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RESEARCH ARTICLE

Assessment of genetic diversity among *Ixora* genotypes through morphological and RAPD molecular marker analysis

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Abstract

Ixora a genus in the Rubiaceae family commonly known as jungle geranium, was the focus of the study. However, classifying *Ixora* species has been challenging due to environmental influences on phenotypic traits. This study explores the genetic diversity and morphological traits of 11 *Ixora* genotypes from various regions in India using 15 RAPD markers and morphological assessments. A total of 210 bands were generated, Unweighted Pair Group Method for Arithmetic Average (UPGMA) Cluster analysis revealed significant genetic relationships among the genotypes. Notably, Genotypes 9, 10 and 11 shared similar banding patterns with marker OPF 02, while Genotypes 4 and 7, despite differing growth habits, showed close genetic ties and shared RAPD markers OPA03 and OPE04. A unique marker (OPA03 - 340 bp) was identified exclusively in Genotype 4. Principal Component Analysis (PCA) indicated that 2 principal components explained 77.074 % of the total variance, highlighting plant height and leaf number as significant traits. Heat map clustering further illustrated phenotypic variability, grouping genotypes based on morphological similarities. This study demonstrates the utility of RAPD markers in distinguishing *Ixora* genotypes and provides valuable insights into morphological traits, guiding future breeding programs and genetic research.

Keywords

Ixora genotypes; genetic diversity; morphology analysis; RAPD markers; genetic relationship

Introduction

The pantropical genus *Ixora* L. is one among the 3 large genus in the Rubiaceae family, consisting of over 530 species worldwide (1, 2). It predominantly grows in humid forests and includes shrubs and small trees. *Ixora* is easily identifiable by its distinctive features, including articulate petioles, hermaphroditic flowers, hypo-crateriform corollas, 2-locular ovaries with a single ovule per locule, bilobed stigma and drupaceous fruits (3). Common names of *Ixora* include Idly poo, Viruchi, West Indian Jasmine, Rangan, Kheme, Ponna, Chann tanea, Techi, Santan, Jarum jarum, Jungle flame, Jungle geranium. The genus is renowned for its ornamental value and is admired for the stunning clusters of flowers in various shades of red, pink, white, yellow and orange (4). Classifying cultivated *Ixora* species has been

challenging and the taxonomy for many species remains inadequate (5). Environmental conditions greatly influence the phenotypical parameters with which it can be classified (6), it limits their effectiveness in distinguishing between populations. DNA-based markers have largely addressed these limitations by enabling direct comparison of DNA markers from individuals across different locations. Extensive artificial selection method and inbreeding have significantly reduced the diversity genetically in many major floricultural crop species (7). Morphological analysis of flower genotypes offers important insights for ornamental plant breeding. Phenotyping of *Ixora* sp. involves studying different physical traits to gain insights into plant diversity and support breeding programs. In the past, flower crops have relied on morphological characteristics to establish relationships between related species (8, 9). Molecular and DNA markers are crucial for characterizing and comprehending the genetic links between cultivars and species. Further, molecular markers serve various purposes including assessing genetic diversity, determining genetic relationships, barcoding, mapping and tagging important genes, developing linkage maps, marker-assisted selection and evaluating genetic fidelity and soma clonal variation in tissue-cultured plants (10). In modern molecular biology research, the use of molecular markers for selection represents a significant advancement for scientists and breeders. Molecular markers have repeatedly been confirmed to be valuable tools for assessing genetic diversity and identifying cultivars in various floricultural crops (11-15). Molecular markers such as random amplified polymorphic DNA (RAPD) are not affected by environmental variation and are commonly used for genetic analysis in various floriculture crops. In the present study, we have used RAPD technique to identify and classify different cultivars of the ornamental *Ixora* species collected from various parts of India. We sampled eleven genotypes and conducted a detailed study of their morphological characteristics. Diversity studies have been conducted and the genotypes have been clustered based on morphological and molecular data.

Materials and Methods

Plant materials

The present study was conducted to study genetic relationship of *Ixora* genotypes at Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore. Different genotypes of *Ixora* plants were collected from different parts of India (Table 1). The *Ixora* plants were planted in red soil at the Department of Floriculture and Landscaping. The experiment design was laid out in Randomized block design with 3 replications. Fresh young leaves of *Ixora* were individually collected from each plant in the early morning during summer in the month of April, 2024 and used for isolation of DNA and the phenotypic traits were taken in subsequent stages during the crop growth.

Phenotyping for morphological traits

The genotypes were phenotyped for various morphological traits. Vegetative parameters such as plant height (cm), number of leaves per plant, leaf length (cm), leaf breadth (cm), plant spread in both North South (N-S) and East West (E-W) (cm) directions, leaf area (cm^2) and chlorophyll index (mg m^{-2}) were recorded (Table 2). Flower parameters including the number of flower cymes per plant, flower diameter (cm), Flower cyme weight (g), number of florets per cyme, hundred florets weight (g), flower bud length (cm), flower bud width (cm) and corolla tube length (cm) were also recorded (Table 3).

Statistical analysis

The data obtained through phenotyping for morphological traits were analyzed using Agriwasp 2.0 with the critical difference and standard error of difference calculated. PCA analysis was conducted using KAU grapes software and heat map clustering was performed with the R program.

DNA isolation and Quantification

The young leaf samples were finely diced and carefully placed into 2 mL microcentrifuge tubes. Further 600 µL of pre-warmed CTAB extraction buffer (approximately at 65 °C)

Table 1. List of genotypes used for RAPD analysis.

Sl. No.	Genotypes	Collected location (latitude and longitude) and Mean Sea level (MSL) (m)	Flower Color
1	Genotype 1	Rajahmundry, Andhra Pradesh	Red
2	Genotype 2	Rajahmundry, Andhra Pradesh	Orange
3	Genotype 3	Rajahmundry, Andhra Pradesh	Yellowish Orange
4	Genotype 4	Rajahmundry, Andhra Pradesh	Pink
5	Genotype 5	Mettupalayam, Coimbatore (11°19'47.0"N 76°58'19.1"E) 321 m MSL	White
6	Genotype 6	Mukkombu, Trichy (10°51'11.9"N 78°40'22.1"E) 81 m MSL	Yellow
7	Genotype 7	Kamalapuram, Salem (11°35'23.9"N 78°04'19.6"E) 298 m MSL	Pink
8	Genotype 8	Mukkombu, Trichy (10°51'11.9"N 78°40'22.1"E) 81 m MSL	White
9	Genotype 9	Mettupalayam, Coimbatore (11°19'47.0"N 76°58'19.1"E) 321 m MSL	Red
10	Genotype 10	Karamadai (11°12'45.8"N 76°57'56.0"E) 369 m MSL	Red
11	Genotype 11	Kamalapuram, Salem (11°35'23.9"N 78°04'19.6"E) 298 m MSL	Yellow

Table 2. Vegetative parameters of *Ixora* genotypes.

Genotypes	Plant Height (cm)	Number of leaves/Plant (nos)	Leaf length (cm)	Leaf breadth (cm)	Plant spread (N-S) (cm)	Plant spread (E-W)	Leaf area (cm^2)	Chlorophyll index (mg m^{-2})
Genotype 1	74.47	104.33	13.98	5.19	65.85	71.08	44.64	39.32
Genotype 2	72.43	96.63	14.97	5.20	55.32	66.67	46.09	40.58
Genotype 3	66.30	80.00	12.12	4.70	44.33	48.89	43.20	30.57
Genotype 4	65.10	96.33	13.05	5.12	46.46	54.67	40.24	42.48
Genotype 5	70.20	70.33	13.46	5.73	43.62	46.78	41.89	33.32
Genotype 6	49.23	54.56	9.98	4.33	42.74	39.89	39.36	36.05
Genotype 7	36.37	44.67	7.32	4.99	28.86	25.67	32.18	32.18
Genotype 8	46.17	53.33	12.03	4.11	37.78	35.78	37.42	34.98
Genotype 9	50.17	59.67	11.67	5.15	46.28	49.87	43.22	43.22
Genotype 10	38.37	45.00	7.98	3.49	32.40	35.67	32.44	32.44
Genotype 11	35.80	35.33	6.08	4.12	29.89	28.67	35.14	35.14
Mean	54.97	67.29	11.15	4.739	43.05	45.76	39.62	36.38
SE(D)	1.25	1.59	0.25	0.09	0.65	0.88	0.72	0.76
CD (5 %)	2.61	3.31	0.52	0.183	1.36	1.84	1.49	1.59

Table 3. Flower parameters of *Ixora* genotype.

Genotypes	Number of flower cymes per plant (nos)	Flower diameter (cm)	Flower cyme weight (g)	Number of florets per cyme (nos)	Hundred florets weight (g)	Flower bud length (cm)	Flower bud width (cm)	Corolla tube length (cm)
Genotype 1	12.17	10.14	8.69	80.50	10.36	5.46	0.35	3.54
Genotype 2	9.69	9.81	8.34	76.50	9.27	4.89	0.26	2.70
Genotype 3	8.00	7.31	7.46	70.50	7.63	4.50	0.25	2.46
Genotype 4	9.50	8.67	6.97	67.50	8.98	4.13	0.14	2.84
Genotype 5	8.50	8.00	7.56	69.50	8.14	4.79	0.13	2.82
Genotype 6	6.94	6.79	7.31	58.50	6.07	4.16	0.14	2.64
Genotype 7	4.28	3.02	6.56	42.27	5.34	3.00	0.17	1.24
Genotype 8	5.51	5.92	6.34	55.03	7.22	4.08	0.11	2.56
Genotype 9	16.52	8.90	9.78	75.50	7.94	5.53	0.30	3.98
Genotype 10	5.51	4.58	5.89	52.50	6.01	4.13	0.19	1.86
Genotype 11	3.71	2.79	3.78	35.50	4.32	2.67	0.12	1.29
Mean	8.21	6.90	7.15	62.16	7.39	4.30	4.30	2.54
SE(D)	0.15	0.15	0.18	1.35	0.14	0.15	0.15	0.039
CD (5 %)	0.32	0.32	0.38	2.81	0.3	0.16	0.16	0.08

was added to the Eppendorf tubes and was followed by mechanical grinding for duration of 2 min. Again 300 µL of CTAB, along with 10 µL of β- Mercapto ethanol were added to the tubes. After incubation period, 600 µL of chloroform: isoamyl alcohol solution (24:1 ratio) was added to each tube and was centrifuged at 10000 rpm for 10 min at 4 °C. The resulting supernatant from each tube was meticulously pipetted out and transferred to new 1.5 mL microcentrifuge tubes. Consequently, 25:24:1 phenol: chloroform: isoamyl alcohol mixture was added to the tubes to facilitate the separation of DNA from secondary metabolites. The solution underwent an additional centrifugation at 10000 rpm for 10 min at 4 °C. These supernatants were then transferred into new 1.5 mL microcentrifuge tubes for further processing. To each tube, 10 µL of sodium acetate was added and thoroughly shaken for a duration of 5 min. The tubes were then supplemented with an equal volume of ice-cold (-20 °C) isopropanol, ensuring thorough mixing and

were left to incubate overnight at 4 °C. On the following day, the tubes were removed and were centrifuged at 12000 rpm for 10 min. In order to eliminate any residual salts, a centrifuge wash with 70 % ethanol was done for duration of 5 min. Once the pellets were completely dried, they were precipitated by adding 50 µL of TE buffer to the tubes. The TE buffer is a standard buffer solution that serves to stabilize and preserve DNA. Following the addition of TE buffer, the tubes were stored at -20 °C, ensuring the long-term stability and accessibility of the extracted genomic DNA for future genetic analyses and experiments. The DNA samples were quantified with NanoDrop Spectrometer based on absorbance measurements at 260 and 280 nm. A ratio of A_{260}/A_{280} near 1.8 indicated the presence of pure DNA. The ratio of OD_{260}/OD_{280} was determined in order to assess the purity and concentration of the DNA sample. DNA concentration was calculated according to the equation of Wilmington (16). Any deviations in these ratios indicated the presence of RNA or phenols, proteins or other contaminants. DNA isolation from those samples was repeated that have shown such deviation value or low quantity (< 150 ng/µL).

PCR analysis using RAPD markers

Totally 50 µL of PCR mixture was used for respective reaction which included 10X PCR buffer, 100 ng of template DNA, 2 units of Taq DNA polymerase, 0.4 Mm of each dNTP, 1.0 µM of primer and 3 mM $MgCl_2$. A total of 15 random sequence primers (Operon Technologies, California, USA) were employed in this study (Table 4). PCR reaction was carried out with the temperature of Initial denaturation at 94 °C for 10 min, denaturation at 94 °C for 45 sec, annealing at 32 °C for 45 sec and extension at 72 °C for 45 sec, final extension at 72 °C for 10 min and the DNA of 1 µL, Primer 1 µL, Master mix 5 µL, Sterile water 3 µL. The gel electrophoresis was performed with 2 % agarose gel with 120V unit for 2 h of the gel was documented using UVITEC gel documentation unit.

Table 4. Details of RAPD primers employed in this study.

Sl. No.	Primer	Sequence (5'-3')	% of G + C	Melting temperature (°C)
1.	OPA 03	AGTCAGCCAC	60	32
2.	OPC 03	GGGGGTCTTT	60	32
3.	OPA 04	AATOGGGCTG	60	32
4.	OPB 04	GGACTGGAGT	60	32
5.	OPC 04	CCGCATCTAC	60	32
6.	OPA 05	AGGGGTCTTG	60	32
7.	OPC 06	GAACGGACTC	60	32
8.	OPF 02	GAGGATCCCT	60	32
9.	OPE 03	CCAGATGCAC	60	32
10.	OPF 03	CCTGATCACC	60	32
11.	OPD 04	TCTGGTGAGG	60	32
12.	OPE 04	GTGACATOCC	60	32
13.	OPF 04	GGTGATOADG	60	32
14.	OPD 05	TGAGCOGACA	60	32
15.	OPE 05	TOAGGGADOT	60	32

Cluster analysis

Cluster analysis was conducted using NTSYSpc software to assess genetic relationships among the genotypes. Data from 210 bands were analyzed using the Unweighted Pair Group Method for Arithmetic Average (UPGMA) and dendrograms were generated to visualize the genetic distances and cluster the genotypes accordingly.

Results and Discussion

Morphological analysis

Eigen values of more than 1 was observed in 2 principal components (PC_1 and PC_2) viz., 12.332, 1.286 respectively and PC_1 contributed 77.074 of the total divergence and PC_2 with 8.04 of the total divergence in this study. Eigen value of less than 1 was observed from PC_3 to PC_{10} with values of 0.751, 0.653, 0.513, 0.202, 0.095, 0.076, 0.051 and 0.04 (Table 5). The % of variation in relation with each principal component could be demonstrated by a scree plot, obtained by a graph between eigen values and principal component numbers. From the graph, it could be observed that the first principal component PC_1 had eigen value 12.332 with 77.1 %. The graph gradually decreased with decreasing eigen value with increasing principal components. The maximum contribution to the variance was due to PC_1 (77.1 %) followed by PC_2 (8 %). The lowest contribution to variance was observed in PC_9 and PC_{10} with 0.3 %. The percentage of variance from PC_3 to PC_8 was 4.7 %, 4.1 %, 3.2 %, 1.3 %, 0.6 % and 0.5 % (Fig. 1). The PC_1 showed maximum contribution of variables on principal components with morphological traits like plant height, number of leaves per plant, leaf length, leaf breadth, plant spread (N-S), plant spread (E-W), chlorophyll index, number of flower cymes per plant, flower diameter, flower cyme weight, number of florets per cyme, hundred florets weight, flower bud length, flower bud width, corolla tube length, flower yield per (Table 6) (Fig. 2). The correlation between variables and principal component of eleven *Ixora* genotypes are represented in (Fig. 3). The previous studies also shows similar results in gerbera for number of flowers and stalk length in which Principal Component Analysis (PCA) demonstrated that the first 4 components explaining 72.3 % of the variation were driven by the number of leaves, flowers and stalk length. Flower yield had a significant positive correlation with the number of leaves and suckers (17).

Table 5. Eigen Values of *Ixora* genotypes.

Principal component	Eigenvalue	% of variance	cumulative % of variance
PC_1	12.332	77.074	77.074
PC_2	1.286	8.04	85.114
PC_3	0.751	4.694	89.809
PC_4	0.653	4.081	93.89
PC_5	0.513	3.206	97.096
PC_6	0.202	1.262	98.358
PC_7	0.095	0.594	98.952
PC_8	0.076	0.478	99.429
PC_9	0.051	0.318	99.748
PC_{10}	0.04	0.252	100

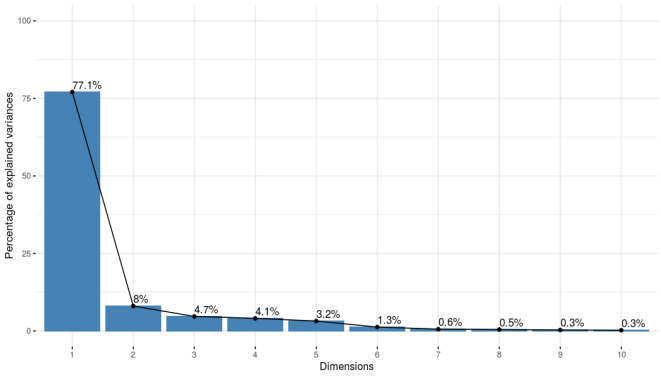

Fig. 1. Graph representing the percentage of variance in different PC.

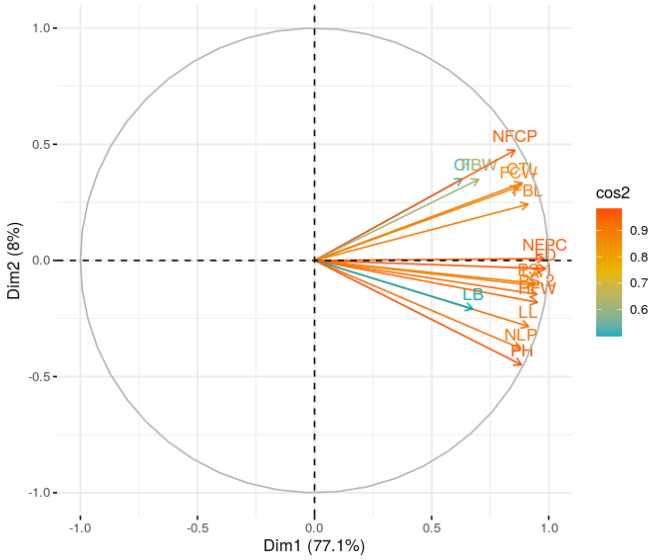

Fig. 2. PCA analysis of different variables.

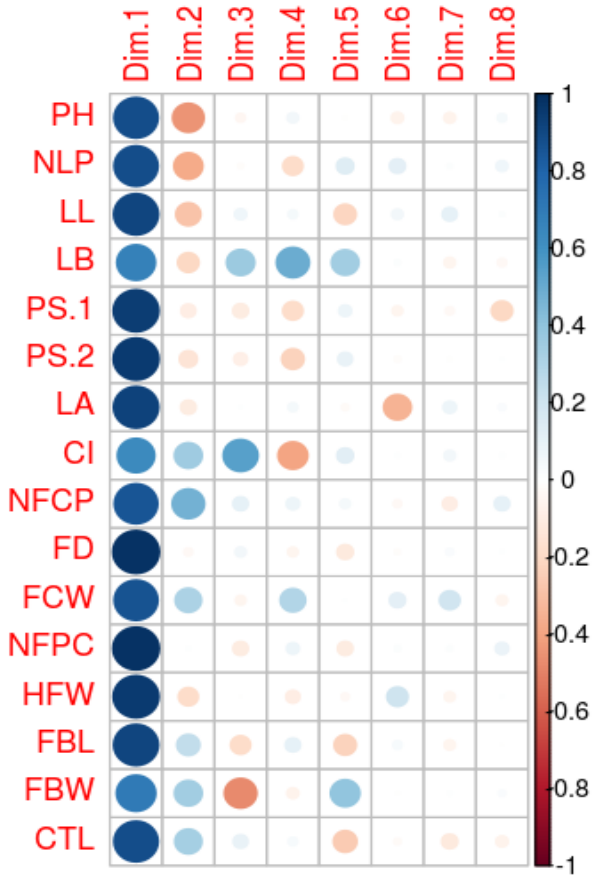

Fig. 3. A correlation plot between variables and principal components.

PH- Plant height (cm), NLP- Number of leaves per plant, LL- Leaf length (cm), LB- Leaf breadth (cm), PS1 - Plant spread (N-S) (cm),PS2- Plant spread (E-W) (cm), LA- Leaf area (cm^2), CI - Chlorophyll index , NFCP - Number of flower cymes per plant, FD - Flower diameter (cm), FCW-Flower cyme weight (g), NFPC- Number of florets per cyme, HFW-Hundred florets weight (g), FBL- Flower bud length (cm), FBW- Flower bud width (cm), CTL- Corolla tube length (cm).

Table 6. % contribution of variables on principal components of *Ixora* genotypes.

Variables	PC_1	PC_2	PC_3	PC_4	PC_5	PC_6	PC_7	PC_8	PC_9	PC_{10}
PH	6.324	15.686	0.235	0.518	0.012	2.21	4.232	2.096	0.018	0.214
NLP	6.285	11.18	0.033	5.076	3.366	6.399	0.126	5.335	0.082	0.297
LL	6.774	6.283	0.596	0.265	9.293	1.646	10.633	0.158	0.004	0.022
LB	3.702	3.381	17.388	37.085	23.542	0.103	3.693	1.918	0.020	0.054
PS.1	7.228	0.765	1.609	5.305	1.038	1.315	1.38	55.374	0.032	0.198
PS.2	7.325	1.66	0.954	7.919	1.805	0.184	0.072	0.019	0.082	0.197
LA	6.945	0.891	0.002	0.279	0.12	59.224	6.626	0.898	0.030	0.007
CI	3.224	9.591	39.896	24.445	2.9	0.023	3.59	0.104	0.033	0.065
NFCP	5.944	17.535	1.458	0.94	0.468	0.522	9.355	14.704	0.012	0.009
FD	7.895	0.099	0.414	0.385	2.737	0.111	0.853	0.03	0.026	0.139
FCW	6.11	7.743	0.347	13.216	0.003	6.691	38.368	4.118	0.024	0.088
NFPC	7.802	0.005	1.515	0.792	2.288	0.143	0.193	8.513	0.022	0.089
HFW	7.347	2.535	0.001	1.25	0.177	20.404	3.081	0.032	0.018	0.119
FBL	6.737	4.535	4.483	1.665	9.694	0.736	3.425	0.002	0.103	0.019
FBW	3.979	9.467	29.797	0.62	30.118	0.01	0.097	0.669	0.009	0.095
CTL	6.378	8.644	1.271	0.239	12.437	0.278	14.278	6.031	0.101	0.014

PH- Plant height (cm), NLP- Number of leaves per plant, LL- Leaf length (cm), LB- Leaf breadth (cm), PS1 - Plant spread (N-S) (cm),PS2- Plant spread (E-W) (cm), LA- Leaf area (cm^2), CI- Chlorophyll index , NFCP - Number of flower cymes per plant, FD - Flower diameter (cm), FCW-Flower cyme weight (g), NFPC- Number of florets per cyme, HFW-Hundred florets weight (g), FBL- Flower bud length (cm), FBW- Flower bud width (cm), CTL- Corolla tube length (cm).

Heat map clustering for morphological traits

The heat map (Fig. 4) provided a clear and effective representation of the variability among the genotypes. This visualization demonstrated how varying genetic backgrounds can result in diverse phenotypic expressions. The analysis revealed that plant height and the number of leaves per plant were red-shaded in Genotypes 1, 2, 3, 4 and 5. This indicates that these genotypes exhibited relatively high values for these traits. Conversely, the number of flower cymes per plant, flower diameter, flower cyme weight, hundred floret weight, flower bud length, flower bud width and corolla tube length were blue-shaded across all the genotypes. Similar findings were reported to facilitate the evaluation of similarity between the studied cultivars of carnation, 20 parameters were selected to generate heat map representations (18). Various size-related parameters, including the area, length and width of both flowers and petals, were grouped within the same node of the upper dendrogram, while shape-related parameters of these structures clustered within a separate node. This suggests that these traits exhibited relatively lower values compared to the red-shaded traits.

The chlorophyll index and leaf area were both blue-shaded and red-shaded in the genotypes, indicating a mix of high and low values for these characteristics. Clustering analysis revealed that Genotypes 1 and 2 as well as Genotypes 3 and 5 were grouped together. These genotypes were identified as tall genotypes. Additionally, Genotypes 6 and 8 were clustered together and they were classified as semi-tall genotypes. Overall, the heat map visualization effectively highlighted the variability among the genotypes, providing valuable insights into the diverse phenotypic expressions associated with different genetic backgrounds.

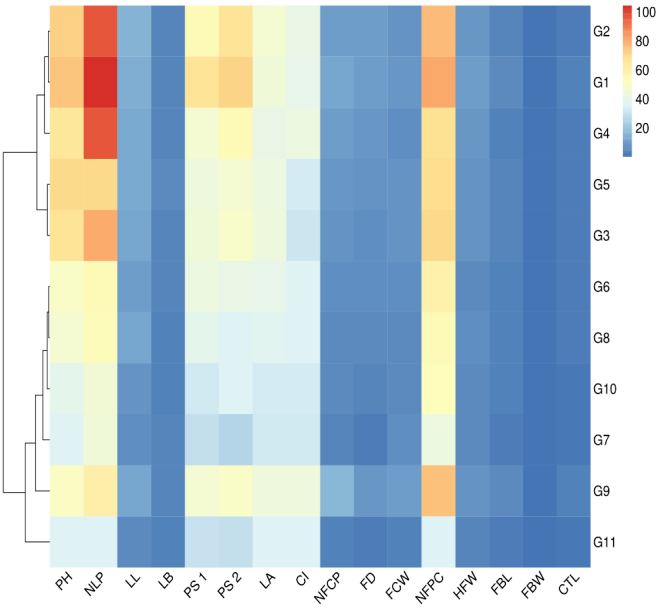

Fig. 4. Heat map representing morphological characteristics in *Ixora* genotypes.

PH- Plant height (cm), NLP- Number of leaves per plant, LL- Leaf length (cm), LB- Leaf breadth (cm), PS1 - Plant spread (N-S) (cm),PS2- Plant spread (E-W) (cm), LA- Leaf area (cm^2), CI- Chlorophyll index , NFCP - Number of flower cymes per plant, FD - Flower diameter (cm), FCW-Flower cyme weight (g), NFPC- Number of florets per cyme, HFW-Hundred florets weight (g), FBL- Flower bud length (cm), FBW- Flower bud width (cm), CTL- Corolla tube length (cm).

Molecular analysis

This study investigates the genetic diversity among 11 *Ixora* genotypes using RAPD (Random Amplified Polymorphic DNA) markers. Despite its popularity, the taxonomy of *Ixora* species has been challenging due to the influence of environmental factors on phenotypic traits, leading to difficulties in accurate classification. The importance of this study lies in its application of RAPD markers, which offer a reliable method for assessing genetic variation and relationships among *Ixora* genotypes. By generating 210 bands and employing cluster analysis, the research uncovers significant genetic relationships that align with certain phenotypic traits, providing a deeper understanding of the genetic makeup of these genotypes

The 5 primers chosen for RAPD analysis were OPA 03, OPA 04, OPC 06, OPF 02 and OPE 04. Fifteen primers were chosen randomly and were subjected to screening, among which 5 delivered optimum RAPD profiles among the cultivars used for study. Therefore, the 5 primers were sent for further analysis. Totally, 210 bands were formed from these primers, with the amplified PCR products ranging from 180 to 700. A phylogenetic tree was constructed by clustering based on the similarity matrix of 11 *Ixora* genotypes (Fig. 5).

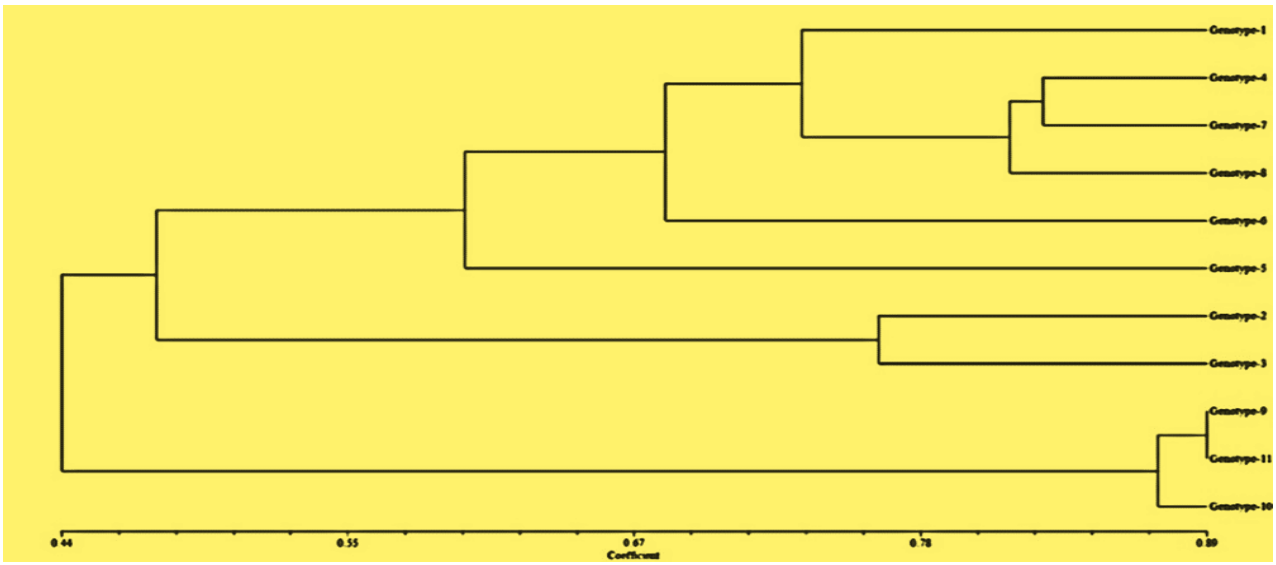

Fig. 5. UPGMA dendrogram of *Ixora* genotypes generated in this study.

Random Amplified Polymorphic DNA (RAPD) analysis was effective in estimating genetic diversity and linkage among plant cultivars. RAPD markers are known to be useful in establishing heritable links / relationships in flower crops like gladiolus (19), canna (20), hibiscus (21, 22), lilium (23), *Dianthus* species (24), Marigold (25). In *Ixora* plants, the genetic diversity was assessed using RAPD primers such as OPU-04, OPU-05, OPU-08, OPU-09, OPU-14 and OPU-15. These 6 primers generated a total of 748 bands. During data combination in the 6 primers, each cultivar produced between 15 and 49 bands. With respect to size, the amplified products diversified between 200 and 3000 base pairs (26).

Cluster analysis revealed that Genotype 4 and Genotype 7 are closely related, both showing pink flower coloration and clustering at a coefficient of 0.31. In the RAPD profiles generated by primer OPA 03 and OPE 04 (Fig. 6), both Genotype 4 and Genotype 7 share common bands at 300 bp and 380 bp. Additionally, Genotype 4 displays an additional band at 340 bp in the profile generated by primer OPA 03 (Fig. 6). The clustering of pink coloured cultivars into a single major node suggests a potential common parentage among them, possibly indicating a shared genetic background that influences flower colour. Similarly, while comparing pink (cv. 5, cv. 8, cv. 12 and cv. 13) and white-flowered cultivar (cv. 3) revealed that despite the difference in flower colour, the 2 dwarf cultivars, cv. 5 and cv. 3, are in the same node within the cultivars of *I. coccinea*. Both also share similar leaf morphology and growth habits (26). Genotype 2 and Genotype 3 exhibit bands at 280 bp, 340 bp, 380bp, 400 bp in the primer OPA 03 profile (Fig. 6). These bands indicate genetic similarity between these 2 genotypes despite their different flower colours.

Genotypes 9, 10 and 11 displayed similar DNA banding patterns when tested with primer OPF 02 with bands appearing at 350 bp, 300 bp, 280 bp, 250 bp, 240 bp, 220 bp and 180 bp (Fig. 7). These genotypes are closely related with both genotypes 9 and 11 producing red coloured flowers. When tested with primer OPE 04, genotypes 10 and 11 showed bands at 300 bp, 280 bp, 240 bp and 200 bp. Furthermore, using primer OPA 03, a 400 bp band was observed in genotypes 9, 10 and 11 (Fig. 6). Genotypes 6, 8 and 9 were found to be unrelated based on cluster analysis. When using Primer OPC 06, these genotypes showed a common band at 400 bp. Additionally, in RAPD profiles produced by primers OPE 04 and OPF 02, genotypes 6, 8 and 9 exhibit bands at 280 bp and 300 bp (Fig. 7).

Conclusion

The morphological traits, as depicted in the heat map, showed clear variability across the genotypes. Genotypes 1, 2, 3, 4 and 5 exhibited relatively high values for plant height and number of leaves per plant, while traits like flower cymes and corolla tube length had lower values across the genotypes. In contrast, RAPD analysis effectively estimated genetic diversity and relationships among various *Ixora* plant cultivars. Genotypes with similar flower colors such as pink or red clustered closely, suggesting potential common parentage. Cluster analysis revealed that genotypes with similar flower colors, such as 4 and 7 as well as 5, 8, 12 and 13 were genetically related, likely due to shared genetic backgrounds. Interestingly, some genotypes with contrasting flower colors, such as Genotypes 2 and 3, exhibited genetic similarity based on common RAPD bands. Conversely, Genotypes 6, 8 and 9 were found to be unrelated despite sharing some RAPD bands. The integration of morphological and RAPD data allowed for a more comprehensive understanding of the genetic diversity, complementing the challenges posed by environmental influences on phenotypic traits. The combined use of morphological and RAPD markers proved to be a powerful approach in elucidating the genetic

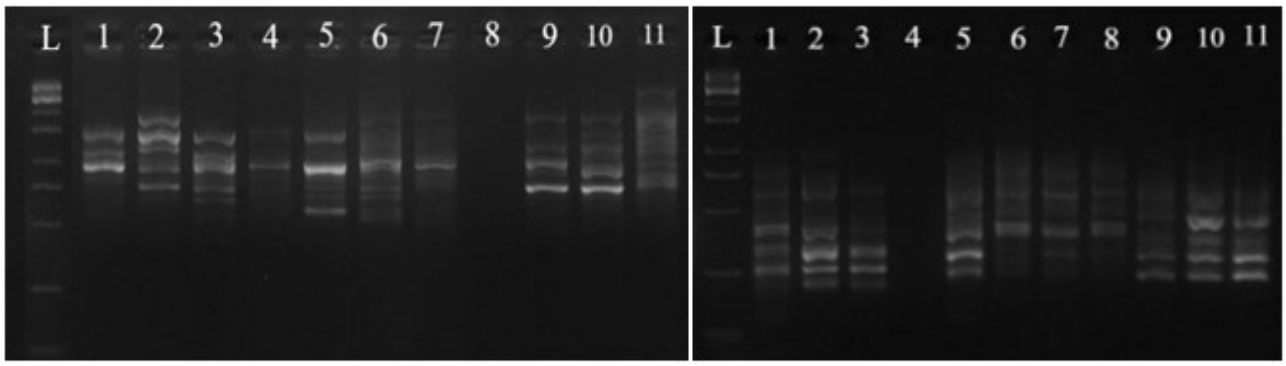

Fig. 6. Electropherogram of *Ixora* genotypes using RAPD OPA 03, OPE 04.

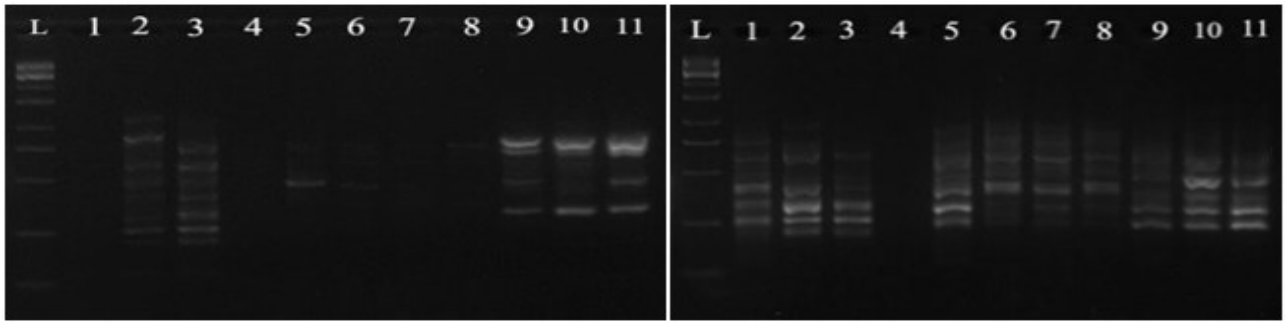

Fig. 7. Electropherogram of *Ixora* genotypes using RAPD OPC 06, OPF 02.

diversity and relationships within this *Ixora* collection, paving the way for improved classification and breeding strategies.

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Authors' contributions

PA carried out the experiment, took observations and analysed the data. MV guided the research by formulating the research concept and approved the final manuscript. NMB contributed by imposing the experiment, helped in editing, summarizing and revising the manuscript VAS helped in securing research funds. KV helped in summarizing and revising the manuscript.

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