Molecular analysis of genetic diversity in Tulip(Tulipa gesneriana L.) cultivation varieties and germplasm resources by SRAP marker

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Research Article

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Abstract

The taxonomy of *Tulipa gesneriana* L. poses a challenging problem as traditional morphological methods no longer suffice to meet the demands. In recent years molecular marker technology has been increasingly utilized for population identification and classification as well as for the analysis of genetic diversity and kinship relationships among tulip cultivation varieties and germplasm resources.To elucidate the genetic diversity of different tulip cultivation varieties and germplasm resources This study was carried out to estimate the genetic diversity kinship relationships between 40 tulipa cultivation varieties and germplasm resources using SRAP marker system .The results showed that out of 43 pairs of primers 21 pairs with high polymorphism were selected and 249 clear and stable bands were amplified including 245 polymorphic bands and Percentage of Polymorphism Bands (PPB) is 98.39%.The genetic similarity index of 40 tulip cultivation varieties and germplasm resources is between 0.5020–0.8675 and the genetic diversity parameters including the Number of alleles (N_a) Effective number of alleles (N_e) Nei's gene diversity index (H) Shannon's information index (I) and polymorphic information content (PIC) are 1.9810 1.5149 0.3042 0.4603 and 0.3212 respectively. This indicates that the genetic diversity of 40 tulip cultivation varieties and germplasm resources is rich .The cluster results analysis and PCoA(principal coordinate analysis) divided 40 tulip cultivars into two major groups A and B (Christmas Magical Banja Luka) with the first group (A) further divided into two subgroups A1 and A2 (Madame Lefeber). These results demonstrate that SRAP can accurately reflect the genetic differences of 40 tulip cultivation varieties and germplasm resources at the molecular level.

1 Introduction

Tulip (*Tulipa gesneriana* L.) is one of the most important ornamental plants worldwide (Neriya et al. 2020). The *Tulipa gesneriana* L. originated from the Pamir Alai and Tien Shan mountain ranges in Central Asia. Their habitat extends from southern Europe NorthAfrica Anatolia and Iran to the north-west of China (Pourkhaloee et al. 2018). Considering a high commercial value due to its various colour long flowering period and pleasing fragrance. *Tulipa gesneriana* L. is of great economic horticultural esthetical ecological conservational and medicinal value. At the same time it also has significant cultural significance in many countries of the word it has been regarded as the national flower by many countries such as Turkey Holland and Iran (Li et al. 2022; Veldkamp et al. 2012; Mu et al. 2020; Marasek-Ciolakowska et al. 2012). Currently 102 accepted taxa with more than 6700 cultivars belong to the genus The vast majority of the cultivars are the hybrids of *Tulipa gesneriana* L. (Ágoston et al. 2020). Due to the high degree of *Tulipa gesneriana* L. hybridization the resulting offspring exhibit extensive traits which hinders the taxonomic classification of the genus (Zonneveld 2009). However their extensive genetic diversity enables them to survive and adapt to environmental changes. Therefore this variation is a crucial goal for the conservation of plant genetic resources (Montalvo et al. 1997). Furthermore in modern plant breeding indigenous plants with extensive variation are considered valuable gene pools for hybrid breeding (Valerie 1999). Therefore understanding the genetic diversity of *Tulip gesneriana* L. genetic

resources is a necessary prerequisite for devising *Tulip gesneriana* L. breeding plans population identification and classification.

With the advancement of molecular biology techniques molecular marker technology has become an efficient means of identifying germplasm resources due to its relatively simple methods high polymorphism and independence from growth environment among other advantages (Alghamedi et al. 2023). sequence-related amplified polymorphism (SRAP) a novel molecular marker employs a unique primer design for amplifying open reading frames. It assimilates the merits of various molecular markers such as restriction fragment length polymorphism (RFLP) (Papa et al. 2020) random amplified polymorphic DNA (RAPD) (Yin et al. 2021) simple sequence repeat (SSR) (James et al. 2020) and amplified fragment length polymorphism (AFLP) (Domblides and Domblides 2023) and possesses characteristics such as simplicity in the operational process clear amplification bands stable outcomes and high reproducibility. Presently it has been extensively employed in the research of genetic mapping for various plant germplasm resources such as *Alfalfa Cultivars* (Shamustakimova et al. 2021) *Lavandula angustifolia* mill (Zagorcheva et al. 2021) *Coffea canephora* (Arun et al. 2020) *Plumbago auriculata* L. (Zagorcheva et al. 2020) and *Triticum aestivum* (Hassan et al. 2020) for the purpose of identifying important trait markers and genetic diversity evaluation. Prior research has indicated that random amplified polymorphic DNA (RAPD) (Qi-fu et al. 2008) inter-simple sequence repeat (ISSR) (Kashin et al. 2016; Kritskaya and Kashin 2019) single nucleotide polymorphism (SNP) (Tang et al. 2013) and expressed sequence tag-SSRs (EST-SSR)(Pourkhaloee et al. 2017) markers can be more effectively utilized for investigating the genetic diversity of tulips. However no studies have been reported on the application of SRAP markers for assessing genetic diversity in *Tulipa gesneriana* L. germplasm resources. The objective of this research is to utilize SRAP molecular markers to evaluate the genetic diversity of 40 tulip cultivation varieties providing a theoretical foundation for population identification and classification kinship analysis and breeding programs in the *Tulipa gesneriana* L. population.

2 Results

2.1. Primer Screening Results

Primers that produced a distinct polymorphic and consistent amplification were choosen for amplification in all accessions. 43 different combinations were used to determine the genetic diversity among the 40 tulip cultivation varieties and germplasm resources. Only 21 SRAP primers showed polymorphism and reproducibility(refer Fig. 1). The following are the combinations: Em1-Me3 Em-1Me4 Em1-Me5 Em1-Me6 Em1-Me7 Em3-Me7 Em4-Me1 Em4-Me10 Em5-Me5 Em5-Me9 Em5-Me10 Em6-Me3 Em6-Me7 Em7-Me2 Em7-Me3 Em9-Me6 Em9-Me7 Em9-Me8 Em10-Me5 Em10-Me6 Em10-Me8.

2.2. SRAP Analysis

SRAP-PCR amplification was conducted on 40 tulip cultivation varieties and germplasm resources to assess their genetic diversity using a set of 21 primer pairs. The results of the amplification are depicted

in the accompanying figure(refer Fig. 2).

The total number of fragments total number of bands percentage of polymorphism bands (PPB) are described in Table 1.A total of 249 fragment were produced from all 21 SRAP primers with an average of 11.9 per marker. The (Em1-Me6) combination produced amaximum of 23 fragment followed by Em3-Me7 and Em5-Me9 which produced 16 fragment. The minimum was 6 fragment which were produced from the Em4-Me1 primer.The polymorphism rate is between 86% and 100%. and the polymorphism rates of a total of 17 pairs of primers including Em1-Me3,Em1-Me4,Em1-Me5,Em1-Me6,Em1-Me7,Em3-Me7,Em4-Me1,Em5-Me10,Em6-Me3,Em7-Me2,Em7-Me3,Em9-Me6,Em9-Me7,Em9-Me8,Em10-Me5,Em10-Me6 and Em10-Me8 are 100%(refer Table 1).

Table 1
Genetic variability among 40 tulip cultivation varieties and germplasm resources as revealed by SRAP markers.

Primer Combination	Total Number of Fragments	Total Number of Bands	percentage of polymorphism bands
Em1×Me3	8	8	100
Em1×Me4	10	10	100
Em1×Me5	10	10	100
Em1×Me6	23	23	100
Em1×Me7	9	9	100
Em3×Me7	16	16	100
Em4×Me1	6	6	100
Em4×Me10	8	8	100
Em5×Me5	9	8	89
Em5×Me9	16	15	94
Em5×Me10	13	13	100
Em6×Me3	10	10	100
Em6×Me7	12	11	92
Em7×Me2	16	16	100
Em7×Me3	11	11	100
Em9×Me6	11	11	100
Em9×Me7	14	14	100
Em9×Me8	8	8	100
Em10×Me5	15	15	100
Em10×Me6	11	11	100
Em10×Me8	14	14	100
Total	249	245	
Average number of bands	11.9	17.1	98.39

The N_a of 40 tulip cultivation varieties and germplasm resources ranges from 1.8571 to 2.0000 with an average of 1.9810 N_e ranges from 1.3261 to 1.6160 with an average of 1.5149 H ranges from 0.2106 to

0.4061 with an average of 0.3042 I ranges from 0.3333 to 0.5930 with an average of 0.4603 PIC ranges from 0.2106 to 0.4915 with an average of 0.3212(refer Table 2).

Table 2
Parameters used for genetic diversity with SRAP markers. N: number of samples N_a: the Number of alleles N_e: Effective number of alleles I: Shannon's Information index for a variety H: Nei's gene diversity index PIC: polymorphic information content.

Number	Primer	N_a	N_e	H	I	PIC
1	Em1×Me3	2.0000	1.5686	0.3453	0.5224	0.3543
2	Em6×Me3	2.0000	1.4838	0.2881	0.4417	0.2881
3	Em7×Me3	2.0000	1.3261	0.2106	0.3333	0.2106
4	Em1×Me7	2.0000	1.4921	0.2919	0.4468	0.2919
5	Em3×Me7	2.0000	1.5243	0.3104	0.4695	0.3104
6	Em6×Me7	1.9167	1.3620	0.2154	0.3376	0.2154
7	Em9×Me7	2.0000	1.5003	0.3015	0.4610	0.3015
8	Em1×Me5	2.0000	1.5722	0.3437	0.5178	0.3437
9	Em5×Me5	1.8889	1.4836	0.3017	0.4592	0.3017
10	Em9×Me6	2.0000	1.7156	0.4061	0.5930	0.4061
11	Em10×Me6	2.0000	1.5052	0.3058	0.4660	0.3058
12	Em4×Me10	1.8571	1.5636	0.3138	0.4560	0.3139
13	Em5×Me10	2.0000	1.5295	0.3135	0.4758	0.4795
14	Em1×Me4	2.0000	1.5476	0.3096	0.4609	0.4915
15	Em4×Me1	2.0000	1.3978	0.2491	0.3927	0.2491
16	Em9×Me8	2.0000	1.6087	0.3566	0.5345	0.3566
17	Em10×Me8	2.0000	1.4767	0.2822	0.4323	0.2822
18	Em7×Me2	2.0000	1.4197	0.2579	0.4031	0.2579
19	Em5×Me9	1.9375	1.3978	0.2439	0.3800	0.2439
20	Em10×Me5	2.0000	1.6160	0.3488	0.5144	0.3488
21	Em1×Me6	2.0000	1.7223	0.3929	0.5685	0.3929
	Average	1.9810	1.5149	0.3042	0.4603	0.3212

2.3. Cluster Analysis

The clustering results depicted in Fig. 3 demonstrate that at a genetic similarity coefficient of 0.590. the 40 materials can be divided into two major categories namely A and B. Category A encompasses 38 tulip cultivation varieties and germplasm resources which can be further subcategorized into A1 and A2 at a similarity coefficient of 0.628. A1 comprises of 37 tulip cultivation varieties and germplasm resources including ‘Barcelona’ ‘Ollioules’, ‘Christmas Marvel’, ‘Royal Ten’, ‘Parade Design’, ‘Leen Van der Mark’, ‘Avignon’, ‘Jaap Groot’, ‘Inzell’, ‘Sweet Prince’, ‘Purple Prince’, ‘Menton’, ‘Avenue’, ‘Muscadet’, ‘Blue Diamond’, ‘Banja Luka’, ‘Pallada’, ‘Lalibela’, ‘Mount Tacoma’, ‘Black Hero’, ‘Oxford's Elite’, ‘Apricot Emperor’, ‘Blushing Lady’, ‘Yellow double’, ‘Red Power’, ‘Yellow Springgreen’, ‘Flaming Parrot’, ‘Parade’, ‘Yellow Pompenette’, ‘Flaming Springgreen’, ‘Aladdin’, ‘Angelique’, ‘Pink Impression’, ‘Abba’, ‘Flash Point’, ‘Peking’, and ‘Carola’. In the A1 subcategory genetic variation between the ‘Barcelona’ ‘Ollioules’ ‘Apricot Empero’ ‘Mount Tacoma’ and other tulip cultivation varieties and germplasm resources is significant indicating a distant genetic relationship. Furthermore the tulip cultivation varieties and germplasm resources ‘Sweet Prince’ and ‘Purple Prince’ are clustered as a group at a similarity coefficient of 0.870. A2 on the other hand comprises of only one tulip cultivation variety and germplasm resource ‘Madame Lefeber’.

There are two tulip cultivation varieties and germplasm resources belonging to category B namely ‘Banja Luka’ and ‘Christmas Magical’(refer Fig. 3).

2.4. Principal Coordinates Analysis

From this Figure it is evident that the individual distances and clustering of the ‘Banja Luka’ and ‘Christmas Magical’ are the highest. Additionally 38 tulip cultivation varieties and germplasm resources are clustered together as one category with ‘Madame Lefeber’ being relatively distant from the other 37 tulip cultivation varieties and germplasm resources in this group. Furthermore there is an overlap in the individual distances of ‘Sweet Prince’ and ‘Purple Prince’(refer Fig. 4).The results of this study are consistent with the results of the clustering analysis.

2.5. Genetic similarity coefficients Analysis

In order to further clarify the genetic diversity among 40 tulip cultivation varieties and germplasm resources this study analyzed the genetic similarity coefficients in the form of a heatmap.Compared with other genetic resources of tulip cultivation varieties and germplasm resources ‘Christmas Magical’ is the lowest genetic similarity coefficients with an additional 39 tulip cultivation varieties and germplasm resources followed by ‘Banja Luka’. Besides that the genetic similarity coefficients between ‘Madame Lefeber’, ‘Peking’ and ‘Caeola’ is relatively small. ‘Christmas Marvel’ and ‘Royal Ten’, ’Inzell’, ’Sweet Prince’ and ‘Purple Prince’, ‘Avenue’ and ‘Muscadet’, ‘Parade’ and ‘Yellow Pompenette’ ‘Abba’ and ‘Flash Point’ The genetic similarity coefficient is relatively high. The results of this study are consistent with the results of the clustering analysis and Principal Coordinates Analysis.

3 Materials and Methods

3.1 Plant Material

The 40 tulip cultivation varieties and germplasm resources (refer Table 3) being tested are sourced from the Key Laboratory of Garden Plants and Ornamental Horticulture in Qinghai Province.

Table 3
Information on 40 tulip cultivation varieties and germplasm resources.

Number	Variety name	Color	Flowering season
1	‘Barcelona’	Pink	Late-flowering
2	‘Ollioules’	Bright pink	Late-flowering
3	‘Christmas Marvel’	Purple	Mid-flowering
4	‘Royal Ten’	Pinkish-purple	Mid-flowering
5	‘Parade Design’	Red	Mid-flowering
6	‘Leen Van der Mark’	Red	Mid-flowering
7	‘Avignon’	Red	Late-flowering
8	‘Jaap Groot’	Yellow	Mid-flowering
9	‘Inzell’	White	Mid-flowering
10	‘Sweet Prince’	Purple	Mid-flowering
11	‘Purple Prince’	Purple	Mid-flowering
12	‘Christmas Magical’	Purple	Mid-flowering
13	‘Menton’	Pink	Late-flowering
14	‘Banja Luka’	Yellow	Mid-flowering
15	‘Avenue’	Red	Mid-flowering
16	‘Muscadet’	Yellow	Mid-flowering
17	‘Blue Diamond’	Purple	Mid-flowering
18	‘Banni Luka’	Yellow	Mid-flowering
19	‘Pallada’	Red	Mid-flowering
20	‘Lalibela’	Red	Mid-flowering
21	‘Mount Tacoma’	White	Late-flowering
22	‘Madame Lefeber’	Red	Early-flowering
23	‘Black Hero’	Purple	Mid-flowering
24	‘Oxford's Elite’	Red	Mid-flowering
25	‘Apricot Emperor’	Apricot	Early-flowering
26	‘Blushing Lady’	Yellow	Mid-flowering
27	‘Yellow double’	Yellow	Mid-flowering

Number	Variety name	Color	Flowering season
28	‘Red Power’	Red	Late-flowering
29	‘Yellow Springgreen’	Yellow and green	Late-flowering
30	‘Flaming Parrot’	Red and purple	Late-flowering
31	‘Parade’	Red	Mid-flowering
32	‘Yellow Pompenette’	Yellow	Late-flowering
33	‘Flaming Springgreen'	White	Late-flowering
34	‘Aladdin’	Red	Mid-flowering
35	‘Angelique’	White and pink	Late-flowering
36	‘Pink Impression’	Pink	Early-flowering
37	‘Abba’	Red	Mid-flowering
38	‘Flash Point’	Pink	Mid-flowering
39	‘Peking’	Red	Late-flowering
40	‘Carola’	Pink	Mid-flowering

3.2 DNA Extraction

Fresh leaf samples of approximately 0.2g were selected for each experimental material and extracted for total DNA using the improved CTAB method (Huang et al. 2000). Following individual extractions the quality and concentration of total genomic DNA for each sample were evaluated using 1.0% agarose gel electrophoresis and a nucleic acid detector. The resulting products were diluted to 80ng/ul and stored at -20℃ for future use.

4.3 Primer Selection and SRAP-PCR Amplification

The SRAP analysis was carried out according to Chen lanyan(Chen et al. 2019) Primer screening was carried out from 43 primer combinations for use in SRAP-PCR amplification(refer Table 4). Total volume of PCR reaction was 20μL containing 2μL 10xPCR Buffer DNA template 80ng 0.4μL for each forward and reverse primers (0.2μmol/μl) 1.6μL Mg2 (2.0mmol/L) 0.16μL dNTPs(0.2mmol/L) 6μL Tap polymerase(1.5U/μL) and 8.44 ddH2O ul to make 20 μL reaction. The PCR amplification protocal included the following steps: Pre-denaturation was performed at 94℃ for 5 minutes followed by denaturation at 94℃ for 1 minute. Annealing was carried out at 35℃ for 1 minute followed by extension at 72℃ for 1 minute. This process was repeated for 5 cycles and then terminated. Denaturation was performed again at 94℃ for 1 minute followed by annealing at 50.5℃ for 1 minute. Extension was carried out at 72℃ for 1 minute for 35 cycles and then extended at 72℃ for 10 minutes. PCR products were checked by electrophoresis on 1% agarose gel in TBE buffer. The bands were visualized under UV light after staining with ethidium bromide.

Table 4
Forward and reverse sequence of the SRAP markers used in the study.

Primer Pair	Forward Sequence	Reverse Sequence
Em1×Me3	GACTGCGTACGAATTAAT	TGAGTCCAAACCGGAAT
Em1×Me4	GACTGCGTACGAATTAAT	TGAGTCCAAACCGGACC
Em1×Me5	GACTGCGTACGAATTAAT	TGAGTCCAAACCGGAAG
Em1×Me6	GACTGCGTACGAATTAAT	TGAGTCCAAACCGGTAG
Em1×Me7	GACTGCGTACGAATTAAT	TGAGTCCAAACCGGTTG
Em3×Me7	GACTGCGTACGAATTGAC	TGAGTCCAAACCGGTTG
Em4×Me1	GACTGCGTACGAATTTGA	TGAGTCCAAACCGGATA
Em4×Me10	GACTGCGTACGAATTTGA	TGAGTCCAAACCGGACC
Em5×Me5	GACTGCGTACGAATTAAC	TGAGTCCAAACCGGAAG
Em5×Me9	GACTGCGTACGAATTAAC	TGAGTCCAAACCGGTCA
Em5×Me10	GACTGCGTACGAATTAAC	TGAGTCCAAACCGGACC
Em6×Me3	GACTGCGTACGAATTGCA	TGAGTCCAAACCGGAAT
Em6×Me7	GACTGCGTACGAATTGCA	TGAGTCCAAACCGGTTG
Em7×Me2	GACTGCGTACGAATTATG	TGAGTCCAAACCGGAGC
Em9×Me7	GACTGCGTACGAATTACG	TGAGTCCAAACCGGTTG
Em9×Me8	GACTGCGTACGAATTACG	TGAGTCCAAACCGGTGT
En10×Me8	GACTGCGTACGAATTTAG	TGAGTCCAAACCGGTGT
Em10×Me5	GACTGCGTACGAATTTAG	TGAGTCCAAACCGGAAG
Em9×Me6	GACTGCGTACGAATTACG	TGAGTCCAAACCGGTAG
Em10×Me6	GACTGCGTACGAATTTAG	TGAGTCCAAACCGGTAG
Em7×Me3	GACTGCGTACGAATTATG	TGAGTCCAAACCGGAAT
Em2×Me1	GACTGCGTACGAATTTGC	TGAGTCCAAACCGGATA
Em6×Me1	GACTGCGTACGAATTGCA	TGAGTCCAAACCGGATA
Em8×Me1	GACTGCGTACGAATTCTG	TGAGTCCAAACCGGATA
Em8×Me3	GACTGCGTACGAATTCTG	TGAGTCCAAACCGGAAT
Em3×Me4	GACTGCGTACGAATTGAC	TGAGTCCAAACCGGACC
Em4×Me4	GACTGCGTACGAATTTGA	TGAGTCCAAACCGGACC

Primer Pair	Forward Sequence	Reverse Sequence
Em5×Me4	GACTGCGTACGAATTAAC	TGAGTCCAAACCGGACC
Em6×Me7	GACTGCGTACGAATTGCA	TGAGTCCAAACCGGTCC
Em7×Me4	GACTGCGTACGAATTCAA	TGAGTCCAAACCGGACC
Em10×Me4	GACTGCGTACGAATTCAG	TGAGTCCAAACCGGACC
Em6×Me8	GACTGCGTACGAATTGCA	TGAGTCCAAACCGGTGC
Em8×Me8	GACTGCGTACGAATTCTG	TGAGTCCAAACCGGTGC
Em3×Me5	GACTGCGTACGAATTGAC	TGAGTCCAAACCGGAAG
Em9×Me5	GACTGCGTACGAATTCGA	TGAGTCCAAACCGGAAG
Em3×Me9	GACTGCGTACGAATTGAC	ACAGTCGAAACCGGTCA
Em6×Me9	GACTGCGTACGAATTGCA	ACAGTCGAAACCGGTCA
Em7×Me9	GACTGCGTACGAATTCAA	ACAGTCGAAACCGGTCA
Em8×Me9	GACTGCGTACGAATTCTG	ACAGTGGAAACGCGTAC
Em3×Me10	GACTGCGTACGAATTGAC	ACAGTGGAAACGCGTAC
Em6×Me10	GACTGCGTACGAATTGCA	ACAGTGGAAACGCGTAC
Em7×Me10	GACTGCGTACGAATTCAA	ACAGTGGAAACGCGTAC
Em9×Me10	GACTGCGTACGAATTCGA	ACAGTGGAAACGCGTAC

4.3. Date Collection

The SRAP data were collected in binary format (fragment absent = 0 fragment present = 1). For subsequent analysis we concatenated all matrices to create a single binary matrix for further analysis (Smith et al. 1997). Genetic diversity was assessed with different parameters such as Percentage of Polymorphism Bands (PPB) Number of alleles (N_a) Effective number of alleles (N_e) Nei's gene diversity index (H) Shannon's information index (I) using POPGENE (v1.32) program (Yeh Francis et al. 1999). The markers' potential to estimate genetic variability was assessed by computing the polymorphic information content (PIC). The PIC was calculated using the formula $PIC = 1 - \sum nj = 1(Pij)^2$ where Pij is the frequency of the ith allele of each marker j and the summation extends towards n alleles for each marke (De Riek et al. 2001). The The Unweighted Pair-Group Method with Arithmetic Mean Algorithm (UPGMA) (Nandi et al. 2001) in NTSYSpc 2.10e software(SeguraAlabart et al. 2022) was used to create the phylogenetic trees. The principal coordinate analysis (PCoA) was used to construct the biplot using PAST 3.11 software. Jaccard similarity coefficients were used to examine data from SRAP markers (Jaccard et al. 1908). Correlation coefficient and genetic distance analysis were used to construct HeatMap using TBtools(v1.098) (Chen et al. 2020).

4 Discussion

Genetic diversity is a fundamental aspect of biodiversity arising from the recombination of genetic material (DNA) through genetic processes such as mutation gene flow and genetic drift. It is a fundamental condition for species to maintain their evolutionary potential and is a crucial factor in the selection of superior breeds (William 1983; Zhang et al. 2012). The genetic diversity of plant species is contingent upon heritable variation within and among populations. This occurrence is attributed to heritable variation in DNA nucleotide sequences chromosomal mutations and recombination during sexual reproduction (Swingland et al. 2001). The evolution and persistence of species within their natural populations are reliant on genetic diversity. Within natural environments both intra- and inter-population genetic diversity may be impacted by alterations in environmental conditions. Populations with high levels of genetic diversity exhibit greater adaptability to habitat loss and environmental change (Tara Hopley et al. 2019). Previous studies on herbaceous plant communities have indicated that an increase in genetic diversity can reduce inter-species competition thereby altering the relationship between plant species diversity and plant productivity. This finding suggests that genetic diversity plays a crucial role in shaping the dynamics of plant communities and has important implications for ecological management and conservation efforts (Schöb et al. 2015). Therefore comprehending the genetic diversity among various species is fundamental to crop improvement genetic resources as well as the basis for population identification and classification protection of biodiversity among others (Bhandari et al. 2017).

Due to the impact of the environment traditional morphological classification is no longer sufficient for species identification and classification. With the advancement of DNA fingerprinting technology diagnostic molecular markers are now widely used for molecular classification variety identification and plant marker-assisted selection (Al-Murish et al. 2013; Hasan et al. 2021). The Sequence-Related Amplified Polymorphism (SRAP) is a relatively new PCR marker technology that has been widely applied in plant germplasm identification variety identification genetic mapping gene cloning and other related fields (Nadeem et al. 2021).

Xuan Dang Thi Kim and colleagues used SRAP markers to explore the genetic diversity of 30 coconut (Cocos nucifera L.) varieties in the Mekong Delta region of Vietnam. They found that the genetic diversity of the 30 coconut varieties was relatively rich with H I and PPB being 0.25 0.21 and 88.89% respectively (Xuan et al. 2022). A. Framarzpour and colleagues analyzed the genetic diversity of 25 (winter/spring) rapeseed (*Brassica napus* L.) cultivars using SRAP markers with H I and PPB values of 0.26 0.4 and 81.74% indicating that rapeseed cultivars have high genetic diversity (Framarzpour et al. 2021). This study used SRAP markers to explore the genetic diversity of 40 tulip cultivars. The values of H I and PPB were 0.3042 0.4603 and 98.39% respectively. The PPB value was much higher than that of coconut (Cocos nucifera L.) varieties and rapeseed (*Brassica napus* L.) cultivars indicating that the 40 tulip cultivation varieties and germplasm resources in this study exhibited rich genetic diversity. The findings of Mahnaz Kiani and colleagues (Kiani et al. 2012) who utilized ISSR markers to analyze the genetic

diversity of 39 tulip materials from the provinces of Khorasan and Yazd in eastern and northeastern Iran align with the conclusion that these materials possess a wealth of genetic diversity.

Clustering analysis is widely used in many areas such as document grouping image recognition web search business intelligence biology information and medicine (Turkoglu et al. 2022). By analyzing the cluster diagram it can be observed that A and B exhibit significant genetic differences and a distant genetic relationship. Specifically the ‘Madame Lefeber’ sub-cluster in A2 forms a distinct group indicating a large genetic difference and remote relationship with the tulip cultivation varieties and germplasm resources in A1. The clustering of the ‘Sweet Prince’ and ‘Purple Prince’ tulip cultivation varieties and germplasm resources at a similarity coefficient of 0.870 both belonging to the purple color group and intermediate-flowered suggests a potential similarity in their genetic background.

The results of the cluster analysis demonstrate that the genetic resources of 40 tulip cultivars and germplasm resources exhibit a rich diversity validated PCoA. The kinship relationships among materials can be visually presented through principal coordinate analysis (PCoA). Smaller spatial distances between individuals indicate higher similarities while larger distances suggest greater genetic differences (Wang et al. 2022). From the spatial distance in the figure it can be seen that tulip cultivation varieties and germplasm resources ‘Banja Luck’, ‘Christmas Magical’, ‘Carola’ ‘Peking’ and ‘Madame Lefeber’ are distantly related to the remaining cultivars while tulip cultivation varieties ‘Sweet Prince’ and ‘Purple Prince’ have a closer genetic relationship between their germplasm resources. The above results are consistent with the analysis results of genetic similarity coefficients and genetic distance HeatMaps. In summary the 40 tulip cultivation varieties and germplasm resources have rich genetic diversity.

5 Conclusion

This study examines 40 tulip cultivation varieties and germplasm resources by employing 21 primer combinations with high polymorphism clear bands and good stability that were selected from 43 pairs of primers. The 40 tulip cultivation varieties germplasm resources were subjected to SRAP-PCR amplification resulting in 245 polymorphic bands. Through the analysis of the polymorphic band percentage genetic diversity parameters cluster dendrogram principal coordinate analysis and correlation heatmap the genetic diversity of the 40 tulip cultivation varieties and germplasm resources was elucidated. The findings of this study provide guidance for tulip breeding germplasm conservation and new variety development while also offering a theoretical basis for tulip germplasm resource classification and identification of genetic relationships.

Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Authors' contributions

All authors contribute to the study conception and design. experimental design was performed by Xiuting Ju, Material preparation,data collection and analysis were performed by Bin Liu, Jiting Tian and Weiqiang Liu. The first draft of the manuscript was written by Douwen Qin and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability

The datasets generated during and analysed during the current study are available from the corresponding author on reasonable request.

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Figures

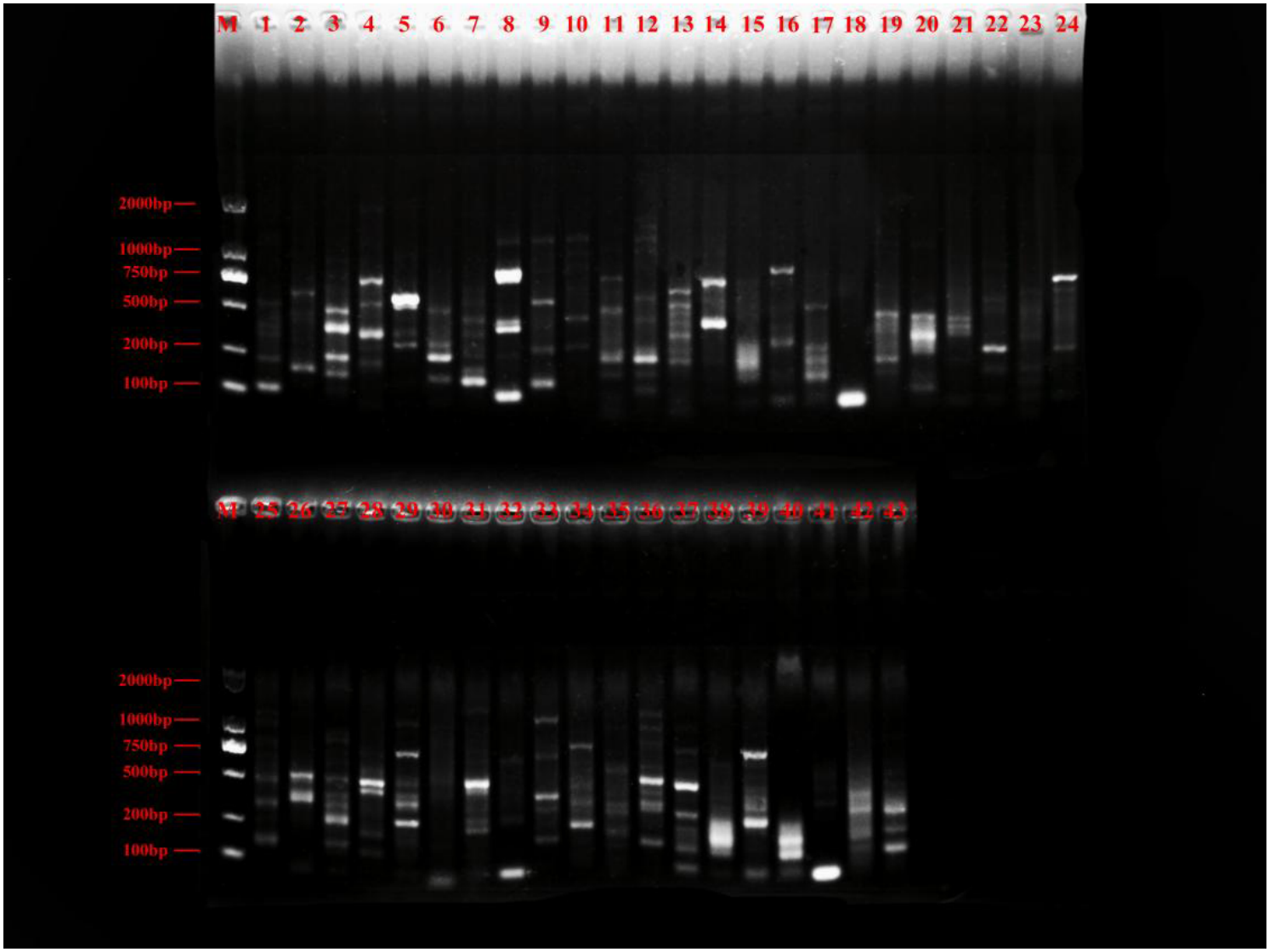

Figure 1

Primer screening results .M: DL2000 DNA Marker 1-43:43 sets of primer combinations

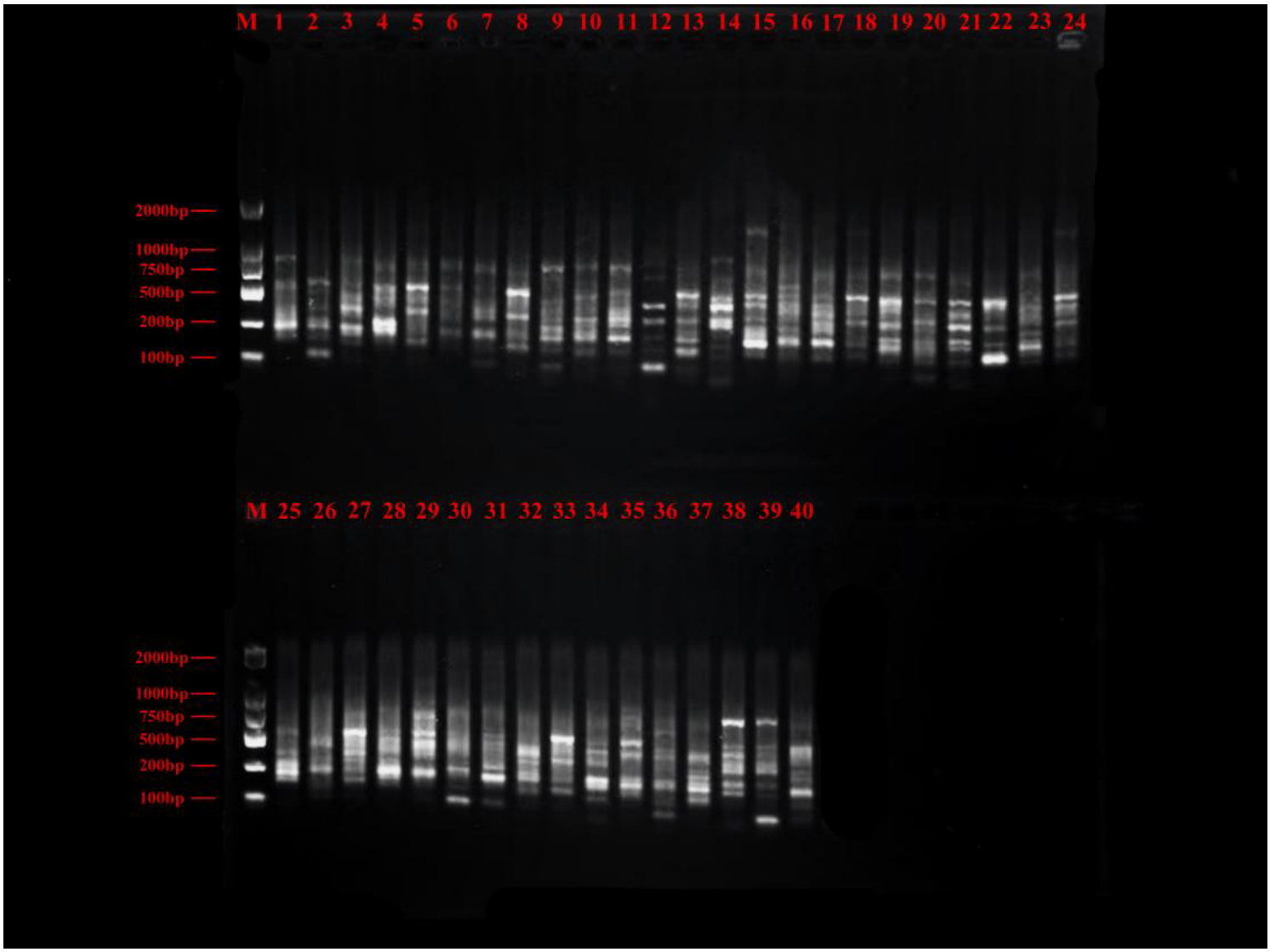

Figure 2

SRAP-PCR amplification of 40 tulip cultivation varieties and germplasm resources DNAs by primer Em10Me5 M:DL2000 DNA Marker 1-40:40 tulip cultivation varieties and germplasm resources

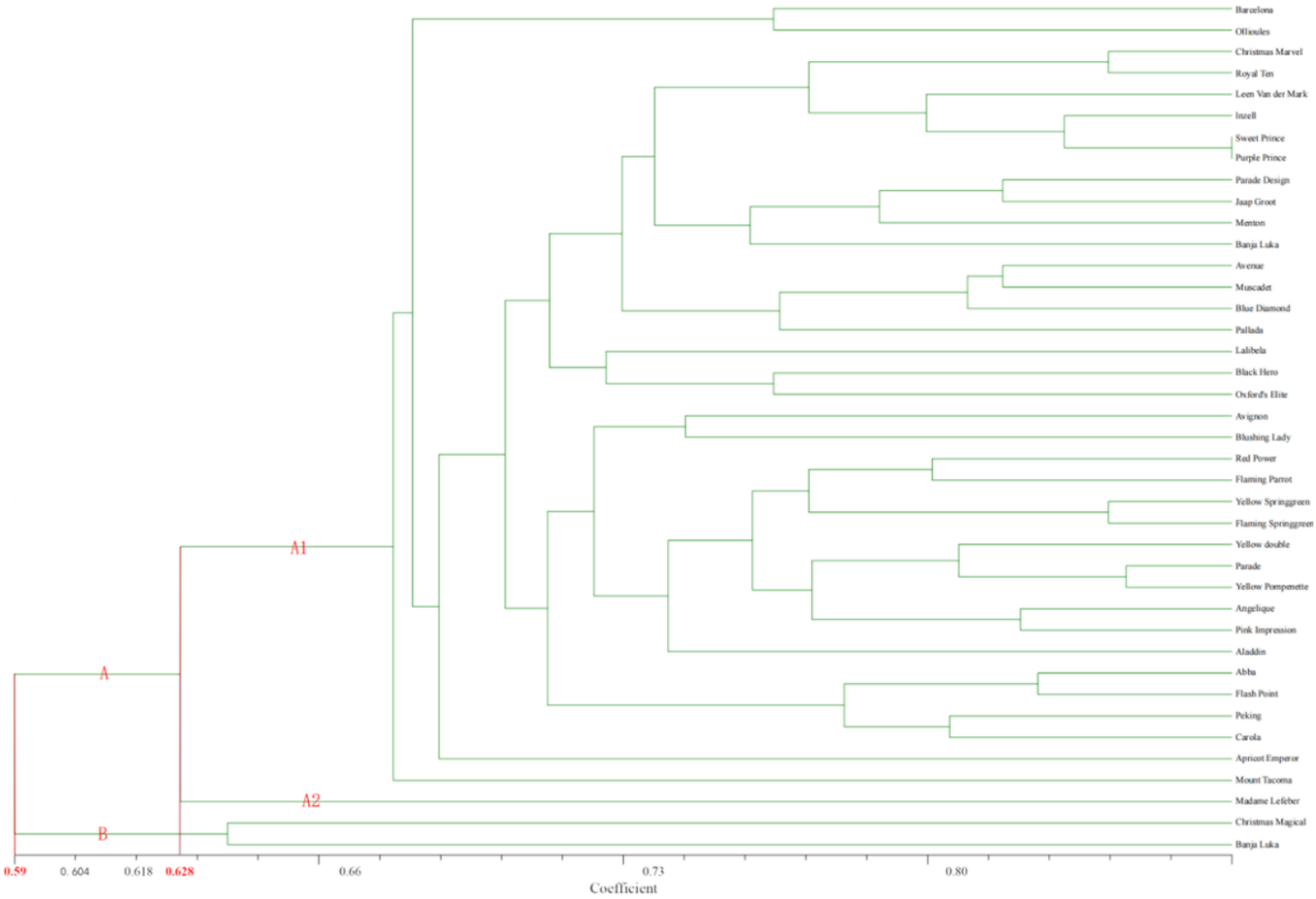

Figure 3

UPGMA clustering dendrogram of 40 tulip cultivation varieties and germplasm resources based on SRAP molecular markers

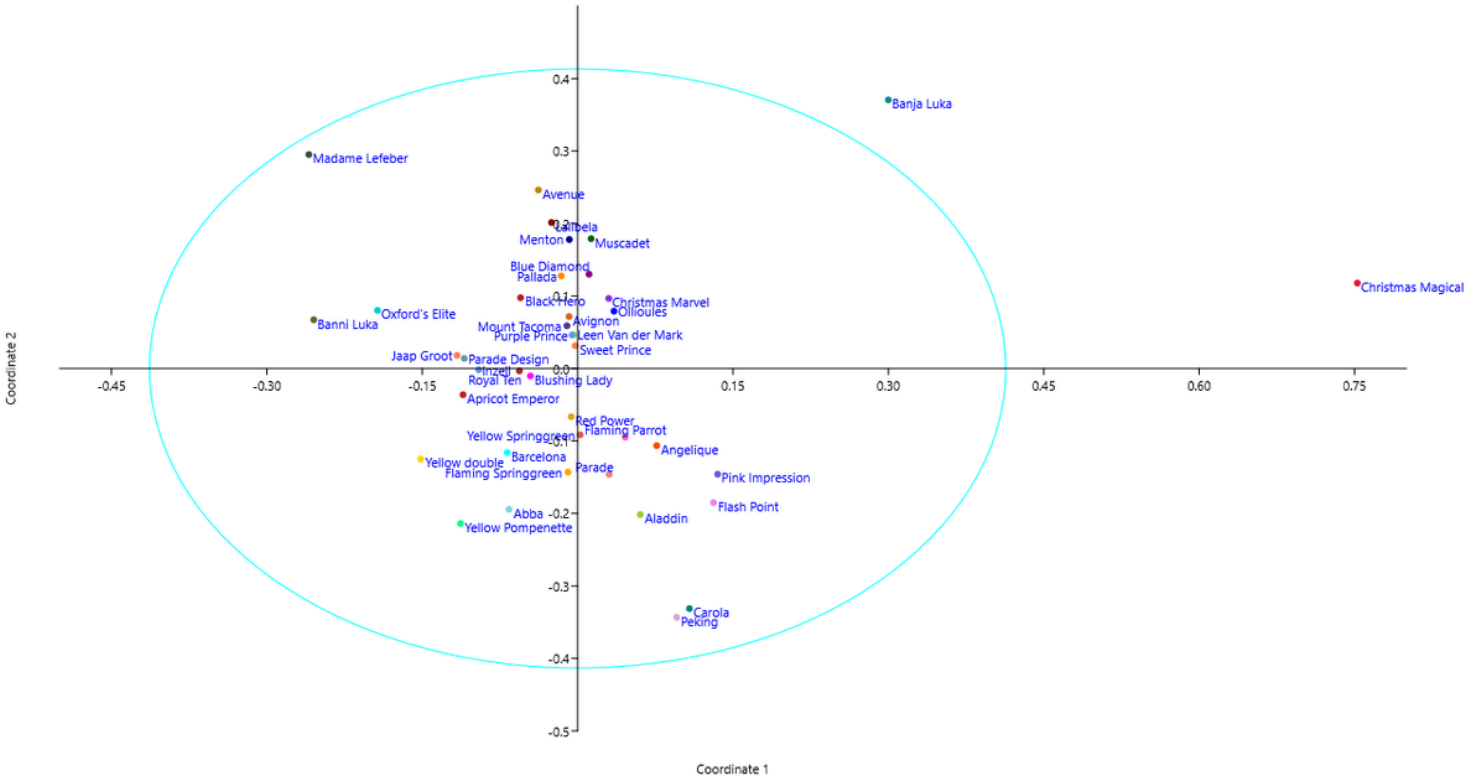

Figure 4

Biplot analysis of 40 tulip cultivation varieties and germplasm resources diversity as inferred from SRAP marker data.

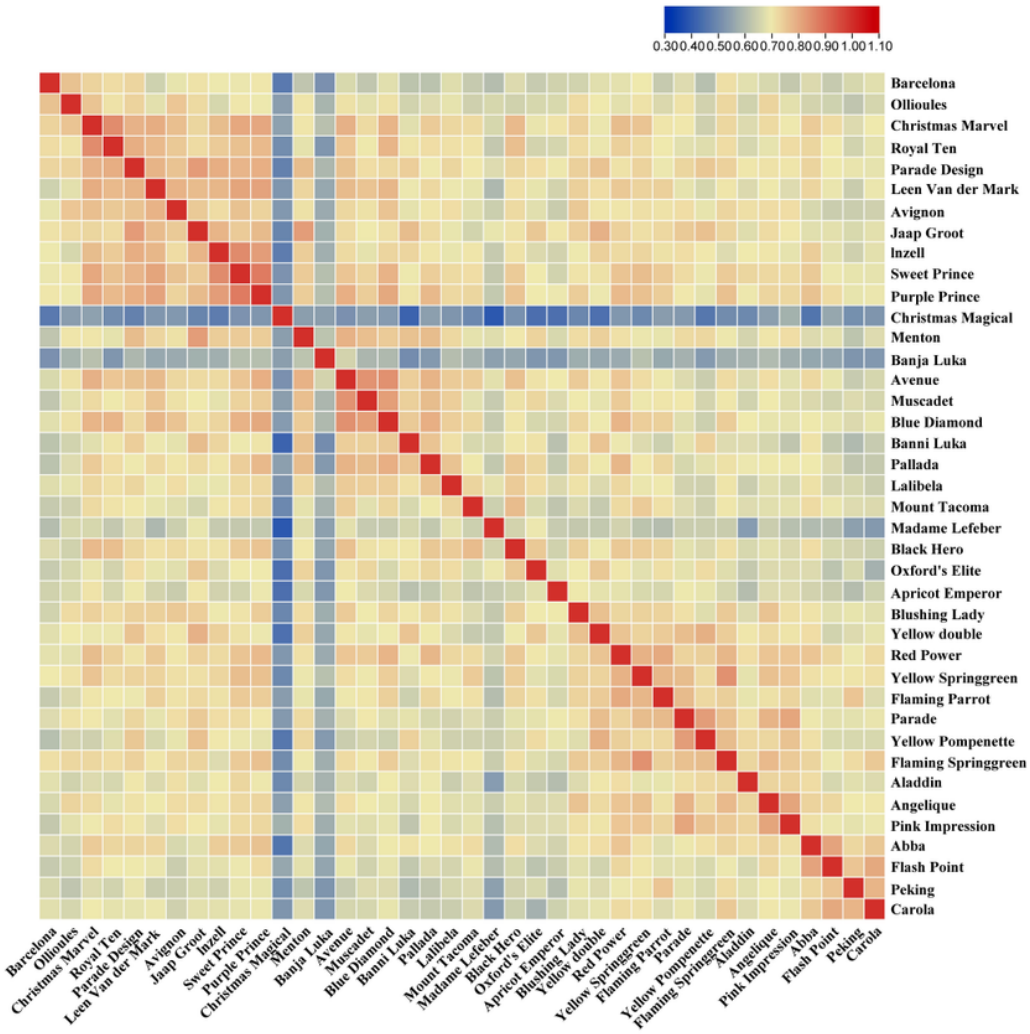

Figure 5

Genetic similarity coefficients of 40 tulip cultivation varieties and germplasm resources.