Multivariate Biplot Analysis for the Diversity in Bread Wheat Genotypes (*Triticum aestivum* L.)

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ABSTRACT

A field study was conducted during (November–April, 2017–2018) at CCSHAU, Hisar, Haryana, Indiaxxx to evaluate wheat genotypes for diversity analysis by multivariate and biplot analysis.xxx The morphological and agronomic attributes of wheat have been evaluated to measure genetic variation and their close relatives. Diversity analysis of bread wheat genotypes was deciphered by fifteen morphological traits utilizing multivariate hierarchical clustering technique and biplot analysis. Multivariate analyses have been applied to measure the diversity in wheat accessions and to evaluate the relative contributions of morphological traits to the total variability in a collection. Recent analyses enable accessions to be classified into clusters as per their similarity indexes. Good amount of variability had been observed for most of the traits. Maximum range was observed for flag leaf area and plant height. The positive correlation of the grain yield with various traits ensured indirect selection for yield can be done by selecting those characters. Significant positive correlation of grain yield exhibited with number of grains spike^{-1}, thousands grain weight and plant height. Multivariate Hierarchical clustering technique by Ward's method expressed three clusters with 20.4, 20.5 and 59.1% of total genotypes. The first principal component accounted for 29.4% with major traits number of tillers plant^{-1}, flag leaf area, biological yield plant^{-1}, grain yield plant^{-1}, weight of grain ear^{-1}, flag leaf length. The contribution of second was 15.7% with major six traits number of grains ear^{-1}, ear weight, weight of grain ear^{-1}, number of spikelet ear^{-1}, grain yield plant^{-1} and number of tillers plant^{-1}.

1. INTRODUCTION

Wheat, an important staple food crop, is preferred over rice and maize as a source of protein in low and middle-income nations (Sansaloni et al., 2020). Being the staple food crop of many countries with diverse climatic conditions, wheat varieties must have enough variability to tolerate to pests, pathogens and abiotic stresses (Ali et al., 2021). Wheat, consumed as whole grains boiled or as bread from its flour in many ways, is not only a staple source of carbohydrates and nutrients for around 40% of the world's population providing 20% of the daily protein and food calories (Lawati et al., 2021). Genetic diversity of plants determines their potential for improved efficiency and hence their use for breeding, which eventually may result in enhanced food production (Rufo et al., 2019). Regular studies of diversity being conducted by breeders to evaluate and utilize the diversity of breeding germplasm, also to determine the uniqueness and distinctiveness among genotypes to safe guard a breeders intellectual property rights (Fu, 2015). In addition, assessment of genetic distance is one of most suitable tools for selection of parents in a wheat crossing scheme for possible yield enhancement (Negisho et al., 2021). A number of suitable methods like principal component analysis, factor analysis and cluster analysis are presently available for the selection of parent, detection of genetic variability, centre of origin, study of interaction among the environments and tracking the course to crop evolution (Mourad et al., 2020). Cluster analysis is a multivariate method, which aims to classify a sample of subjects based on a set of measured variables into a number of different groups such that similar subjects are placed in the same group (Giraldo et al., 2019). It sorts genotypes into groups, or clusters, so the degree of association will be strong between members of the same cluster and weak between members of different clusters. The cluster analysis was performed using a measure of similarity levels and Euclidean distance (Sant'Anna et al., 2020). Different researcher grouped bread wheat genotypes using cluster analysis. Principal component analysis makes it possible to transform a given set of characteristics (variables), which are mutually correlated, into a new system of characteristics, known as principal components, which are not correlated (Ahmadizadeh et al., 2019). The obtained variables may also be used for further analysis, where the assumption of no co-linearity is required. Moreover, the analysis is characterized by the fact that it includes the total variance of variables, explains maximum of variance within a data set, and is a function of primary variables (Wani et al., 2018). Hierarchical clustering is the most widely used approach in the analysis of crop genetic diversity. Several hierarchical clustering methods, including single linkage, complete linkage, simple average, median, un weighted paired group method using arithmetic averages (UPGMA) and Ward's of minimum variance have been used (Pixley et al., 2018). Each of these approaches has some distinctive features. However the grain yield influenced by spikelet per spike, number of effective tillers per plant, grain numbers per spike that is why statistical techniques correlation studies and principal component analysis provide reliable assessment of complex relationships among various traits (Wouw et al., 2009, Ahmad et al., 2017; Fayaz et al., 2019). Diversity of bread wheat genotypes had been deciphered by multivariate hierarchical clustering by considering fifteen morphological traits simultaneously along with the biplot analysis utilizing two principal components.

2. MATERIALS AND METHODS

A total of forty-four bread wheat genotypes including four popular checks were evaluated under field trials laid out in a Randomized Block Design (RBD) with three replications in the research field area of Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar during the November–April, 2017–2018. Recommended agronomic practices have been applied to ensure good harvest of wheat yield. Five randomly selected competitive plants for each genotype observed for altogether fifteen important morphological traits *viz* DH: Days to heading, NTP: No of tillers plant^{-1}, FLL: Flag leaf length (cm), FLB: Flag leaf breadth (cm), EW: Ear weight (g), FLA: Flag leaf area (cm²), PH: Plant height(cm), EL: Ear length (cm), NGE: Number of grains ear^{-1}, GWE: Weight of grain ear^{-1} (g), NSE: Number of spikelet ear^{-1}, GW: Thousand grain weight, GY: Grain yield plant^{-1} (g), BY: Biological yield plant^{-1} (g), HI: Harvest index (%). Variability among genotypes as per theses traits exhibited by Radar and Bar charts. Descriptive measures of variability viz., range, mean and standard error, Skewness, Kurtosis and CV calculated to summarize variation in genotypes. Reputed statistical software SAS version 9.3 along with JMP 9 was exploited for analysis and graphical presentations.

3. RESULTS AND DISCUSSION

3.1. *Variability among genotypes for morphological traits*

Good amount of variability had been observed among the studied genotypes as depicted by in Figure 1. Around the circle in Radar chart 44 genotypes were depicted and the values for the traits for a particular genotype were depicted by the point's concentric circles. The radar diagram was utilized by Porter and Niksiar, 2018 to express the variability. Descriptive measures of variability *viz* mean, range, standard error, and coefficient of variation for traits have been presented in Table 1. Highest range recorded
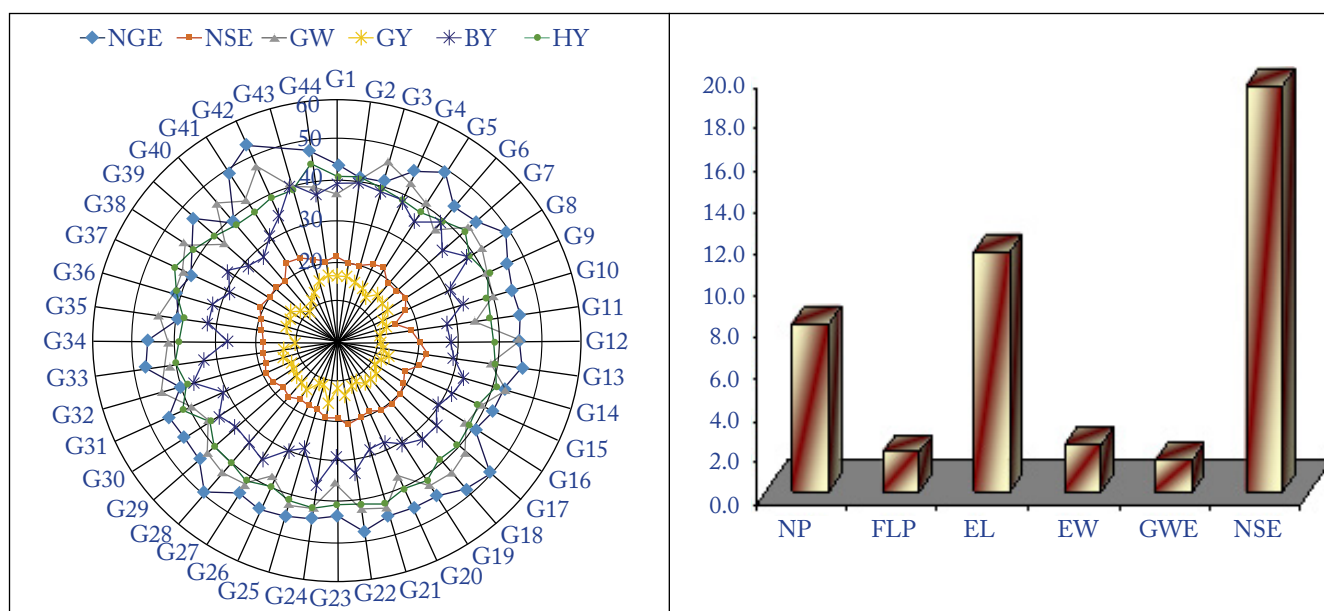
Figure 1: Diversity among genotypes for morphological traits explanined by radar and bar chart

Table 1: Descrptive measures of variability among traits

Traits	DH	NTP	FLL	FLB	FLA	PH	EL	EW	NGE	GWE	NSE	GW	GY	BY	HI
Mean	95.76	8.04	22.70	1.89	33.22	101.66	11.53	2.23	45.02	1.51	19.47	40.93	12.92	32.48	39.81
SD	4.19	1.53	3.26	0.22	7.01	5.45	1.12	0.38	3.29	0.29	1.51	3.11	1.62	3.83	1.66
SE	0.631	0.23	0.491	0.034	1.06	0.82	0.17	0.057	0.49	0.04	0.23	0.47	0.24	0.58	0.23
Min	85.67	5.9	17.23	1.32	19.16	90.17	9.36	1.42	38.33	1.04	14.89	34.29	10.37	26.79	36.67
Max	101.33	11.3	29.09	2.29	50.64	110.2	13.75	3.3	53.22	2.33	22.56	47.91	16.23	40.12	44.56
Range	15.66	5.4	11.86	0.97	31.49	20.03	4.39	1.88	14.89	1.29	7.67	13.62	5.86	13.33	7.89
CV	4.38	19.03	14.36	11.64	21.10	5.36	9.71	17.04	7.31	19.21	7.76	7.60	12.54	11.79	4.17

SD: Standard Deviation; SE: Standard Error; Min: Minimum; Max: Maximum

for flag leaf area (31.49) and plant height (20.03). Such significant range of variations provides an ample scope for yield improvement. Similar results were earlier recorded by Ahmadizadeh et al., 2019. The phenotypic values for days to heading varied from 85.67 for G17 to 101.33 days for genotype G34. Among the experimental set tallest genotype was G3 expressed maximum plant height (110.2 cm) while the maximum spike length was observed at G39 (13.67 cm) i.e. comparable to the G41 (13.75 cm) one of the check. Shortest height of 90.17 cm was recorded by genotype G14. Highest thousand grains weight was shown by genotype G3 (46.05) near to G42 values i.e. check. Sufficient variability among genotypes provided ample scope for selection of superior and desired genotypes by the concerned breeders (Awan et al., 2015; Ahmad et al., 2017; Lawati et al., 2021).

3.2. Association analysis

Most of the morphological traits exhibited a significant correlation coefficient values among them. The grain yield plant[-1] is observed to have significant positive association with number of effective tillers plant[-1], number of grains plant[-1], thousand grain weights as expected (Mourad et al., 2020). Grain yield plant[-1] has further been observed to have positive association with ear weight and grain weight ear[-1] as well. Earlier Tsonev et al. (2021) have recorded the similar pattern of significant positive correlation with grain yield. The negative association of harvest index with biological yield was in agreement with the studies of Mecha et al., 2017. The characters exhibiting strong positive association with grain yield indicate that selection based on morphological traits could definitely lead to improvement in grain yield and therefore based on the results obtained in the present investigation it is suggested that number of grains spike[-1], hundred grain weight, number of effective tillers plant[-1] coupled with spike weight and grain weight spike[-1] be given emphasis for future improvement programme focusing on grain yield (Table 2).

Table 2: Correlation coefficients to study association analysis

	NTP	FLL	FLB	FLA	PH	EL	EW
DH	-0.0746	-0.2692	-0.0841	-0.1578	-0.0165	0.1072	-0.3738
NTP		0.4916	0.0787	0.3986	0.4971	0.3962	0.1906
FLL			0.0795	0.7396	0.5642	0.0334	0.2738
FLB				0.5519	-0.1874	0.5018	0.1687
FLA					0.2780	0.2631	0.4309
PH						0.0124	0.0853
EL							0.1636
EW							
NGE							
GWE							
NSE							
GW							
GY							
BY							

Table 2: Continue...

	NGE	GWE	NSE	GW	GY	BY	HI
DH	-0.1956	-0.4569	0.0706	-0.2349	-0.0001	0.0150	-0.0398
NTP	0.0766	0.2812	0.0070	0.2101	0.7373	0.7875	-0.0057
FLL	0.2999	0.3638	-0.2653	-0.0932	0.3214	0.3261	0.0436
FLB	-0.1235	0.0366	-0.1052	0.1147	0.1215	0.1058	0.0483
FLA	0.3606	0.3937	-0.0568	0.0599	0.3841	0.3863	0.0447
PH	0.0619	0.1457	-0.0989	-0.1089	0.2803	0.3385	-0.1146
EL	-0.1238	0.1283	0.2650	0.3469	0.3036	0.3160	0.0142
EW	0.5964	0.8541	0.4515	0.1548	0.2285	0.2484	-0.0419
NGE		0.6108	0.2911	0.0146	0.0594	0.1083	-0.1313
GWE			0.3889	0.2005	0.2663	0.3064	-0.0746
NSE				0.1545	0.0838	0.0996	-0.0326
GW					0.1263	0.1303	0.0129
GY						0.9402	0.3317
BY							-0.0071

Harvest index shows here negative correlation with the number of spikelet per ear and days to heading which is in agreement to the results of Mecha et al. (2017).Plant height has significant and positive association with biomass yield plant while number of spikelet per ear has a negative correlation with the plant height. Positive correlation of grain yield observed with number of grains per spike, thousand grains weight and plant height (Tsonev et al., 2021). Days to heading and Plant height exhibit positive and significant correlation which is in accordance to the studies of Phougat et al., 2017, Wani et al., 2018; Giraldo et al., 2019.

3.3. Multivariate hierarchical clustering of genotypes

Using a univariate statistical analysis and standard deviations for each one of trait does not provide a complete insight into the complex analysis (Bhatta et al., 2018). Multivariate statistical methods are appropriate tools for the analysis of the complex structure (Ahmadizadeh et al., 2021). The hierarchical cluster analysis is a simple way of grouping the set of selected genotypes as per their similarities based on 15 morphological variables. Multivariate techniques

have been used to estimate the genetic divergence between accessions, like biometric models estimated by the Euclidean Distance and hierarchical grouping methods (Ali et al., 2021). Hierarchical clustering techniques have long been the most popular clustering method with Ward's method and average linkage probably being the best available (Awan et al., 2015). Three clusters of genotypes had been observed nine, nine and twenty six amounts to 20.4, 20.5 and 59.1 percent of total genotypes (Figure 2).

3.4. Biplot analysis

Results of the principal component analysis (PCA) indicated that the first two components were important in explaining the variation among the 44 accessions studied and cumulatively accounted for 45.1% of the total phenotypic variation (Table 3). The first principal component (PC) accounted for 29.4% of the total variation. It illustrated the variations in number of tillers plant^{-1}, flag leaf area, biological yield plant^{-1}, grain yield plant^{-1}, Weight of grain ear^{-1}, flag Leaf length primarily (Rufo et al., 2019). Principal component two contributed 15.7% to the total variation. Six variables, including Number of grains ear^{-1}, ear weight, weight of grain ear^{-1}, number of spikelets ear^{-1}, Grain yield plant^{-1} and number of tillers plant^{-1} were to contribute more
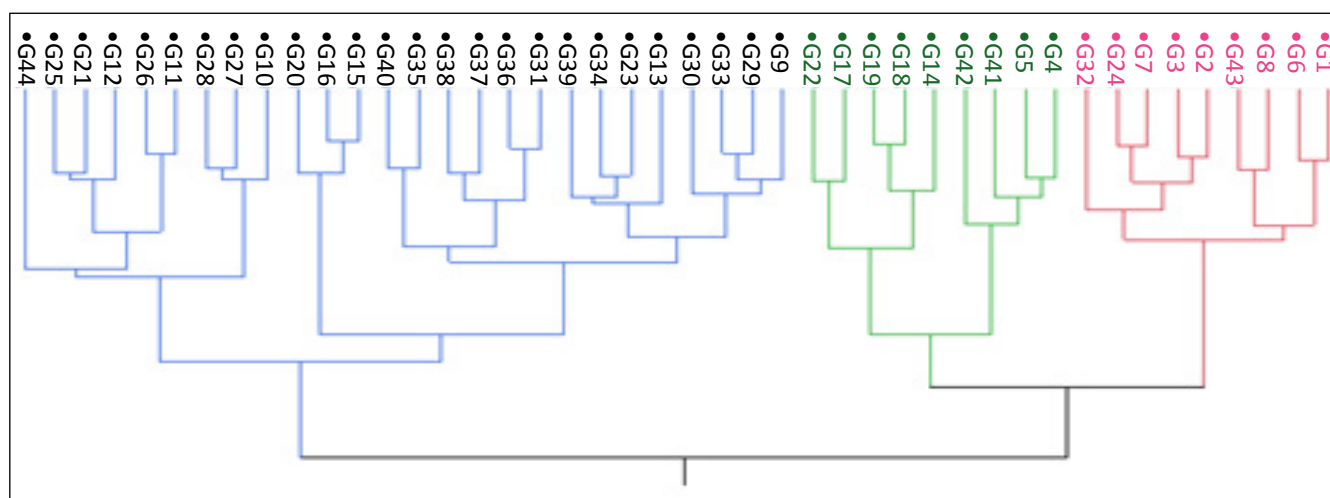

Figure 2 : Hierarchical clustering of genotypes based on fifteen morphological traits as per ward's method

Table 3: Loadings of traits as per first two principal components

Traits	PCA1	PCA2
DH	-0.1439	0.2692
NTP	0.3610	0.2886
FLL	0.3167	0.0772
FLB	0.1289	0.0961
FLA	0.3522	0.0264
PH	0.2115	0.1867
EL	0.1905	0.1591
EW	0.3152	-0.4084
NGE	0.2144	-0.4143
GWE	0.3351	-0.3924
NSE	0.0942	-0.3007
GW	0.1126	-0.0605
GY	0.3451	0.2994
BY	0.3582	0.2709
HI(%)	0.0182	0.1367
variance	29.36	15.68

to second PC. Out of the 15 traits evaluated, 09 were found to contribute most to the first two principal components (Table 3) and were therefore considered most discriminate to summarize phenotypic variation among the accessions through hierarchical cluster analysis. The biplot analysis is an appropriate method to analyse interaction between genotypes and traits and narrowing down the number of traits to the ones contributing a major portion to the variability as discussed by Negisho et al. (2021). The first two components explained 45.05% of the total variation in genotypes (Figure 3). The high positive correlation has been observed between HI, GY, BY, NTP and FLA in the positive direction. The genotype, G 1 had the highest values for grain yield plant^{-1} (15.82 g) and biological yield plant^{-1} (39.09) and number of tillers plant^{-1} (10.71). Though G 5 achieved maximum grain weight ear^{-1} (2.04) and ear weight (2.83) of was highest among genotypes. The difference between the biplot origin and genotype position in the biplot is the vector length of the genotype and it is a measure of the distinctiveness of the genotype from other genotypes as reviewed by Joshi and Okuno (2010). In the biplot vectors of traits showing acute angles are positively correlated whereas those showing obtuse or straight angles are negatively correlated and those with right angles have no
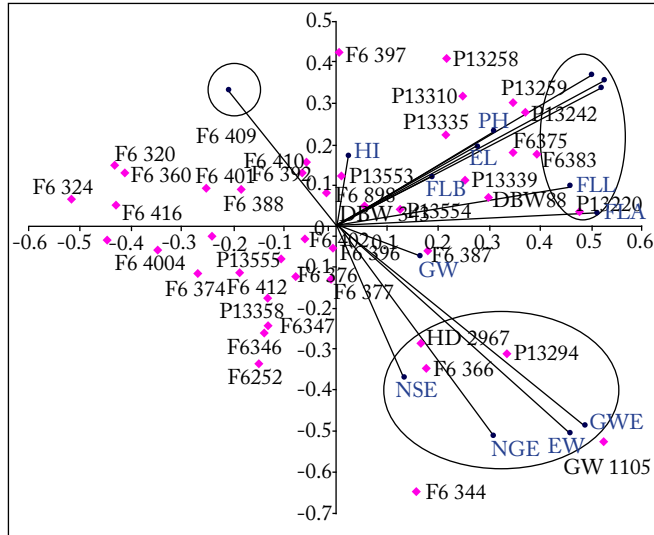
Figure 3: Biplot analysis of morphological traits of evaluated wheat genotypes

correlation. The genotypes having long length of the vector have higher or extreme values for one or more characters. Selection among such genotypes may be performed either for further trials or for their use as parents in breeding programs (Sant'Anna et al., 2020).

4. CONCLUSION

Grain yield plant^{-1} had a positive correlation with number of tillers per plant, flag leaf area, plant height, ear length, grain weight ear^{-1} and thousand grain weight. These traits are most yield determiner traits found in the study. Simultaneous selection among such traits will improve the grain yield in wheat. Selection of genotypes as parents from this set would be beneficial for a breeding programme. Multivariate with biplot analysis would be more appropriate for diversity analysis for efficient selection of promising genotypes irrespective of crops.

5. REFERENCES

Ahmad, S., Pasha, I., Saeed, M., Shahid, M., 2017. Principal component analysis and correlation studies of spring wheats in relation to cookie making quality. International Journal of Food Properties 20(10), 2299–2313.

Ahmadizadeh, M., Shahbazi, H., Valizadeh, M., Zaefizadeh, M., 2019. Genetic diversity of durum wheat landraces using multivariate analysis under normal irrigation and drought stress conditions International Journal of Irrigation and Water Management 6(10), 001–009.

Ali, N., Hussain, I., Ali, S., Khan, N.U., Hussain, I., 2021. Multivariate analysis for various quantitative traits in wheat advanced lines. Saudi Journal of Biological Sciences 28(1), 347–352.

Awan, S.I., Ahmad, S.D., Ali, M.A., Ahmed, M.S., Rao, A., 2015. Use of multivariate analysis in determining characteristics for grain yield selection in wheat. Sarhad Journal of Agriculture 31(2), 139–150.

Bhatta, M., Morgounov, A., Belamkar, V., Poland, J., Baenziger, P.S., 2018. Unlocking the novel genetic diversity and population structure of synthetic Hexaploid wheat. BMC Genomics 19, 1–12.

Desheva, G., Kyosev, B., 2015. Genetic diversity assessment of common winter wheat (*Triticum aestivum* L.) genotypes. Emirates Journal of Food and Agriculture 27, 283–290.

Fayaz, F., Aghaee, S.M., Talebi, R., Azadi, A., 2019. Genetic diversity and molecular characterization of iranian durum wheat landraces (*Triticum turgidum* durum (Desf.) Husn.) Using DArT Markers. Biochemical Genetics 57(1), 98–116.

Fu, Y.B., 2015. Understanding crop genetic diversity under modern plant breeding. Theoretical and Applied Genetics 128, 2131–2142.

Giraldo, P., Benavente, E., Manzano-Agugliaro, F., Gimenez, E., 2019. Worldwide research trends on wheat and barley: A bibliometric comparative analysis. Agronomy 9, 352.

Joshi, B.K., Okuno, K., 2010. A genotype by trait biplot analysis for multiple traits-based selection of genotypes of Tartary buckwheat Fagopyrum 27, 13–19.

Kumar, V., Pandey, S., Shukla, R.S., Rani, A., 2020. Multivariate analysis for morphological traits in newly developed cytoplasmic lines of wheat. International Achieves of Applied Science Technology 11(1), 116–121.

Lawati, A.H.A., Nadaf, S.K., Saady, N.A.A., Hinai, S.A.A., Almamari, A., Maawali, A.A., 2021. Multivariate analyses of indigenous bread wheat (*Triticum aestivum* L.) landraces of Oman. Emirates Journal of Food and Agriculture 33(6), 483–500.

Mecha, B., Alamerew, S., Assefa, A., Dutamo, D., Assefa, E., 2017. Correlation and path coefficient studies of yield and yield associated traits in bread wheat (*Triticum aestivum* L.) genotypes. Advances in Plants and Agriculture Research 6(5), 1–10.

Mourad, A.M.I., Belamkar, V., Baenziger, P.S., 2020. Molecular genetic analysis of spring wheat core collection using genetic diversity, population structure, and linkage disequilibrium. BMC Genomics 21, 434.

Negisho, K., Shibru, S., Pillen, K., Ordon, F., Wehner, G., 2021. Genetic diversity of Ethiopian durum wheat landraces. PLoS ONE 16(2), e0247016.

Nielsen, N.H., Backes, G., Stougaard, J., Andersen, S.U., Jahoor, A., 2014. Genetic diversity and population structure analysis of European hexaploid bread wheat

(*Triticum aestivum* L.) varieties. PLoS One 9(4), e94000.

Phougat, D., Panwar, I.S., Saharan, R.P., Singh, V., Godara, A., 2017. Genetic diversity and association studies for yield attributing traits in bread wheat [*Triticum aestivum* (L.) em. Thell]. Research on Crops 18(1), 139–144.

Pixley, K.V., Salinas-Garcia, G.E., Hall, A., Kropff, M., Ortiz, C., Bouvet, L., Suhalia, A., Vikram, P., Singh, S., 2018. CIMMYT's seeds of discovery initiative: harnessing biodiversity for food security and sustainable development. Indian Journal of Plant Genetic Resources 31(1), 1–10.

Porter, M.M., Niksiar, P., 2018. Multidimensional mechanics: Performance mapping of natural biological systems using permutated radar charts. PLoS ONE 13(9), e0204309.

Rufo, R., Alvaro, F., Royo, C., Soriano, J.M., 2019. From landraces to improved cultivars: Assessment of genetic diversity and population structure of mediterranean wheat using SNP markers. PLoS One 14, e0219867.

Sansaloni, C., Franco, J., Santos, B., 2020. Diversity analysis of 80,000 wheat accessions reveals consequences and opportunities of selection footprints. Nature of Communications 11, 4572.

Sant'Anna, I.D.C., Silva, G.N., Carneiro, V.Q., Pontes, D.S., Nascimento, M., Cruz, C.M., 2020. Comparison of projection of distance techniques for genetic diversity studies. Acta Science Agronomy 42, e42483.

Tsonev, S., Nikolai, K.C., Gallina, M., Anna, D., Elena, G.T., 2021. Genetic diversity and population structure of bread wheat varieties grown in Bulgaria based on microsatellite and phenotypic analyses. Biotechnology & Biotechnological Equipment 35(1),1520–1533.

Wani, S.H., Sheikh, F.A., Najeeb, S., Sofi, M., Iqbal, A.M., Kordrostami, M., Parray, G.A., Jeberson, M.S., 2018. Genetic variability study in bread wheat (*Triticum aestivum* L.) under temperate Conditions. Current Agriculture Research 6, 268–277.