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ARTICLE

Morphological and SRAP-based genetic diversity analysis of *Zinnia elegans* Jacq. accessions

Análise morfológica e da diversidade genética baseada em SRAP de acessos de *Zinnia elegans* Jacq.

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Abstract
Zinnia elegans Jacq. is ornamental plant commonly cultivated in Indonesia. This species has various flower shapes and colors and is easy to be cultivated in any environmental conditions. This study aimed to determine the genetic diversity of 18 *Z. elegans* accessions based on morphological and SRAP molecular markers. The research was arranged a completely randomized design (CRD) with 18 accessions as treatments and three replications within each treatment. SRAP analysis was performed using five primer combinations of C1 (Me6-Em6), C2 (Me8-Em4), C3 (Me8-Em7), C4 (Me9-Em4), and C5 (Me10-Em4). Meanwhile, *Z. elegans* descriptor from UPOV was used in morphological analysis for quantitative and qualitative traits. The result showed a dendrogram with two main branches of group A and B for morphological traits and SRAP markers with a similarity coefficient of 0.18-0.78 and 0.46-0.80, respectively. SRAP markers significantly correlated with six morphological traits, such as ray floret apex (RFA), ray floret length (RFL), leaf length (LL), leaf width (LW), stem length (SL), and ray floret width (RFW). Five morphological traits that had high GCV and strong SRAP correlation were prioritized for selection. Five SRAP combination primers can be used for molecular marker resources of Zinnia breeding.
Keywords: molecular marker, ornamental plant, phylogeny, variability, accession.

Resumo
Zinnia elegans Jacq. é uma planta ornamental comumente cultivada na Indonésia. A espécie possui vários formatos e cores de flores e é fácil de ser cultivada em quaisquer condições ambientais. Este estudo teve como objetivo determinar a diversidade genética de 18 acessos de *Z. elegans* com base em marcadores morfológicos e moleculares SRAP. A pesquisa foi organizada em um delineamento inteiramente casualizado (CRD) com 18 acessos como tratamentos e três repetições dentro de cada tratamento. A análise SRAP foi realizada usando cinco combinações de primers de C1 (Me6-Em6), C2 (Me8-Em4), C3 (Me8-Em7), C4 (Me9-Em4) e C5 (Me10-Em4). Enquanto isso, o descritor *Z. elegans* da UPOV foi usado na análise morfológica para características quantitativas e qualitativas. O resultado mostrou um dendrograma com dois ramos principais do grupo A e B para características morfológicas e marcadores SRAP com um coeficiente de similaridade de 0,18-0,78 e 0,46-0,80, respectivamente. Os marcadores SRAP correlacionaram-se significativamente com seis características morfológicas, como ápice do florete radial (RFA), comprimento do florete radial (RFL), comprimento da folha (LL), largura da folha (LW), comprimento do caule (SL) e largura do florete radial (RFW). Cinco características morfológicas que apresentaram alto VGC e forte correlação com SRAP foram priorizadas para seleção. Cinco primers de combinação SRAP podem ser usados como marcadores moleculares para o melhoramento de zínias.
Palavras-chave: marcador molecular, planta ornamental, filogenia, variabilidade, acesso.

Introduction

Zinnia elegans Jacq. is one of ornamental plants that grow a lot in Indonesia and is known as *'kembang kertas'*. This species is known as ornamental plant with various flower colors and easy to grow in all environment conditions (Pongoh and Paat, 2022). *Z. elegans* has economic value as an ornamental plant, grown as bedding, cut flowers and flowering potted plant (Sharaf-Eldien et al., 2017). Subgenus *Zinnia* L. divided into some species, including *Z. peruviana, Z. elegans, Z. haageana,* and *Z. angustifolia.* Plant breeders in Europe intensively selected and crossed the species to produce a variety of cultivars with various colors, shapes, and flower sizes (Song et al., 2025). The variation of *Z. elegans* can also help scientists understand its adaptability or tolerance toward environmental stress that has posed considerable emphasis in line with climate change and sustainable agricultural practices (Toscano and Romano, 2021).

Z. elegans has numerous morphological traits that are influenced by cultivation methods and environmental factors (Martins et al., 2021); therefore, molecular approach is needed to determine plant diversity. The benefit of using molecular markers is to determine genetic diversity and population genetics, which is important for breeding program, conservation, protection, and the introduction of endangered and important plant species. Among the many types available, such as SSR (Simple Sequence Repeat) and InDel (Insertion/Deletion) markers, each has its own strengths and limitations. In this study, we chose to use SRAP (Sequence-Related Amplified Polymorphism) markers based on both practical and technical considerations.

SRAP markers were specifically developed to target coding regions in the genome, which are the area most likely to influence important agronomic and morphological traits. Unlike SSR or InDel markers, which usually require prior genomic information and custom primer design, SRAP markers can be used without any sequence information. This makes them particularly suitable for ornamental species like *Z. elegans*, where genomic resources are often still limited. SRAP markers target open reading frames in the genome and have some advantages, such as the reproducibility, cost effectiveness, and no requirement for prior sequence information, providing an appropriate tool for assessing genetic diversity in different plant species (Bidyananda et al., 2024). SRAP markers have proven to be highly polymorphic and effective in capturing meaningful genetic variation.

Molecular approaches have a correlation with some morphological traits; there are several previous studies indicating a correlation between SRAP markers and morphological traits. SRAP markers effectively identify associations between quantitative traits in watermelon genotypes (AbdoliNasab and Ramini, 2020), corelated to agronomical traits in *Nigella sativa* (Golkar and Nourbakhsh, 2009), yield of F1 crosses in

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eggplant (Annepu et al., 2023), genetic investigations of Cyclamen germplasm (Cornea-Cipcigan et al., 2023), *Nelumbo nucifera* Gaertn. Cultivars (Nakkuntod and Luanglue, 2024), and genetic diversity among *Dianthus* accessions (Fu et al., 2008). Considering these advantages, SRAP provided a practical and reliable method for exploring genetic relationships in our study material.

The first stage of the *Z. elegans* breeding program for ornamental purposes is the characterization of many accessions to get essential data regarding the genetic diversity of *Z. elegans,* which is crucial for the successful conservation and management of this species. Analysis of genetic diversity using morphological and SRAP marker will contribute to breeding programs for new cultivars with favorable traits and improved commercial quality and performance of *Z. elegans*. In addition to expanding the current knowledge, this study provides a point of reference for future studies involving other ornamental species, contributes to floral biodiversity conservation, and promotes floral biodiversity. This study aimed to determine the genetic diversity of 18 *Z. elegans* accessions based on morphological and SRAP molecular markers.

Material and Methods

The research was conducted at CV Tani Organik Merapi (TOM), Yogyakarta, Indonesia for accession source and the Plant Breeding Laboratory, Department of Agronomy, Universitas Gadjah Mada for molecular analysis. The research was carried out from August 20 to November 10, 2024. The materials of this research were eighteen accessions of *Z. elegans* (Fig. 1) consisting of six flower color group (white group (A1-A2), yellow group (A3-A5), orange group (A6-A7), red group (A8-A10), purple group (A11-A12), and red-purple group (A13-A18) based on RHS color chart. The variables observed in this research were quantitative and qualitative traits. The quantitative traits include leaf length, leaf width, flower head diameter, stem length, number of ray floret, ray floret length, ray floret width, and disc diameter. The qualitative traits including some sub-traits consist of leaf undulation (absent, medium, strong), leaf area of anthocyanin (absent, medium, large), leaf length/width ratio (very low, medium, very high), flower head type (single, semi-double, double), ray floret curvature (incurving, straight, reflexing), ray floret curved axis (distal quarter, distal half), ray floret apex (acute, rounded, truncate, emarginate, dentate), ray floret color based on RHS color chart, and disc color based on RHS color chart (UPOV, 2022). Thus, a total of 17 traits were evaluated using UPOV descriptor for *Z. elegans* (UPOV, 2022). Each variable was observed from three different plants that had the same flower color and flower head type. All accessions were observed at the same time.

DNA extraction was carried out using CTAB method (Doyle and Doyle, 1990) by isolating fresh and healthy leaf. The DNA palette was diluted with deionized distilled water for the amplification procedure. A total of 10 primer combinations were prescreened using DNA samples of three accessions with different flower color. Out of the 10 primer combinations, five primer combinations that generated clear polymorphic and reproducible bands were selected to evaluate polymorphism in 54 DNA samples of 18 accession. All SRAP primers used in this study were universal primers originally designed by Li and Quiros (2001) that have been widely applied in genetic diversity studies across plant species. Each SRAP primer consists of forward primer (Me-series) and a reverse primer (Em-series). Details of five primer combinations are shown in Table 1 (Maulani et al., 2023).

The annealing temperature was optimized within the range of 35 °C to 50 °C, after which the temperature producing polymorphic bands was chosen as the annealing temperature. DNA amplification was conducted using PCR (Thermal Cycler BIORAD). The amplification reaction stage was pre-denaturation at 94 °C for 5 min; followed by 5 cycles of denaturation at 94 °C for 1 min, annealing at 35 °C for 1 min, extension at 72 °C for 1 min; then 35 cycles of denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min, and extension at 72 °C for 1 min; concluding with a final extension at 72 °C for 8 min. Gel electrophoresis was conducted at 100 volts for 60 minutes with 1.5% agarose gel in TBE buffer, observed under UV light, and images were recorded with a digital camera. The bands were assigned binary value, with 1 indicating amplification and 0 indicating no amplification.

Table 1. SRAP primers used in this research

Code	Primer combinations	Sequence	
		Forward	Reverse
MC1	Me6-Em6	5'-TGAGTCCAAACCGGACA-3'	5'-GACTGCGTACGAATTGCA-3'
MC2	Me8-Em4	5'-TGAGTCCAAACCGGACT-3'	5'-GACTGCGTACGAATTTGA-3'
MC3	Me8-Em7	5'-TGAGTCCAAACCGGACT-3'	5'-GACTGCGTACGAATTCAA-3'
MC4	Me9-Em4	5'-TGAGTCCAAACCGGAGG-3'	5'-GACTGCGTACGAATTTGA-3'
MC5	Me10-Em4	5'-TGAGTCCAAACCGGAAA-3'	5'-GACTGCGTACGAATTTGA-3'

The research was arranged in a completely randomized design (CRD) including 18 accessions with three replications within each accession. 18 accessions were grouped based on flower head color, namely white group (A1, A2), yellow group (A3, A4, A5), orange group (A6, A7), red group (A8, A9, A10), purple group (A11, A12), and red-purple group (A13, A14, A15, A16, A17, A18). The sample plants for each accession were selected based on uniform growth criteria consisting of the second to fourth flower with the same flower head diameter. The data collected were analyzed using NTSYSpc version 2.10e software for dendrogram of similarity; iMEC (an online marker efficiency calculator) for polymorphic information content (PIC), effective multiplex ratio (E), resolving power (R), discriminating power (D), and heterozygosity index (H) (Amiryousefi et al., 2018); GenAlEx 6.51b2 software for PCA and AMOVA; and R Studio for correlation heatmap; and ANOVA.

An Analysis of variance was conducted using F-test to examine statistically significant differences across genotypes for quantitative traits. Duncan's multiple range test with $p<0.05$ was utilized to assess genotypic variations. To estimate genetic variability between accessions, genotypic variance (σ^2g) and phenotypic variance (σ^2p) were calculated following the method described by Syukur et al. (2015). The formulas used were as follows:

1. **Genotypic variance (σ^2g)** = $\frac{MSg - MSe}{r}$
2. **Environmental variance (σ^2e)** = MSe
3. **Phenotypic variance (σ^2p)** = $\sigma^2g + \sigma^2e$
4. **Genotypic coefficient of variation (GCV)** = $\left(\frac{\sqrt{\sigma^2g}}{\bar{x}}\right) \times 100$
5. **Phenotypic coefficient of variation (PCV)** = $\left(\frac{\sqrt{\sigma^2p}}{\bar{x}}\right) \times 100$

Remarks:
MSg: the genotypic mean square
MSe: the error mean square
R: replication
$\bar{x}$: the mean of the evaluated trait

Variability was interpreted based on the classification by Deshmukh et al. (1986), as cited in Maretta et al. (2020), where both GCV and PCV values are categorized into high (> 20%), medium (10% to 20%), and low (< 10%).

Results and Discussion

Plant diversity lays the foundation for breeding programs, providing genetic variability necessary to enhance crop resilience, yield, and

quality as well as adaptability to changing environmental conditions (Hassani et al., 2020). The purpose of genetic diversity conservation is preserving unique traits within species that can be used for developing new hybrid (Golkar and Nourbakhsh, 2019). Targeting the conservation of plant biodiversity through unique ornamental and ecological plants has prioritized identifying and conserving genetic variability, such as in special examples like *Z. elegans* (Wu et al., 2019). The materials of this research were 18 accessions of *Z. elegans* (Fig. 1) that had variability on morphological traits. Morphological traits are divided into quantitative and qualitative traits. There are eight quantitative traits and nine qualitative traits (UPOV, 2022).

Fig. 1. Flower phenotypic of 18 *Z. elegans* accessions.
Note: A1-A2 white group; A3-A5 yellow group; A6-A7 orange group; A8-A10 red group; A11-A12 purple group; A13-A18 red-purple group (based on RHS color chart)

Variability of eight quantitative traits in 18 *Z. elegans* accessions was indicated by the GCV and PCV criteria (Table 2). Five quantitative traits had high criteria for GCV, including stem length, leaf length, leaf width, number of ray florets, and ray floret width. Flower head diameter contributed to a low GCV, being the lowest between all traits. The PCV criteria are identical to the GCV criteria for all traits. High genetic diversity enhances the probability of successful selection methods for plant characters, since it gives a selection in plant breeding through a higher frequency of genes. High PCV and GCV criteria for quantitative traits could be used for the selection program (Maretta et al., 2020). Sleper and Poehlman (2006) indicate that the efficiency of selection is dependent upon the within-plant variance of a population and the between-plant variation is influenced by the environmental factors. Morphological traits are partially related to variance resulting from environmental factor. The absence of morphological markers for assessing diversity requires supplementary markers, such as SRAP genetic markers, to enhance the investigation of species diversity.

Table 2. Variability of quantitative character in 18 *Z. elegans* accessions.

Trait	MS	σ^2p	σ^2g	σ^2e	GCV	GCV Criteria	PCV	PCV Criteria
Stem Length	63.21	21.11	21.05	0.06	55.27	High	55.35	High
Leaf Length	10.35	3.50	3.42	0.08	21.34	High	21.58	High
Leaf Width	5.49	1.89	1.80	0.09	33.44	High	34.29	High
Flower Head Diameter	105.29	35.17	35.06	0.11	9.97	Low	9.99	Low
Number of Ray Florets	9069.92	3026.36	3021.78	4.58	111.43	High	111.51	High
Ray Floret Length	9.40	3.21	3.09	0.12	11.34	Medium	11.55	Medium
Ray Floret Width	0.26	0.10	0.08	0.02	27.94	High	31.14	High
Disc Diameter	9.25	3.11	3.07	0.03	11.30	Medium	11.36	Medium

Note: MS = Mean Square; σ^2p = Phenotypic Variance; σ^2g = Genetic Variance; σ^2e = Environmental Variance; GCV = Genotypic Coefficient of Variance; PCV = Phenotypic Coefficient of Variance; GCV and PCV Criteria = High (> 20%), Medium (10% to 20%), Low (< 10%) (Deshmukh et al. (1986); (Maretta et al., 2020)).

The results of the diversity analysis on qualitative traits are presented in Table 3, showing the proportion of each sub-trait. There are variabilities in three leaf traits and six floral traits. Each trait has several sub-traits described according to UPOV. The existence of proportions for several sub-traits indicates that there are variabilities in these characters. Based on qualitative trait characterization, 72.22% of accessions have moderate leaf undulation, 55.56% of accessions do not have leaf area of anthocyanin coloration at base, and 61.11% of accessions have moderate leaf length/width ratio. Based on flower parts, 61.11% of accessions have single flower head type, 44.44% of accessions have straight ray floret curvature, 77.78% of accessions have distal quarter ray floret curved axis, 38.89% of accessions have dentate ray floret apex, and 33.33% of accessions have red-purple group color of ray floret and disc. This shows that these traits can be used to identify diversity in *Z. elegans* accessions.

Table 3. Variability of qualitative character in 18 *Z. elegans* accessions.

Character	Sub-character	Proportion (%)	Character	Sub-character	Proportion (%)
Leaf Undulation	1. Absent	22.22	Ray Floret Apex	1. Acute	5.56
	2. Medium	72.22		2. Rounded	11.11
	3. Strong	5.56		3. Truncate	11.11
Leaf Area of Anthocyanin	1. Absent	55.56		4. Emarginate	33.33
	2. Medium	22.22		5. Dentate	38.89
	3. Large	22.22	Flower Color	1. White Group	11.11
Leaf Length / Width Ratio	1. Very low	22.22		2. Yellow Group	16.67
	2. Medium	61.11		3. Orange Group	11.11
	3. Very high	16.67		4. Red Group	16.67
Flower Head Type	1.Single	61.11		5. Purple Group	11.11
	2. Semi-double	27.78		6. Red-Purple Group	33.33
	3. Double	11.11	Disc Color	1.Yellow Group	27.78
Ray Floret Curvature	1. Incurving	38.89		2. Orange Group	11.11
	2. Straight	44.44		3. Red Group	16.67
	3. Reflexing	16.67		4. Purple Group	11.11
Ray Floret Curved Axis	1. Distal quarter	77.78		5. Red-Purple Group	33.33
	2. Distal half	22.22			

The dendrogram shown represents a hierarchical clustering analysis on the coefficient of similarity of eighteen accessions based on qualitative traits (Fig. 2). Morphological data grouped the accessions into two clusters (A and B), displaying a similar but not identical pattern to the SRAP-based genetic dendrogram. The values for the coefficient of similarity between samples were plotted along the x-axis in the range of 0.18 to 0.78, indicating the difference in such magnitude between the samples on phenotypic level. Cluster A consists of sixteen accessions, indicating a higher degree of similarity between them, while distinctly separating from Cluster B (B1 and B2). Dendrogram shows a genetic difference from Cluster A. A1, A3, and A5 had high similarity, with coefficient of 0.78, meaning that 78% qualitative traits of those accessions were same. The difference between the three accessions is in the ray floret color and the disc color, which were observed using the RHS color chart. The ray floret color of A1, A3, and A5 is grouped in the white group 155A, yellow group 3D, and yellow group 7A, respectively. Meanwhile, the disc color of A1, A3, and A5 is grouped in the yellow group 150D, yellow group 3B, and yellow group 13A, consecutively.

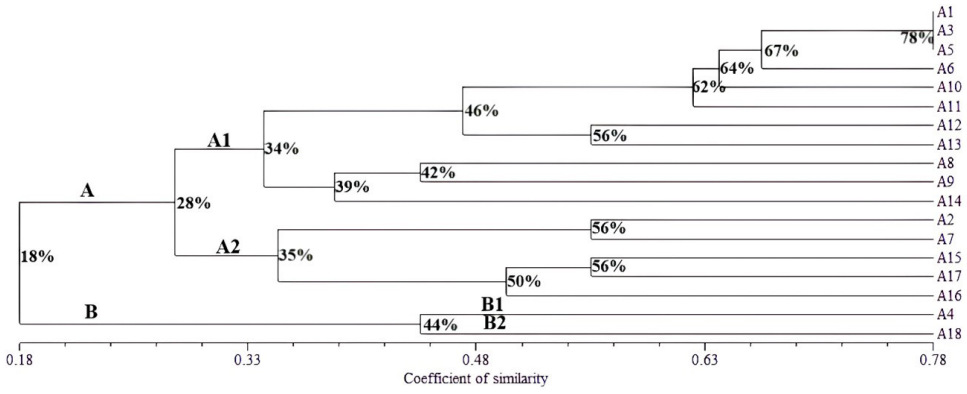

Fig. 2. Dendrogram of Clustering 18 *Z. elegans* Accessions Based on Qualitative Characters

Hierarchical clustering analysis, frequently visualized in dendrograms, has become a common method in studies of genetic diversity, as it can reveal the relationships between genotypes based on the degree of commonality of genotypes that provide specific phenotypic or genetic marker traits. This approach is used to find genetic resources that contribute to the improvement of agricultural crops and biodiversity (Darkwa et al., 2020).

Table 4. SRAP primer profile of 18 *Z. elegans* accessions

Primer combination	Size (bp)	TNB	NPB	PBB (%)	PIC	E	R	D	H
Me6-Em6	100-1200	15	15	100	0.35	5.39	8.33	0.87	0.46
Me8-Em4	100-1000	18	18	100	0.34	5.61	5.89	0.90	0.43
Me8-Em7	100-1500	11	10	91	0.35	3.72	4.56	0.89	0.45
Me9-Em4	150-1300	14	14	100	0.35	4.83	6.33	0.88	0.45
Me10-Em4	100-700	9	9	100	0.35	3.06	3.44	0.89	0.45

Note: TNB: total of number bands; NPB: number of polymorphic bands; PPB: percentage of polymorphic bands; PIC: polymorphic information content; E: effective multiplex ratio; R: resolving power; D: discriminating power; H: expected heterozygosity.

Five primer combinations out of ten primers were selected for analyzing 18 *Z. elegans* accessions. SRAP primer profiles of 18 *Z. elegans* accessions (Table 4) were analyzed using iMEC (an online marker efficiency calculator). Based on Table 4, five SRAP primer combinations amplified a wide range fragment from 100 to 1500 bp. The total number of bands (TNB) values ranged from 9 to 18 bands with percentage of polymorphic band of 91% and 100%. The percentage of polymorphic bands indicates the diversity level, in which high percentage of polymorphic bands illustrates high level of diversity. The polymorphic information content (PIC) describes the hybrid population diversity. The PIC values of five primer combinations are 0.34 and 0.35, indicating that all samples have moderate diversity because the value is close to the maximum value (0.5). A dominant marker with equal distribution of alleles in the population has a higher value, with a maximum of 0.5 (Chesnokov and Artemyeva, 2015).

The highest value of the effective multiplex ratio (E) was Me8-Em4 (5.61), and the lowest was Me10-Em4 (3.06). The value of E parameter indicates that the primer combinations are appropriate for analyzing interspecific and intraspecific genetic diversity (Singh et al., 2014). The resolving power (R) of this study had a wide range of 3.44 (Me10-Em4) to 8.33 (Me6-Em6). A high R value shows that the various primer combinations can distinguish the cultivars (Maulani et al., 2023). The highest value of discriminating power (D) parameter was 0.90 (Me8-Em4) and the lowest was 0.87 (Me6-Em6). The resolving power (R) and the discriminating power (D) parameters describe the ability of markers to distinguish the *Z. elegans* accessions. The expected heterozygosity (H) is a common statistic for evaluating genetic variation within populations (Harris and DeGiorgio, 2017). The value of H parameter of this study ranged from 0.43 (Me8-Em4) to 0.46 (Me6-Em6), indicating that the genetic variation between 18 *Z. elegans* accession was relatively high.

Figure 3 describes the cluster of 18 *Z. elegans* accessions based on SRAP markers. The SRAP markers used resulted in a high number polymorphic bands, which were more than 9 bands per primer combinations, and revealed 100% polymorphism bands of five primer combinations on Table 1. Research results indicated that the genetic similarity coefficient was 0.8 (the nearest) and 0.46 (the farthest) with two cluster (A and B). There are two major groups, namely group A consisting of fifteen accessions and group B consisting of three accessions. In the first group (A), A7 was separated from other accessions in the same group with coefficient of 0.47. A5 and A10 were the most similar accessions with coefficient of similarity of 0.8. In the second group (B), A15 was separated form A17 and A18 with coefficient of similarity of 0.51. Genetic markers are more sensitive to measure plant diversity than morphological traits. Genetic study techniques such as Sequence-Related Amplified Polymorphism SRAP markers are used extensively, which is known for rapidness of polymorphism detection and easiness. The integration between SRAPs and morphological traits enables an integrative assessment of molecular and phenotypic variation relevant to phylogenetic inference and population structure analysis (Zhang et al., 2021). In peas, polymorphism and good diversity indication determined using SRAP was higher when compared with SSR marker (Abd El-Fatah and Nafea, 2020).

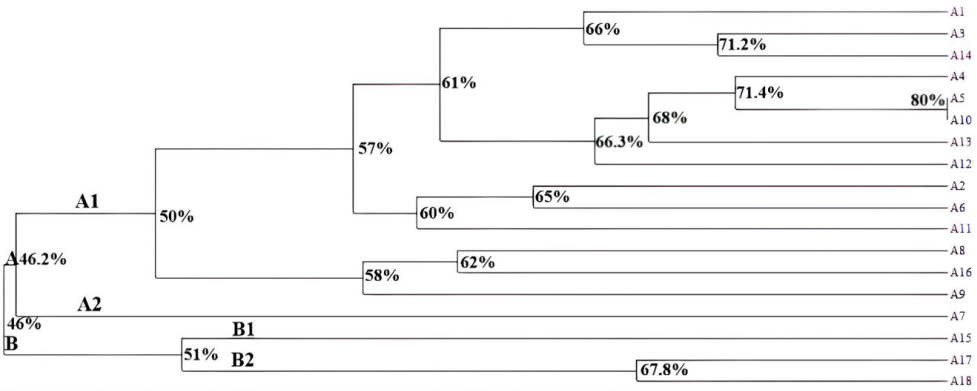

Fig. 3. Dendrogram of Clustering 18 *Z. elegans* Accessions Based on SRAP Marker

Previous studies have shown that the correlation between morphological data and molecular markers (such as SRAP) varies depending on species and environmental conditions. For example, in *Chrysanthemum morifolium*, the clustering results from morphological data formed six groups, while SRAP analysis formed three main clusters. This difference is due to the deeper genetic information captured by SRAP, compared to morphological traits that can be influenced by the environment (Hodaei et al., 2019).

The PCA is represented by the plot (Fig. 4) to show what groups are related and clustered together based on their pairwise similarities. Population in this research was divided by flower color, such as white group (A1, A2), yellow group (A3, A4, A5), orange group (A6, A7), red group (A8, A9, A10), purple group (A11, A12), and red-purple group (A13, A14, A15, A16, A17, A18) (Fig. 4). The white group is clustered in the lower-left region, suggesting high similarity within this group. There is greater spread within the yellow group, and they are middle-right, resulting in some variance. The lower-central part of the plot displays a relatively tight cluster, which represents orange group. The red group appear in the upper-left section because it has a unique separation from other groups. In contrast, the purple group is distributed through the lower-middle area still in accordance with moderate heterogeneity within the group. Finally, the red-purple group is in both upper-left and upper-right areas of the plot, indicating some internal diversity but still has clear separation from other groups

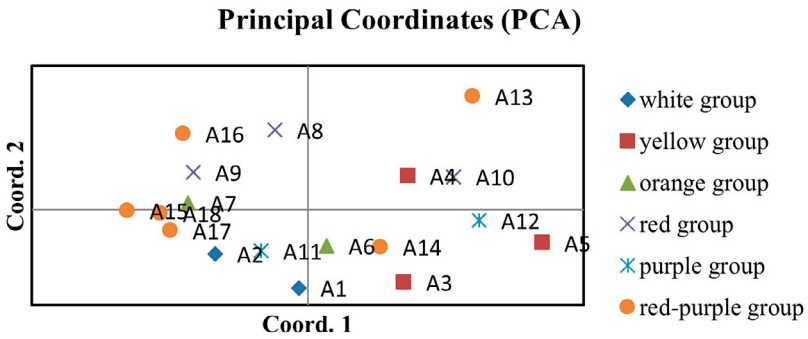

Fig. 4. PCA result of 18 *Z. elegans* accessions.

Most samples within a group are close together, indicating high similarity within the group. Accession within their group that are in the same quadrant include, A1 and A2 (white group); A3 and A5 (yellow group); A8 and A9 (red group); and A15, A17, and A18 (red-purple group). Some accessions are positioned further away from their groups, potentially indicating unique trait or variation. These patterns indicate the presence of structured variation between the samples, which may reflect underlying biological, genetic, or environmental factors.

The correlation of morphological and SRAP data is presented in the form of a heatmap in Fig. 5. This heatmap shows the pairwise correlation between variables. Based on Fig. 5, the number of ray florets has strong positive correlation with flower head type and ray floret curvature. Then, leaf width has a strong negative correlation with leaf length/width ratio. The positive correlation between molecular marker and morphological trait can determine the usage of SRAP primer combination to identify specific morphological trait.

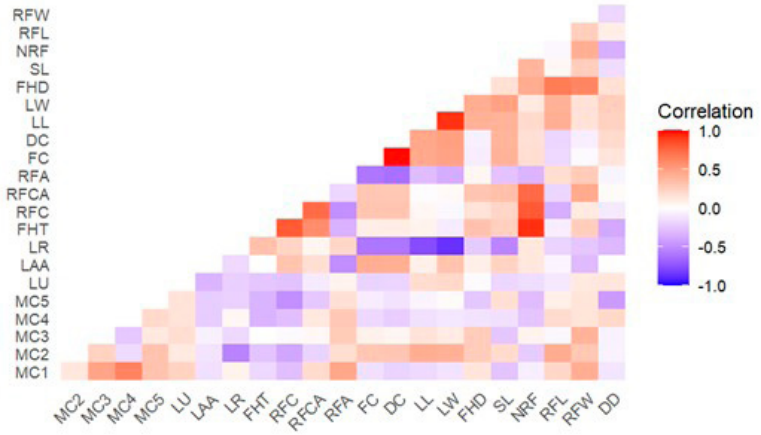

Fig. 5. Correlation Heatmap of Morphological and SRAP Data

Note: MC1 = Marker combination 1; MC2 = Marker combination 2; MC3 = Marker combination 3; MC4 = Marker combination 4; MC5 = Marker combination 5; LU = Leaf undulation; LAA = Leaf area of anthocyanin; LR = Leaf length/width ratio; FHT = Flower head type; RFC = Ray floret curvature; RFCA = Ray floret curved axis; RFA = Ray floret apex; FC = Flower color; DC = Disc color; LL = Leaf length; LW = Leaf width; FHD = Flower head diameter; SL = Stem length; NRF = Number of Ray Florets; RFL = Ray floret length; RFW = Ray floret width; DD = Disc diameter

MC1 – MC 5 are primer combinations for SRAP analysis (Table 1). The primer combinations used in this research had positive correlation with some morphological trait, even though it was not the strong correlation. MC 1 and MC 4 has a high correlation with ray floret apex (RFA). MC 2 has a high correlation with ray floret length (RFL) and has a positive correlation with other traits, such as leaf length (LL), leaf width (LW), stem length (SL), and ray floret width (RFW), correlated to flower and leaf dimension. MC 3 has a high correlation with ray floret width (RFW). MC 5 has a high correlation with stem length (SL). Based on the results, each primer combinations can determine specific morphological traits. However, the five primer combinations do not have a positive correlation with the flower structure traits, such as flower shape and flower color. This can happen because the flower structure is regulated by the MADS Box domain protein, whereas SRAP is not specifically designed to read or detect the MADS Box because the basic principle of this technique is to amplify general open reading frames (ORFs).

Morphological traits combined with SRAP markers can offer an integrated approach to assess genetic diversity, enabling a better understanding of genotype-phenotype relationships (Hassani et al., 2020) and understanding of gladiolus diversity support targeted breeding programs aimed at cultivar improvement, conservation, and the development of superior ornamental genotypes for hybridization efforts (Jadhav et al., 2025). In Egyptian bread wheat genotypes, SRAP molecular markers associated with genetic diversity and/or marker-trait associations for spike length, plant height and tillers /plant have been revealed (Khaled et al., 2021). SRAP marker is closely related to double flower trait in Petunia hybrid (Liu et al., 2016).

This partial overlap is often explained by the presence of gene flow between varieties recorded by SRAP but not phenotypically, as well as selection pressures that change phenotypic expression more than actual genetic composition. Biologically, the partial overlap between morphological and SRAP clusters shows that, even if there is still a lot of genetic variety, morphological traits can converge as a result of artificial selection in ornamental plant cultivation. For example, SRAP markers demonstrated a greater resolution in identifying genetic links in Dianthus plants than morphology, which is more prone to environmental fluctuation and unable to differentiate between varieties' geographical origins (Fu et al., 2008). To provide more comprehensive picture of the genetic relationships and breeding of ornamental plants, it is crucial to combine morphological and SRAP analysis. These findings highlight the importance of molecular markers in assessing genetic diversity between *Z. elegans* accessions and indicate that molecular marker-based assessment of diversity can be helpful for breeding programs seeking to select resilient and other favorable phenotypes.

Conclusions

Genetic diversity analysis is the initial stage of breeding program that can be done by analyzing morphological and molecular traits. The genetic diversity of 18 *Z. elegans* accessions based on morphological and SRAP markers produced a dendrogram with two main branches with a similarity coefficient of 0.18-0.78 and 0.46-0.8, respectively. Based on the heatmap, five SRAP primer combinations used can identify certain morphological traits. The highest positive correlation in each primer combination is MC 1 and MC 4 with ray floret apex (RFA); MC 2 with ray floret length (RFL), leaf length (LL), and leaf width (LW); MC 3 with ray floret width (RFW); and MC 5 with stem length (SL). Five morphological traits that had high GVC and strong SRAP correlation were prioritized for selection. Five SRAP combination primers can be used for molecular marker resources of Zinnia breeding.

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Author Contribution

MFS: Conceptualization, Resources, Methodology, Writing – Review & Editing. **AP:** Conceptualization, Methodology, Writing – Review & Editing. **YAP:** Methodology, Writing – Review & Editing. **ES:** Methodology, Writing – Review & Editing.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement

All the research data is contained in the manuscript.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that the use of AI and AI-assisted technologies was not applied in the writing process.

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