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Phylogenetic diversity and relationships among sorghum genotypes of breeding collection

Y. Honcharov[1], O. Yalanskyi[2], L. Prysiazhniuk[3], I. Dikhtiar[3], S. Melnyk[3], S. Hryniv[3], M. Tahantsova[3] and N. Holichenko[3]

[1]Research Institute of Agrarian Business, Tokova Str. 2A, UA52502 Vesele village Dnipro region, Ukraine
[2]Institute of Grain Crops of NAAS of Ukraine, Laboratory of Sorghum Crop Breeding, Volodymyra Vernadskoho Str. 15, UA49000 Dnipro, Ukraine
[3]Ukrainian Institute for Plant Variety Examination, Henerala Rodimtseva Str. 15, UA03041 Kyiv, Ukraine
*Correspondence: prysiazhniuk_l@ukr.net

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Abstract. Sorghum is gaining prominence as a biofuel crop, currently taking the position as the second-largest source of grain-based ethanol after maize. Estimation of genetic diversity to identify groups with similar genotypes is important for conserving, evaluating and utilizing genetic resources. This study aimed to evaluate the genetic diversity and population structure in different genotypes of sorghum collection for breeding purposes to improve cultivars. There were investigated thirty-one sorghum genotypes of different origin. The genetic diversity of sorghum genotypes was assessed by five SSR markers. To evaluate morpho-agronomical traits, days to flowering, plant high, 1,000 seeds weight and yield were studied. As the result of analysis, four cluster groups were formed based on Roger's & Tanimoto dissimilarity. These cluster groups included from three to sixteen sorghum genotypes, one genotype K2105 formed the separate cluster. The Shannon index calculated based on SSR markers was 1.99. Two principal components explained approximately 63% of the total variance. The greatest effect of year weather conditions was observed on the trait 'days to flowering'. The plant height was affected by the genotype of grain sorghum. The yield and 1,000 seed weight were affected by weather conditions, but the impact rate was significantly lower than the effect of days to flowering. The correlation between SSR markers and trait 'days to flowering' based on distances matrices was weak, but significant ($r = 0.1$). Thus, obtained results can be utilized for revealing genetic variation and identifying slightly different genotypes in a sorghum breeding program.

Key words: *Sorghum bicolor* L., SSR markers, genetical distances, morpho-agronomical traits, principal components.

INTRODUCTION

Grain sorghum (*Sorghum bicolor* L.) is considered as one of the most important cereals and a potential energy crop. It is a recognized biomass crop suitable for biofuel production because of two critical factors: high biomass production and efficient water

use. This crop is a short-day C4 plant, and its easy adaptability to hot and dry agroecology makes it a climate change-responsive crop (Sari, 2023). Estimation of genetic diversity to identify groups with similar genotypes is important for conserving, evaluating and utilising genetic resources, for studying the diversity of different germplasm as possible sources of genes that can improve the performance of cultivars, and for determining the uniqueness and distinctness of the phenotypic and genetic constitution of genotypes (Geleta et al., 2006). It is proved that flowering plays a vital role in the life cycle of seed-propagated plants as it crucially adapts the plant to its environment, aligning vegetative and reproductive growth phases with the local climate (El Mannai et al., 2011; Wang et al., 2020).

Measurement of phenotypic and genotypic variance in field trials is a common approach to examine the genetic differences among genotypes (Yoshida & Yoshida, 2004). Effective and modern method of new crop cultivars production is MAS-selection (Marker-Assisted Selection). MAS-selection with DNA-markers allows to conduct the selection for the improvement a trait directly through favourable genotype screening and to make comparative evaluation of the allelic status of DNA markers (Goncharov et al., 2016). SSR (Single Sequence Repeats) markers have characteristics affecting comprehensively of genome coverage, discrimination ability, reproducibility, speed, and cost of data generation and scoring that impact applications of the individual technologies by plant breeders (Smith et al., 2000).

Soil and climate conditions in the South of Ukraine are quite different compared to Western European countries, China or Mexico. Therefore, their experience cannot be applied to Ukraine. This stipulates for the study of sorghum growing in the specific warm and arid conditions of southern Ukraine (Bazaluk et al., 2021). The Steppe zone of Ukraine has the longest growing season, but receives the lowest precipitation (from 450 mm along the Forest-Steppe margin to 300 mm at the south part) and often suffers from drought (Prysiazhniuk et al., 2023). The phenotypic and genotypic variance among grain sorghum genotypes based on the field performance will supply valuable information to the plant breeder. Combining the molecular data analysis and phenotypic data performance will be effective for determining promising genotypes in future sorghum breeding programs. Thus, aimed to evaluate the genetic diversity and population structure in different genotypes of sorghum collection for breeding purposes to improve cultivars.

MATERIALS AND METHODS

Plant material and laboratory measurements

Thirty-one grain sorghum genotypes (28 cultivars and 3 lines) of different origin were used in this study (Table 1).

The laboratorial studies were performed in 2016 at SE Institute of Grain Crops of National Academy of Agrarian Sciences (Dnipro, Ukraine). DNA was isolated from seedlings by CTAB (cetriltrimethylammonium bromide) method according to plant DNA isolation protocol (Kalendar, 2019). Five SSR markers were used for the study of DNA polymorphism in grain sorghum genotypes: Sb4-32, Sb4-121, Sb6-57, Sb6-84 and Sb4-15. PCR was performed according to Smith et al. (2000) and Goncharov et al. (2016). The PCR products were separated on a horizontal agarose gel (3%) by the electrophoresis device Sub-cell GT (Bio-Rad, USA) at 5 V cm^{-1}. For the visualization of

DNA fragments, 5 μl L^{-1} of ethidium bromide was added to Tris-borate buffer. The results of separation were analyzed by the visualization system GelDocTM (Bio-Rad, USA) (Goncharov et al., 2016).

Table 1. Origins and type of material

No.	Name	Type	Maturity group	Origin
1	O-1112	Cultivar	Medium	Unknown
2	Dn-13f	Line	Medium	Synelnykove (Ukraine)
3	K-2105	Cultivar	Medium	South Africa
4	K-1677	Cultivar	Medium	Unknown
5	K-1043	Cultivar	Early	Unknown
6	Henicheske 11	Cultivar	Early	Henichesk (Ukraine)
7	Henicheske 209	Cultivar	Early	Henichesk (Ukraine)
8	Vinets	Cultivar	Early	Henichesk (Ukraine)
9	Perlyna	Cultivar	Early	Henichesk (Ukraine)
10	Kolor	Cultivar	Early	Henichesk (Ukraine)
11	Henicheske 200	Cultivar	Early	Henichesk (Ukraine)
12	K-3043	Cultivar	Early	Brazil
13	K-815	Cultivar	Early	Unknown
14	K-1260	Cultivar	Early	China
15	K-141 Feterita early	Cultivar	Early	USA
16	K-142 Feterita early	Cultivar	Early	USA
17	Hrand	Cultivar	Early	Synelnykove (Ukraine)
18	Dniprovskyi 39f	Cultivar	Early	Synelnykove (Ukraine)
19	Dn-37s	Line	Very early	Synelnykove (Ukraine)
20	Dn-37f	Line	Very early	Synelnykove (Ukraine)
21	K-2812 Feterita early	Cultivar	Very early	Unknown
22	K-2792 Feterita	Cultivar	Very early	Sudan
23	Feterita 204 short	Cultivar	Very early	Unknown
24	K-105	Cultivar	Very early	Unknown
25	K-2459/11	Cultivar	Very early	Turkmenistan
26	Dniprovskyi 39	Cultivar	Very early	Synelnykove (Ukraine)
27	I-488376 Z-150	Cultivar	Very early	USA
28	Dniprovskyi 30	Cultivar	Very early	Synelnykove (Ukraine)
29	Eritreia	Cultivar	Very early	Henichesk (Ukraine)
30	K-3732	Cultivar	Very early	Unknown
31	Henicheske 129	Cultivar	Very early	Henichesk (Ukraine)

Field measurements and environments

The field experiment was carried out during 2021–2023 with the same experiment design each year at pilot plots of Synelnykove breeding station of SE Institute of Grain Crops of National Academy of Agrarian Sciences (Dnipro region, Ukraine) (48°21'07" N, 35°27'11" E). To evaluate morpho-agronomical traits, days from emergence to flowering, plant height, 1,000 seed weight and yield were studied. The cultivars and lines were planted in three-row plots with a density 200 thousand plants per hectare with three replications. A spacing between the rows was 0.45 m. The plot size was 30 m^2. Data was recorded from continuous sampling of 10 plants of each row, and mean value of each trait was used for analysis. The weather conditions rates were

provided by Sinelnykove weather station which is located around 8 km from pilot plots (48°19'04" N, 35°30'43" E) (Table 2).

Table 2. The amounts of precipitation and air temperature during 2021–2023 grain sorghum growing season

Month	Amounts of precipitation, mm				Air temperature, °C			
	Normal daily average	2021	2022	2023	Nornal daily average	2021	2022	2023
April	28.3	54.4	56.6	100.7	12.7	8.0	9.9	10.3
May	46.0	27.9	19.2	29.1	16.0	15.8	15.4	16.4
June	59.0	202.5	20.4	29.1	19.6	19.5	22.2	20.3
July	56.0	70.8	35.1	42.1	21.3	23.6	22.4	23.0
August	37.0	51.1	46	29.9	20.6	22.8	24.6	24.3
September	36.0	23.9	34.5	13.8	15.4	13.8	15.2	19.3

Statistical analysis

The coefficient of agrometeorological indicators from daily average amounts during 2021–2023 was computed using the equation below:

$$Dc = \frac{X_i - \overline{X}}{\sigma} \quad (1)$$

where Dc – deviation coefficient; X_i – indicator of current weather; X – daily average amounts; σ – mean-square deviation. The rate of deviation coefficients was determined according to scale: Dc = 0-1 – close to normal conditions; Dc = 1-2 – strong different conditions; $Dc > 2$ – close to unique conditions (Yeremenko et al., 2017).

SSR data were scored with regard to the presence or absence of bands for each genotype. PIC (Polymorphism Information Content) was calculated via the equation:

$$PIC_i = 1 - \sum_{i=1}^{n} P_i^2 \quad (2)$$

where n – the total number of different alleles at a locus, and P_i – the frequency of the i^{th} allele (Lee & Park, 2017).

Genetic dissimilarities (GD) based on SSR markers between pairs of grain sorghum genotypes were measured by the Roger's & Tanimoto coefficient. Cluster analysis was carried out using UPGMA (the Unweighted Pair Group Method) by XLSTAT software of MS Excel (trial version). The population structure of 31 grain sorghum genotypes according to their origins was established using Shannon's information index by GenAlEx 6.503 (Suvi et al., 2020).

The significant differences and the factors impact on morpho-agronomical traits were determined by ANOVA using STATISTICA 12.0 software (trial version). Principal component analysis (PCA) was applied to extract information from multivariate dataset of morpho-agronomical traits by XLSTAT software of MS Excel (trial version). GD on the basis of principal components were measured by Ward's method with Euclidean distances. Mantel test was used to examine correlations between distance matrices of principal components on the basis of morpho-agronomical traits and SSR markers (Prysiazhniuk et al., 2022).

RESULTS AND DISCUSSION

As result of SSR analysis of 31 grain sorghum genotypes by 5 markers, 46 alleles were observed. The numbers of alleles per locus ranged from 7 (Sb4-32) to 13 (Sb4-15), and the average number of alleles per locus was 9.2. Table 3 shows the number of alleles, the range of alleles size and frequency, and PIC for each SSR marker.

Table 3. Characteristics of five SSR loci analyzed

SSR	Number of alleles	Alleles size, bp	Alleles frequncy	PIC
Sb4-32	7	156–190	0.03–0.23	0.83
Sb4-121	9	197–249	0.03–0.29	0.83
Sb6-57	8	250–294	0.03–0.26	0.82
Sb6-84	9	157–205	0.03–0.19	0.81
Sb4-15	13	570–741	0.03–0.19	0.90

PICs of each locus ranged from 0.81 (Sb6-84) to 0.90 (Sb4-15) with a mean value of 0.84. Thus, the obtained high PIC values, indicate that the identified alleles are evenly represented among grain sorghum genotypes. Profiles of five SSR markers were collectively able to discriminate all studied genotypes.

A UPGMA dendrogram was constructed for the 31 grain sorghum genotypes based on the 5 SSRs, in which the Roger's & Tanimoto dissimilarity coefficients ranged from 0.08 (Henicheske 209 and Henicheske 11, K-2812 Feterita early and Feterita 204 short) to 0.36 (observed between the majority of studied genotypes) (Fig. 1).

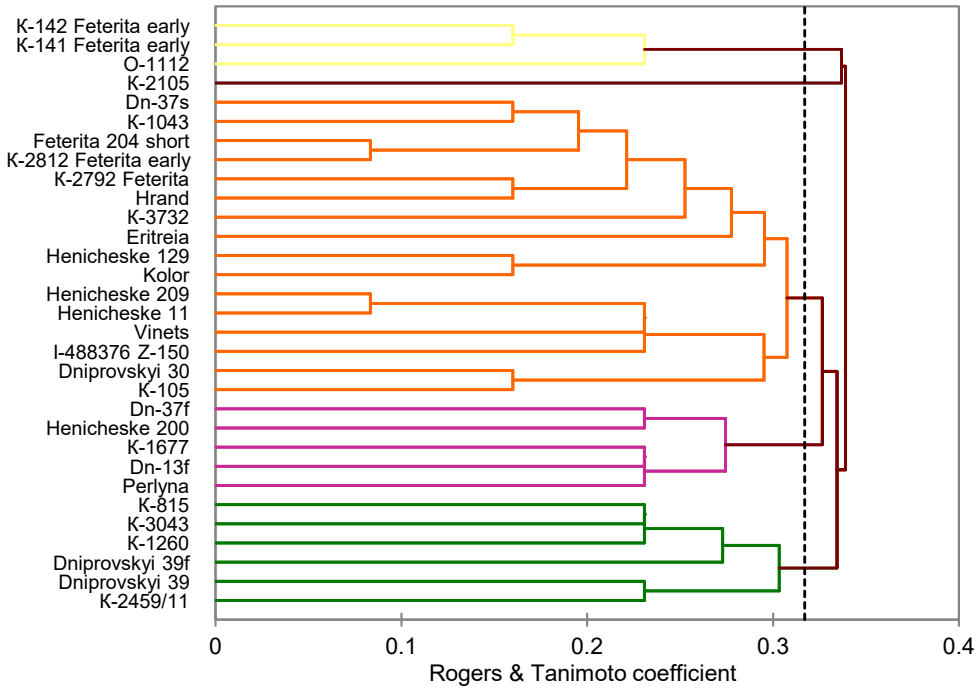

Figure 1. A dendrogram showing the genetic dissimilarity based on the five SSR markers among 31 grain sorghum genotypes.

The 31 grain sorghum genotypes were divided into 4 groups, K-2105 cultivar formed separate cluster. The largest group included 16 genotypes of grain sorghum analyzed in this study. Other groups formed with five and six genotypes. The smallest group included three genotypes with dissimilarity coefficients ranged from 0.16 to 0.23. The obtained results shown that grain sorghum genotypes distributed into clusters according to both their origin and maturity groups.

In order to study variation within and among the populations of grain sorghum genotypes Shannon's information index was calculated on the basis identified alleles by five SSR markers. For nine populations according to grain sorghum origin, Shannon's information index within the population was 1.13, among the population was 0.86. In the case of dividing the grain sorghum genotypes according their maturity group into 4 populations, the variation within the population was 1.58, among the population was 0.41. The total value of Shannon's information index for both cases of was 1.99. Thus, genetic diversity analysis of 31 grain sorghum genotypes indicated the highest genetic diversity within the populations. However, the genetic diversity of grain sorghum genotypes among genotypes based on their origin was higher than based on maturity group.

The results of SSR analysis obtained in this study were comparable to the previously published reports in sorghum. Thus, the results provided by Smith et al. (2000) showed that profiles from the 15 SSR loci were collectively able to discriminate among all fifty but a single pair of grain sorghum inbred lines. The PIC values for SSR loci ranged from 0.78 to 0.35 with a mean value of 0.58. There was demonstrated the associations among inbred lines mostly on the basis of origin. Geleta et al. (2006) reported that the 10 microsatellite loci used produced 43 polymorphic bands across 45 sorghum accessions. The PIC values for SSR loci ranged from 0.52 for Sb4-32 and Sb6-342 loci to 0.79 for Sb4-22, while one primer pair (Sb4-15) was found to be monomorphic. The Shannon diversity index based on data set obtained by SSR markers was 0.539. The cluster analysis shown that there was more grouping based on the site of collection. Adugna (2014) studied the eight cultivated sorghum landrace populations in the center of origin (Ethiopia). Seven phenotypic indicators and 12 highly polymorphic sorghum SSR markers were utilized. It was shown that each of the 12 SSR loci demonstrated high levels of polymorphism. The PIC values varying from 0.38 to 0.85. The 160 individuals were grouped into three major clusters according to their maturity group and origin. AMOVA analysis revealed that 54.44% of the variation occurred within populations, 32.76% among populations within regions, and 12.8% among the regions of origin.

Although, the cluster analysis results of this study are very similar to the published reports, the differences in allele sizes and PIC were observed. The high levels of allelic diversity of SSR markers observed in this study probably were associated with the extensive range of genetic diversity represented in the panel of sorghum genotypes.

Significant variation was observed among the sorghum genotypes concerning the measured morpho-agronomical characteristics. The average number of days to flowering varied from 53.50 to 75.60 days. Plant height was as small as 74.60 cm and as large as 173.00 cm. The 1,000 seed weight was in the range of 12.50 to 37.90 g. Grain yield ranged from 0.84 to 8.33 t ha^{-1} (Table 4).

Table 4. Simple descriptive statistics and principal component factor loadings of the morpho-agronomical traits

Descriptive statistics indicators	Days to flowering	Plant height, cm	1,000 seed weight, g	Yield, t ha^{-1}
Mean	62.93	108.79	23.97	4.40
Minimum	53.50	74.60	12.50	0.84
Maximum	75.60	173.00	37.90	8.33
Std.Dev.	4.98	22.95	6.03	1.72
Coeficient of variation, %	7.91	21.10	25.16	39.05
Standard error of mean	0.52	2.38	0.63	0.18
PCA1	0.173	0.604	0.730	0.721
PCA2	0.924	-0.099	-0.381	0.247

In PCA, two principal components explained approximately 63% of the total variance among the 31 grain sorghum genotypes, using an eigenvalue greater than one as a measure for the significance of a principal component. PC1 describes 36.18% of variabilities in studied morpho-agronomical traits. The 1,000 seed weight and grain yield contributed the largest factor loadings (0.73 and 0.72, respectively) for PCA1. Thus, PC1 can be interpreted as the productivity of studied sorghum genotypes. The PCA2 describes 26.75% of variability in the morpho-agronomical traits. For PCA2 the trait 'days to flowering' and 1,000 seed weight contributed the largest share of the variation (0.92 and 0.31). The PCA2 determines the length of growing season. Fig. 2 shows the distribution of 31 grain sorghum genotypes which were clustered into four main groups on the PC1 and PC2 plot.

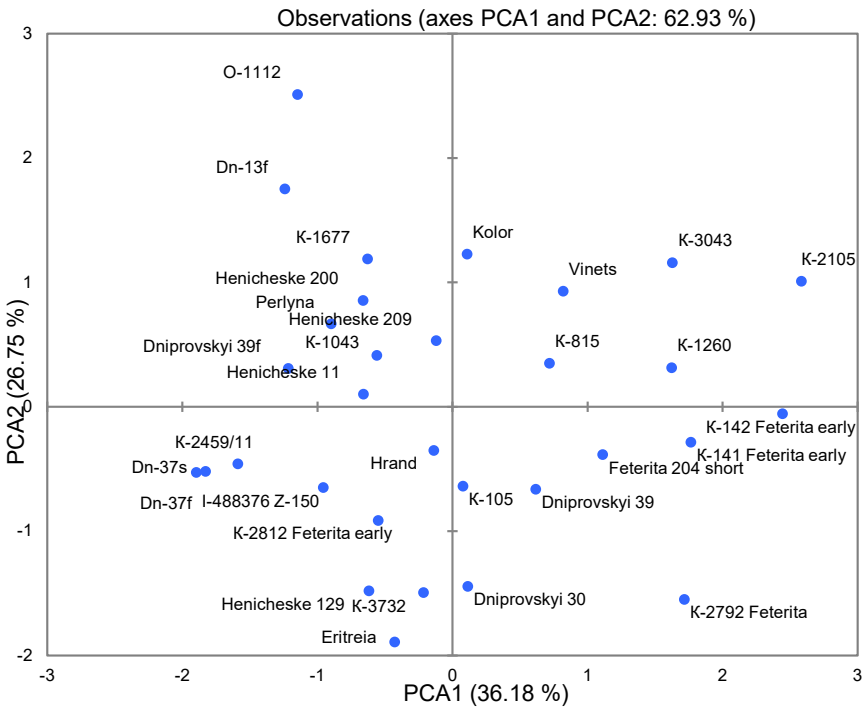

Figure 2. Score plot of the two first principal components (PCA1 and PCA2) showing the position of 31 grain sorghum genotypes.

Majority of grain sorghum genotypes were pooled in groups. genotypes were clearly separated from the groups. The genotypes O-1112, Dn-13f and K-1677, which were characterized by the greatest number of days from emergence to flowering and belong to the medium maturity group were clearly separated from the groups of other sorghum genotypes. The similar distribution was observed for Eritreia, K-3732 and Henicheske 129 genotypes of the very early maturity group, which had the shortest period of time from emergence to flowering. There was not significant correlation in all pairs of morpho-agronomical traits based on the average values for three years. This can be explained by the fact that the weather conditions in 2021-2023 varied significantly. Thus, calculated deviation coefficients (*Dc*) show that the air temperature in July of 2021 was extremely high (*Dc* = 2.3) while the amounts of precipitation was characterized as close to normal. In 2022–2023 the highest values of the air temperature were observed during August (*Dc* was 2.2 and 2.0 in 2022 and 2023, respectively). The amounts of precipitation were closed to normal during these years (Table 5).

Table 5. Deviation coefficients for the air temperature and amounts of precipitation during grain sorghum growing season in Dnipro region of Ukraine

Month	Air temperature			Amounts of precipitation		
	2021	2022	2023	2021	2022	2023
April	-2.4	-1.4	-1.2	0.9	0.9	2.4
May	-0.5	-1.4	1.0	-1.6	-2.4	-1.5
June	-0.1	2.1	0.6	1.7	-0.5	-0.4
July	2.3	1.1	1.7	0.9	-1.3	-0.9
August	1.2	2.2	2.0	1.5	1.0	-0.8
September	-0.7	-0.1	1.7	-1.2	-0.1	-2.1

The lack of precipitation was observed in May of 2022 (*Dc* = -2.4). The tendency of the precipitation shortage was in 2021 and 2023 as well. However, according to *Dc* for the air temperature, only 2023 is characterized with drought in May. It was reported that the production of sorghum is afected by drought stress during both pre-fowering (panicle development) and postflowering stage (between flowering and grain development) (Abreha et al., 2022). As calculated *Dc* demonstrate, the drought weather conditions were observed from June to August in 2022. As Wang et al. (2020) shown the relationship of trait 'days to flowering' with yield of grain sorghum hybrids was frequently positive but varied across the genetic background. In this study, the greatest effect of year weather conditions was observed on the trait 'days to flowering' (Table 6).

Table 6. Mean squares from the analysis of variance of morpho-agronomical traits of grain sorghum genotypes evaluated during 2021–2023[1]

Effect	DF[2]	Days to flowering	Plant height	1,000 seed weight	Yield
Intercept	1	3,683,362	11,006,426	534,480.7	18,032.97
Genotype (G)	30	677	16121	1,072.2	64.55
Year weather conditions (Y)	2	1,127	19	40.5	18.69
G*Y	60	4	16	20.4	12.45
Error	837	2	2	1.6	0.37

[1] Significant at 0.05 probability levels; [2] DF – Degree of freedom.

The plant height was not affected by the year weather condition, only the genotype of grain sorghum impacted on this trait. In contrast, the yield and 1,000 seed weight were affected by weather conditions, but the impact rate was significantly lower than the effect of days to flowering. The results of Yoshida & Yoshida (2004) studies shown that the environmental factors affected the days to flowering more strongly than genetic factors. As Wang et al. (2020) reported, while flowering and tillering are traits with high repeatability, they may interact in a complex way with the growing environment to affect the timing and intensity of water stress during the crop life cycle.

Thus, taking into account the weather condition impact on studied morpho-agronomical traits, Pearson correlation coefficients between morpho-agronomical traits were calculated for each experimental year. There was determined that correlation was significant ($p = 0.05$) between 1,000 seed weight and yield of grain sorghum genotypes in 2023 ($r = 0.36$). The weak correlation between 1,000 seed weight and yield can be explained by the presence of few lines among the studied sorghum genotypes, which cannot be estimated by the grain yield.

Considering that the trait 'days to flowering' contributed the largest share of the variation for PCA2, the UPGMA dendrogram was constructed for the 31 grain sorghum genotypes based on this trait (Fig. 3).

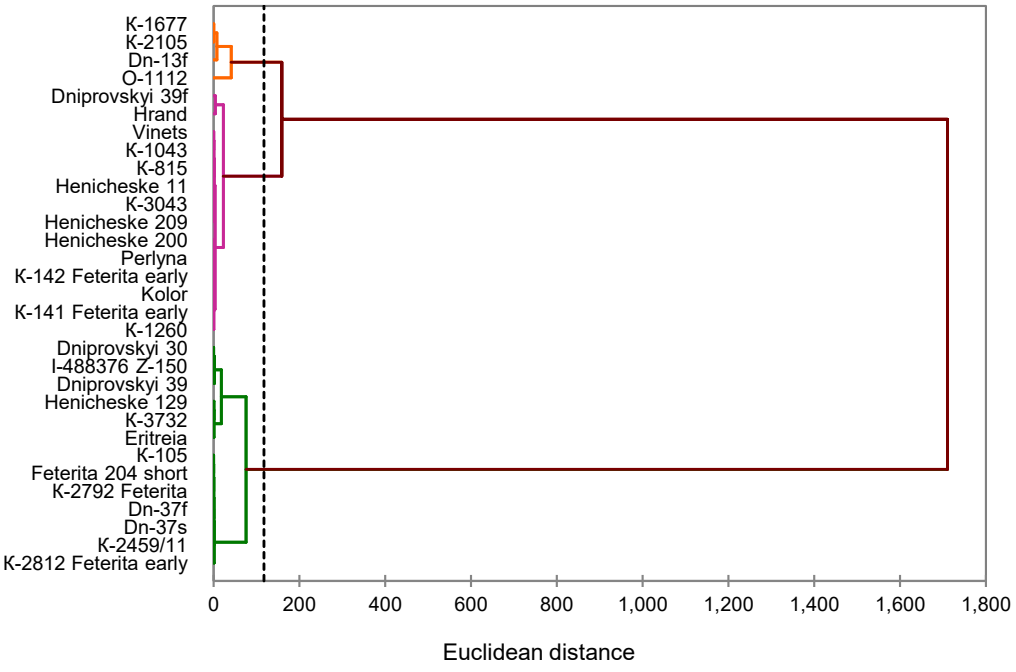

Figure 3. A dendrogram showing the genetic dissimilarity based on trait 'days to flowering'.

According to the obtained results, there were 3 cluster groups. The largest group included 14 of grain sorghum genotypes. Other groups formed with 4 and 13 genotypes. The present distribution shown that grain sorghum genotypes formed the cluster groups according to the maturity groups. As El Mannai et al. (2011) reported that under controlled day-length conditions, a total of seven flowering time loci were identified. Recently,

some agronomically important genes such as those affecting biomass and flowering time have been isolated using genetic mapping and comparative genome studies between sorghum. It is known that DNA markers are used to facilitate sorghum breeding for the agronomic traits (Kawahigashi et al., 2022). As result of Mantel test for the correlation between Roger's & Tanimoto GD and the Euclidean distances based on trait 'days to flowering' was weak, but significant ($r = 0.1$, $p = 0.05$). There was not significant correlation between GD matrices based on SSR markers and other morpho-agronomical traits. The similar results were obtained by Yoshida & Yoshida (2004). They investigated the correlation between the result of Spearman rank dissimilarity analysis with SSR markers and that of a combination for four, five and six phenotypic data. All combinations of four, five and six phenotypic data gave results highly correlated with SSR marker data. However, in contrast to this study, none of the dissimilarity distance matrices based on single phenotypic data showed a significant correlation with the dissimilarity distance matrix of SSR markers. Possible reasons for the non-association in grain sorghum genotypes were the limited number of SSR markers and different types of material (lines and hybrids) and high phenotypic diversity observed in this study that PCA shown.

CONCLUSIONS

In this study, five SSR markers successfully distinguished all the 31 grain sorghum genotypes. In clustering analysis, the 31 sorghum genotypes were classified into four groups based on SSR markers, K-2105 cultivar formed separate cluster. In PCA, the first PC represented the productivity of studied sorghum genotypes, and the second PC determines the length of growing season. By assessment of the rate of factors impact it was found that the trait 'days to flowering' is affected the most by the year weather conditions. Breeders are greatly concerned about these effects since comprehending the genetics of flowering is crucial for adjusting the life cycle of sorghum to its various growing environments. The plant height is determined only by the genotype of grain sorghum. In Mantel test, distance matrices of UPGMA clusters based on trait 'days to flowering' and SSR markers showed statistically significant, but weak correlation. It, might be related with the limited number of this trait specific markers. This information will be valuable for sorghum breeding programs.

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