



Integrative analysis of phenotypic and ISSR marker-based genetic diversity in *Tabernaemontana divaricata* genotypes

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ABSTRACT

Tabernaemontana divaricata (L.) R.Br. ex Roem. & Schult. is an important ornamental and medicinal shrub exhibiting considerable phenotypic variability, yet its genetic diversity remains underexplored. The present study aimed to assess genetic variability among twelve genotypes using an integrated approach combining morphological traits and Inter Simple Sequence Repeat (ISSR) markers. Fourteen quantitative and qualitative morphological traits were evaluated to estimate phenotypic and genotypic coefficients of variation, heritability, and genetic advance. High variability, heritability, and genetic advance were observed for key traits such as chlorophyll content, flower bud length, and yield, indicating strong additive gene action and selection potential. Principal component analysis revealed that five components explained 88.20% of total variation, with vegetative vigor, physiological efficiency, and floral traits contributing significantly. Cluster analysis based on morphological data grouped genotypes into three distinct clusters. Molecular characterization using 13 ISSR primers generated 215 bands, of which 191 were polymorphic, with an average polymorphism of 88.50%. The polymorphic information content (PIC) ranged from 0.16 to 0.94, indicating moderate to high marker informativeness. UPGMA clustering based on ISSR data also grouped genotypes into three major clusters, revealing substantial genetic divergence. Similarity coefficients ranged from 0.181 to 0.625, reflecting moderate genetic diversity among genotypes. The concordance between morphological and molecular analyses highlights the effectiveness of integrated approaches in genetic diversity studies. Overall, the study demonstrates significant genetic variability in *T. divaricata*, providing valuable insights for germplasm conservation, selection of divergent parents, and development of improved genotypes for floricultural and pharmaceutical applications.

Key words: Molecular characterization, ISSR, genetic diversity, genotypes, phenotypes.

INTRODUCTION

Tabernaemontana divaricata (L.) R.Br. ex Roem. & Schult., an evergreen ornamental shrub belonging to the family Apocynaceae is widely distributed across tropical and subtropical regions. It is extensively cultivated in countries such as India, Sri Lanka, Thailand, Malaysia and Vietnam. In India, the species is predominantly grown in southern states including Tamil Nadu, Karnataka, Andhra Pradesh, Kerala and Odisha where it exhibits profuse year-round flowering and plays a significant role in loose flower markets (Prachayasakul *et al.*, 18). The plant holds considerable ornamental, cultural and medicinal importance. Its attractive pinwheel-shaped white flowers, pleasant fragrance and extended shelf life make it suitable for garlands, religious offerings and landscape use. Unlike seasonal Jasmine species such as *Jasminum sambac* and *Jasminum auriculatum*, *T. divaricata* flowers continuously throughout the year, making it a valuable

substitute during peak demand periods. Beyond its ornamental value, *T. divaricata* is a rich source of diverse phytochemical constituents particularly indole alkaloids, flavonoids, terpenoids, phenolic compounds and glycosides which contribute to its wide spectrum of pharmacological activities (Kalaimagal *et al.*, 9). Several studies have reported its analgesic, anti-inflammatory, antimicrobial, antioxidant, anticancer and wound-healing properties thereby highlighting its significance in traditional and modern medicine (Ghosh *et al.*, 6). The presence of bioactive alkaloids such as voacangine and coronaridine further enhances its therapeutic potential. Additionally, the plant exudes a milky latex containing biologically active compounds which has applications in traditional remedies and potential pharmaceutical uses. From an industrial perspective, the fragrant flowers of *T. divaricata* have potential applications in the perfumery and essential oil industry, although it remains underutilized compared to other aromatic crops. The latex and secondary metabolites also hold promise for biotechnological and pharmaceutical industries indicating its scope as a multipurpose crop bridging floriculture and medicinal plant sectors (Naidoo *et al.*, 16). Despite

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its multifaceted utility, *T. divaricata* remains under exploited as a commercial floricultural and medicinal crop. The presence of considerable phenotypic variability among genotypes particularly in floral morphology, growth habit, fragrance intensity and other characters suggests a rich underlying genetic diversity. However, morphological characterization alone is often insufficient due to environmental influences and phenotypic plasticity necessitating the use of molecular tools for precise genetic analysis. Among various DNA-based marker systems, Inter Simple Sequence Repeat (ISSR) markers have emerged as a highly informative, reproducible and cost-effective technique for genetic diversity studies (Kumar *et al.*, 11). ISSR markers target the microsatellite regions (simple sequence repeats) distributed throughout the genome. They utilize single primers anchored at the 3' or 5' end of repeat sequences (such as di-, tri- or tetra-nucleotide repeats) to amplify the intervening DNA segments through polymerase chain reaction (PCR) (Sharma *et al.*, 21). The polymorphism arises due to variations in the length of the inter-repeat regions leading to differences in banding patterns among genotypes. This dominant marker system does not require prior sequence information offers high genome coverage and exhibits better reproducibility compared to RAPD markers, making it particularly suitable for underutilized and non-model plant species. Integrating molecular characterization with morphological variability enables a comprehensive understanding of genotype performance. Such combined approaches are essential for identifying elite genotypes with superior ornamental traits, enhanced metabolite

profiles and improved adaptability. Furthermore, assessing both phenotypic and genotypic variance facilitates accurate selection and classification of germplasm (Gholami *et al.*, 5). However, reports on ISSR-based molecular characterization of *T. divaricata* are limited and studies linking genetic diversity with phytochemical composition and functional traits remain scarce. Understanding the genetic structure and variability of available genotypes is crucial for germplasm conservation, marker-assisted selection and future breeding programmes aimed at developing high-yielding, stress-tolerant and metabolite-rich cultivars.

Therefore, the present study aims to assess the genetic diversity among *Tabernaemontana divaricata* genotypes using ISSR markers in conjunction with morphological evaluation, to provide a scientific basis for their conservation, genetic improvement and effective utilization in floriculture, pharmaceutical and industrial applications.

MATERIALS AND METHODS

The plant material for the present study consisted of one-year-old genotypes of *Tabernaemontana divaricata* sourced from the germplasm collection maintained at the Department of Floriculture and Landscape Architecture, Tamil Nadu Agricultural University, Coimbatore. These genotypes were originally collected from various regions across Tamil Nadu and subsequently assembled and conserved in the departmental germplasm repository.

The details of the genotypes used in the study are represented in Table 1.

Table 1: List of genotypes *Tabernaemontana divaricata* used in the study.

Accession No.	Types	Location of collection			Photograph
		Area	Latitude	Longitude	
Acc-01	Single (Dwarf plants)	Thondamuthur, Coimbatore	10.9899°N	76.8406°E	
Acc-02	Single (Blunt buds)	Thiyagarayanagar, Chennai	13.0418°N	80.2341°E	
Acc-03	Single (Variegated leaves)	Thimmavaram, Chengalpet	12.7155°N	79.9577°E	

Contd...

Genetic Diversity in Tabernaemontana divaricata

Table 1 contd...

Accession No.	Types	Location of collection			Photograph
		Area	Latitude	Longitude	
Acc-04	Single (Pointed buds)	Kollam, Kerala	8.8932°N	76.6141°E	
Acc-05	Semi-double (Two- whorled flowers)	Thondamuthur, Coimbatore	10.9899°N	76.8406°E	
Acc-06	Single (Round petals)	Periyakrishnapuram, Salem	11.6548°N	78.4312°E	
Acc-07	Double (Pointed buds)	Kalpakkam, Chengalpet	12.5238°N	80.1568°E	
Acc-08	Double (Wavy petals)	Vadavalli, Coimbatore	11.0268°N	76.9058°E	
Acc-09	Double (Scented flowers)	Madhavaram, Chennai	13.1488°N	80.2306°E	
Acc-10	Double (Saucer shaped flowers)	Medavakkam, Chennai	12.9171°N	80.1923°E	
Acc-11	Double (Greenish buds)	Mettupalayam, Coimbatore	11.3028°N	76.9383°E	
Acc-12	Double (Fringed petals)	Thoppampatti, Coimbatore	11.0939°N	76.9283°E	

Total Phenotypic traits recorded 14 traits were recorded namely plant height (cm), plant spread (cm), number of branches per plant, leaf length(cm), leaf width(cm), leaf area(cm²), chlorophyll content (SPAD), number of flowers per plant, flower bud length(cm), flower bud diameter(cm), stalk length(cm), number of petals, fragrance scoring and yield per plant (g/plant).

Phenotypic and genotypic variances were estimated according to the formula given by Lush (14). Genotypic variance (σ^2_g) = $MS_1 - MS_2 / r$ Where, MS_1 = Mean sum of squares for genotypes. MS_2 = Mean sum of squares for error or error variance, r = Number of replications. Phenotypic variance (σ^2_p) = $\sigma^2_g + \sigma^2_e$. Where, σ^2_g = Genotypic variance. σ^2_e = Error variance.

The Phenotypic and Genotypic Coefficient of Variations were worked out as per the methods suggested by Burton (2).

$$\text{Phenotypic coefficient of variation} = \frac{\sqrt{(\sigma^2_p)}}{\text{General mean}} \times 100$$

$$\text{Genotypic coefficient of variation} = \frac{\sqrt{(\sigma^2_g)}}{\text{General mean}} \times 100$$

$$\text{Environmental coefficient of variation} = \frac{\sqrt{(\sigma^2_e)}}{\text{General mean}} \times 100$$

PCV and GCV were classified as noted below and suggested by Sivasubramaniam and Madhava Menon (22).

GCV and PCV	Category
Less than 10%	Low
10-20%	Moderate
More than 20%	High

Heritability in the broad sense (h^2) was estimated based on the formula proposed by Lush (14) and expressed in per cent. Heritability, (h^2) = $(\sigma^2_g) / (\sigma^2_p) \times 100$ Where, (σ^2_g) - Genotypic Variance, (σ^2_p) - Phenotypic Variance. As suggested by Johnson *et al.*, (8), heritability values are categorized as follows:

Heritability	Category
Less than 30%	Low
30 – 60%	Moderate
More than 60%	High

Genetic advance was estimated by the following formula as per the method suggested by Johnson *et al.* (8). Genetic advance = $(\sigma^2_g) / (\sqrt{\sigma^2_p}) \times xk$. Where, σ^2_g - Genotypic variance $\sqrt{(\sigma^2_p)}$ - Phenotypic standard deviation, k - Selection differential, the value of which is 2.06 at 5% selection intensity. Genetic advance as per cent of mean = $GA / \text{General mean} \times 100$. The range of genetic advance as per cent

of mean was classified as suggested by Johnson *et al.* (8)

Genetic advance	Category
0-10	Low
10-20	Moderate
> 20	High

Total genomic DNA was extracted using the CTAB method as described by Doyle and Doyle (3) with minor alterations applied as necessary to optimize the protocol for *Tabernaemontana divaricata*.

100 mg of sectioned leaf fragments was transferred into pre-labelled 2 ml sterile Eppendorf tubes. The tubes were sealed with Parafilm and kept in a thermocol container filled with liquid nitrogen to rapidly freeze and harden the tissue. This step was essential due to the presence of latex in *Tabernaemontana divaricata* leaves which can interfere with grinding and cause DNA degradation. Subsequently, 250 μ l of pre-warmed CTAB extraction buffer was added to each tube. Sterilized stainless steel beads (6.3 mm diameter) were put into the tubes for mechanical disruption. Tissue lyser was utilized to agitate the tissues at a frequency of 1800 for 40 seconds. Later, the final volume was adjusted to 1500 μ l by adding 1350 μ l of CTAB buffer along with a pinch of polyvinylpyrrolidone (PVP) and 20 μ l of β -mercaptoethanol. The tubes were incubated in a preheated water bath at 65 °C for 1 hour with gentle mixing every 15 minutes to ensure effective lysis. The supernatant was carefully transferred to a fresh tube and an equal volume of chloroform: isoamyl alcohol (24:1) was added and centrifuged at 12,000 rpm for 10 minutes at 4 °C to separate the aqueous phase containing genomic DNA and was pipetted out and moved to a sterile Eppendorf tube. To precipitate DNA, an equal volume of pre-chilled isopropanol was added to the Eppendorf and incubated overnight at 4°C. After incubation, DNA was pelleted out by centrifugation at 12,000 rpm for 15 minutes at 4°C. After discarding the supernatant, the pellet was centrifuged at 12,000 rpm for 10 minutes at 4°C after being twice cleaned with 70% ethanol. After the supernatant was decanted, the DNA pellet was dried to remove any remaining ethanol and dissolved in 40 μ l of nuclease free water. It was stored at -20°C for further use.

The integrity of the isolated genomic DNA was evaluated by electrophoresis on a 0.8% agarose gel prepared in 1 $\times$ TAE buffer and run at 70 V. Gels were stained with ethidium bromide (3 μ l/100ml) to visualize nucleic acids, and the DNA bands were observed using a Bio-Rad Gel Doc™ EZ Imager (Model 1708270). DNA quality was assessed

based on band morphology, wherein intact samples exhibited a single, high-molecular-weight band, while degraded samples appeared as smeared profiles.

The isolated DNA samples were quantified using a Nano Drop spectrophotometer (Citizen) by measuring absorbance at 260 nm and 280 nm. The purity of DNA was assessed based on the OD 260/280 ratio, which ranged from 1.63 to 1.88 indicating acceptable purity for downstream applications. The DNA was then diluted to a working concentration of 50 ng/μl for Polymerase Chain Reaction (PCR) analysis and stored at -20°C until further use.

Genomic DNA was subjected to polymorphism analysis using Inter Simple Sequence Repeat (ISSR) markers to elucidate the genetic diversity and molecular variation among the studied *Tabernaemontana divaricata* genotypes. Totally 13 primers were used and are represented in the Table 2.

As indicated in the Table 3 reaction conditions and ideal concentrations of several components namely, template DNA, MgCl₂, dntps, Taq DNA polymerases and primers were prepared in 10 μl of reaction mixture for each PCR analysis.

With few modifications, the PCR amplification for ISSR markers was carried out in accordance with Williams *et al.* (25). The following program depicted in Table 4 was used to carry out DNA amplification in a thermal cycler (T100 Thermal Cycler from Bio-Rad).

The amplified PCR products were resolved by electrophoresis on a 1% agarose gel prepared in 1× TAE buffer. Each DNA sample was thoroughly combined with 6X gel loading dye prior to loading to facilitate tracking and visualization. A 1 kb DNA

ladder was included alongside the samples and served as a molecular size standard for accurate estimation of fragment sizes. Electrophoresis was performed in a horizontal gel system at a constant voltage of 70 V for 30 minutes. After separation, the gel was visualized using a UV transilluminator and images were captured with a gel documentation system under ultraviolet illumination to document the banding patterns.

Table 3: PCR Components and their concentration.

PCR components	Volume for one reaction (μl)
Taq buffer with MgCl ₂	1.5 μl
dNTP's	0.5 μl
Primer	2.0 μl
Template DNA (20-30ng/100μl)	2.0 μl
Taq DNA polymerase	0.2 μl
Sterile distilled water	3.8 μl
Total reaction volume(μl)	10 μl

Table 4: PCR amplification programming.

Sl. No.	Step	Temperature (°C)	Time	No. of cycles
1.	Initial denaturation	95	2 min	
2.	Final denaturation	95	45 sec	
3.	Primer annealing	40	30 sec	35
4.	Initial extension	72	45 sec	
5.	Final extension	72	10 min	

Table 2: ISSR Primers used in the study for analysis of genetic diversity.

Primer Code	Primer sequence (5'-3')	Tm°	No. of bases	GC content (%)
UBC-807	AGAGAGAGAGAGAGAGT	42.5	17	47
UBC-823	TCTCTCTC TCTCTCTCC	47.5	17	52.9
UBC-846	CACACACACACACART	53.7	18	44.4
UBC-853	TCTCTCTC TCTCTCTCRT	54	18	44.4
UBC-855	ACACACAC ACACACACYT	60.2	18	44.4
UBC - 1	ACACACACACACACACT	49.3	17	47
UBC -809	AGA GAG AGA GAG AGA GG	46.6	17	52.9
UBC -810	GAGAGAGAGAGAGAGAT	42.9	17	47
UBC- 825	ACA CAC ACA CAC ACA CT	49.3	17	47
UBC- 848	CAC ACA CAC ACA CAC ARG	50.6	18	50
UBC 2	GAGAGAGAGAGAGAGAT	44.64	17	47
UBC 808	AGAGAGAGAGAGAGAGC	47.05	17	52.94
UBC 866	CTC CTCCTCCTCCTCCTC	54.88	18	66.6

The data on the gel produced by all 13 ISSR primers were scored manually. For data analysis only bands that were distinct and repeatable are used. The band profiles were given binary scores, which ranged from 1 to 0. An amplified band was given a score of “1” for presence and a score of “0” for absence. The intensity of created bands was not taken into account while scoring. Data matrices were then acquired and assembled for additional analysis in an MS-Excel (Version 2024) sheet.

Polymorphic information content (PIC) was calculated as per the formula of Roldán-Ruiz *et al.* (20) $PIC = 2f_i(1-f_i)$. Where, f_i is the frequency of the amplified allele and $1-f_i$ is the frequency of null allele.

$EMR = n \times \beta$ [$\beta = np / (np + nnp)$]. Where, ‘n’ is the average number of bands amplified by accession to a specific system marker (multiplex ratio) and β is the number of polymorphic loci (np) divided by the sum of the number of polymorphic loci (np) and the number of non-polymorphic loci (nnp) (Weir, 25).

$MI = EMR \times PIC$. Marker index is the product of polymorphic information content (PIC) and (EMR) effective multiplex ratio (Powell *et al.*, 17).

The morphological data recorded during the experiment were subjected to statistical analysis using the KAU-GRAPES software developed by Kerala Agricultural University (KAU), Thrissur, Kerala, India. The program was employed for analysis of variance (ANOVA) and computation of critical difference (CD) at 5% level of significance for comparing treatment means. Correlation analysis, Principal Component analysis and Cluster analysis were also performed with KAU-GRAPES. Molecular data was analysed using the Numerical Taxonomic and Multivariate Analysis System (NTSYS-pc) software tool, version 2.2 (Rohlf, 19). Jaccard’s coefficient (Jaccard, 7) based on the SIMQUAL function was used to create the similarity matrix from binary data and the Unweighted Pair Group Method using Arithmetic Averages (UPGMA) (Sokal and Sneath, 23) based on the SAHN (J) function of NTSYS was used to create

the dendrogram. A dendrogram and a similarity matrix were used to graphically depict the output data.

RESULT AND DISCUSSION

The variability of eight characters based on phenotypic and genotypic coefficients of variation are furnished in Table 2. Coefficient of variation was computed for valid comparison between the characters, which were recorded in different units. The phenotypic coefficient of variation ranged from lowest of 21.85% (flower bud diameter) to highest 84.90% (chlorophyll content). In all traits, PCV values were only slightly higher than GCV, indicating minimal environmental influence. The genotypic coefficient of variation ranged from lowest of 21.84% (flower bud diameter) to highest 84.70% (chlorophyll content).

The estimates of genotypic and phenotypic coefficients of variation (GCV and PCV) indicated substantial variability among *Tabernaemontana* genotypes. Chlorophyll content showed the highest GCV and PCV, reflecting wide genetic variability and strong selection potential. High variability was also observed for leaf area, flower bud yield and flower bud diameter, suggesting their importance in breeding. Moderate variability was recorded for plant height and flower bud length, while leaf length exhibited the least variation, indicating relative uniformity. The marginal difference between PCV and GCV across traits suggests minimal environmental influence and a predominance of genetic factors.

The estimates of broad-sense heritability and genetic advance as percentage of mean are presented in Table 5. Heritability ranged from the lowest of 0.83% (number of petals) to the highest of 0.99% (Plant height, Chlorophyll content, flower bud diameter and Flower bud length). The genetic advance as percentage of mean (GAM) ranged from the highest of 174.09% (number of petals) to the lowest of 44.95% (Flower bud diameter). GAM was high for number of petals (174.09) followed by Flower

Table 5: Phenotypic and genotypic coefficient of variation.

S. No.	Traits	Grand mean $\pm$ SE	GCV %	PCV %	Heritability (%)	GAM %
1.	Plant height	123.16	35.04	35.15	0.99	71.98
2.	Leaf length	11.61	22.87	23.73	0.92	45.42
3.	Leaf area	3.82	24.36	25.48	0.91	48.00
4.	Chlorophyll content	10.90	84.70	84.90	0.99	174.09
5.	Flower bud length	53.17	48.95	48.96	0.99	100.82
6.	Flower bud diameter	51.40	21.84	21.85	0.99	44.95
7.	Number of petals	1.09	39.65	43.49	0.83	74.95
8.	Flower bud yield	17.26	39.55	40.16	0.96	80.23

bud length (100.82), flower bud yield (80.23), Number of petals (74.48) and plant height (71.98).

The present study revealed uniformly high heritability across key traits in *Tabernaemontana*, indicating predominant control by additive genetic factors and strong potential for selection in breeding programs. Similar trends have been reported in chrysanthemum, where heritability values exceeding 96% for floral and growth traits, along with high genetic advance, suggest significant scope for improvement (Kumar *et al.*, 11). Likewise, cut chrysanthemum genotypes showed very high heritability (up to 99.97% for flower diameter) with high GAM, supporting effective direct selection. In roses, traits such as petal number and flower diameter also exhibited high heritability (>80%), with narrow-sense estimates for petal number ranging from 0.85 to 0.91, indicating strong genetic control and breeding potential (Kılıç *et al.*, 10).

Assessment of correlation among the morphological characters are depicted in the Fig. 1. The first principal component (Dim 1) is predominantly associated with vegetative growth traits showing strong positive correlations with plant height and plant spread, thereby indicating that this axis represents overall plant vigor and morphological size. The second component (Dim 2) is largely defined by chlorophyll content, reflecting the physiological efficiency and productivity potential of the genotypes. The third component (Dim 3) has positive correlation with yield, while the fourth component (Dim 4) is mainly influenced by the

number of petals, representing floral morphological diversity. The subsequent components contribute less to the overall variation and account for minor or residual traits.

PCA indicated that a few key traits predominantly govern morphological variability among genotypes. Vegetative traits such as plant height and spread (Dim 1) were major contributors, serving as reliable indicators of plant vigor and important selection criteria. Chlorophyll content (Dim 2) reflected physiological efficiency and productivity potential, while number of flowers (Dim 3) highlighted the role of plant architecture. Floral traits like petal number (Dim 4) contributed to ornamental quality. As these components explain most of the variability, they can be effectively used as selection indices for identifying superior genotypes and guiding breeding programs for both yield and aesthetic value.

Principal Component Analysis (PCA) of the morphological traits in *T. divaricata* genotypes resulted in five principal components (PCs) with eigenvalues greater than one (Table 6). These five PCs together explained 88.20% of the total variability observed among the genotypes. Out of these, the first three components alone contributed to 71.07 % of the variation. Among the components, PC1 accounted for the highest proportion of variability (43.25%), followed by PC2 (17.36%) and PC3 (10.45%). The fourth and fifth components contributed 9.25% and 7.87%, respectively. From the sixth component onwards, the contribution of each PC dropped below 6%.

The PCA biplot showed that plant height, spread and leaf traits were major contributors to PC1, representing vegetative growth and canopy development, while chlorophyll content and floral traits

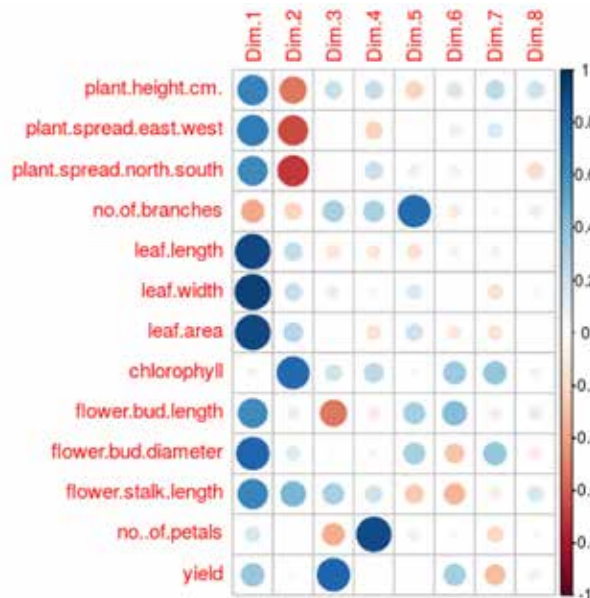


Fig. 1. Correlation plot variables v/s PCs.

Table 6: Estimation of eigen value, percentage of variance and cumulative variance of *T. divaricata* genotypes.

Principal component	Eigenvalue	% Variance	Cumulative variance
PC1	5.62	43.25	43.25
PC2	2.25	17.36	60.62
PC3	1.35	10.45	71.07
PC4	1.20	9.25	80.32
PC5	1.02	7.87	88.20
PC6	0.68	5.26	93.46
PC7	0.57	4.42	97.89
PC8	0.15	1.19	99.08
PC9	0.08	0.61	99.69
PC10	0.03	0.26	99.96
PC11	0.00	0.03	100

dominated PC2, indicating physiological efficiency and reproductive growth. Branch number exhibited an opposite orientation, suggesting a negative correlation with vigor traits. Genotypes Acc-04 and Acc-01 were clearly separated, indicating distinct trait profiles, whereas Acc-03 and Acc-05 showed weaker associations with vigor and chlorophyll. Most accessions (Acc-06, Acc-07, Acc-08, Acc-10 and Acc-12) clustered near the origin, reflecting balanced performance, while Acc-09 showed moderate alignment with PC2. Overall, Acc-04 and Acc-01 represent divergent phenotypes, while centrally clustered genotypes exhibit generalist traits useful for breeding. Similar PCA-based differentiation has been reported in *Jasminum sambac* (Ganga *et al.*, 4) and roses (Luhmann *et al.*, 13), highlighting its effectiveness in identifying diverse and promising genotypes.

The PCA biplot (Fig.2) of the 12 genotypes based morphological parameters data displays the distribution of accessions along the first two principal components (PC1 and PC2) which together accounted for 60.62% of the total variance. The genotypes are widely distributed across all four quadrants indicating considerable genetic diversity among them. Acc-01 and Acc-04 are positioned away from the origin towards the negative PC1 axis. Acc-08, Acc-06 and Acc-12 are placed in the positive PC1, forming a closely associated group. Acc-02 and Acc-09 are located near the central axis. Acc-03 occupies the negative PC2. Acc-10 and Acc-11 are positioned in the lower positive PC1 and negative PC2 quadrant, while Acc-07 lies in the positive PC1 region near the axis. Acc-02 is located close to the origin.

The dendrogram based on morphological parameters resulted in three major clusters (Fig.3). In this analysis, Acc-01, Acc-03 and Acc-04 form a distinct group. Second cluster is formed by Acc-05, Acc-06, Acc-08, Acc-02 and Acc-07 which merge at intermediate distances. The third cluster comprises Acc-09, Acc-12, Acc-10 and Acc-11 which are grouped

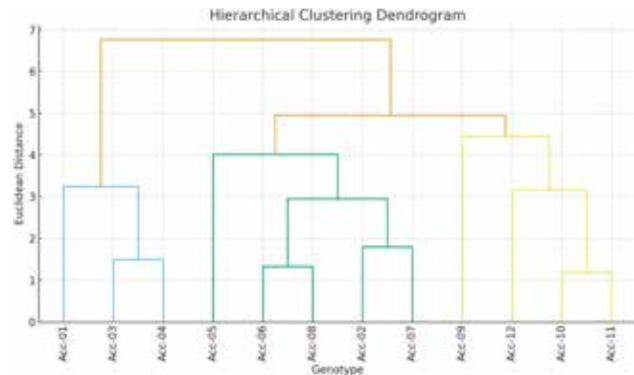


Fig. 3. Clustering dendrogram based on morphological parameters.

together at relatively shorter distances within the subgroup, at higher distances, these three major groups merge, showing the broader genetic diversity among the accessions.

The dendrogram based on morphological traits revealed three distinct clusters, indicating considerable variability among the genotypes. Cluster I (Acc-01, Acc-03 and Acc-04) showed close resemblance but remained distinct, suggesting potential uniqueness. Cluster II (Acc-05, Acc-06, Acc-08, Acc-02 and Acc-07) exhibited moderate divergence, indicating relatedness with retained variability. Cluster III (Acc-09, Acc-12, Acc-10 and Acc-11) showed the highest similarity, merging at shorter distances and representing closely related morphotypes. At higher linkage distances, all clusters merged, highlighting overall genetic diversity. These patterns are useful for selecting divergent parents to enhance heterosis and genetic gain in breeding programs.

Out of 13 ISSR markers tested all most all produced clear and reproducible bands and major 4 are depicted in Figure 4. Polymorphism and efficiency parameters of ISSR markers is depicted in Table 7. The number of alleles generated by these ISSR markers was found to range from 11 to 21, with marker UBC-846 giving the highest (21) and marker UBC 1 giving the lowest number of amplicons (11). A total of 215 bands were produced with the different markers out of which 191 (14.69 %) bands were polymorphic. The percentage of polymorphism ranged from 81.82 % for UBC-1 to 100% for UBC 808 with an average of 88.50 % polymorphism per marker. To determine PIC values of each marker, the mean of PIC value was analyzed for all loci. The highest PIC value of 0.94 (UBC 808) and the lowest of 0.165 (UBC 1), with an average PIC value per marker of 0.48 were obtained. In this study, the highest effective multiplex ratio (EMR) 158.33 was observed with the marker UBC

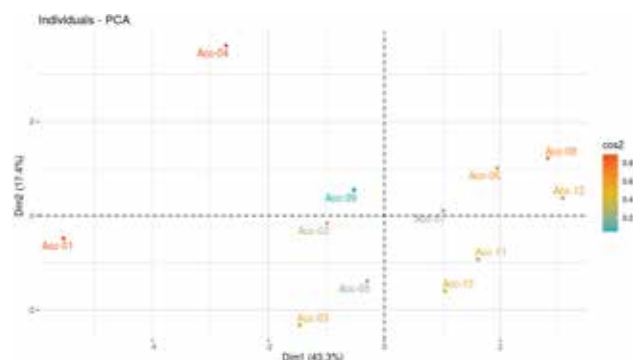


Fig. 2. PCA Biplot.

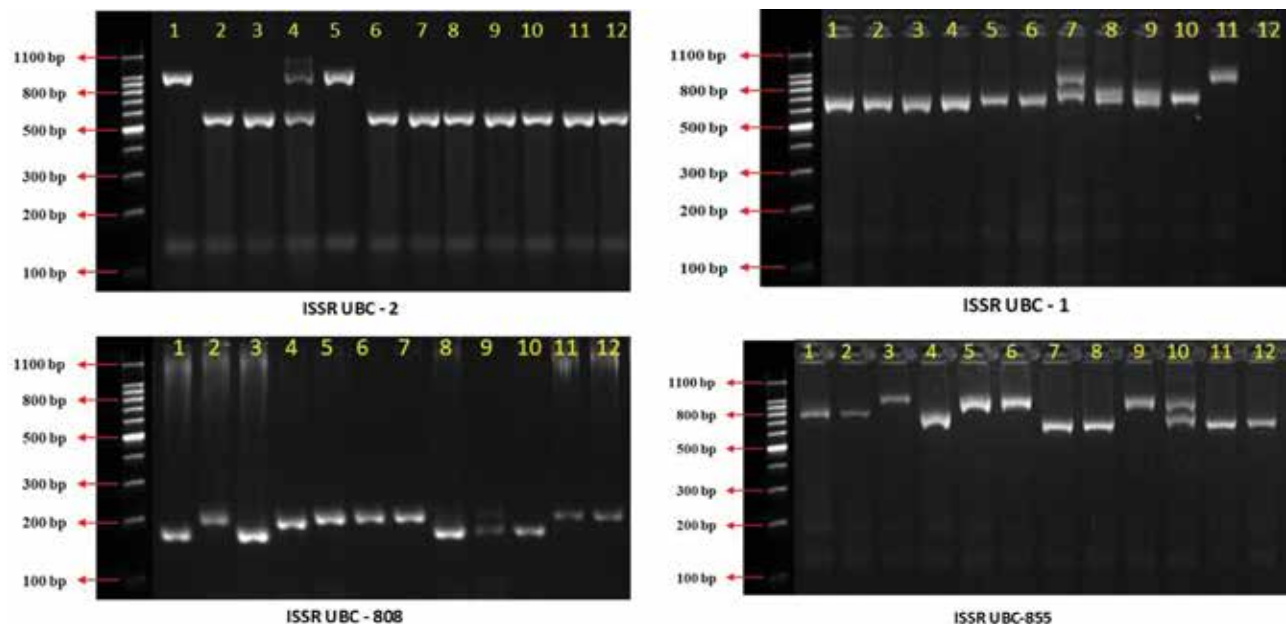
Table 7: Evaluation of polymorphism and efficiency parameters of ISSR markers used for genetic diversity analysis in *T. divaricata* genotypes.

Primer	Total alleles	Number of polymorphic loci	Polymorphic percentage (PM%)	Polymorphic information content (PIC)	Effective multiplex ratio (EMR)	Marker Index (MI)
UBC-807	20	16	88.89	0.494	133.33	65.00
UBC-823	17	15	88.24	0.498	125.00	62.50
UBC-846	21	19	90.48	0.499	158.33	78.50
UBC-853	18	16	88.89	0.494	133.33	65.00
UBC-855	13	11	84.62	0.473	91.67	43.33
UBC - 1	11	9	81.82	0.165	75.00	13.00
UBC -809	20	18	90.00	0.500	150.00	75.00
UBC -810	18	16	88.89	0.494	133.33	65.00
UBC- 825	20	18	90.01	0.495	150.00	73.50
UBC- 848	20	18	90.1	0.495	150.00	73.50
UBC 2	13	11	84.62	0.355	91.67	32.50
UBC 808	13	13	100.00	0.94	91.67	45.00
UBC 866	13	11	84.00	0.473	91.67	43.33
Total	215	191				
Avg/primer	16.53	14.69	88.50	0.48	121.12	56.78

846 and the lowest EMR of 75.00 were observed with the marker UBC 1, with an average EMR of 121.12 per marker. To determine the general usefulness of the marker system used, the MI (marker index) for each ISSR marker was calculated. The highest MI was observed with the marker UBC-846 (78.50) and

lowest in the marker UBC 1 (13.00) with an average MI of 56.78 per marker.

Plant genotype characterization is essential for supporting breeding programs. With advances in molecular biology, DNA and protein-based markers provide reliable assessment of genetic

**Fig. 4.** PCR amplification of ISSR markers.

diversity, as they are not influenced by environmental factors. Unlike morphological traits, which may be misleading, molecular data offer more precise insights into intra- and inter-specific diversity, and genotypes that appear similar morphologically may differ genetically. Hence, molecular characterization is more effective for the conservation and utilization of genetic resources. Among these, Inter Simple Sequence Repeat (ISSR) markers are widely used due to their high reproducibility, cost-effectiveness and ability to generate abundant polymorphism without prior sequence information. They are highly efficient in assessing genetic diversity, population structure, cultivar identification and phylogenetic relationships in both wild and cultivated species (Luhmann *et al.*, 13).

Polymorphic Information Content (PIC) measures the effectiveness of genetic markers in differentiating genotypes. In this study, PIC values of ISSR markers ranged from 0.16 (UBC-1) to 0.94 (UBC-808), with a mean of 0.48, indicating moderate to high marker informativeness. Markers with PIC >0.50 are highly informative, while those between 0.25–0.50 are moderately informative. Accordingly, UBC-808 was highly efficient in distinguishing *Tabernaemontana* genotypes, whereas UBC-1 was less informative. Similar high PIC values for ISSR markers have been reported in *Jasminum sambac* (Ganga *et al.*, 2021) and *Rosa* spp. (Luhmann *et al.*, 13), confirming their effectiveness in assessing genetic variability. Variations in PIC values reflect differences in the ability of markers to detect allelic diversity and their utility in genetic diversity studies, germplasm characterization and molecular breeding (Borner and Branchard, 1).

The dendrogram obtained through Agglomerative Hierarchical Clustering (AHC) analysis using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) based on ISSR markers resulted in three major clusters (Fig. 5). Cluster I consists of genotypes Acc-08, Acc-03, Acc-01, Acc-10 and Acc-09. Cluster I was sub-divided into Sub-Cluster I-a and Sub-Cluster I-b. Sub-Cluster I-a consists of genotypes Acc-08 and Acc-03, which are the closest pair within this group. Sub-Cluster I-b consists of genotype Acc-01, which subsequently joins with the sub-cluster formed by Acc-10 and Acc-09. Cluster II consists of genotypes Acc-07, Acc-04, Acc-02, Acc-12 and Acc-11. Cluster II was sub-divided into Sub-Cluster II-a and Sub-Cluster II-b. Sub-Cluster II-a consists of genotypes Acc-07 and Acc-04 which show the highest similarity within this cluster. Sub-Cluster II-b consists of genotypes Acc-02, Acc-12 and Acc-11. Within this, Sub-Cluster II-b1 consists of Acc-12 and Acc-11, which are closely related and

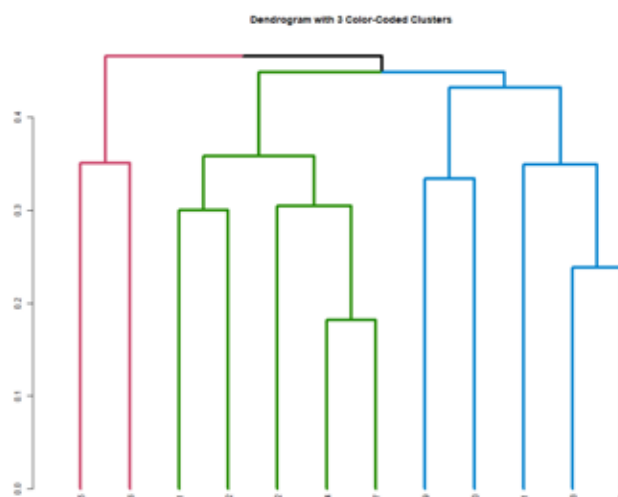


Fig. 5. Hierarchical clustering dendrogram based on ISSR markers.

they subsequently merge with Acc-2. Cluster III consists of two genotypes Acc-06 and Acc-05 which are highly similar to each other but most distinct from the rest of the genotypes.

The similarity matrix was developed using the NTSYS-PC 2.02 software and the dendrogram was constructed for the given genotypes using Jaccard's similarity coefficient. The results of the similarity coefficient are presented in Table 8. The UPGMA algorithm was employed for grouping all genotypes based on their similarity values. The similarity coefficient ranged from 0.181 to 0.625. The lowest similarity coefficient (0.181) was recorded between Acc-07 and Acc-04 whereas the highest similarity coefficient (0.625) was observed between Acc-05 and Acc-11. Most of the similarity coefficients were in the range of 0.4–0.5.

UPGMA-based Agglomerative Hierarchical Clustering (AHC) of *Tabernaemontana* genotypes revealed three distinct clusters. Cluster III comprised Acc-6 and Acc-5, indicating close similarity. Cluster I included Acc-8, Acc-3, Acc-1, Acc-10 and Acc-9, with sub-grouping of closely related pairs (Acc-8 and Acc-3) and their association with the remaining genotypes. Cluster II consisted of Acc-7, Acc-4, Acc-2, Acc-12 and Acc-11, where Acc-7 and Acc-4 formed a tight subgroup, while Acc-12 and Acc-11 clustered together before merging with Acc-2. Similar clustering patterns have been reported in chrysanthemum where ISSR markers grouped cultivars into distinct clusters and subclusters (Michałowska and Lema-Rumińska, 2019) and Iranian terrestrial orchids where ISSR-based UPGMA analysis identified multiple clusters useful for understanding population structure and conservation planning (Gholami *et al.*, 5).

Table 8: Similarity matrix of *T. divaricata* genotypes using ISSR marker.

	Acc-01	Acc-02	Acc-03	Acc-04	Acc-05	Acc-06	Acc-07	Acc-08	Acc-09	Acc-10	Acc-11	Acc-12
Acc-01	0											
Acc-02	0.43	0										
Acc-03	0.43	0.39	0									
Acc-04	0.43	0.30	0.48	0								
Acc-05	0.43	0.40	0.38	0.5	0							
Acc-06	0.43	0.43	0.40	0.39	0.35	0						
Acc-07	0.43	0.30	0.48	0.18	0.5	0.31	0					
Acc-08	0.43	0.27	0.23	0.37	0.40	0.36	0.30	0				
Acc-09	0.43	0.43	0.40	0.39	0.5	0.38	0.45	0.43	0			
Acc-10	0.43	0.39	0.36	0.48	0.51	0.47	0.48	0.31	0.33	0		
Acc-11	0.43	0.36	0.54	0.31	0.62	0.54	0.31	0.43	0.52	0.40	0	
Acc-12	0.43	0.36	0.47	0.39	0.56	0.52	0.39	0.43	0.58	0.47	0.3	0

Jaccard's similarity coefficients among *Tabernaemontana* genotypes indicated that Acc-07 and Acc-04 were the most genetically divergent, while Acc-05 and Acc-03 showed the highest similarity. Most genotype pairs exhibited moderate similarity, reflecting balanced genetic diversity within the population. Comparable ranges have been reported in garden cress (*Lepidium sativum*), where ISSR-based similarity values ranged from 0.11 to 0.89 (Kumar and Yadav, 12) and in roses, with values between 0.22 and 0.68, indicating moderate to high diversity among accessions (Luhmann *et al.*, 13).

This study concluded that phenotypic and ISSR marker based genetic diversity of *Tabernaemontana divaricata* provide scientific conservation, genetic improvement, industrial application and effective utilization in floriculture.

AUTHOR'S CONTRIBUTION

Conceptualization of research (SPT, SG); Designing of the experiments (SG, SPT); Contribution of experimental materials (SG, SPT, MV, NS); Execution of field/lab experiments and data collection (DLN); Analysis of data and interpretation (DLN); Preparation of the manuscript (DLN); Review of the final draft and corrections (SPT, SG, MV, NS).

DECLARATION

The Authors declare there is no conflict of interest.

ACKNOWLEDGEMENTS

Authors would like to Thank department of floriculture and landscape architecture and Centre of excellency in biotechnology for providing assistance in carrying out the research work.

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(Received : July, 2026; Revised : August, 2026;
Accepted : October, 2026)