



SCIREA Journal of Agriculture

<http://www.scirea.org/journal/Agriculture>

Sptemper 27, 2016

Volume 1, Issue1, October 2016

EVALUATION OF GENETIC DIVERSITY OF EINKORN GENOTYPES MAINTAINED IN THE NATIONAL COLLECTION OF BULGARIA BY MULTIVARIATE ANALYSIS

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ABSTRACT:

The knowledge about of genetic diversity of einkorn wheat (*Triticum monococcum* L.) genotypes is a key to reliable and sustainable production of the food crops adapted to diverse conditions. Twenty two einkorn accessions from the National genebank of Bulgaria were carried out in the experimental field of IPGR-Sadovo, Bulgaria during 2013-2015 growing seasons. Twelve agronomical traits were included in the study. Significant genotypic differences were observed for all studied traits indicating considerable variations among genotypes for each character. Grain yield per plant correlated positively with thousand kernel weight and grain weight per spike. The results from stepwise regression analysis showed that grain weight per spike and number of productive tillers per plant had justified approximately 72% of grain yield variations. Principal Component analysis was applied to group accessions according to similarity on the basis of twelve traits in five components in the factor plane. The first five components explained 82.32% of total variation in the experiment. Cluster analysis based on the five factors grouped the genotypes into four groups. Genotypes in the fourth cluster had the highest mean with respect to

first factor. Members of this group can use for increase in number of grain per spike, thousand grain weight and grain yield per plant in breeding programs.

Key words: einkorn, genetic diversity, correlation, stepwise regression, PC-analysis

Abbreviation: LSL - length of second leaf (cm), WSL - width of second leaf (cm), TNTP - total number of tillers per plant, NPPT - 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) and TGW - thousand grain weight (g)

INTRODUCTION

Einkorn wheat *Triticum monococcum* L. ($2n=2x=14$, AABB) is one of the earliest domesticated crops. However, it was abandoned for cultivation before the Bronze Age and has infrequently been used in wheat breeding [1]. Nowadays, einkorn is grown on a limited regions in Western Turkey, on the Balkan Peninsula, in Italy, Spain, Switzerland, Germany and Austria [2, 3], thanks to its rusticity and adaptation to harsh climates [4]. The attention toward this ancient species has also been renewed by the increasing demand for traditional and healthy 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 [4-6]. Assessing diversity of einkorn accessions collected in different countries and stored in diverse genebanks would considerably facilitate the evaluation of useful traits and utilization in wheat improvement. Various genetic resources were tested in different country for their agronomic performance in organic agriculture and their nutritional value [7-13]. Diversity studies of einkorn wheat based on morphological traits, isozymes and molecular markers have generally concerned a reduced number of accessions [14]. Germplasm improvement and genetic diversity is a key to reliable and sustainable production of the food crops. The evaluation of phenotypic diversity to identify groups with similar genotypes is important for utilizing genetic resources for investigation the diversity of breeding materials [15]. Criteria for the estimation of genetic diversity can be morphological traits and the success of a breeding program depends upon the amount of genetic variability of these traits present in the plant materials [16]. The uses of

multivariate techniques are an important strategy for germplasm classification and study of genetic relationships among genotypes [17, 18].

In Bulgaria, einkorn was grown as forage crop until the 60's in the Eastern Rhodopes, in the South-East in the regions of Elhovo, Yambol and Nova Zagora, and in the South-West, in the region of Radomir. Collecting missions of wheat relatives carried out in Bulgaria by the Institute of Plant Genetic Resources (IPGR) during 1998–1992 reported only wild einkorn. Cultivated einkorn has been recently re-introduced in the Rhodopes and Balkan Mountains with grain and bread now being sold as dietetic foods [4, 19]. Einkorn collection as genetic resources is also maintained at long-term storage condition in the National genebank of Bulgaria [20].

The aim of this study was to evaluate the genetic diversity of 22 einkorn genotypes maintained in the national collection of Bulgaria by using of multivariate analysis.

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 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).

Statistical analyses were performed using the statistical program SPSS 19.0. The mean data were subjected to analysis of variance to test the level of significance among the genotypes for different characters according to Steel and Torrie [21]. Phenotypic correlations were calculated by using phenotypic variances and covariance [22]. Stepwise regression analysis was applied for determination of the best model, which accounted for variation exists in grain yield as dependent variable. Principal component analysis (PC-analysis) was applied to group accessions according

to similarity on the basis of twelve traits (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, grain yield per plant and thousand grain weight) in five components in the factor plane and the related clusters were plotted based on the main components.

RESULTS

Analysis of variance

Mean squares of 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 (g), GYP - grain yield per plant and TGW - thousand grain weight showed highly significant differences between genotypes (Table 1).

Correlation between investigated characters

Pearson's correlation coefficients between 12 characters are presented in Table 2. Width of the second leaf showed significant negative correlation with number of productive tillers per plant. Total number of tillers per plant correlated positively with number of productive tillers per plant (0.673) and negatively with plant height (-0.579). Number of productive tillers per plant was in negative relationship with plant height (-0.507) at $p \leq 0.05$. Spike length with awn correlated positively with spike length without awn (0.423). Number of spikelets per spike showed significant positive and negative correlation with number of grains per spike (0.441) and thousand grain weight (-0.570). The correlations between grain weight per spike, grain yield per plant and thousand grain weight were positive and significant at the 0.01 level. Grain yield per plant also correlated positively with thousand kernel weight (0.621) (Table 2).

Stepwise regression analysis

Stepwise regression analysis for grain yield as dependent variable and agronomic traits as independent variables in 22 einkorn wheat genotypes was applied (Table 3). Results showed that grain weight per spike and number of productive tillers per plant remained in the last model.

They explained 72% of variation in the yield. The standard linear regression equation for grain yield per plant (GYP) was:

$$\text{GYP} = -5.88 + 10.76 * \text{GWS} + 0.28 * \text{NPTP}$$

Principal component analysis

PC-analysis was applied to arrange accessions by their similarity. The values of the five components to each of the study parameters were calculated empirically (Table 4). The analysis showed that the first component explained 24.57 %, the second- 21.32%, the third- 17.36%, the fourth - 10.68% and the fifth- 8.39% of the total variation. Five factors explained total 82.32% of the variation in the experience. First factor had an important role to justify alteration of grain weight per spike, grain yield per plant and thousand grain weight. Second factor was in positive correlation with total and productive number of tillers per plant and negative with plant height. The factorial coefficients for spike length without awn, number of spikelets per spike and number of grains per spike were high and positive for the third factor. In the fourth factor that had explained 10.68% of alteration, width of second leaf had the greatest effect, while length of second leaf had the highest effect for the fifth factor (Table 4).

Cluster analysis based on the five factors grouped the genotypes into four groups at 25% linkage distance (Fig. 1). Average of factors for each cluster is shown in table 5. In the first cluster, six accessions were classified including 27.27% of total genotypes. Accessions in this cluster were in the highest rate with respect to fourth factor. Second group comprised 5 genotypes including 28.13% of total genotypes. The varieties in this cluster had almost similar values for second and fifth factors. In the third group, 7 genotypes were classified including 31.8% of total genotypes. These genotypes were superior with respect to spike length without awn, number of spikelets per spike and number of grains per spike. In the fourth group, 4 accessions were classified 18.18% of total genotypes. Genotypes in this cluster had highest mean with respect to first factor (Fig.1, Table 5).

DISCUSSION

The knowledge about the extent of variability among wheat genotypes is a high value for the genetic improvement programs and the efficient genetic diversity utilization of plant materials [16, 23, 24]. Some researchers emphasize the importance of the assessment of the levels and patterns of genetic diversity in crop breeding [18, 23, 25, 26]. Guzy et al. [27] reported a high variability for yield within einkorn. Empilli et al. [8] observed high variation for earliness and plant height in 1039 accessions of *T. monococcum* L. from different geographical origins. Brandolini et al. [11] found significant differences among 169 einkorn accessions samples from diverse origins for heading date, number of spikelets per spike, seed length, protein content and SDS sedimentation volume. Butnaru et al. [12] recorded a large variation for grain number per spike and grain weight in a collection of 37 einkorn accessions collected in Romania and Hungary.

In the present study analysis of variance showed that there is considerable variability among einkorn genotypes in all of the investigated characters. This indicated the existence of high genetic diversity among investigated landraces and also reflected the broad ranges observed for each trait [28-31]. Environment mean squares were also significant for all the traits except grain yield per plant. G x E interaction was significant for all the traits except for width of second leaf, grain weight per spike and grain yield per plant. However, the extent of effect of genotype, environment and genotype x environment varied [32, 33].

The association of structural elements of yield with grain yield and the interrelationships among the components assumes special importance as the basis for selecting high yielding genotypes. In this study, simply correlation analysis determined that grain weight per spike is the most important crop characteristic for yield improvement. Thousand grain weight is the next important trait, respectively. The other traits correlated no significantly with grain yield per plant.

Stepwise regression analysis is a multiple statistical method that can screen or select the most important variables through a dependent variable such as the grain yield [34]. It is a semi-automated process of building a model by successively adding or removing variables based solely on the t-statistics of their estimated coefficients. In order to remove effect of non-effective characters in regression model on grain yield, stepwise regression was used [35, 36]. Results of stepwise regression analysis showed that, grain weight per spike and number of productive tillers per plant remained in the final model. Particularly, 47% of the variation in grain yield was explained by grain weight per spike, 72% was contributed collectively by grain weight per spike

and number of productive tillers per plant (Table 3). Thus in this study these two traits had the greatest impact on the grain yield.

Correlation analysis helps to determination effective traits in order to indirect selection superior genotypes [37]. On the other hands, principal component analysis is suitable multivariate technique in identify and determination of independent principal components that are effective on plant traits separately. Therefore, correlation and principal component analysis helps breeders to genetic improvement traits such as yield that have low heritability specifically in early generations via indirect selection for traits effective on this [38-41].

The PCA and cluster analysis are preferred tools for morphological characterization of genotypes and their grouping on similarity basis based on this approach [18, 42-44]. According to Moetamadipoor et al. [45] factor analysis is crucially important in identification of independent factors which separately affect main plant traits. Considering use of varimax rotation in this technique, which leads to maximizing variance between factors, the factors which justify higher percent of the variation between traits are more important and they should be considered in breeding programs. The results obtained from PC-analysis showed that the first five components explained 82.63% of total variation. First factor determining 24.57 % of the variation had an important role to justify alteration of grain weight per spike, grain yield per plant and thousand grain weight. Increasing grain weight per spike and thousand grain weight would be the most effective way of increasing yield.

Cluster analysis based on PCA is a more precise indicator of differences among wheat genotypes than cluster analysis (not based on PCA) [46]. In this study it grouped the accessions into four groups. Genotypes in the first cluster had the highest mean with respect to fourth factor. Genotypes of the second cluster can be used for increase in total and productive tiller per plant and reduced the plant high. Genotypes in the third cluster can be used to receive big and productive spikes. Genotypes in the fourth cluster had the highest mean with respect to first factor. Members of this group can use for increase in number of grain per spike, thousand grain weight and grain yield per plant in breeding programs.

CONCLUSION

Analysis of variance revealed considerable variability among einkorn genotypes in all of the investigated characters. Simply correlation analysis determined that grain weight per spike is the most important crop characteristic for yield improvement, following by thousand grain weight. Stepwise regression analysis showed that, grain weight per spike and number of productive tillers per plant explained 72% of the variation in grain yield. With respect to the results of PC-analysis, the first factor could be introduced as an effective factor in increasing yield. It accounted for 24.57 % of the total changes that this factor had a positive relationship with grain weight per spike, grain yield per plant and thousand grain weight. Grouping of genotypes by multivariate methods in the study is of practical value for the breeding programs.

Table 1. Mean squares of investigated traits for twenty two einkorn genotypes

Sources of variation	Genotype (G)	Environmental (E)	Interaction (GxE)
<i>df</i>	21	1	21
LSL	114.41*	7684.88***	136.96***
WSL	0.58***	20.63***	0.29
TNTP	930.92***	38428.18***	716.66*
NPTP	340.59***	12635.75***	206.56***
PH	1846.03***	29596.10***	277.85***
SLWA	65.70***	61.94***	11.93***
SLWOA	42.01***	458.59***	26.53*
NSS	392.99***	10527.96***	85.09***
NGS	402.98***	21780.25***	207.98***
GWS	0.58***	48.71***	0.06
GYP	631.76*	944.75	514.69
TGW	337.83***	196.12**	110.72***

*p<0.05, **p<0.01, *** p<0.001

Table 2. Phenotypic correlation coefficients of investigation yield components in einkorn wheat

	LSL	WSL	TNTP	NPTP	PH	SLWA	SLWOA	NSS	NGS	GWS	GYP	TGW
LSL	1											
WSL	0.109	1										
TNTP	-0.073	-0.240	1									
NPTP	-0.042	0.438*	0.673*	1								
PH	0.260	0.044	0.579*	0.507*	1							

	LSL	WSL	TNTP	NPTP	PH	SLWA	SLWOA	NSS	NGS	GWS	GYP	TGW
			*									
SLWA	0.185	0.249	-0.297	-0.309	0.145	1						
SLWOA	-0.055	-0.128	0.017	0.150	0.247	0.423*	1					
NSS	-0.123	0.141	-0.112	0.082	-0.058	0.237	0.332	1				
NGS	-0.320	0.161	-0.019	-0.100	0.204	0.127	0.418	0.441*	1			
GWS	0.059	-0.032	0.030	-0.146	0.231	-0.036	0.009	-0.235	0.376	1		
GYP	0.140	-0.391	0.334	0.396	.071	-0.135	0.151	-0.121	0.104	0.689**	1	
TGW	0.213	-0.142	-0.003	-0.118	0.147	-0.211	-0.313	-0.570**	-0.245	0.779**	0.621**	1

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

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

Table 3. Stepwise regression analysis for grain yield (dependent variable) and other agronomic traits (independent variable) in einkorn wheat genotypes

Model	R Square	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Correlations			Collinearity Statistics	
		B	Std. Error	Beta			Zero-order	Partial	Part	Tolerance	VIF
		1	(Constant)	-0.87			2.05		-0.42	0.68	
	GWS	9.68	2.32	0.68	4.17	0.00	0.68	0.68	0.68	1.0	1.00
2	(Constant)	-5.88	1.95		-3.02	0.01					
	GWS	10.76	1.75	0.76	6.16	0.00	0.68	0.82	0.75	0.98	1.02
	NPTP	0.28	0.07	0.51	4.14	0.00	0.39	0.69	0.50	0.98	1.02

Table 4. Weighted factors (PC1, PC2, PC3, PC4 and PC5) of descriptive characteristics on the rotated matrix with five factors

Characters	Principal component (PC)				
	1	2	3	4	5
LSL	0.126	-0.069	-0.093	0.051	0.870
WSL	-0.094	-0.128	0.062	0.900	0.091
TNTP	0.157	0.872	-0.012	-0.132	-0.042

Characters	Principal component (PC)				
	1	2	3	4	5
NPTP	-0.004	0.799	0.111	-0.465	0.027
PH	0.218	-0.831	0.170	-0.177	0.140
SLWA	-0.097	-0.241	0.531	0.283	0.488
SLWOA	0.028	-0.072	0.825	-0.302	0.125
NSS	-0.281	0.086	0.702	0.161	-0.139
NGS	0.305	-0.098	0.698	0.222	-0.489
GWS	0.968	-0.114	0.016	0.103	-0.089
TGW	0.819	0.257	0.097	-0.359	0.124
GYP	0.804	-0.106	-0.500	-0.060	0.144
% of variance explained	24.57	21.32	17.36	10.68	8.39
Cumulative variance, %	24.57	45.89	63.25	73.93	82.32

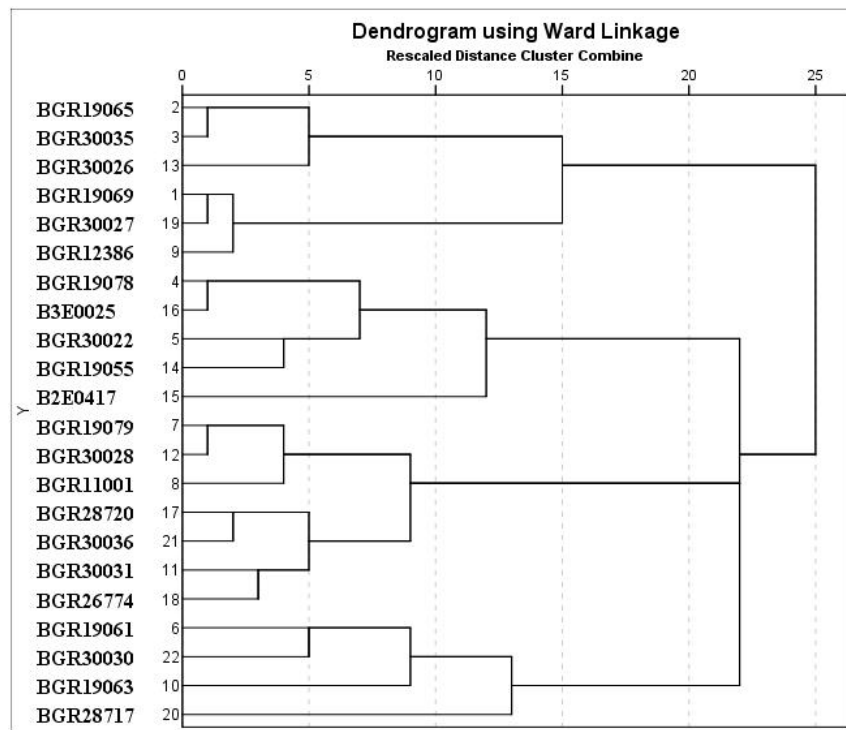


Fig.1. Tree diagram of 22 genotypes for 5 extracted factors using hierarchical cluster analysis (Ward's method and Euclidean distance)

Table 5. The average of traits for achieved groups from cluster analysis based on factor analysis in 22 einkorn wheat genotypes

	Factor 1	Factor 2	Factor 4	Factor 5
Cluster 1	-0.625	-0.076	1.083	0.457
Cluster 2	-0.469	0.674	-1.031	0.614
Cluster 3	0.017	-0.293	-0.435	-0.907
Cluster 4	1.493	-0.214	0.427	0.134

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