense and genetic advance expressed as percentage of mean

Variability plays an important role in crop breeding. An insight into the magnitude of variability present in crop species is of utmost importance as it provides the basis for selection (30). Highly significant differences and wide ranges of variation for all characters indicated the presence of sufficient variability among the genotypes (31-33).

In present study, 22 genotypes of einkorn wheat were studied to assess their genetic potential. The analysis of variance is presented in **Table 1**. Coefficient of variation ranged from 5.79 to 20.86%. The most relative variable character during the period of study was total number of tillers per plant (20.86 %), following to number of productive tillers per plant (19.23 %) and grain yield per plant (18.01 %). The values of these coefficients confirm that these traits are more susceptible to change under the influence of different factors (34). Relatively the least variable for the period of study indicated plant height (5.79%) (**Table 1**).

Table 1. Analysis of variance for various quantitative traits in einkorn wheat genotypes

Trait	N	Minimum	Maximum	Mean	Std. Deviation	Variance	CV, %	MS
LSL	22	20.68	27.58	23.06	1.78	3.18	7.73	57.2*
WSL	22	0.96	1.45	1.16	0.13	0.02	10.96	0.29***
TNTP	22	12.67	25.53	18.08	3.77	14.21	20.86	255.81***
NPTP	22	10.50	19.67	14.39	2.77	7.66	19.23	137.88***
PH	22	112.10	139.43	124.16	7.19	51.76	5.79	983.46***
SLWA	22	14.40	19.81	16.63	1.35	1.83	8.12	32.85***
SLWOA	22	7.17	10.11	8.73	0.78	0.61	8.99	11.069***
NSS	22	31.05	42.03	36.19	3.30	10.92	9.13	196.49***
NGS	22	29.77	40.70	35.53	3.35	11.19	9.42	201.49***
GWS	22	0.57	1.03	0.74	0.13	0.02	17.24	0.29***
GYP	22	4.82	10.09	7.45	1.34	1.80	18.01	32.35***
TGW	22	20.00	32.50	24.90	3.06	9.38	12.30	168.91***

The values of mean square for all twelve characters showed highly significant differences between einkorn accessions (**Table 1**). This means the existence of a high degree of genetic variability in the material to be exploited in breeding program (20, 35, 36).

Environmental variances ranged from 0.06 (grain weight per spike) to 39.72 (plant

height). Genotypic variance varied between 0.01 (width of second leaf and grain weight per spike) and 52.43 (plant height). Relatively the highest phenotypic variance values of 92.21 for plant height, 39.77 for number of grain per spike and 39.57 for total number of tillers per plant were recorded in the study (**Table 2**).

Table 2. Genetic parameters of various yield components of 22 einkorn wheat genotypes

Trait	EV	GV	PhV	GCV	PhCV	H, %	GAM, %
LSL	35.31	1.22	36.52	4.78	26.20	3.33	1.80
WSL	0.10	0.01	0.11	8.94	28.32	9.97	5.82
TNTP	26.85	12.72	39.57	19.73	34.80	32.15	23.04
NPTP	15.24	6.81	22.05	18.14	32.63	30.90	20.77
PH	39.78	52.43	92.21	5.83	7.73	56.86	9.06
SLWA	1.93	1.72	3.64	7.88	11.48	47.15	11.15
SLWOA	0.92	0.56	1.48	8.61	13.94	38.10	10.94
NSS	6.93	10.53	17.46	8.97	11.55	60.31	14.35
NGS	30.25	9.51	39.77	8.68	17.75	23.92	8.75
GWS	0.05	0.01	0.07	15.58	34.98	19.83	14.29
GYP	7.81	1.36	9.17	15.68	40.67	14.87	12.46
TGW	13.56	8.63	22.19	11.80	18.92	38.89	15.16

Genotypic and phenotypic coefficients of variation are used to measure the variability that exists in a given population (23). Deshmukh et al. (37) classified PhCV and GCV values as high (>20%), moderate (10-20%) and low (<10%). In the present study high PhCV and moderate GCV were observed in traits: total number of tillers per plant (GCV=19.73%, PhCV=34.80%), number of productive tillers per plant (GCV=18.14%, PhCV=32.63%), grain weight per spike (GCV=15.58%, PhCV=34.98%), grain yield per plant (GCV=15.68%, PhCV=40.67%). Moderate PCV and GCV were found for thousand grain weight (GCV =11.80% and PhCV=18.92%). Moderate PhCV and low GCV were recorded for spike length with awns (GCV =7.88% and PhCV=11.48%), spike length without awns (GCV =8.61% and PhCV=13.94%), number of spikelets per spike (GCV =8.97% and PhCV=11.55%), number of grain per spike (GCV =8.68% and PhCV=17.75%). Low PhCV and GCV showed plant height (GCV =5.83% and PhCV=7.73%). Low GCV and high PhCV were registered for length and width of second leaf (**Table 2**). The PhCV values were higher than GCV values for all the traits which reflect the influence of environment on the expression of traits (33, 38-40).

Heritability estimates provide the information about index of transmissibility of the quantitative characters of economic importance and are essential for an effective crop breeding

strategy. The magnitude of heritability also helps in predicting the behavior of succeeding generations by devising the appropriate selection criteria and assessing the level of genetic improvement (36, 41). The higher the heritability estimates, the simpler are the selection procedures (25). In our study high estimates of heritability in broad sense (above 60%) were recorded for number of spikelets per spike – 60.31% (**Table 2**). According to Gashaw et al. (2010) characters with high heritability are predominantly controlled by genetic factors. Thus, we could anticipate genetic advance under selection for these characters from different types and intensities of selection. Moderate broad sense heritability estimates (30-60%) were exhibited for total and productive tillers per plant (32.15% and 30.90%), plant height (56.86%), spike length with and without of awns (47.15% and 38.10%) and thousand grain weight (38.89%) which suggested that selection should be delayed to more advance generations for these traits (40). Low heritability (<30%) was recorded for length and width of second leaf (3.33% and 9.97%), number of grain per spike (23.92%), grain weight per spike (19.83%) and grain yield per plant (14.87%) (**Table 2**). According to the same authors this implies that selection may be considerably difficult due to the masking effect of the environment for these traits.

The expected genetic advance expressed as a percentage of the mean varied between 1.80%

and 23.04%. Genetic advance as percentage of mean was high for total number of tillers per plant (23.04%) followed by number of productive tillers per plant (20.77%). It was moderate for spike length with and without of awns (11.15% and 10.94%), number of spikelets per spike (14.35%), grain weight per spike (14.29%), grain yield per plant (12.46%) and thousand grain weight (15.16%). The low genetic advance was recorded for the remaining traits (length and width of second leaf, plant height and number of grain per spike) (**Table 2**).

The heritability value alone provides no indication of the amount of genetic progress that would result in selecting the best individual, but heritability estimates along with the genetic advance is considered more useful (41, 42). In this study high heritability accompanied with moderate expected genetic advances in the case of number of spikelets per spike indicated that most likely the heritability was due to additive gene effect and selection may be effective in early generations for this trait (40). High values for the genetic advance expressed as a percentage of the mean and moderate values for the heritability in broad sense were observed in the traits total number of tillers per plant and number of productive tillers per plant. Moderate estimate for both heritability and genetic advance was registered for spike length with awns, spike length without awns and thousand grain weight. Moderate heritability accompanied with low expected genetic advances was computed for plant height. Low heritability coupled with moderate genetic advance was observed for grain weight per spike and grain yield per plant. Low values for both heritability and genetic advance were recorded for length of

second leaf, width of second leaf and number of grain per plant that may be due of the presence of non-additive type of genetic action.

Associations among characters

Grain yield is a complex trait influenced by a number of yield contributing traits. Information on the association of morpho-agronomic traits with grain yield forms a basis for any successful breeding programme (43). Pearson's correlation coefficients between twelve characters are presented in Table 3. Number of productive tillers per plant showed significant negative and positive correlation with width of second leaf (-0.460*) and total number of tillers per plant (0.969**). Plant height correlated positively with length of second leaf (0.460*) and negatively with both total and productive number of tillers per plant (-0.506*, -0.496*). Spike length without awns was in positive relationship with spike length with awns (0.625**) at $p \leq 0.01$. Number of spikelets per spike correlated negatively with length of second leaf (-0.463*) and positively with spike length without awn (0.693**). Number of grain per spike showed significant positive correlations with spike length without awn (0.705**) and number of spikelets per spike (0.679). The correlation between grain yield per plant and grain weight per spike was positive and significant at the 0.01 level (0.607**). The relationships between thousand grain weight, number of spikelets per spike, grain weight per spike and grain yield per plant were also significantly (-0.606**, 0.748** and 0.472*) (**Table 3**). Increase of thousand grain weight is associated with decrease of number of spikelets per spike and increase of grain weight per spike and grain yield per plant.

Table 3. Phenotypic correlation coefficients of investigated characters in einkorn wheat

	LSL	WSL	TNTP	NPTP	PH	SLWA	SLWO A	NSS	NGS	GWS	GYP	TGW
LSL	1											
WSL	-0.064	1										
TNTP	-0.097	-0.377	1									
NPTP	-0.084	-0.460*	0.969**	1								
PH	0.460*	0.032	-0.506*	-0.496*	1							
SLWA	0.213	0.245	-0.275	-0.272	0.087	1						
SLWOA	-0.029	0.102	-0.089	-0.103	0.283	0.625**	1					
NSS	-0.463*	0.260	0.075	0.035	-0.089	0.261	0.693**	1				
NGS	-0.204	0.131	-0.105	-0.078	0.151	0.258	0.705**	0.679**	1			
GWS	0.015	-0.068	-0.223	-0.149	0.208	-0.103	-0.043	-0.160	0.316	1		
GYP	-0.031	-0.401	0.401	0.534*	-0.128	-0.221	-0.013	-0.093	0.183	0.607**	1	
TGW	0.323	-0.136	-0.218	-0.148	0.168	-0.197	-0.381	-0.606**	-0.249	0.748**	0.472*	1

*Correlation is significant at the 0.05 level (2-tailed).

**Correlation is significant at the 0.01 level (2-tailed).

Correlation studies along with path analysis provide a better understanding of the

association of different characters with grain yield. Path coefficient analysis measures the

direct influence of one variable upon the other, and permits separation of correlation coefficients into components of direct and indirect effects. Portioning of total correlation into direct and indirect effects provide actual information on contribution of characters and thus form the basis for selection to improve the yield (44). In our study of path coefficient analysis, grain yield represented the dependent variable and the remaining estimated characters (length of second leaf, width of

second leaf, total number of tillers per plant, number of productive tillers per plant, plant height, spike length with awn, spike length without awn, number of spikelets per spike, number of grains per spike, grain weight per spike, thousand grain weight and grain yield per plant) were the independent ones. Direct and indirect effects of these components determined on grain yield and their contribution ratios were presented in **Table 4**.

Table 4. Direct (Bold and Underline) and indirect effect of 11 characters (independent variables) on grain yield per plant (dependent variable) in 22 einkorn genotypes. The last column shows genotypic correlations (r_{ph}) of independent variables with grain yield per plant

Traits	LSL	WSL	TNTP	NPTP	PH	SLWA	SLWOA	NSS	NGS	GWS	TGW	Total indirect effect	r_{ph}
LSL	<u>0.018</u>	-0.005	0.133	-0.159	-0.058	-0.056	-0.015	0.046	0.048	0.010	0.006	-0.049	-0.031
WSL	-0.001	<u>0.079</u>	0.516	-0.877	-0.004	-0.064	0.053	-0.026	-0.031	-0.045	-0.003	-0.481	-0.402
TNTP	-0.002	-0.030	<u>-1.371</u>	1.848	0.064	0.072	-0.047	-0.007	0.025	-0.147	-0.004	1.772	0.401
NPTP	-0.002	-0.036	-1.329	<u>1.907</u>	0.062	0.071	-0.054	-0.004	0.019	-0.098	-0.003	-1.373	0.534**
PH	0.008	0.003	0.693	-0.945	<u>-0.126</u>	-0.023	0.149	0.009	-0.036	0.137	0.003	-0.002	-0.128
SLWA	0.004	0.019	0.377	-0.519	-0.011	<u>-0.261</u>	0.328	-0.026	-0.061	-0.068	-0.004	0.040	-0.221
SLWOA	-0.001	0.008	0.121	-0.197	-0.036	-0.163	<u>0.525</u>	-0.069	-0.167	-0.028	-0.007	-0.538	-0.013
NSS	-0.008	0.021	-0.103	0.068	0.011	-0.068	0.364	<u>-0.100</u>	-0.161	-0.105	-0.012	0.006	-0.094
NGS	-0.004	0.010	0.143	-0.150	-0.019	-0.067	0.370	-0.068	<u>-0.237</u>	0.208	-0.005	0.419	0.182
GWS	0.000	-0.005	0.306	-0.284	-0.026	0.027	-0.022	0.016	-0.075	<u>0.657</u>	0.014	-0.049	0.608**
TGW	0.006	-0.011	0.299	-0.281	-0.021	0.051	-0.200	0.061	0.059	0.491	<u>0.019</u>	0.454	0.473*

Direct effects obtained in path analysis showed that maximum positive direct effect on grain yield per plant was contributed mostly by number of productive tillers per plant (1.907), following by grain weight per spike (0.657) and spike length without awns (0.525). This means that a slight increase in one of these traits may directly contribute to grain yield. On the other hand, the maximum negative direct effect was exhibited by total number of tillers per plant (-1.371), followed by spike length with awns (-0.261) and number of grain per spike (-0.237). Total number of tillers per plant had positive indirect effect on yield grain per plant via number of productive tillers per plant, (1.907), respectively. Opposite, number of productive tillers per plant had negative indirect effect on yield grain per plant via total number of tillers per plant (-1.329). Width of second leaf and plant height had positive indirect effect on grain yield per plant via total number of tillers per plant (0.516, 0.693), while negative effect via number of productive tillers per plant (-0.877, -0.945). Spike length

with awns had negative indirect effect on grain yield per plant via number of productive tillers per plant (-0.519).

In order to remove effect of non-effective characteristics in regression model on grain yield, stepwise regression was used (45). In stepwise regression analysis, grain yield plant as dependent variable and other traits as independent variables were considered. The results showed that grain weight per spike, number of tillers per plant and total number of tillers per plant with $R^2 = 85.30\%$, had justified the maximum of yield changes (**Table 5**). According to Nasari et al. (46) existence of significant R square in a successful regression equation indicates the effectiveness of the traits to increase grain yield. It is observed that grain weight per spike was the most important character followed by number of productive tillers per plant and total number of tillers per plant. Particularly, 36.9% of the variation in grain yield was explained by grain weight per spike, 76.70% by grain weight per spike and

number of productive tillers per plant altogether and 83.50% was contributed collectively by grain weight per spike, number of productive tillers per plant and total number

of tillers per plant. The best prediction equation, based on the final step of stepwise regression analyses, was determined as follows:

$$\text{GYP} = -2.033 + 6.411 \cdot \text{GWS} + 0.830 \cdot \text{NTP} - 0.399 \cdot \text{TNTP}.$$

Table 5. Stepwise regression of grain yield (dependent variable) and other traits (independent variables) in einkorn wheat

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Correlations			Collinearity Statistics		R Square	Adjusted R Square
	B	Std. Error	Beta			Zero-order	Partial	Part	Tolerance	VIF		
1	(Constant)	2.722	1.401	1.942	0.066						0.369	0.337
	GWS	6.369	1.863	0.607	0.003	0.607	0.607	0.607	1.000	1.000		
2	(Constant)	-2.466	1.261	-1.957	0.065						0.767	0.743
	GWS	7.364	1.173	0.702	0.000	0.607	0.821	0.694	0.978	1.023		
	NTP	.309	.054	0.638	0.000	0.534	0.795	0.631	0.978	1.023		
3	(Constant)	-2.033	1.101	-1.847	0.081						0.835	0.808
	GWS	6.411	1.072	0.611	0.000	0.607	0.816	0.572	0.874	1.144		
	NTP	0.830	0.196	1.713	0.001	0.534	0.706	0.404	0.056	17.981		
	TNTP	-0.399	0.146	-1.123	0.014	0.401	-0.541	-0.261	0.054	18.506		

CONCLUSION

The present study showed the existence of wide ranges of variations for all of the traits among einkorn wheat genotypes. High heritability accompanied with moderate expected genetic advances in the case of number of spikelets per spike indicated that most likely the heritability was due to additive gene effect and selection may be effective in early generations for this trait. High values for the genetic advance and moderate values for the heritability in broad sense were also observed in the traits total number of tillers per plant and number of productive tillers per plant. Maximum positive direct effects on grain yield per plant were contributed mostly by number of productive tillers per plant and grain weight per spike, while the maximum negative direct effect was exhibited by total number of tillers per plant. Stepwise regression analysis also showed that grain weight per spike, number of tillers per plant and total number of tillers per plant had justified the maximum of yield changes.

ABBREVIATION

LSL - length of second leaf (cm), WSL - width of second leaf (cm), TNTP - total number of tillers per plant, NTP - number of productive tillers per plant, PH - plant height (cm), SLWA

- spike length with awn (cm), SLWOA - spike length without awn (cm), NSS - number of spikelets per spike, NGS - number of grains per spike, GWS - grain weight per spike (g), GYP - grain yield per plant (g), TGW - thousand grain weight (g), MS - Mean squares, CV - coefficient of variation (%). EV - Ecological variance, GV - Genotypic variance, PhV - Phenotypic variance, PhCV - Phenotypic coefficient of variability, GCV - Genotypic coefficient of variability, H - Heritability (%), GA - Genetic advance (%) and GAM - Genetic advance in percentage of means (%)

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