The Genetic Structure and Marker- Morphological Trait Associations in Forage Kochia Population Grown in Central Anatolia

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Abstract

Forage kochia, a naturally growing and semi-shrub in Türkiye's flora, tolerates adverse soil and climatic conditions. In the research, the morphological and yield values of the forage kochia populations collected from 5 different locations in Konya were examined during 2018–2019. According to morphological and yield values, we determined 80 plants, 76 plants with excellent yield potential, and four outgroup plants for molecular studies. A total of 250 polymorphic fragments were obtained from these 80 plants. In our study, the average PIC value was 0.322, and the mean MI value was 8.99. Genetic diversity parameters of the populations were obtained using the GenAlEx program, and it was found that the mean He was 0.209, and the percentage of polymorphic loci was 81.20%. According to the results of AMOVA, among-population variation was 9%, while within-population variation was 91%. The dendrogram obtained as a result of the study determined that the genetic distance between plants varied between 0.63 and 0.90. According to the similarity index used in the study, it was stated that there was a high degree of similarity (90%) between 3212 and 5419 coded plants. Furthermore, it was noted that the markers related with plant height were associated with canopy diameter, number of main branches, and leaf color. The results show us that these populations are a treasured gene resource for plant breeding.

Introduction

Forage kochia (*Kochia prostrata* (L) Scrad Syn. *Bassia prostrata* (L.) A.J. Scott), is a semi-shrub belonging to the *Chenopodiaceae* family. It grows naturally in Türkiye's flora and shows the distribution in Europa and Asia (Acar 2013; Anonymous 2019). Due to forage kochia being a xerophyte plant, it can be easily grown at temperatures such as -32°C to + 40°C and in regions with low annual precipitation after planting (Çetik 1985; Harrison et al. 2000; Acar and Dursun 2010). Because of its drought and salinity tolerance, pasture improvement has been carried out using forage kochia on dry pastures in the western part of the USA, on ranges with 70–110 mm rainfall in Jordan, and on salt-affected fields in Russia (Blauer et al. 1993; Harrison et al. 2000; Shamsutdinov and Shamsutdinov 2009; Bailey et al. 2010). In addition, financial products can be obtained, and the feed inputs used in livestock can be reduced using forage kochia to improve arid and salty pastures in Türkiye.

There are different ecotypes of forage kochia and variations in plant height, stem color and diameter, leaf size, maturation, growth period, and soil adaptation characteristics of these ecotypes (Kitchen and Monsen 2008). In addition, Koç Koyun and Acar (2021) stated that the forage kochia populations grown in Konya, Türkiye, have high variation in terms of morphological properties. Although there are different ecotypes in our country since forage kochia is the natural plant of our country, there is no steppe grass variety in Türkiye. However, the "Immigrant" variety (*Kochia prostrata* subsp. *virescens*) was developed in the USA in 1984, and the "Snowstorm" variety (*K. prostrata* subsp. *grisea*) was created in 2012 (Tilley et al. 2012). In addition to these varieties, other forage kochia lines used in pasture improvement are BC-118, Pustinny-Select, KZ-6X, Otavny-Select, Sahro-Select (Bailey et al. 2009; Waldron et al. 2013).

Today, breeding programs supported by molecular markers are seen as necessary to shorten the breeding period in classical plant breeding. Molecular markers provide information about the genetic structure of the plant and are used in phylogenetic studies, heterosis studies, genetic diversity studies, and breeding backcrosses (Semagn et al. 2006, Nadeem et al. 2018). In addition, many different molecular markers such as RFLP, AFLP, ISSR, SSR, ESTs, DArt, and SNPs are used to determine the degree of similarity in plants (Khan et al. 2014). ISSR applications, one of these markers widely used for various research purposes, do not require pre-sequence information and, at the same time, constitute an effective marker system that is easy and inexpensive to apply. However, studies worldwide to determine the genetic relationships of *Kochia* sp. species are minimal.

The Immigrant variety, which was developed from the forage kochia brought to the Americas by introduction, had limited potential for use as a pasture plant there. For this reason, 200 pasture-type Kochia ecotypes were collected from Uzbekistan and 192 from Kazakhstan, which have the gene center of forage kochia, in 1999 (Waldron et al. 2001; 2002). Molecular and cytological analyses were carried out on the appropriate material for the first time, with the selections made from these collected materials. Working on the same material, Lee et al. (2005) used the RAPD and RAPD-derived STS methods. In this study, the genetic relationships of 9 *K. americana* (USA origin), 20 *K. prostrata* ('Immigrant' cultivar, 1 Uzbekistan genotype, 18 Kazakhstan genotype), and 7 *K. scoparia* (USA origin) plants were determined. In addition, limited chloroplast and rDNA and GISH analysis were also included in the same study. According to the results obtained from the survey, interspecies hybridization was not found. Apart from this study, no

molecular characterization and breeding studies have been found in our country or elsewhere in the world. In light of this information, this research is a first in our country and the world by studying the material of Anatolian origin.

Molecular markers alone are insufficient in agricultural product development, and morphological markers are also needed to determine the plant's yield potential (Chahal and Gosal 2002). Therefore, in our study, 14 to 16 plants from each population, a total of 76 forage kochia, were determined, which were superior in terms of morphology and yield characteristics of five forage kochia populations grown in Konya conditions (Koç Koyun and Acar 2021). Furthermore, in this study, PCR-based ISSR molecular markers determined the genetic relationships of these plants and the relationships between some morphological traits and ISSR markers for the first time.

Material and Methods

Morphological studies

In 2016, the seeds of populations collected from 5 locations in Konya, the predominant province in Central Anatolia, given in Table 1, were grown in a greenhouse. Then, seedlings with varying phenotypes were selected within the population, and seedling heights did not exceed 15 cm. These seedlings were planted in PanAgro Aslım Farm Kasinhani (37° 43' 11" N, 32° 37' 52" E), Konya, in 2017 for use as a material in the trail. The parcel length was 10 m in the study, and each parcel had two rows. In planting, row spacing was 1.40 m, and intrarow was 1.00 m. The sprinkler irrigation was done once after sowing. There was no fertilization during and after planting. The weed control in the area was provided by hoe in total four times, including twice in 2018 and 2019.

Table 1
The populations codes, places, and status of culture

Pop Kodu	District Collected Locations	Altitude (m)	Status of Culture
1P	Karapinar: Karapinar Kartal Kayalari	1000	Grown naturally for long years in there
2P	Karatay: Bahri Dagdas International Agricultural Research Institute	1006	Planted in 2014 from collected KOP region
3P	Selcuklu: Campus Beltway	1126	Grown naturally for long years in there
4P	Selcuklu: Ardicli Rural	1160	Grown naturally for long years in there
5P	Selcuklu: S.U.F.A. Forage Kochia Demonstration Garden	1130	Planted in 2013 from collected Konya region

According to soil analysis results, the soil of the experimental field is slightly alkaline (pH 7.8) and has high organic matter (5.41%). The field has a problematic soil, with a very high lime content, extreme salinity (1003,75 µS cm^{-1} EC), and boron toxicity (57,356 mg kg^{-1}). The other soil parameters are given in Table 2. The soil texture is clay loam, and potassium, calcium, and magnesium content are at excess levels.

Table 2
Soil properties of the experimental field

Soil Parameter	Value	Category	Soil Parameter	Value	Category
Organic Matter (%)	5.410	High	Cu (mg kg^{-1})	0.343	Sufficient
K (%)	0.058	Excess	Fe (mg kg^{-1})	9.67	Sufficient
Ca (%)	0.371	Excess	Zn (mg kg^{-1})	0.333	Insufficient
Mg (%)	0.069	Excess	Mn (mg kg^{-1})	2.360	Medium

[1]The soil analysis was made by the Research Laboratory of the Department of Soil Science and Plant Nutrient, Faculty of Agriculture, Selcuk University

Data Collection

We investigated the morphological properties between June and November in this research from between 2018 and 2019. Regrowth in spring of the plants was determined using a 1–9 scale [1: Very early (late March-early April); 3: Early (after 15 April); 5: Intermediate (early May); 7: Late (after 15 May); 9: Very late (June) or dead]. (Özköse 2012). We defined the blooming period as showing at least one flower in the plant within the forage kochia populations. We determined this time in plants by Scoring 1–11 (1: end of June- early July, 3: end of July- early August, 5: end of August-early September, 7: end of September- early October, 9: end of October- early November, 11: end of November) (Koç Koyun and Acar 2021). We also observed lower and upper branch color (1: Yellow, 3: Orange, 5: Light Brown, 7: Red, 9: Pink, 11: Purple), leaf color (1: Red-Grey, 3: Greyish Green, 5: Bluish green, 7: Green) and hairiness (1: Very rare or absent, 3: Infrequent, 5: Moderate, 7: Frequent, 9: Very Frequent) by Scoring. Moreover, we specified the plant height (cm), canopy diameter (cm), number of branches, and stem diameter (mm). We measured the plant height from the soil surface (Van Riper and Owen 1964; Tamkoç 1992) and canopy diameter for the maximum diameter (Acar et al. 2019). According to Aygun and Olgun (2018), we counted the number of branches. We surveyed stem diameter 5 cm from the soil surface via digital calipers.

Statistical Analysis

In the study, two-year averages of the plants in all populations were taken, and the plants with the highest values were selected from each population. Then, to examine their morphological proximity selected plants were subjected to descriptive statistics and cluster analysis in the JMP7 software package program (Koç Koyun and Acar 2021).

Molecular research

In this study, at the end of the second year (2019), we selected for molecular studies 14 to 16 plants that have completed their development and are highly productive, morphologically different from each population. A total of 76 genotypes from 400 plants were used. In the study, four-winged saltbush [*Atriplex canescens* (Pursh) Nutt.] and sugar beet (*Beta vulgaris* L.) were used as outgroup plants, two plants from each of their species, a total of 4 plants, since they are in the same family.

Molecular studies in the research were carried out in the Molecular Genetics and Biotechnology Laboratory of the Faculty of Agriculture of Selcuk University. First, DNA isolation was performed according to the method of Doyle and Doyle (1990) and with the 2X CTAB method. Then, the concentration of isolated DNA samples was measured at different wavelengths using the Thermo Scientific µDropTM Plate N12391 Spectrophotometer. It was also visualized on the gel to see if the DNA was recovered definitively and to determine that it was not fragmented. Finally, the necessary 1.0% agarose gel was prepared, and DNA samples were loaded into the wells and carried out in electrophoresis (Thermo EC250-90) at 70 volts for 60 minutes.

Though preliminary tests were conducted using 50 primers, the final analysis used nine polymorphic ISSR primers (Khan et al. 2015; Pandey et al. 2019). Amplification reactions were performed in Techne-512 thermocycler, and the total reaction volume was 12 µl. The reaction mixture contained 1.25 µl of 10X *Taq* PCR buffer containing $(NH_4)_2 \cdot SO_4$ without $MgCl_2$, 1.25 µl of 25 mM $MgCl_2$,

0.2 µl of 25 mM dNTPs, 0.15 µl of 5 U/µl Taq DNA polymerase, 0.25 µl of 10 µM ISSR primer and 1 µl of 50 ng/µl DNA. The sequences of ISSR primers and their G + C ratios, and the PCR reaction cycles useded in this study are given in Table 2.

Table 2
ISSR primers sequences their G + C ratios, and PCR reaction cycles

ISSR primers	Primer sequences	G + C ratio (%)	Initial denaturation	First Step[1]	Second Step[2]	Final Extensions
ISSR M2	5'-ACCACCACCACCACCACCG – 3'	68.4	95 °C	60.8	58.8	72 °C
ISSR M3	5'-AGCAGCAGCAGCAGCAGCC – 3'	68.4	-3 min	63.1	61.1	-10 min
ISSR M6	5'-GTCACCACCACCACCACCACCAC-3'	65.2		68.0	66.0	
ISSR M8	5'-ACACACACACACACACACG-3'	52.6		56.7	54.7	
ISSR M12	5'-GACACGACACGACACGACAC-3'	61.4		61.0	59.0	
ISSR M13	5'-CACACACACACARG – 3'	53.6		44.8	42.8	
ISSR M16	5'-CAC ACA CAC ACA CAC AGC – 3'	55.6		56.0	54.0	
ISSR M17	5'-CAGCACACACACACACACA-3'	52.6		56.7	54.7	
ISSR M18	5'-CGTCACACACACACACACA – 3'	52.6		57.0	55.0	
[1] Denaturation/annealing/primer extension 15 cycles 95°C—1 min/Tm— 1 min/72°C—2 min						
[2] Denaturation/annealing/primer extension 25 cycles 95°C—1 min/Tm— 1 min/72°C—2 min						

After the amplification, PCR products were split by electrophoresis (Thermo EC250-90) in 1.5% agarose gel with 1X TBE buffer at 70 V for 2 h and 100 V for 1 h. The gel was stained using ethidium bromide and snapped under Trans illuminator UV light provided by Vilber Lourmat Gel Documentation System. A 100 bp plus Thermo Scientific DNA ladder was used as a standard marker for quantifying different ISSR-based gel products (Gwanama et al. 2000; Brown and Myers 2002; Decker-Walters et al. 2002; Decker-Walters et al. 2004; Kwon et al. 2004).

Statistical Analyses

Statistical analyses and evaluations made within the scope of molecular studies were examined under three headings: genetic diversity analysis, genetic structure analysis, and determining the relationships between some morphological traits and ISSR markers.

Genetic diversity analysis

The polymorphism ratio of the primers and populations was obtained from the ratio of the number of polymorphic bands obtained to the total number of scorable bands. PIC (Polymorphism Information Content), RP (Resolving Power), EMR (Effective Mutliplex Ratio), and MI (Marker Index) values giving information about the effectiveness of ISSR primers were calculated using the formulas below (Chesnokov and Artemyeva 2015).

$PIC_{(\text{dominant marker})} = 2f(1-f)$

f: Allele frequency

$$RP = \sum Ib$$

$Ib = 1 - 2(0.5 - p)$

p: Allele frequency ratio

$$\mathbf{EMR}=np\left(\frac{np}{n}\right)$$

np: Number of polymorphic bands

n: Total number of bands

$$\mathbf{MI}=PIC\times EMR$$

Na (No. of Different Alleles), Ne (No. of Effective Alleles), I (Shannon's Information Index), He (Expected Heterozygosity), uHe (Unbiased Expected Heterozygosity), and Polymorphic locus percentage were calculated from the genetic diversity parameters of the populations made using the GenAlEx program (Peakall and Smouse 2006).

Genetic structure analysis

AMOVA (molecular variance analysis) and Nei genetic distance were determined using the GenAlEx program (Peakall and Smouse 2006).

The repetitive bands obtained from dominant-marker-based ISSR applications were recorded as 1 and 0 in each gel, where '1' indicates the band's presence, '0' suggests the band's absence, and '9' shows the missing value. The Cluster analysis of the obtained data was made in the NTSYSpc-2.10e (Numerical Taxonomy and Multivariate Analysis System) package program, and Principal Coordinate Analysis (PCoA) was performed in the Minitab 16 package program (Labate 2000).

A matrix was created so that '1' indicates the presence of the band, '0' is the band's absence, and '-9' is the missing value by using STRUCTURE 2.3.4. package program. The created files' ΔK table (K = 1; K = 10) was obtained with the STRUCTURE HARVESTER application over the internet (Earl and vonHoldt 2012).

Determining the relationships between some morphological traits and ISSR markers

In determining the relationship between some plant characteristics and ISSR markers, 250 markers obtained from 9 primers were selected as independent variables. Morphological traits (spring regrowth, flowering time, plant height, canopy diameter, number of main branches, stem diameter, lower and upper branch color, leaf color, and hairiness) were chosen as dependent variables. Multiple regression analysis (Stepwise MRA) was performed in the SPSS 15 package program to determine the relationships between traits. In addition, the correlation between the dependent variable morphological characteristics was determined using the JMP 7 package program (Virk et al. 1996).

Results

Morphological studies

The morphological values, standard deviation (SD), coefficient of variation (CV), and mean values of 76 genotypes with superior yield potential selected using the average of the data obtained in 2018 and 2019 are given in Table 3. In addition, the dendrogram obtained using morphological data with these selected genotypes is shown in Fig. 1.

Table 3
The morphological properties of selected superior genotypes among forage kochia population, and their mean, SD and CV

Pops. Code	Geno. code	Regrow. in Spring (Scor.[1])	Bloom. Period (Scor.[2])	Plant Height (cm)	Canopy Diame. (cm)	Number of Main Branches	Stem Diamet. (mm)	Lower Branch Color (Scor.[3])	Upper Branch Color (Scor.[3])	Leaf Color (Scor.[4])	Leaf hairiness (Scor.[5])
1P	113	4.00	6.00	16.00	25.00	2.00	1.10	6.50	8.00	500	7.00
	1113	4.00	5.00	10.50	29.00	2.00	1.16	0.67	1.00	2.50	3.50
	1115	3.00	5.00	11.50	36.00	4.00	1.13	3.50	1.00	3.00	3.25
	1116	3.00	5.00	61.60	63.25	7.50	2.41	3.75	4.00	7.00	6.50
	125	5.00	5.00	17.50	27.00	5.00	0.70	5.00	9.00	6.50	7.25
	1215	4.00	6.00	25.50	20.00	5.00	3.54	2.50	1.00	4.50	6.00
	1219	4.00	1.00	36.50	93.00	16.00	1.40	4.00	0.50	4.50	3.50
	1220	3.00	5.00	42.00	50.50	7.50	3.05	7.75	2.00	4.50	6.50
	134	4.00	8.00	36.00	59.00	12.00	1.33	9.00	9.00	6.50	6.50
	1313	1.00	4.00	68.00	106.00	17.50	2.66	7.00	1.00	6.00	7.00
	1315	1.00	4.00	67.60	78.50	29.00	2.46	3.00	2.00	7.00	7.50
	1316	1.00	4.00	74.55	101.50	19.50	2.03	5.00	1.00	8.00	7.00
	1410	3.00	3.00	22.50	50.00	700	9.50	7.25	4.00	5.00	6.25
	1414	5.00	7.00	9.75	10.00	5.00	3.00	6.50	5.00	6.50	7.00
2P	215	1.00	4.00	47.00	43.00	10.50	3.50	3.75	1.00	5.00	7.50
	216	1.00	4.00	60.50	95.50	13.00	2.21	4.75	1.00	5.50	7.00
	2118	4.00	5.00	15.00	18.00	7.00	2.00	3.75	4.00	6.00	7.00
	2120	8.00	7.00	12.00	18.00	7.00	3.00	3.75	0.50	1.50	3.50
	226	3.00	3.00	22.00	36.00	6.00	2.75	6.50	4.00	5.00	7.00
	2216	2.00	6.50	38.75	42.50	9.50	6.18	6.50	4.00	8.00	6.50
	2217	2.00	5.00	57.50	90.00	23.00	1.65	6.50	1.00	7.00	7.00
	2218	3.00	4.00	52.50	62.00	7.00	3.23	7.50	1.00	7.00	7.50
	2311	3.50	6.00	42.00	40.00	6.00	287	2.25	1.00	5.00	6.00
	2312	4.00	6.00	31.00	30.75	4.50	1.94	1.00	1.00	4.00	7.00
	2313	1.00	5.00	62.50	71.50	8.00	5.55	3.00	4.00	6.00	7.00
	2314	4.00	7.00	10.75	20.00	5.00	7.00	5.50	4.00	6.50	7.00
	2413	5.00	7.00	17.00	19.00	2.00	3.00	2.50	4.00	5.00	7.00
	2414	1.00	9.00	14.50	5.00	2.00	1.00	4.00	4.00	2.00	7.50
	2418	4.00	3.00	28.00	42.00	2.00	3.00	4.75	1.00	4.50	6.50
	2420	3.00	7.00	26.50	18.00	2.50	2.59	5.50	1.00	5.00	6.50
3P	317	4.50	4.00	34.50	47.00	5.50	3.39	4.75	2.00	6.00	6.00

Pops. Code	Geno. code	Regrow. in Spring (Scor.[1])	Bloom. Period (Scor.[2])	Plant Height (cm)	Canopy Diame. (cm)	Number of Main Branches	Stem Diamet. (mm)	Lower Branch Color (Scor.[3])	Upper Branch Color (Scor.[3])	Leaf Color (Scor.[4])	Leaf hairiness (Scor.[5])
	319	4.00	4.00	47.00	53.50	9.00	1.63	7.00	1.00	5.00	7.00
	3111	4.00	3.00	24.00	30.00	5.00	2.66	9.50	10.00	5.00	6.50
	3113	2.00	1.00	20.50	58.00	9.00	5.00	6.50	0.85	4.00	7.00
	3212	5.00	8.00	8.00	14.50	4.00	5.00	7.00	11.00	4.50	7.00

[1]Scoring 1: Very early; 3: Early; 5: Intermediate; 7: Late; 9: Very late or dead.

[2]Scoring 1: end of June- early July, 3: end of July- early August, 5: end of August-early September, 7: end of September- early October, 9: end of October- early November, 11: end of November

[3]Scoring 1: Yellow, 3: Orange, 5: Light Brown, 7: Red, 9: Pink, 11: Purple),

[4]Scoring 1: Red-Grey, 3: Greyish Green, 5: Bluish green, 7: Green

[5]Scoring 1: Very rare or absent, 3: Infrequent, 5: Moderate, 7: Frequent, 9: Very Frequent

Table 3
Cont.

Pops. Code	Geno. code	Regrow. in Spring (Scor.[1])	Bloom. Period (Scor.[2])	Plant Height (cm)	Canopy Diame. (cm)	Number of Main Branches	Stem Diamet. (mm)	Lower Branch Color (Scor.[3])	Upper Branch Color (Scor.[3])	Leaf Color (Scor.[4])	Leaf hairiness (Scor.[5])
3P	3214	4.00	3.00	13.25	40.00	7.00	3.00	4.00	5.00	4.00	6.50
	3215	4.00	5.00	55.05	58.00	3.50	3.45	5.00	1.00	5.00	7.00
	3216	3.00	5.00	59.55	59.00	7.50	1.56	8.00	1.00	5.00	7.00
	333	4.00	1.00	31.50	59.00	10.00	0.90	5.50	1.00	4.00	6.50
	336	4.00	5.00	73.35	78.50	6.00	5.75	7.00	5.00	7.05	7.50
	3314	3.00	1.00	21.00	55.00	7.00	6.00	5.50	4.00	6.00	7.25
	3319	4.00	5.00	25.00	50.00	8.00	1.60	5.00	8.00	5.00	7.00
	346	4.00	7.00	12.00	23.00	2.00	7.00	6.00	7.00	4.00	7.00
	348	4.50	8.00	9.25	11.00	2.00	3.00	3.50	1.00	5.50	7.00
	3415	3.00	6.00	45.50	61.00	8.00	3.62	4.00	1.00	6.00	5.50
	3418	1.00	5.75	62.75	104.00	35.50	3.36	3.50	1.00	8.00	7.00
4P	416	2.00	5.00	18.50	42.00	7.00	4.00	2.67	0.50	2.50	3.50
	418	3.00	7.00	6.00	14.00	1.00	0.50	4.67	0.50	2.50	3.50
	4113	4.00	7.00	21.00	34.00	4.00	5.00	6.50	8.00	5.50	7.00
	4114	3.00	5.00	8.50	12.00	2.00	3.00	6.50	6.00	4.50	7.50
	425	4.00	5.00	39.00	33.50	10.00	2.04	5.00	2.00	5.00	6.75
	429	3.00	7.00	16.00	28.00	8.00	7.00	9.00	9.00	5.50	7.00
	4215	4.00	3.00	56.50	34.00	10.50	1.70	3.75	1.00	6.00	6.50
	4216	4.00	5.00	19.00	40.00	10.50	4.80	5.00	4.00	5.50	7.00
	434	3.00	4.50	54.50	84.00	7.50	2.15	5.00	5.00	6.00	6.50
	438	2.00	4.00	43.50	26.50	7.50	2.07	4.25	5.00	6.00	6.50
	4319	2.00	6.00	26.25	48.00	10.00	1.25	6.50	4.00	8.00	7.00
	4320	4.00	6.00	17.00	33.00	4.00	3.00	5.50	4.00	8.00	6.50
	446	5.00	7.00	6.50	8.00	2.00	5.00	3.00	1.00	5.50	6.00
	4420	3.00	7.00	34.00	36.50	5.00	5.02	3.75	7.00	5.50	6.50
5P	513	3.00	5.00	23.00	50.00	2.00	6.00	8.50	4.00	8.00	6.50
	514	2.00	4.00	57.50	62.00	10.00	2.22	8.63	2.00	5.50	6.00
	516	3.00	4.00	41.50	43.00	4.50	3.45	3.25	6.00	6.05	6.00
	5116	5.00	5.00	8.50	5.00	2.00	1.00	5.25	4.00	6.00	7.00
	526	5.00	8.25	11.75	14.50	3.50	2.41	5.00	4.00	5.00	7.50
	527	4.00	8.00	9.00	13.00	2.00	5.00	4.00	4.00	4.50	7.00

Pops. Code	Geno. code	Regrow. in Spring (Scor.[1])	Bloom. Period (Scor.[2])	Plant Height (cm)	Canopy Diame. (cm)	Number of Main Branches	Stem Diamet. (mm)	Lower Branch Color (Scor.[3])	Upper Branch Color (Scor.[3])	Leaf Color (Scor.[4])	Leaf hairiness (Scor.[5])
	5210	4.00	5.00	20.00	38.00	3.00	1.75	3.75	7.00	5.00	7.00
	5211	1.00	5.00	34.50	46.50	15.00	1.51	6.50	1.00	5.00	7.25
	531	3.00	5.00	56.00	56.50	5.50	2.35	7.50	1.00	3.00	7.00
	532	3.00	6.50	42.00	52.00	4.00	2.44	8.50	5.00	5.00	7.00
	533	2.00	4.00	54.00	60.00	5.50	2.64	6.50	4.00	4.00	6.75

[1]Scoring 1: Very early; 3: Early; 5: Intermediate; 7: Late; 9: Very late or dead.

[2]Scoring 1: end of June- early July, 3: end of July- early August, 5: end of August-early September, 7: end of September- early October, 9: end of October- early November, 11: end of November

[3]Scoring 1: Yellow, 3: Orange, 5: Light Brown, 7: Red, 9: Pink, 11: Purple),

[4]Scoring 1: Red-Grey, 3: Greyish Green, 5: Bluish green, 7: Green

[5]Scoring 1: Very rare or absent, 3: Infrequent, 5: Moderate, 7: Frequent, 9: Very Frequent

Table 3. Cont.

Pops. Code	Geno. code	Regrow. in Spring (Scor.[1])	Bloom. Period (Scor.[2])	Plant Height (cm)	Canopy Diame. (cm)	Number of Main Branches	Stem Diamet. (mm)	Lower Branch Color (Scor.[3])	Upper Branch Color (Scor.[3])	Leaf Color (Scor.[4])	Leaf hairiness (Scor.[5])
5P	539	2.00	11.00	30.00	64.00	4.00	2.00	2.50	4.00	8.00	7.50
	541	4.00	7.75	13.75	22.50	3.00	2.42	6.25	9.00	4.00	7.00
	542	4.00	5.00	26.75	39.00	4.00	4.00	9.00	4.00	4.50	8.00
	5410	3.00	3.00	39.25	58.50	9.00	1.30	9.00	4.00	5.50	6.50
	5419	3.00	5.00	32.50	65.00	10.00	5.15	6.25	0.50	2.50	3.50
Mean		3.28	5.24	32.18	44.27	7.36	3.11	5.34	3.49	5.28	6.53
SD		1.28	1.87	19.02	24.61	5.94	1.78	1.99	2.74	1.47	1.08
CV (%)		38.96	35.67	59.11	55.58	80.70	57.19	37.32	78.37	27.93	16.48

[1]Scoring 1: Very early; 3: Early; 5: Intermediate; 7: Late; 9: Very late or dead.

[2]Scoring 1: end of June- early July, 3: end of July- early August, 5: end of August-early September, 7: end of September- early October, 9: end of October- early November, 11: end of November

[3]Scoring 1: Yellow, 3: Orange, 5: Light Brown, 7: Red, 9: Pink, 11: Purple),

[4]Scoring 1: Red-Grey, 3: Greyish Green, 5: Bluish green, 7: Green

[5]Scoring 1: Very rare or absent, 3: Infrequent, 5: Moderate, 7: Frequent, 9: Very Frequent

The dendogram was first divided into two main groups and the main groups were branched into 5 different groups (Fig. 1). Plants in group A: 113, 125, 1414, 2118, 226, 2314, 3111, 3212, 3214, 333, 3319, 346, 4113, 4114, 429, 4216, 4320, 4420, 5116, 5210, 532, 541 and 542. In group A, 3P (Campus Beltway-Selçuklu) and 4P (Ardıçlı Rural-Selçuklu) are the dominant populations with 26% each. Plants in group B: 1215, 2312, 2413, 2414, 2414, 2420, 348, 446, 526 and 527. In group B, Bahri Dağdaş I.A.R.I. Population is the 2nd population with a rate of 44%. Plants in group C are: 1113, 1115, 1219, 2120, 416, 418 and 5419. In this group, Karapinar Kartal Kayalari Population, which is the 1st population, is the dominant population with 37.5%. Plants in group D are: 1116, 1220, 134, 1410, 215, 2216, 2218, 2311, 2313, 2418, 317, 319, 3113, 3215, 3216, 336, 3314, 3415, 425, 4215, 434, 438, 4319, 513, 514, 516, 5211, 531, 533, 539 and 5410. In group D, 3P (Campus Beltway-Selçuklu) and 5P (S.U.F.A Forage Kochia Demonstration Garden) are dominant with a rate of 25%. In group E; there are plants coded 1313, 1315, 1316, 216, 2217 and 3418 and this group consists of the most superior types among 76 plants in terms of morphological characteristics, and within this group, 1P is included with a rate of 50%. 1P has a minimum of 11% and a maximum of 50% in all groups and is the most common population in terms of morphological characteristics among the other populations.

Molecular research

Genetic Diversity Analysis

PIC (Polymorphism Information Content), RP (Resolving Power), EMR (Effective Mutliplex Ratio), and MI (Marker Index) values, as well as the number of polymorphic bands and polymorphism rate, which provide information about the efficiency calculations of ISSR primers, are given in Table 4.

Table 4
Polymorphism data and efficiency analysis of ISSR primers

ISSR Primers	Polymorphic Bands	PIC	RP	EMR	MI
ISSR-M2	15	0.264	6.84	15.00	3.95
ISSR-M3	34	0.273	12.46	34.00	9.28
ISSR-M6	32	0.348	31.33	32.00	11.14
ISSR-M8	27	0.302	10.86	27.00	8.14
ISSR-M12	32	0.323	17.90	32.00	10.34
ISSR-M13	24	0.354	11.84	24.00	8.49
ISSR-M16	33	0.329	15.74	33.00	10.87
ISSR-M17	19	0.355	9.80	19.00	6.74
ISSR-M18	34	0.352	19.75	34.00	11.95
Mean/Total*	250*	0.322	15.17	27.78	8.99

In the study, 9 ISSR primers were used, a total of 250 fragments were scored from 80 plants used in the project, and all the scored fragments were polymorphic. Therefore, our study found the polymorphism rate to be 100%. The PIC value used to evaluate the degree of polymorphism was found to be 0.322 on average in our study. RP value is a parameter showing the discrimination power of the marker and was found to be 15.17 on average in our study. The mean EMR value was 27.78, and the mean MI value was 8.99 in our study.

Table 5
Mean and standard errors of genetic diversity parameters in GenAlEx program

Pop Kodu	Name of Populations	Na	Ne	I	He	uHe	% P
1P	Karapinar Kartal Kayalari	1.708 ± 0.044	1.341 ± 0.020	0.345 ± 0.014	0.217 ± 0.010	0.226 ± 0.011	84.40
2P	Bahri Dagdas I.A.R.I	1.628 ± 0.048	1.302 ± 0.018	0.321 ± 0.014	0.199 ± 0.010	0.206 ± 0.010	80.00
3P	Campus Beltway-Selçuklu	1.724 ± 0.044	1.332 ± 0.019	0.342 ± 0.014	0.214 ± 0.010	0.221 ± 0.010	86.00
4P	Ardıçlı Rural-Selçuklu	1.624 ± 0.049	1.348 ± 0.019	0.350 ± 0.014	0.222 ± 0.010	0.231 ± 0.011	80.80
5P	S.U.F.A Forage Kochia Demonstration Garden	1.528 ± 0.053	1.301 ± 0.019	0.308 ± 0.015	0.194 ± 0.010	0.200 ± 0.011	74.80
Mean		1.642 ± 0.021	1.325 ± 0.009	0.333 ± 0.006	0.209 ± 0.005	0.217 ± 0.005	81.20 ± 1.95
Na = No. of Different Alleles							
Ne = No. of Effective Alleles = $1 / (p^2 + q^2)$							

I = Shannon's Information Index = -1* (p * Ln (p) + q * Ln(q))

He = Expected Heterozygosity = 2 * p * q

uHe = Unbiased Expected Heterozygosity = (2N / (2N-1)) * He

P = Polymorphic locus percentage

Genetic diversity parameters of the populations using the GenAlEx program are given in Table 5. In the study, Na (no. of different alleles) averaged 1.642, Ne (no. of effective alleles) averaged 1.325, I (Shannon's Information Index) 0.333, He (Expected Heterozygosity) 0.209, uHe (Unbiased Expected Heterozygosity) 0.217 and polymorphic locus ratio was 81.20%. Campus Beltway-Selcuklu Population (3P) had the highest number of different alleles (Na; 1.724) and polymorphic loci (P; 86%) compared to other populations, while Ardıçlı Rural- Selcuklu Population (4P) had the highest number of effective alleles (Ne; 1. 348), Shannon's Information Index (I; 0.350), He (Expected Heterozygosity; 0.222) and uHe (Unbiased Expected Heterozygosity; 0.231).

Genetic Structure Analysis

The molecular analysis of variance obtained using 9 ISSR primers is given in Table 6, and the Nei Genetic Distance of Populations is shown in Table 7. In addition, the dendrogram obtained according to UPGMA clustering analysis is given in Fig. 2, the principal coordinate analysis (PCoA) performed in NTSYS 2.10e program is shown in Fig. 3, the ΔK graph obtained with STRUCTURE HARVESTER application is given in Fig. 4, and the grouping is given in Fig. 5.

It was determined that the genetic distance between the selected plants from the population was between 0.63 and 0.90 when the dendrogram obtained according to the UPGMA cluster analysis in Fig. 2 was examined. As a result of the cluster analysis, the plants were divided into two main branches and formed different branches within themselves. In the first branch, there are 6 genotypes (425, 429, 4215, 434, 438, and 4320) belonging to the 4th Population (Ardicli Rural- Selcuklu), and it can be stated that it has a 63% closeness with the other plants examined in the study. According to the cluster analysis, the second branch includes the

other plants in the study. The fact that 70 forage kochia selected in the survey are in the same branch with sugar beet (*Beta vulgaris*) and four-winged saltbush (*Atriplex canescens*) may be since the primers we used in the study are not sequences specific to forage kochia but a general sequence.

Sugar beet varieties in the same family (*Amaranthaceae* Syn. *Chenopodiceae*) as the forage kochia used as an outgroup plant in the study shows about 70% similarity with forage kochia and four-winged saltbush indicates approximately 73% similarity. Sugar beet varieties showed 83% similarity among themselves, while plants belonging to the four-winged saltbush showed about 85% similarity among themselves.

According to the similarity index used in the study, the genotype coded 3212 belonging to the 3rd Population (Campus Beltway-Selcuklu), and the plant code 5419 belonging to the 5th population (SUFA Forage Kochia Demonstration Garden) were found to be highly similar (90%). This situation may be due to the geographical proximity of these two populations

According to the results of AMOVA, while among populations variation was 9%, within populations variation was 91%, which was considerably higher than among populations variation (Table 6).

Table 6
AMOVA with 76 superior genotypes selected from five different forage kochia populations

Source	df	SS	MS	Est. Var.	%		Value	P (rand > = data)
Among Pops	4	464.698	116.175	4.537	9			
Within Pops	71	3357.196	47.284	47.284	91	PhiPT	0.088	0.001
Total	75	3821.895		51.821	100			
PhiPT = AP / (WP + AP) = AP / TOT								
AP = Est. Var. Among Pops, WP = Est. Var. Within Pops								

Table 7
Pairwise Population Matrix of Nei Genetic Distance using GenAlEx program.

	Karapinar Kartal Kayalari (1P)	Bahri Dagdas I.A.R.I (2P)	Campus Beltway-Selcuklu (3P)	Ardiçli Rural-Selcuklu (4P)	S.U.F.A. Forage Kochia Demonstration Garden (5P)
1P	0.000				
2P	0.053	0.000			
3P	0.051	0.038	0.000		
4P	0.040	0.055	0.044	0.000	
5P	0.067	0.031	0.035	0.061	0.000

The Nei Genetic Distance of the forage kochia populations in Table 7 varies between 0.031 and 0.067, and it can be stated that these populations are genetically close to each other.

According to the results of PCoA analysis, plants belonging to the 1st Population (Karapinar Kartal Kayalari) show a closer but scattered distribution compared to other plants. In contrast, plants in other populations are clustered closer to each other (Fig. 3). This situation can be interpreted that the 1st Population may have been geographically separated from different regions, and these plants may have exchanged genes within themselves. It should also be noted that 2nd Population (Bahri Dagdas I.A.R.I.) and 5th Population (S.U.F.A. Forage Kochia Demonstration Garden) consist of mixed populations collected in the KOP region and Konya region, respectively. For this reason, it should be taken into consideration that there may be seeds of plants belonging to 3rd

Population (Campus Beltway-Selcuklu) and 4th Population (Ardicli Rural-Selcuklu) among the plants of 2nd Population (Bahri Dagdas I.A.R.I.) and 5th Population (S.U.F.A. Forage Kochia Demonstration Garden).

When the grouping of forage kochia populations was performed according to the Bayesian model using the STRUCTURE HARVESTER application, the highest ΔK value was obtained at K = 2 (Fig. 4). As a result of the analysis, 76 forage kochia with superior potential were divided into two subgroups (Fig. 5). The first group, the red group, included 33 plants, while the green group included the remaining 43 plants. According to the population distribution, the red group, which is the first group, consists of the Karapinar Kartal Kayalari Population (1P) (14 superior plants), two plants from the Bahri Dagdas I.A.R.I. Population (2P), five plants from the Campus Beltway- Selcuklu Population (3P), 11 plants from the Ardicli Rural-Selcuklu Population (4P), and only 514 coded plants from the S.U.F.A. Forage Kochia Demonstration Garden Population (5P). The second group, the green group, includes 14 plants from the Bahri Dagdas I.A.R.I. Population (2P), 11 plants from the Campus Beltway- Selcuklu Population (3P), three plants from the Ardicli Rural- Selcuklu Population (4P), and 15 plants from the S.U.F.A. Forage Kochia Demonstration Garden Population (5P).

Association between some morphological traits and ISSR markers

The relationships between ISSR markers and some morphological traits determined using multiple regression analysis (Stepwise MRA) are given in Table 8. Stepwise Multiple Regression Analysis was performed using 250 polymorphic markers obtained from 9 ISSR markers selected as independent variables. Some relationships are statistically significant at 5% level, while others are statistically significant at 1% level. When the values given in Table 8 are examined, it is noteworthy that more than one marker is associated with morphological traits.

Table 8
Relationships between some morphological traits and ISSR markers and Multiple Regression Analysis (Stepwise MRA) Coefficients

Traits	ISSR Markers	Standard Error	Standardized Beta (β) Coefficient	*t*	*P*	*r*	R^2	(ANOVA) F	(ANOVA) *P*
Regrowth in Spring	ISSR-M8_14	0.036	0.311	3.229	0.002	0.299[a]	0.089	7.258	0.009[a]
	ISSR-M18_3	0.202	-1.309	-3.403	0.001	0.412[b]	0.169	7.448	0.001[b]
	ISSR-M18_25	0.209	1.083	2.814	0.006	0.498[c]	0.248	7.895	0.000[c]
	ISSR-M12_25	0.256	0.716	3.394	0.001	0.545[d]	0.298	7.517	0.000[d]
	ISSR-M12_14	0.246	-0.553	-2.615	0.011	0.600[e]	0.360	7.875	0.000[e]
Blooming Period	ISSR-M6_2	1.035	-0.293	-2.700	0.009	0.317[a]	0.101	8.287	0.005[a]
	ISSR-M6_28	0.573	0.223	2.052	0.044	0.387[b]	0.150	6.428	0.003[b]
Plant Height	ISSR-M18_20	0.840	0.315	2.987	0.004	0.311[a]	0.096	7.897	0.006[a]
	ISSR-M6_26	6.559	0.346	3.250	0.002	0.417[b]	0.174	7.680	0.001[b]
	ISSR-M6_16	4.136	-0.290	-2.711	0.008	0.500[c]	0.250	8.015	0.000[c]
Canopy Diameter	ISSR-M18_3	4.417	2.080	4.763	0.000	0.397[a]	0.158	13.885	0.000a
	ISSR-M18_18	4.539	-1.718	-3.932	0.000	0.494[b]	0.244	11.793	0.000[b]
	ISSR-M6_16	4.896	-0.337	-3.446	0.001	0.558[c]	0.311	10.827	0.000[c]
	ISSR-M6_26	7.795	0.300	3.068	0.003	0.626[d]	0.392	11.421	0.000[d]
Number of Main Branches	ISSR-M18_20	0.176	0.573	8.072	0.000	0.660[a]	0.435	57.063	0.000[a]
	ISSR-M6_31	1.062	0.432	5.105	0.000	0.686[b]	0.471	32.524	0.000[b]
	ISSR-M17_8	0.830	-1.356	-2.511	0.014	0.708[c]	0.502	24.146	0.000[c]
	ISSR-M17_7	-	-	-	-	0.729[d]	0.532	20.138	0.000[d]
	ISSR-M6_17	1.105	0.451	4.844	0.000	0.748[e]	0.560	17.799	0.000[e]
	ISSR-M6_16	0.937	-0.291	-3.748	0.000	0.765[f]	0.585	16.201	0.000[f]
	ISSR-M13_6	0.784	1.098	3.732	0.000	0.785[g]	0.616	15.598	0.000[g]
	ISSR-M13_3	0.775	-0.944	-3.236	0.002	0.809[h]	0.655	15.891	0.000[h]
	ISSR-M17_7$_{removed}$	-	-	-	-	0.801[i]	0.642	17.430	0.000[i]
	ISSR-M17_9	1.104	2.431	3.379	0.001	0.819[j]	0.671	17.084	0.000[j]
	ISSR-M17_5	0.859	-1.259	-2.272	0.026	0.834[k]	0.695	16.703	0.000[k]

Regrowth in Spring $_{(dependent\ variables)}$:

[a]: Predictors: (Constant), M8_14,

[b]: Predictors: (Constant), M8_14, M18_3,

[c]: Predictors: (Constant), M8_14, M18_3, M18_25,

[d]: Predictors: (Constant), M8_14, M18_3, M18_25, M12_25,

[e]: Predictors: (Constant), M8_14, M18_3, M18_25, M12_25, M12_14

Blooming Period (dependent variables):

[a]: Predictors: (Constant), M6_2,

[b]: Predictors: (Constant), M6_2, M6_28

Plant Height (dependent variables):

1. [a]. Predictors: (Constant), M18_20,
2. [b]. Predictors: (Constant), M18_20, M6_26,
3. [c]. Predictors: (Constant), M18_20, M6_26, M6_16

Canopy Diameter (dependent variables):

[a]: Predictors: (Constant), M18_3,

[b]: Predictors: (Constant), M18_3, M18_18,

[c]: Predictors: (Constant), M18_3, M18_18, M616,

[d]: Predictors: (Constant), M18_3, M18_18, M6_16, M6_26

Number of Main Branches (dependent variables):

[a]: Predictors: (Constant), M18_20,

[b]: Predictors: (Constant), M18_20, M6_31,

[c]: Predictors: (Constant), M18_20, M6_31, M17_8,

[d]. Predictors: (Constant), M18_20, M6_31, M17_8, M17_7,

[e]: Predictors: (Constant), M18_20, M6_31, M17_8, M17_7, M617,

[f]: Predictors: (Constant), M18_20, M6_31, M17_8, M17_7, M6_17, M6_16,

[g]: Predictors: (Constant), M18_20, M6_31, M17_8, M17_7, M6_17, M6_16, M13_6,

[h]: Predictors: (Constant), M18_20, M6_31, M17_8, M17_7, M6_17, M6_16, M13_6, M13_3,

[i]: Predictors: (Constant), M18_20, M6_31, M17_8, M6_17, M6_16, M13_6, M13_3,

[j]: Predictors: (Constant), M18_20, M6_31, M17_8, M6_17, M6_16, M13_6, M13_3, M17_9,

[k]: Predictors: (Constant), M18_20, M6_31, M17_8, M6_17, M6_16, M13_6, M13_3, M17_9, M17_5

Table 8
Cont.

Traits	ISSR Markers	Standard Error	Standardized Beta (β) Coefficient	*t*	*P*	*r*	R^2	(ANOVA) F	(ANOVA) *P*
Stem Diameter	ISSR-M6_10	0.494	-0.258	-2.311	0.024	0.230[a]	0.053	4.118	0.046[a]
	ISSR-M6_13	0.463	-0.232	-2.081	0.041	0.325[b]	0.106	4.316	0.017[b]
Lower Branch Color	ISSR-M13_10	0.409	1.007	2.219	0.030	0.258[a]	0.067	5.280	0.024[a]
	ISSR-M13_20	0.522	-2.053	-3.503	0.001	0.369[b]	0.136	5.760	0.005[b]
	ISSR-M13_7	0.472	1.291	2.449	0.017	0.450[c]	0.203	6.103	0.001[c]
Upper Branch Color	ISSR-M3_26	0.544	1.051	3.596	0.001	0.249[a]	0.062	4.893	0.030[a]
	ISSR-M3_19	0.545	-0.877	-2.988	0.004	0.378[b]	0.143	6.081	0.004[b]
	ISSR-M6_22	0.621	-0.232	-2.159	0.034	0.442[c]	0.195	5.811	0.001[c]
Leaf Color	ISSR-M18_19	0.273	1.856	4.249	0.000	0.384[a]	0.147	12.766	0.001[a]
	ISSR-M18_31	0.406	-3.086	-4.614	0.000	0.558[b]	0.311	16.504	0.000[b]
	ISSR-M6_12	-	-	-		0600[c]	0.360	13.528	0.000[c]
	ISSR-M18_32	0.495	3.257	3.982	0.000	0.632[d]	0.400	11.814	0.000[d]
	ISSR-M18_4	0.380	-1.687	-2.690	0.009	0.670[e]	0.449	11.417	0.000[e]
	ISSR-M6_19	0.284	-0.332	-3.450	0.001	0.697[f]	0.486	10.862	0.000[f]
	ISSR-M6_12$_{removed}$	-	-	-	-	0.688[g]	0.473	12.556	0.000[g]
	ISSR-M6_26	0.446	0.198	2.112	0.038	0.711[h]	0.505	11.724	0.000[h]
Leaf Hairiness	ISSR-M6_21	0.310	0.590	4.429	0.000	0.320[a]	0.102	8.420	0.005[a]
	ISSR-M8_16	0.203	-2.557	-3.951	0.000	0.394[b]	0.155	6.716	0.002[b]
	ISSR-M8_22	0.201	2.243	3.499	0.001	0.456[c]	0.207	6.284	0.001[c]
	ISSR-M6_20	0.334	-0.511	-3.564	0.001	0.520[d]	0.270	6.566	0.000[d]
	ISSR-M6_2	0.535	-0.275	-2.825	0.006	0.588[e]	0.346	7.417	0.000[e]
	ISSR-M6_30	0.311	-0.210	-2.140	0.036	0.622[f]	0.387	7.260	0.000[f]

Stem Diameter $_{(dependent\ variables)}$:

[a]: Predictors: (Constant), M6_10

[b]: Predictors: (Constant), M6_10, M6_13

Lower Branch Color $_{(dependent\ variables)}$:

[a]: Predictors: (Constant), M13_10

[b]: Predictors: (Constant), M13_10, M13_20

[c]: Predictors: (Constant), M13_10, M13_20, M13_7

Upper Branch Color (dependent variables):

[a]: Predictors: (Constant), M3_26

[b]: Predictors: (Constant), M3_26, M3_19

[c]: Predictors: (Constant), M3_26, M3_19, M6_22

Leaf Color (dependent variables):

[a]: Predictors: (Constant), M18_19

[b]: Predictors: (Constant), M18_19, M18_31

[c]: Predictors: (Constant), M18_19, M18_31, M6_12

[d]: Predictors: (Constant), M18_19, M18_31, M6_12, M18_32

[e]: Predictors: (Constant), M18_19, M18_31, M6_12, M18_32, M18_4

[f]: Predictors: (Constant), M18_19, M18_31, M6_12, M18_32, M18_4, M6_19

[g]: Predictors: (Constant), M18_19, M18_31, M18_32, M18_4, M6_19

[h]: Predictors: (Constant), M18_19, M18_31, M18_32, M18_4, M6_19, M6_26

Leaf Hairiness (dependent variables):

[a]: Predictors: (Constant), M6_21

[b]: Predictors: (Constant), M6_21, M8_16

[c]: Predictors: (Constant), M6_21, M8_16, M8_22

[d]: Predictors: (Constant), M6_21, M8_16, M8_22, M6_20

[e]: Predictors: (Constant), M6_21, M8_16, M8_22, M6_20, M6_2

[f]: Predictors: (Constant), M6_21, M8_16, M8_22, M6_20, M6_2, M6_30

Table 9
Correlation matrix between some morphological traits

	1	2	3	4	5	6	7	8	9
2	0.187								
3	-0.551**	-0.336**							
4	-0.567**	-0.443**	0.818**						
5	-0.510**	-0.303*	0.604**	0.716**					
6	0.012	0.049	-0.120	-0.049	-0.104				
7	-0.054	-0.140	0.106	0.141	0.009	0.153			
8	0.235*	0.272*	-0.325**	-0.302**	-0.289*	0.169	0.334**		
9	-0.249*	0.019	0.383**	0.362**	0.365**	0.114	0.141	0.160	
10	-0.178	0.099	0.194	0.024	0.066	0.097	0.292*	0.350*	0.494*

1: Regrowth in Spring; 2: Blooming Period; 3: Plant Height; 4: Canopy Diameter; 5: Number of Main Branches; 6: Stem Diameter; 7: Lower Branch Color; 8: Upper Branch Color; 9: Leaf Color; 10: Leaf Hairiness

*: $p < 0.05$; **: $p < 0.01$

Figure 6 shows that the markers associated with plant height (ISSR-M18_20, ISSR-M6_26, ISSR-M6_16) are also associated with plant diameter, number of main branches, and leaf color. In addition, in our study, it was determined that there was a statistically significant correlation between these traits and plant height at 1% level (Table 9).

Discussion

Forage kochia provides fodder for animals during the summer dry feed period when other plants that are tolerant to drought and salinity dry up. In addition, since it does not burn during this period, it is a semi-shrub species that can be used for different purposes, such as fire prevention strips (Koç Koyun and Acar 2022). In this study, which was carried out to examine the genetic relationships of superior genotypes selected from different forage kochia populations, the morphological characteristics of the selected plants were compared with the findings of Koç Koyun and Acar (2021) working in the same region and other researchers working with forage kochia (McFarland et al. 1990; Harrison et al. 2000; Clements et al. 2020; Lauriault and Waldron 2020) were determined. These differences may be due to the use of superior genotypes in our study and caused by the plants grown under abiotic stress due to the arid climate of our study area and the extreme structure of the soil, such as being saline and calcareous.

Using molecular markers in plant breeding offers a practical and effective breeding program. For this reason, the discussions on the genetic relationships of the research are given under sub-headings.

Comparison of Morphological and Molecular Dendograms

In the dendrogram obtained using morphological data, the most phenotypically similar plants were 3319 and 5210 from the 3rd Population and 5th Population, respectively, while the most distant plants were 113 and 1116 from the 1st Population. On the other hand, when the dendrogram obtained by molecular methods was analyzed, although the genotypes coded 3212 and 5419 belonging to the 3rd Population and 5th Population, respectively, showed 90% genetic similarity. However, these plants were morphologically in groups A and C, respectively. In addition, in the molecular dendrogram, six plants belonging to the 4th Population (Ardicli Rural-Selcuklu) showed 63% similarity with other forage kochia. Still, in the dendrogram made with morphological values, these plants were in groups A (429 and 4320) and D (425, 4215, 434, and 438). Therefore, when the dendrograms obtained using morphological and molecular data are compared, it can be stated that the differences between the kinship ties of the plants, as seen in 3212 and 5419, may cause differences in the phenotypes of plants with similar genetic

structure due to the pressure of environmental conditions such as salinity, excessive lime and boron toxicity (Koç Koyun et al. 2023).

Genetic Diversity Analysis

Genetic diversity is the variation of heritable characteristics present in a population of one species and serves as a way for populations to adapt to changing environments (Xu et al. 2016). Afonso et al. (2019), who conducted a study on PIC, RP, EMR, and MI values that provide information about the efficiency of ISSR primers, determined the average PIC value as 0.44, the average EMR value as 0.24, the average MI value as 0.12 and average RP value as 3.78 in a study on Manihot populations using ISSR molecular markers. Chesnokov and Artemyeva (2015) reported that the PIC value in dominant markers was between 0.00 and 0.50. Since the marker system we used in our study was dominant, our findings are within the range of Chesnokov and Artemyeva (2015).

Harris and DeGiorgio (2017) stated that Expected Heterozygosity or H value called gene diversity is a published statistic used to evaluate within-population variation. Shuyskaya et al. (2015) determined the Expected Heterozygosity of *Bassia sedoides* populations using ISSR molecular markers as 7.9% in the Makan population and 4.5% in the Podolsk population. In the same study, the polymorphic marker rate of the Makan population was 20%, while this value was recorded as 13% in Podolsk populations. Afonso et al. (2019), working on Manihot populations, found Na (number of different alleles) 1.67, Ne (number of effective alleles) 1.46, H (Nei genetic diversity) average 0.26, Shannon's Information Index (I) 0.38, Hs (intrapopulation genetic diversity) 0.587. In the same study, the rate of polymorphic markers ranged between 48% and 92%. Yilmaz (2020), who studied 94 laurels belonging to Türkiye, recorded H (genetic diversity) as 0.31 and I (Shannon's Information Index) as 0.46.

Genetic Structure Analysis

Analysis of Molecular Variance is a method to detect population differentiation utilizing molecular markers (Excoffier et al. 1992). This procedure was initially implemented for DNA haplotypes but applied to any marker system. Also, AMOVA is a powerful tool that can help support hypotheses of population structure due to clonal reproduction or isolation without making assumptions about Hardy-Weinberg equilibrium (Kamvar et al. 2023). Mengistu and Messersmith (2002), who conducted research on *Kochia scoparia*, determined among-population variation as 10% and within-population variation as 90% according to AMOVA results. Yilmaz (2020), working on four different laurel populations, found that among populations variation (4%) was lower than within populations variation (96%). Golkar and Nourbakhsh (2019) used SRAP and SCoT molecular marker systems on black cumin populations and found that among-population variation was 12.83%, and within-population variation was 87.17%.

Genetic polymorphism is a heterozygous DNA variation in more than 1% of the population (Stankiewicz and Lupski 2012). Ray and Roy (2007) reported that the family *Chenopodiaceae* includes *Amaranthoideae* (*Amaranthus gangeticus, A. paniculatus, A. viridis, A. hypochondriacus, A. caudatus, A. cruentus, Celosia cristata Telanthera philoxeroides*) and *Chenopodioideae* [*Basella rubra, Chenopodium album, Spinacia oleraceae, Beta vulgaris* (now included in *Betoideae* subclass)] using 11 ISSR primers. In the study, a total of 177 ISSR fragments were obtained, and the polymorphism rate of those in the subclass *Chenopodioideae* was 97%. In contrast, 98% polymorphism rate was determined in the subclass *Amaranthoideae*. The genetic similarity between the plants varied between 0.06 and 0.85. The dendrogram obtained from the research was divided into two main branches. *C. cristata* and *T. philoxerosides* of the subclass *Amaranthoideae* formed a separate branch. The rest of the species in the dendrogram were included in the second branch, and this branch showed a distinction according to the subclasses *Amaranthoideae* and *Chenopodioideae*.

Lee et al. (2005) analyzed the degree of affinity of *Kochia prostrata*, *K. americana*, and *K. scorpia* species using RAPD markers. In the study, 458 polymorphic bands were obtained from 9 plants of *K. americana*, 20 plants of *K. prostrata*, and seven plants of *K. scorpia* species. While there were 80 RAPD markers specific for *K. scorpia*, 54 and 55 species-specific RAPD markers were identified for *K. americana* and *K. prostrata*, respectively. It was stated that each species formed a different cluster with RAPD markers. Shuyskaya et al. (2015) used 4 ISSR primers to examine the degree of relatedness of the Makan and Podolsk populations of *Bassia sedoides* in the South Urals region. They obtained 29 bands, eight of which were polymorphic. The polymorphism rate in the Makan population was higher than the polymorphism rate of the Podolsk population. In the same study, the Nei Genetic Distance between the two populations was 0.151. Qari et al. (2019) examined the genetic variation of *B. indica* collected from 3 different regions of Egypt (the northern coastal region, the Delta region, and the Upper Egypt region) in the summer of 2018. In the study, 4

RAPD primers were used, and a total of 26 bands were scored, 9 of which were polymorphic. *B. indica* populations from the Delta region and Upper Egypt region showed 74% and 53% similarities, respectively, with the North Coast region. The differences observed in the populations might be caused by the differences in the soil structures of the area where the plants grow.

Motawei and Al-Ghumaiz (2012) used 15 primers and obtained an 85.7% polymorphism rate in their ISSR study with six forage grasses. In the study, the genetic distance between plants was expressed as 0.41 to 0.92. While the varieties of *Lolium perenne* showed 92% similarity among themselves, the variety Niva of *Dactylis glomerata* showed 90% similarity with these perennial grasses. In addition, the variety Niva is 41% similar to the Takepo variety of *D. glomerata*. This situation may be because Niva and Takepo varieties originated from the Czech Republic and New Zealand, respectively. Arslan and Tamkoc (2011) reported that the molecular genotyping is essential in determining the proper starting material for plant breeding, aimed at determining the genetic relatedness and intraspecific genetic diversity between *Poa angustifolia* and *P. trivialis* species. A total of 401 bands, 363 of which were polymorphic (90.52%), were obtained using 20 selected ISSR primers. In the study, the intraspecific polymorphism rate of *P. angustifolia* was 64.98%, while the intraspecific polymorphism rate of *P. trivialis* was 43.22%.

A population's genetic structure can broadly be defined as the amount and distribution of genetic variation within and between populations (Lübeck 2004). Pandey et al. (2019) stated that 25 genotypes were divided into two subgroups according to STRUCTURE HARVESTER program in their study to examine the degree of relatedness of watermelon and fodder watermelon genotypes originating from Türkiye, Turkmenistan, and Saudi Arabia. Golkar and Nourbakhsh (2019) examined the degree of relatedness of black cumin populations with SRAP and SCoT molecular markers and divided the populations into subgroups with the STRUCTURE HARVESTER program. The study stated that the populations were grouped into six subpopulations using the SRAP molecular marker system. In comparison, the SCoT molecular marker system was divided into four subpopulations. Yilmaz (2020), who studied the molecular characterization of 94 laurel plants in 4 different regions of Türkiye (Mediterranean, Aegean, Black Sea, and Marmara), stated that laurel populations were grouped into two subgroups as a result of STRUCTURE clustering analysis.

Association between some morphological traits and ISSR markers

It should be remembered that economically essential traits such as yield, plant height, and disease resistance are polygenic quantitative traits (Chahal and Gosal 2002; Semagn et al. 2010). Furthermore, it has been stated that each gene affecting quantitative traits shows an additive effect in the formation of the character (Gökçora 1973; Şehirali and Özgen 2002).

Vafaee et al. (2017), who examined the relationship between 43 SNPs and 41 SSR markers and morphological traits in grape populations, found that cluster length and fruit length were associated with the same marker (Vrzag79-246) as a result of multiple regression analysis. Similarly, Ipek et al. (2015), working on olive populations, examined the relationship between 168 polymorphic markers obtained from 8 AFLP markers and morphological traits and found that two different markers affect more than one trait. In a study conducted on cherry populations, it was reported that four other ISSR markers were associated with fruit color. In comparison, the same 3 ISSR markers were associated with fruit harvest time and water-soluble dry matter (Ganopoulos et al. 2011).

The tendency of genes on the same chromosome to pass as a group during gamete formation is defined as linkage. Since these genes acting as a group will be passed on to the offspring as a unit, these genes are also called linked genes (Gökçora 1973; Genç and Yağbasanlar 1998; Chahal and Gosal 2002). In addition, many genes control quantitative characters, and these genes can be linked to desirable or undesirable traits (Chahal and Gosal 2002). For example, as a result of GWAS studies in soybean (Contreras-Soto et al. 2017), plant height and seed weight traits (Contreras-Soto et al. 2017), and technical length trait in flax (Soto-Cerda et al. 2014) were reported to be controlled by genes linked to 6 traits (Zhang et al. 2018).

Pleiotropy, defined as the effect of one gene on more than one different phenotypic trait (Hämälä et al. 2020), is often confused with linkage (Genç and Yağbasanlar 1998). In linkage, traits are transmitted to offspring together because they are located on the same chromosome, whereas in pleiotropy, affected traits may not be governed by linked genes. For example, branching, an undesirable quality with pleiotropic effects in sunflowers, is controlled by the B locus. The same study by Bachlava et al. (2010)

reported that B locus also affects seed morphological traits and oil content. In addition, it was stated that the dwarfism gene in broad beans has a pleiotropic effect and causes dark green leaves in the plant (Hughes et al. 2020).

The effect of markers on more than one trait in the results we obtained in our study may be due to linkage or pleiotropic effect, or it may be due to the correlation between the traits we studied (Culp et al. 1979; Vafaee et al. 2017).

Conclusion

In this study in which the genetic relationships of forage kochia populations were examined, although Karapınar Kartal Kayaları Population (1P) is geographically separated from other regions in terms of Nei genetic distance, it is genetically close to SUFA Forage Kochiaa Demonstration Garden Population (5P) because this species is cross-fertilization by the wind. In addition, according to the results of molecular variance analysis, within-population variation (91%) is considerably higher than among-population variation, which may be due to the fertilization biology of the plant. When the relationships between some morphological traits and ISSR markers were examined, the markers associated with plant height (ISSR-M18_20, ISSR-M6_26, ISSR-M6_16) were also associated with plant diameter, number of main branches, and leaf color. For this reason, we can state that steppe grass breeding studies should continue with superior types selected from each population. Since the morphological markers used in classical breeding methods take longer to reach the desired result, biotechnological processes are nowadays emphasized in breeding to shorten this period. Therefore, although the studies in breeding forage kochia are limited and the genes identified for this species are few, it is possible to achieve success in breeding in a shorter time with molecular approaches (QTL mapping, Marker-Assisted Selection, etc.) in this genomic era.

Declarations

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Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Nur KOÇ KOYUN, Erdoğan E. HAKKI and Ramazan ACAR. The first draft of the manuscript was written by Nur KOÇ KOYUN, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Figures

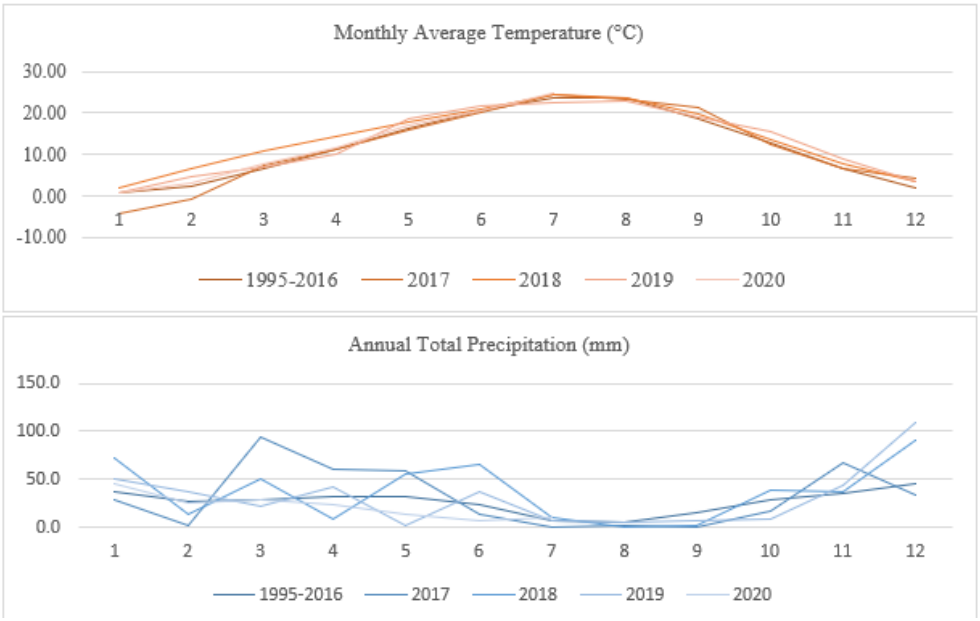

Figure 1

Climate characteristics of the experimental field in Konya-Türkiye

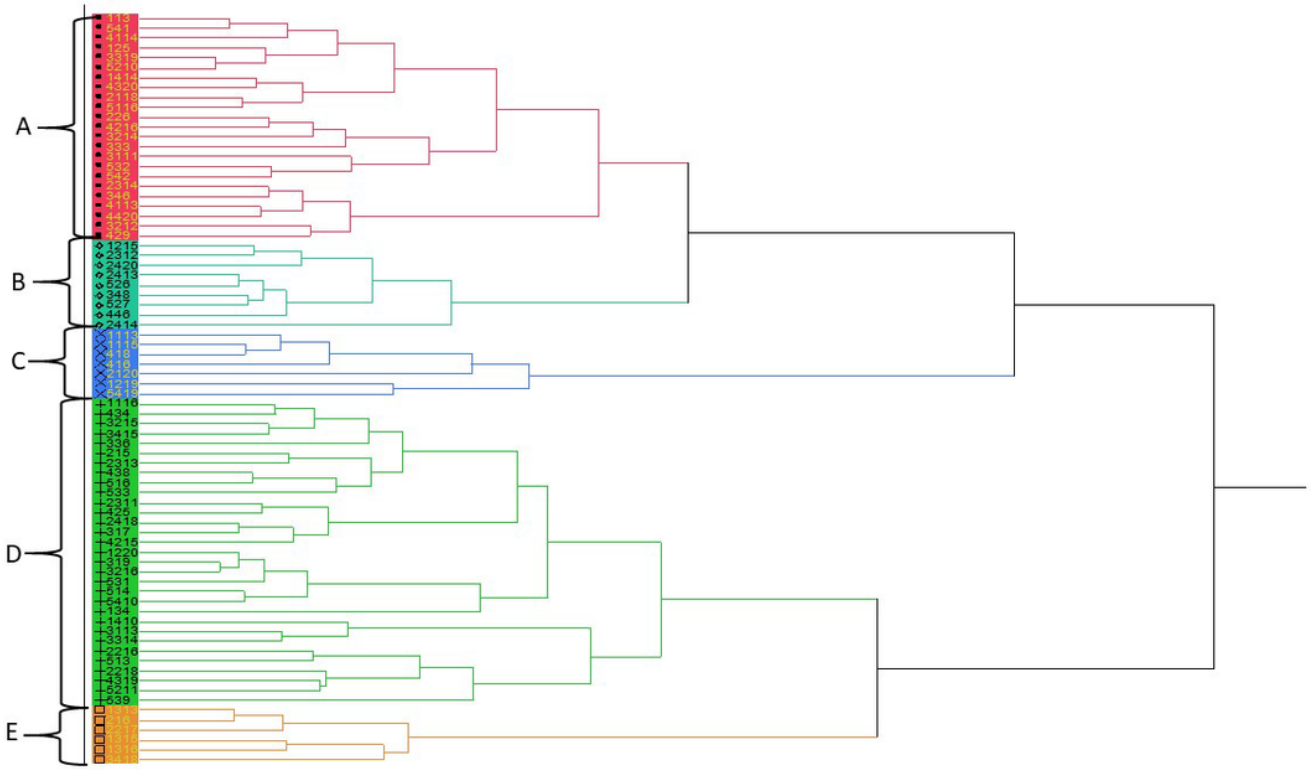

Figure 2

Figure 1. The dendrogram was made with morphological data of selected plants from forage kochia populations using JMP 7 statistical program

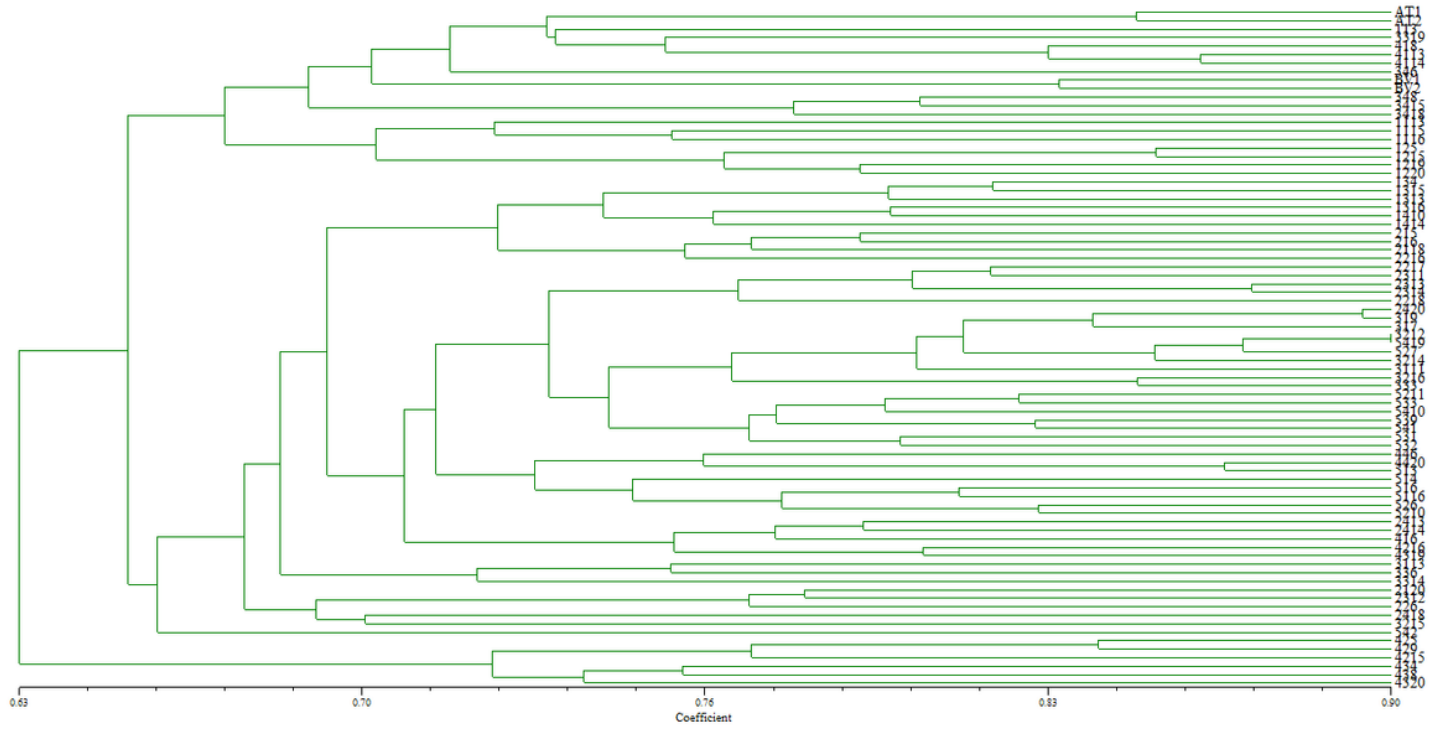

Figure 3

Figure 2. The dendrogram was obtained by UPGMA clustering analysis using 9 ISSR primers with selected superior genotypes from forage kochia populations and outgroups (AC: *Atriplex canescens* and BV: *Beta vulgaris*)

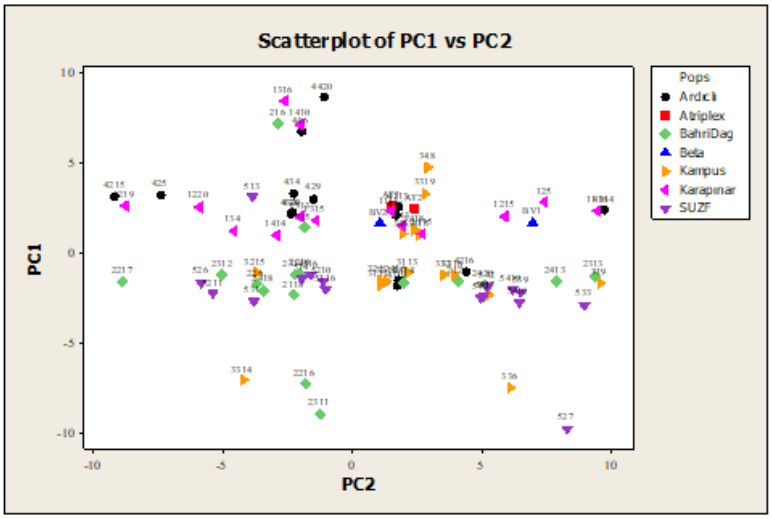

Figure 4

Figure 3. Principal coordinate analysis (PCoA) in Minitab 16 shows the relationship between 76 superior genotypes and outgroup plants selected from five different forage kochia populations.

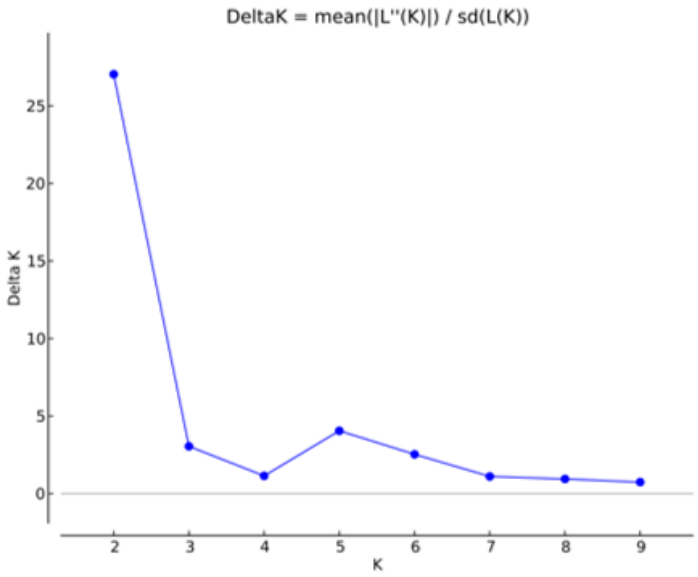

Figure 4. ΔK graph of 76 superior forage kochia genotypes in STRUCUTRE HARVESTER application

Figure 5

See image above for figure legend

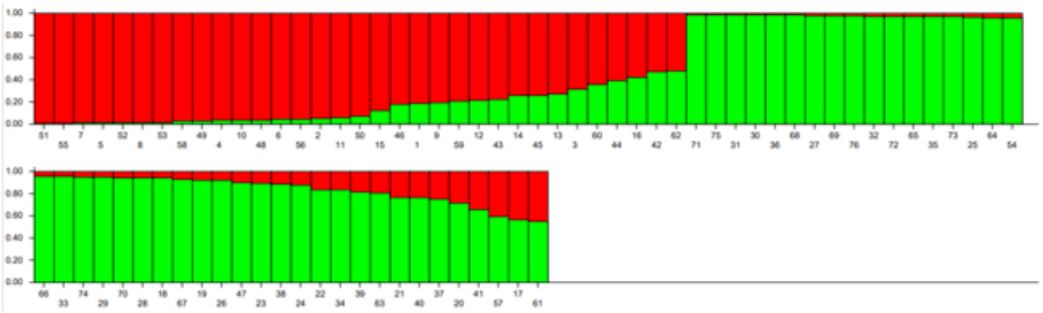

Figure 6

Figure 5. STRUCTURE cluster analysis of 76 superior forage kochia genotypes (K=2)

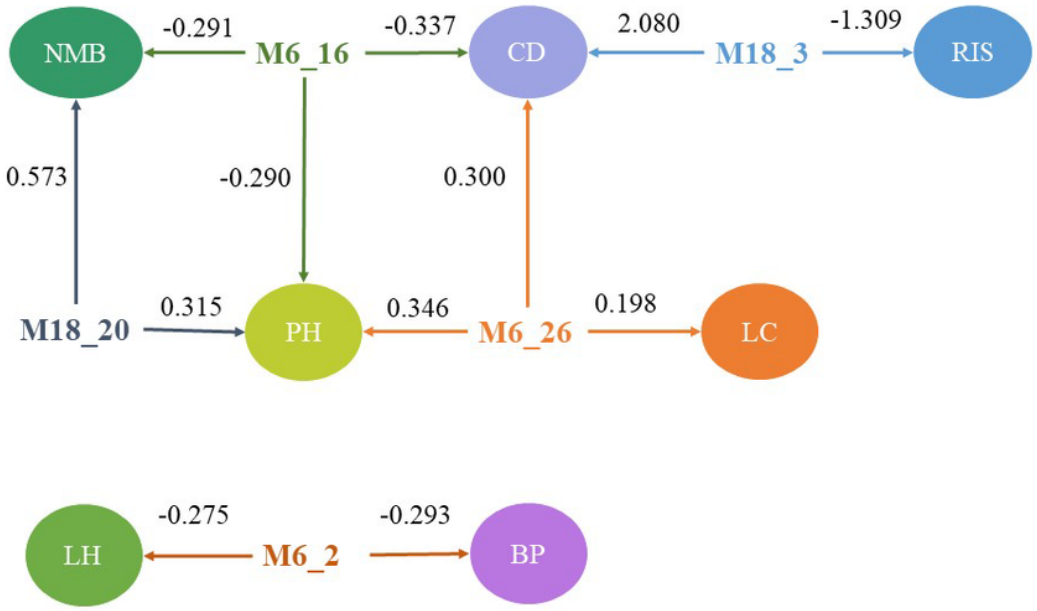

Figure 7

Figure 6. Markers associated with more than one morphological trait and their associations with these traits, RIS: Regrowth in Spring; BP: Blooming Period; PH: Plant Height; CD: Canopy Diameter; NMB: Number of Main Branches; LC: Leaf Color; LH: Leaf Hairiness (β Coefficient are given)