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Evaluation of genetic diversity of 12 gerbera varieties (*Gerbera* L.) based on morphology and RAPD marker

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Abstract. This study focuses on evaluating the genetic diversity of 12 Gerbera varieties from farms in Da Lat city (Lam Dong province) based on 9 morphological characteristics and 10 RAPD primers. A higher different level between varieties was observed by using RAPD markers compared to morphological markers. A total of 110/135 polymorphic bands were generated, which occupied 12.2%. The average of PIC and Rp value are 0.48 and 11.39, respectively. The genetic similarity coefficient based on morphological analysis and RAPD data was 0.98-0.99 and 0.46-0.82, respectively, which showed that RAPD primers are useful for detecting genetic polymorphism. The results from morphological data showed that XB, HX and DX2 varieties are suitable for the cut flower market; meanwhile, DD1 and DD2 varieties are characterized by large flower size, DD2 and DX2 have long and wide petals. The RAPD analysis results indicated that OPE02, OPE08 and OPE13 generated a high polymorphic rate (100%), while OPG16 primer showed the lowest polymorphism percentage (52.9%). This study demonstrates that the combination of phenotypic and genotypic methods provides valuable insights into the understanding of genetic diversity among species.

Keywords: Gerbera, genetic diversity, morphological characteristics, molecular marker, RAPD marker

1 Introduction

Gerbera belongs to the Asteraceae family, including 30 species that originate from Africa, Madagascar, Asia, and South America [1]. *Gerbera jamesonii* L. is one of the top ten cut flowers in the world after *Chrysanthemum, Rosa, Lilium,* and *Dianthus caryophyllus* [2]. With a short life cycle (8 months), this plant is highly economic valuable for decorative use at events. It has a long vase life, which makes it ideal for long-distance shipping and export markets.

Gerbera jamesonii L. is a perennial herbaceous and suitable for home decoration or artistic flower arrangement. Leaf has deeply lobed, pubescent leaves that emerge from a basal rosette, reaching up to 40 cm in length. Juvenile leaves are round, but adult leaves are distinguished by slight incisions or divisions of their margins. Furthermore, leaf blades vary in size and color depending on specific cultivars [3]. Some leaves have axils where the flower buds form, grow into a huge scape, and exhibit a terminal inflorescence known as a capitulum. The floral stem is slightly hairy; its length and diameter vary depending on cultivar, plant age, and growth conditions. Some long -stem cultivars reach a height and are appropriate 60 cm and use as cut flowers, while the most compact cultivars are utilized as pot flowers [3]. Gerbera plants are available in a diverse palette of colors, such as red, cream, orange, pink, and some cultivars presenting cream in the middle flowers [4].

The ornamental market is extremely dynamic and demands constant novelties, so genetic improvement programs are imperative to develop new cultivars, such as improving various traits, novel colors, form size, and vase life of flowers that meet customer's demands are necessary [5].

An important tool for crop improvement programs is genetic diversity analysis. Accurate assessment of the levels of genetic diversity can be invaluable in crop breeding for identifying diverse parental plants to create segregating progenies with maximum genetic variability for further selection, as well as analyzing genetic variability in cultivars [6].

The assessment of genetic diversity can be based on various criteria such as morphological characteristics or molecular markers. This allows breeders to determine genotypes which used for plant breeding programs [7]. The analysis of agromorphological traits represents a fundamental step in determining species and is relatively simple to perform. This analysis of variability based on this type of feature alone is not conclusive, given the limited number of traits. Indeed, the number of studies that have evaluated the genetic diversity based on morphological characteristics has been conducted [3,8].

Among the available strategies for assessing genetic variability, molecular markers is considered the most widely applicable because these markers are best suited for understanding the genome and may be used in paternity testing, genetic variability characterization, the elucidation of genetic relationships between genotypes, developing methods for the maintenance of genetic variability existing in germplasm banks, and identifying genes or combinations of features related to key traits of biological and agronomic interest [9].

Random Amplified Polymorphic DNA (RAPD) is a molecular marker technique that has been widely used since 1990. RAPD markers were used for identifying polymorphisms and interspecific and intraspecific pepper hybrids [10,11], analyzing genetic diversity in eggplant [12], black pepper [13], banana [14], and analyzing genetic diversity [15]. The main advantages of this technique are that they are fast and does not require a previous DNA sequence. Chung et al. [16] have successfully employed RAPD to analyze the genetic diversity among 24 genotypes of *Gerbera hybrida*. Da Mata et al. [17] evaluated 42 Barterton daisy varieties based on 12 RAPD markers. The present study focuses on evaluating the genetic variability of 12 Gerbera varieties based on morphological characteristics and RAPD markers and using them in further breeding and genetic studies.

2 Materials and Methods

2.1 Materials

Twelve Gerbera varieties from farms in DaLat city (Lam Dong province) were collected including Song Hy Do (SHD), Song Hy Tim (SHT), Xanh Bo (XB), Cam Nhuy Xanh (CX), Do Nhuy Den La Nhan (DD1), Do Nhuy Den (DD2), Vang Nhuy Den (VD), Vang Nhuy Xanh (VX), Do Nhuy Xanh Gion (DX1), Do Nhuy Xanh (DX2), Hong Nhuy Den (HD), and Hong Nhuy Xanh (HX) and used for this study (Figure 1, Table 1).

2.2 Genetic diversity based on morphological characteristics

The data were collected randomized manner with 5 replications of each accession. Assessment began when the plants reached the commercial stage. Analysis of the ten quantitative parameters was performed. The measurement of the quantitative traits was performed with criteria

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such as plant height (cm): from the plant base to the apical tip, number of leaves, width of leaves (cm): the maximum width of the leaf blade, leaf length (cm): distance from the petiole- lamina junction to the leaf apex, petiole length (cm): from basal insertion point to the lamina, inflorescence diameter (mm): the maximum diameter of the capitulum, scape length (cm): from the plant base to the receptcle, ray floret length (mm): from its base at the receptacle to its tip, ray florest width (mm): the maximum width of the ligule.

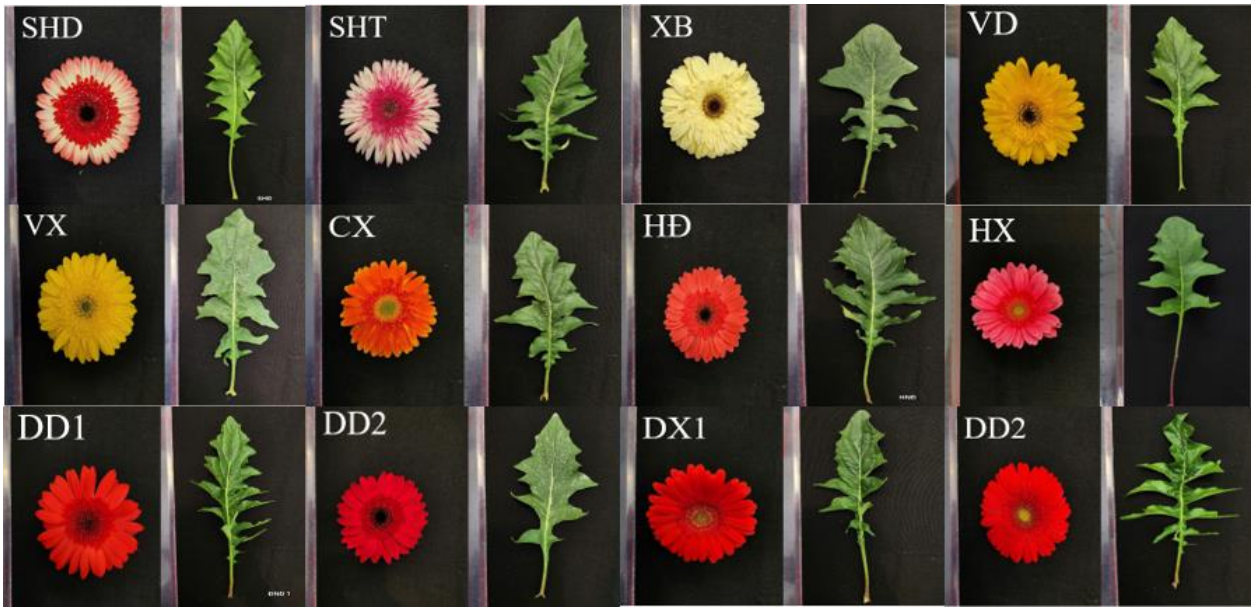

Fig. 1. Flower and leaf samples were Gerbera varieties collected from farms in Da Lat city

Table 1. Classification for 12 Gerbera accession

Varieties name	Plant code	Inflorescence type	Flower color	References
Song Hy Do (Sorbet)	SHD	Semi-double	White	[18]
Song Hy Tim (Artist)	SHT	Semi-double	White	[19]
Xanh Bo (Cream endura)	XB	Double	Yellow	[19]
Vang Nhuy Den (GoldenG.)	VD	Semi-double	Yellow	[3]
Vang Nhuy Xanh (Dana ellene)	VX	Double	Yellow	[19]
Cam Nhuy Xanh (Neoma Agrihorti)	CX	Semi double	Orange	[5]
Hong Nhuy Den (Aqua melone)	HD	Semi double	Pink	[18]
Hong Nhuy Xanh (Pink Elegance)	HX	Double	Pink	[3]
Do Nhuy Den La Nhan (Ramboginii)	DD1	Semi-double	Red	[16]
Do Nhuy Den (Stanza)	DD2	Double	Red	[18]
Do Nhuy Xanh Gion (Cariba)	DX1	Semi double	Red	[17]
Do Nhuy Xanh (Florense)	DX2	Semi-double	Red	[16]

The quantitative traits were analysed by Minitab 21. Clustering was performed using Unweighted Pair-Group Method Using an Arithmetic Average (UPGMA).

2.3 Anthocyanin content

A mortar and pestle were used to grind 0.5g flower sample which using liquid nitrogen. The fine powder was transferred into 5ml extraction solution (99 methanol: 1 HCl v/v) in order to extract anthocyanins. After 24 hours in dark conditions at 4°C, the mixture was centrifuged at 13,000 rpm for 20 mins. The supernatant was transferred to a fresh tube, and the total anthocyanin content was measured according to the method described by [20] using a spectrophotometer. The measurement of anthocyanin was conducted using the equation Q_{At} = A530 × M−1; Q_{At} = amount of At, M = fresh weight (g) of the plant material used for extraction. Extracted samples of each transformed plant were analysed in triplicate.

2.4 DNA extraction and RAPD analysis

Young leaf (0.5 g) was used to extracted following the DNA Dneasy Plant Pro kit (QIAGEN) from producer. After extraction, DNA concentration from each genotype was checked by nanodrop. The PCR reaction of RAPD was carried out in 10 µl of reaction mixture consisting 5 µl 2 × Phanta flash master mix (Vazyme), 1 µl DNA (100ng/µl), 1 µl primer RAPD (100 pmol) (Vazyme) and 3 µl distilled water. PCR amplification was performed in a DNA thermal cycler (DNA Dneasy Plant Pro kit, QIAGEN), which was programmed for initial DNA denaturation at 95°C for 5 min, followed 35 cycles of 15 seconds denaturation at 95°C, annealing at 55° – 60 °C for 40 seconds, and extension at 72°C for 5 mins, with a final extension at 72°C for 10 mins The RAPD primer sequences was showed in Table 2.

Amplified products were resolved by electrophoresis on 2% agarose gel in tris-acid acetic EDTA (TAE) buffer stained with Invitrogen™ BlueJuice™ Gel Loading Buffer (10 ×) carefully loaded a molecular ladder into the first lane, followed by plant samples. The gel was run at 100V for 90 mins, following the termination of electrophoresis the DNA bands were observed on the MultiDoc UVP system (UVP GelStudio PLUS, AnalytikJena).

Data scoring and analysis

Scoring bands were done on the basis of their presence (1) or absence (0) in the gel. The genetic associations were evaluated by calculating Jaccard's similarity coefficient for pair-wise comparisons based on the proportion of shared bands produced by primers. The similarity matrix was subjected to the cluster analysis of the unweighted pair group method with arithmetic averages (UPGMA), and a dendrogram was generated by using NTSYS-pc version 2.1 [21]. PIC (Polymorphic Information Content) value provides information on the ability of the primer to generate polymorphism between the studied accessions. It is calculated by the following formula:

PIC (i) = 1 – Σ Pij^2 [22], where Pij is the frequency of the band revealed by the jth primer. The resolving power of the primer was calculated using the formula: resolving power (Rp) = Σ Ib (band informativeness). Band informativeness was calculated for each band scored by the primer individually. Ib = 1-[2(0.5-p)], where p is the proportion of occurrence of bands in the varieties out of the total number of genotypes [23].

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Table 2. List of primers used for RAPD amplification in 12 Gerbera varieties [24].

Primers	Sequences	Tm (℃)
OPE 02	5′-GGTGCGGGAA-3′	34
OPE 08	5′-TCACCACGGT-3′	32
OPE 13	5′-CCCGATTCGG-3′	34
OPE 14	5′-TGCGGCTGAG-3′	34
OPF 18	5′-TTCCCGGGTT-3′	32
OPG 10	5′-AGGGCCGTCT-3′	34
OPG 03	5′-GAGCCCTCCA-3′	34
OPG 13	5′-CTCTCCGCCA-3′	34
OPG 16	5′-AGCGTCCTCC-3′	34
OPG 17	5′-ACGACCGACA-3′	32

3 Results and Discussion

3.1 Genetic analysis of morphological characteristics

The investigation of morphological characteristics of twelve Gerbera genotypes for flower color, inflorescence flower, plant height, number of leaves, leaf width, leaf length, petiole length, inflorescence diameter, scape length, and ray floret width is shown in Tables 3 and 4.

Flower color is a pivotal trait determining the aesthetic and commercial value of ornamental plants. Consequently, its accurate and efficient characterization is a fundamental prerequisite for successful genetic improvement and breeding programs. Based on colorimeter measurements and RHSCC, 811 chrysanthemum resources were divided into eight color groups: white, yellow, purple, light yellow, dark, red, orange, and brown [25]. In this study, the flower color of 12 Gerbera varieties is shown in Table 1. Flower color was classified into 5 groups, including white (SHD and SHT), yellow (XB, VD and VX), orange (CX), pink (HD and HX) and red (DD1, DD2, DX1 and DX2). Inflorescence types of 12 gerbera varieties were divided into 2 groups such as semi-double inflorescence (SHD, SHT, VD, CX, HD, DD1, DX1 and DX2) and double inflorescence (XB, VX, HX and DD2). Differences in inflorescence type and flower color show that the 12 Gerbera cultivars represent various genotypes.

The variation in the ten numerical morphological parameters was assessed by the coefficient of variation (CV), which ranged from 3.28% to 13.05% across all varieties. Number of leaves showed the highest variation (13.05%), while plant height, leaf length, leaf width, petiole length, flower stem length, flower diameter, petal length, petal width, and anthocyanin content showed the lower coefficient of variation (<10%). In plant breeding programs, these indicators may be selected for uniformity and consistency for large-scale breeding programs (Tables 3 and 4). For the cut flower market, XB, HX, and DX2 varieties are considered suitable for the vase flowers; while DD1 and DD2 were chosen for large flowers; DD2 and DX2 are also suitable for the long and wide petals in breeding programs.

Table 3. Plant height, number of leaves, leaf length, leaf width and petiole length of 12 Gerbera accessions

Number	Accessions	Plant height (cm)	Number of leaves (leaf)	Leaf length (cm)	Leaf width (cm)	Petiole length (cm)
1	**SHD**	34.0± 1.58^{ab}	16.2 ± 1.30^{b}	36.6 ± 1.19^{abc}	12.4 ± 1.14^{ef}	9.4 ± 0.55^{c}
2	**SHT**	28.6 ± 0.55^{de}	9.6 ± 0.89^{de}	29.2 ± 0.91^{fgh}	14.0 ± 0.79^{bcde}	5.2 ± 0.84^{f}
3	**XB**	33.4 ± 3.49^{bc}	9.8 ± 1.64^{de}	40.1 ± 2.41^{a}	16.5 ± 2.35^{a}	11.4 ± 0.89^{ab}
4	**VD**	31.4± 0.89^{bcd}	22.0 ± 1.87^{a}	31.2 ± 1.30^{efgh}	11.5 ± 0.50^{f}	10.4 ± 1.14^{bc}
5	**VX**	37.7 ± 1.04^{a}	10.4 ± 1.14^{de}	38.6 ± 1.39^{ab}	16.1 ± 0.74^{ab}	7.2 ± 0.84^{ef}
6	**CX**	17.8 ± 2.17^{h}	14.2 ± 3.19^{bc}	28.4 ± 2.61^{h}	11.4 ± 1.67^{f}	7.0 ± 1.00^{ef}
7	**HD**	26.2 ± 0.76^{ef}	12.6 ± 1.52^{bcd}	31.6 ± 2.70^{efgh}	12.6 ± 1.19^{def}	10.0 ± 1.00^{bc}
8	**HX**	26.8 ± 1.30^{ef}	15.6 ± 1.52^{bc}	29.0 ± 0.71^{gh}	11.0 ± 0.71^{f}	12.4 ± 0.55^{a}
9	**DD1**	30.7± 1.92^{bcd}	10.0 ± 1.00^{de}	33.6 ± 1.52^{cde}	15.0 ± 0.71^{abcd}	6.0 ± 1.00^{ef}
10	**DD2**	21.0 ± 1.00^{gh}	12.2 ± 1.48^{cd}	35.8 ± 0.84^{bcd}	13.2 ± 0.84^{cdef}	9.0 ± 0.71^{cd}
11	**DX1**	29.9± 2.33^{cde}	8.0 ± 1.58^{e}	32.3 ± 2.17^{defg}	15.2 ± 0.91^{abc}	6.2 ± 0.84^{ef}
12	**DX2**	23.2 ± 1.92^{fg}	9.8 ± 1.64^{de}	32.9 ± 0.89^{cdef}	11.5 ± 0.79^{f}	10.2 ± 0.45^{bc}
Mean		**28.4**	**12.5**	**33.3**	**12.5**	**8.7**
Standard deviation		**5.76**	**4.09**	**3.98**	**2.14**	**2.37**
Minimum		**17.8**	**8.0**	**28.4**	**11.4**	**5.2**
Maximum		**37.7**	22.0	40.1	16.5	12.4
CV (%)		**5.81**	**13.05**	**4.72**	**7.71**	**10.29**

Different letters indicate a statistically significant difference ($p<0.05$). ± standard deviation.

Table 4. Flower stem length, flower diameter, petal length, petal width and anthocyanin content of 12 Gerbera accessions

Number	Accessions	Flower stem length (cm)	Flower diameter (mm)	Petal length (mm)	Petal width (mm)	Anthocyanin content (g/FW)
1	**SHD**	50.2 ± 1.30^{de}	10.9 ± 0.65^{abc}	46.0 ± 2.24^{ab}	9.8 ± 0.45^{abc}	2.9 ± 0.07^{e}
2	**SHT**	42.9 ± 0.74^{fg}	11.3 ± 0.30^{ab}	42.0 ± 2.74^{ab}	8.2 ± 0.45^{c}	1.1 ± 0.15^{f}
3	**XB**	61.6 ± 0.90^{a}	10.8 ± 0.45^{abc}	40.0 ± 0.00^{bc}	10.0 ± 0.00^{abc}	1.1 ± 0.05^{f}
4	**VD**	49.4 ± 1.67^{de}	9.7 ± 0.34^{cd}	34.4 ± 2.61^{c}	11.2 ± 1.10^{ab}	1.4 ± 0.05^{f}
5	**VX**	51.7 ± 1.57^{cd}	11.3 ± 0.67^{ab}	41.0 ± 4.18^{bc}	11.0 ± 2.24^{ab}	1.5 ± 0.12^{f}
6	**CX**	42.5 ± 2.83^{g}	10.6 ± 0.22^{bcd}	44.0 ± 5.48^{ab}	10.0 ± 0.00^{abc}	3.4 ± 0.05^{e}
7	**HD**	44.8 ± 1.30^{fg}	9.4 ± 0.65^{d}	34.2 ± 2.39^{c}	9.8 ± 0.45^{abc}	2.7 ± 0.06^{e}
8	**HX**	56.0± 2.12^{ab}	9.8 ± 0.27^{cd}	44.0 ± 1.00^{ab}	8.6 ± 0.55^{c}	2.8 ± 0.02^{e}
9	**DD1**	46.6 ± 1.52^{ef}	12.0 ± 0.61^{a}	45.0 ± 5.00^{ab}	10.0 ± 0.00^{abc}	11.6 ± 0.25^{d}
10	**DD2**	50.8 ± 2.17^{cd}	11.3 ± 1.20^{ab}	49.0 ± 4.18^{a}	11.4 ± 1.34^{a}	33.6 ± 0.69^{a}
11	**DX1**	44.6 ± 0.96^{fg}	11.2 ± 0.76^{ab}	41.0 ± 2.24^{bc}	10.0 ± 0.00^{abc}	25.3 ± 0.96^{b}

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Number	Accessions	Flower stem length (cm)	Flower diameter (mm)	Petal length (mm)	Petal width (mm)	Anthocyanin content (g/FW)
12	**DX2**	54.1 ± 2.25^{bc}	10.8 ± 0.27^{abc}	47.0 ± 2.74^{ab}	9.4 ± 0.89^{bc}	21.2 ± 0.33^{c}
Mean		**49.6**	**10.8**	**39.8**	**10.0**	**9.05**
Standard deviation		**5.77**	**0.92**	**0.53**	**0.12**	**0.21**
Minimum		**42.5**	**9.4**	**34.2**	**8.6**	**1.5**
Maximum		**61.6**	**12.0**	**49.0**	**11.4**	**33.6**
CV (%)		**3.28**	**4.92**	**6.82**	**6.03**	**3.84**

Different letters indicate a statistically significant difference ($p<0.05$). ± standard deviation.

Flower color is one of the vital criteria in classifying flower varieties. The differences in flower colors are due to various pigments, which are classified into three main groups: carotenoids, flavonoids, and betalains [26]. From varieties to pigment analysis, understanding the pigment in flowers is useful for plant breeders. A few experiments were conducted to evaluate genetic diversity using pigment colors. Anthocyanin content ranged from 1.12 to 11.58, with an average of 9.03. Anthocyanin content in the red group was highest (11.6-33.6 g/FW) for DD1, DX2, DX1, and DD2; following orange (3.4 g/FW for CX); and pink (2.7 g/FW for HD and 2.8 g/FW for HX). Likewise, anthocyanin content in the white and yellow groups was the least (1.1 -1.5 g/FW). The color variety of gerbera is attributed to both anthocyanins and carotenoids [28]. In some Gerbera cultivars, total anthocyanin was identified as a key factor influencing the color transition from yellow to orange [27]. Chen et al. [28], analysed the flower color of ten Gerbera varieties through microscopic observation, and colorimetric analysis. The results indicated that anthocyanin serves as the principal pigments in red, purple, and orange-red flowers. Our results were the same as a previous study by Zhou Y et al. [27], who reported that the highest anthocyanin content was found in red flowers (49.9 g/FW), followed by purple flowers (24.5 g/FW) and orange flowers (10.8 g/FW). This finding will provide insights into Grebera color characteristics, which will assist in screening breeding materials with execellent flower color characteristics. The results also indicated that DD2, DX1, and DX2 genotypes are suitable for selecting color varieties.

The genetic variation of 12 Gerbera varieties assessed based on 10 criteria is shown in Table 5. The results showed that genetic similarity coefficients ranged from 0.95 to 1.0, indicating a very high level of similarity in the comparative data. Figure 2 presents a dendrogram illustrating genetic relationships among 12 varieties of Gerbera, which are separates into three primary clusters. Cluster I is the larger of the two main clusters, containing eight accessions: SHT, SHD, DX2, DX1, DD1, DD2, VD, and VX. This group represents a broad collection of related varieties. Within cluster I, DD1 and DD2 genotypes are extremely similar because they are very close (0.00); SHD - DX1 and VD-VX also show high genetic similarity; while SHT and DX2 showed moderate similarity, but more distant from the other members of group I. For cluster II, this is a smaller group, containing three accessions CX, HD and HX, which are more closely related to each other than they are to any variety in group I. Within cluster II, the varieties CX and HD are the most related pair in this group. Cluster III comprised only one accession from the XB varieties.

Table 5. Genetic similarity values of 12 Gerbera varieties on the Jaccard's coefficient based on the morphological traits data.

Varieties	SHT	SHD	XB	CX	VD	VX	DD1	DD2	DX1	DX2	HD	HX
SHT	1.00											
SHD	0.99	1.00										
XB	0.99	0.97	1.00									
CX	0.99	0.99	0.96	1.00								
VD	0.99	0.99	0.98	0.99	1.00							
VX	1.00	0.99	0.98	0.99	1.00	1.00						
DD1	0.99	0.99	0.98	0.99	0.99	1.00	1.00					
DD2	0.99	0.99	0.98	0.99	1.00	1.00	1.00	1.00				
DX1	0.99	1.00	0.97	0.99	0.99	0.99	1.00	1.00	1.00			
DX2	0.99	0.99	0.99	0.99	0.99	0.99	0.99	1.00	0.99	1.00		
HD	0.99	0.98	0.95	1.00	0.99	0.99	0.99	0.98	0.99	0.98	1.00	
HX	0.99	0.99	096	0.99	0.99	0.99	0.99	0.99	0.99	0.98	0.99	1.00

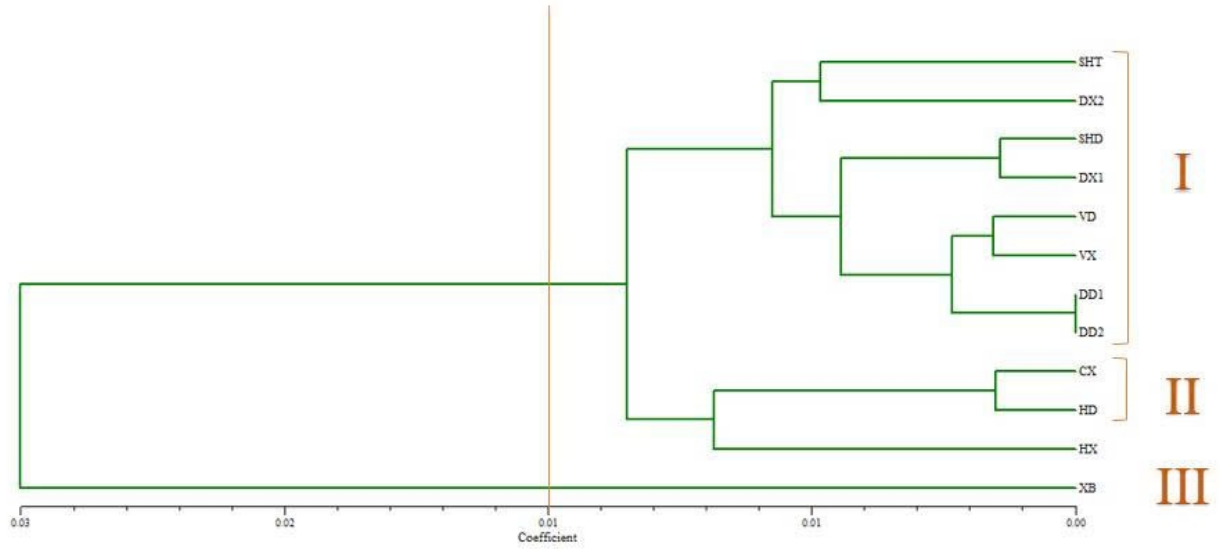

Fig. 2. Dendrogram illustrating genetic relationships among 12 varieties of Gerbera varieties collected from DaLat farms. The dendrogram was generated by UPGMA cluster analysis calculated from morphological trait

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There are many studies on evaluating genetic diversity based on agromorphological characteristics and phylogenetic relationships between species have been demonstrated. Sudré et al. [29] used multivariate techniques to evaluate the genetic divergence among 56 accessions of "chili" and sweet pepper. The results were possible to separate the accessions into eight distinct groups, indicating that there is genetic variability for the evaluated traits. Bertini et al. [30] studied the genetic divergence of five coriander genotypes (*Coriandrum sativum* L.) and successfully used multi-categorical variables in the discrimination of genotypes. Amorin et al. [31] confirmed the genetic divergence of fifteen sunflower accessions using twelve agronomical characteristics. These examples provide evidence for the efficiency of multivariate analysis in the genetic discrimination of individuals and clustering; thus, homogeneity can be exhibited within groups, and heterogeneity can be exhibited between groups. However, a critical limitation of the assessment of genetic divergence based on morphological and phenological traits is time-consuming and may be affected by environmental factors. Therefore, molecular markers provided a more efficient and reliable alternative for studying genetic variability

3.2 Genetic diversity based on RAPD markers

To detect polymorphism, ten random decamer primers were used for RAPD analysis of different genotypes. Out of ten random primers screened, one primer did not show any amplification for any of the genotypes (OPF18). Almost all varieties amplified were more than ten by all primers. The DNA amplification and polymorphism generated among different varieties of 12 Gerbera using random primers are presented in Figure 3 and Table 6.

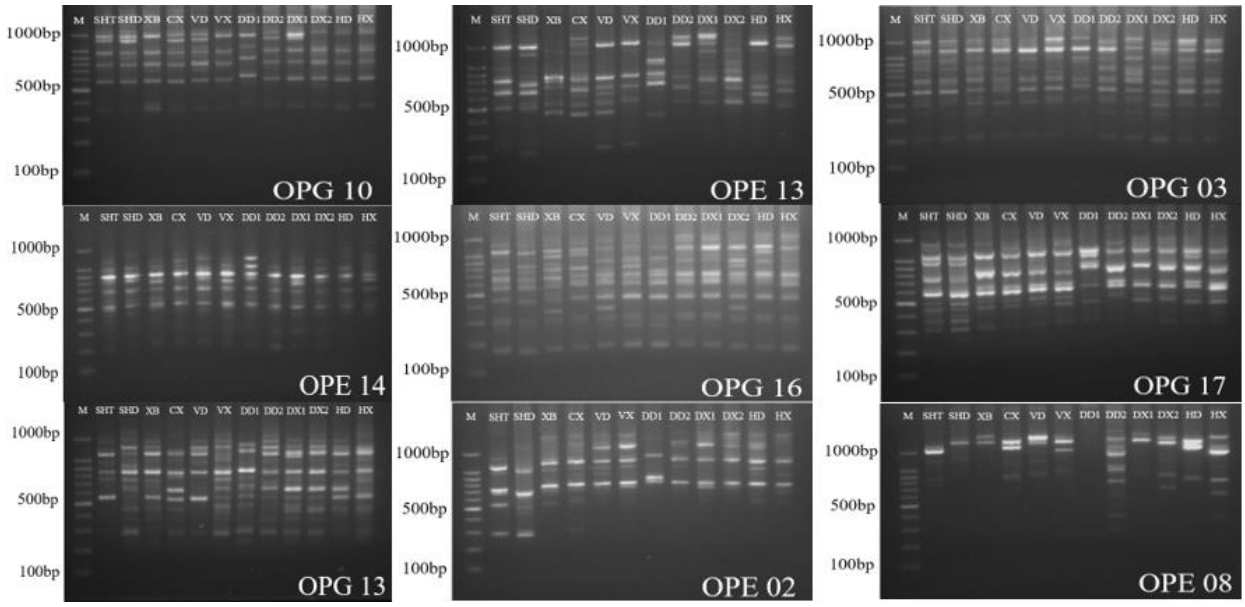

Fig. 3. RAPD bands generated by RAPD primers with their polymorphisms. M: 1kb ladder (Gelpilot, QIAGEN)

Nine RAPD primers produced distinct, clearly visible polymorphisms and were thus used for the genetic relationship analysis. A total of 135 bands were generated from 09 primers, 12 to 17 bands were generated by different primers, with an average of 15 bands per primer. The highest

number of bands (17) was amplified by primer OPG13 and OPG16, respectively. Out of 110 bands were polymorphic, while 25 bands were monomorphic, resulting in 82.4% polymorphism among the varieties. The percent of polymorphism ranged from 52.9% to 100% for the 12 varieties of Gerbera; the maximum level of polymorphism was 100% produced by OPE02, OPE08, and OPE13. The minimum level of monomorphism was 52.9% produced by OPG16 (Table 6). For RAPD markers, the (PIC) values ranged from 0.38 to 0.5, and the mean of PIC per primer for polymorphic bands was 0.48. The highest PIC value (0.5) was observed with primer OPE14, OPG10, and OPG17, respectively. The lowest PIC value (0.38) was found with primer OPE08 (Table 6). In RAPD marker analysis, the PIC value is a vital metric for evaluating and selecting primers. This value reflects the diversity and frequency of alleles among the samples. A higher PIC value is considered a more effective primer for analyzing genetic variation [32]. Resolution power (Rp) analysis is used to determine the effectiveness of the primer in producing bands. Each primer has a value of Rp, which ranged from 6.5 to 14.67 with an average of 11.39 per primer. The highest Rp value was generated by the OPG13 primer with a value of 14.67, while the lowest value was observed in OPE08 with a value of 6.5 (Table 6). Based on the overall polymorphism parameters analysed, the most effective primers in producing polymorphic bands in Gerbera genotypes were OPE02, OPE13, OPG13, and OPG17.

Table 6. Amplified DNA bands and polymorphism generated in 12 accession of *Gerbera* L. using 9 RAPD markers

STT	Primers	Total number of amplified fragments	Number of polymorphic bands	Number of polymorphic bands	Percentage of polymorphic loci (%)	PIC	Resolving power (Rp)
1	OPE 02	15	15	0	100.0	0.49	13.17
2	OPE 08	13	13	0	100.0	0.38	6.50
3	OPE 13	16	16	0	100.0	0.49	14.00
4	OPE 14	14	10	4	71.4	0.50	10.50
5	OPG 10	12	11	1	91.7	0.50	10.83
6	OPG 03	16	11	5	68.8	0.49	12.17
7	OPG 13	17	13	4	76.5	0.49	14.67
8	OPG 16	17	9	8	52.9	0.49	7.50
9	OPG 17	15	12	3	80.0	0.50	13.17
Total		**135**	**110**	**25**			
Average		**15**	**12.22**		**82.4**	**0.49**	**11.39**

Cluster analysis

The average linkage between the varieties was used for constructing a phylogenetic tree. The relationship among the 12 Gerbera varieties used during the present investigation is presented in Figure 4. The hierarchical cluster analysis identified four major clusters at a similarity coefficient of 0.32. Cluster I comprised two genotypes, SHT and SHD with a low similarity value (0.2). This means both genotypes have close relationship. Whereas eight varieties (XB, VX, DX2, DX1, DD2, CX, HD, and VD) at a similarity coefficient of 0.29 were divided into two

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subclusters. Subcluster I included five varieties (XB, VX, DX2, DX1, and DD2), they again separated into 3 groups, and VX and DX2 were close to each other. Subcluster II comprised CX, HD, and VD varieties, where CX and GD have a closed relationship. Cluster III comprised HX. Varieties DD1 were the out-group.

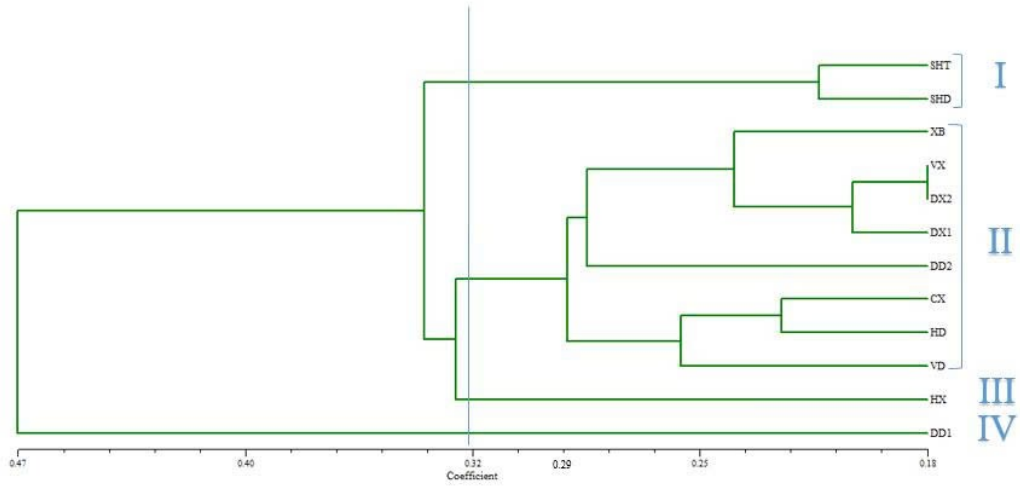

Fig. 4. Dendrogram illustrating genetic relationships among 12 varieties of Gerbera varieties collected from farms in Dalat city (Lam Dong province). The dendrogram was generated by UPGMA cluster analysis calculated from 135 RAPD bands produced by 9 RAPD primers.

Similarity Matrices

Similarity matrix of 12 Gerbera varieties revealed the relationship among them (Table 7). The similarity indices between various accessions ranged from 0.46 to 0.82. A maximum similarity value of 0.82 was found at DX2 and VX varieties, whereas HX and DD1 varieties were observed to be genetically most diverse with a similarity value of 0.46.

Table 7. Genetic similarity values of 12 Gerbera varieties on the Jaccard's coefficient based on the RAPD data.

Varieties	SHT	SHD	XB	CX	VD	VX	DD1	DD2	DX1	DX2	HD	HX
SHT	1.00											
SHD	0.79	1.00										
XB	0.64	0.67	1.00									
CX	0.67	0.69	0.67	1.00								
VD	0.66	0.65	0.72	0.77	1.00							
VX	0.68	0.75	0.80	0.67	0.73	1.00						
DD1	0.50	0.63	0.52	0.48	0.55	0.61	1.00					
DD2	0.64	0.63	0.62	0.68	0.69	0.75	0.47	1.00				
DX1	0.70	0.69	0.77	0.71	0.75	0.81	0.55	0.74	1.00			
DX2	0.62	0.74	0.71	0.67	0.70	0.82	0.56	0.74	0.78	1.00		
HD	0.55	0.67	0.72	0.78	0.71	0.73	0.48	0.69	0.72	0.75	1.00	
HX	0.60	0.66	0.64	0.68	0.65	0.67	0.46	0.60	0.66	0.69	0.77	1.00

The analysis of morphological traits is a foundational step and cost-effective method for plant breeding programs. However, molecular analysis provides an essential tool for quantifying genetic variability within and between genotypes and for elucidating the geographical and environmental effects on phenotype. Furthermore, molecular data are useful for validating morphological models. Da Mata et al. [17] and Rezende et al. [33] used RAPD markers for the genetic diversity analysis, whereas de Pinho et al. [3] and Gong and Deng [34] used EST-SSR markers to analyse the genetic variability for Gerbera. Priyanka Prajapati et al. [24] analysed 12 Gerbera genotypes (*Gerbera jamesonii* Bolus ex by RAPD markers to evaluate genetic divergence. Consequently, the combination of molecular and morphological analyses is a complementary strategy in the characterization of Gerbera accessions and is valid for identifying novel cultivars. According to D'Imperio et al. [35], the two methods are incomplete because the molecular data are less relevant for the identification of cultivars by farmers and agronomists compared to phenotypic data. In contrast, morphological data are incomplete without a determination of the molecular basis of a trait, given the influence of environmental pressures on phenotypic expression.

4 Conclusion

Based on phenotypic evaluation, it is possible to conclude the following: depending on the demands of consumers, 12 Gerbera varieties were divided into different groups such as DD2 and DX1 for bright colors; large flowers and width petal; XB, HX and DX2 for cut flowers; the vaireties DD1 and DD2 for large flowers; DD2 and DX2 for flower size breeding. This result indicated that RAPD showed HX, and DD1 have different genetic characteristics, compared to XB, DX2, DX1, DD2, CX, HD, and VD varieties. The combination of agromorphological and molecular analyses helps to better distinguish the genetic diversity of species, providing a more comprehensive and in-depth understanding of genetic variation within a population or between different individuals in groups.

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Declarations

Conflict of interest all authors have no interests to declare.

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