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Original Research Paper

Diversity analysis in Hibiscus (*Hibiscus* spp.) germplasm

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ABSTRACT

There is a wide array of germplasm availability in hibiscus throughout the globe. India, harbours a rich wealth of hibiscus, including *H. rosa-sinensis, H. fragilis, H. storckii, H. schizopetalous*, etc. These species serve as crucial repositories of genes responsible for various traits, including biotic and abiotic stress tolerance, colour intensity, petal arrangement, and hardiness. Safeguarding these genetic resources is essential for their utilization in future breeding programme. In this study, 19 hibiscus germplasm underwent precise classification based on 23 vegetative and floral characters following distinctness, uniformity, and stability (DUS) test guidelines of Protection of Plant Varieties and Farmers' Rights Authority, New Delhi, India. Dissimilarity calculations, hierarchical cluster analysis, network plot analysis, and principal component analysis (PCA) were employed to assess the extent of diversity among the germplasm. The highest diversity was observed in variety June's Joy. The hierarchical cluster analysis grouped the species into four major clusters, validated by linear discriminant analysis. Notably, *Hibiscus rosa-sinensis* germplasm grouped in the same cluster for flower colour. PCA results mirrored the dendrogram pattern, with June's Joy standing out distinctly. While, no single morphological trait can singularly differentiate all germplasms, the collection of a comprehensive passport data incorporating various traits is imperative for advancing further studies in hibiscus genetic diversity and breeding programme.

Keywords: Genetic diversity, hierarchical cluster analysis, network plot analysis, PPV&FRA, principal component analysis

INTRODUCTION

The genus *Hibiscus*, family Malvaceae, comprising more than 400 species of flowering plants that are native to warm temperate, sub-tropical and tropical regions throughout the world (Edmonds, 1991). The ancestors of today's modern hibiscus hybrids were interspersed all around the globe. The eight *Hibiscus* species *viz*., *Hibiscus rosa-sinensis*, *H. lilliflorus*, *H. storckii*, *H. fragilis*, *H. scnizopetalus*, *H. genevieve*, *H. arnottianus* and *H. kokio*, are considered to be the ancestors of the modern exotic *Hibiscus* which were originally native to Mauritius, Madagascar, Fiji, Hawaii, and either China or India (Ayanbamiji et al., 2012).

Studies have proved the presence of antioxidant, antifungal, and antimicrobial properties in flowers of *Hibiscus* genus (Khristi & Patel, 2016). India is one of the key cultivators of the *Hibiscus*, but with the increasing demand for novel *Hibiscus* cultivars in the world flower market, a huge number of varieties are being bred every year with novel flowering, foliage and other growth traits. Therefore, precise identification and characterization of those cultivars is necessary for variety identification, regulation, and protection of plant breeder's rights. Plant characterization helps us by providing the detailed description about material, which is vital for its conservation, management, and utilization of the material.

Morphological classification helps to identify the internal and external structure of the various plants that helps the botanists to improve their analytical approaches. It also helps to co-relate different varieties of same species as well as different species on the basis of morphological parameters. In order to classify the array of germplasm the DUS (distinctive, uniform and stable) testing is done in many floricultural crops like tuberose, marigold, rose, jasmine, lily, chrysanthemum, bougainvillea, carnation, gerbera, orchid, etc. It is useful for variety identification, variety registration and plant variety protection (PVP) Act, varietal information system and variety classification into different groups, and genetic resources (Jyoti et al., 2015).

Plant breeders have traditionally chosen plants based on visible or measurable characteristics such as flower colour, seed colour, leaf form, fruit shape, stem length, and so on (Jiang, 2013). Such descriptors are the qualities that are highly heritable, ranging from morphological markers, and are expressed in all contexts to establish distinctions or similarities in phenotypic attributes of each accession. Classification and evaluation based on phenotypic features is a quick, simple, and useful guidance in selecting parents for hybridization (Brown & Caligari, 2008).

Considering the above-mentioned rationale, the present study was aimed to evaluate Hibiscus germplasm based on DUS descriptors in order to classify *Hibiscus* germplasm for future breeding programme.

MATERIALS AND METHODS

Plant material

A total of 19 *Hibiscus* germplasm (15 from *Hibiscus rosa-sinensis* namely Brilliant, Celia, Cinnamon Girl, Double Peach, Elephant Ear, Eureka, Golden Doublon, Holly's Pride, Isobel Beard, June's Joy, Red Dragon, Sudarshan Chakra, Valentine's Day, Viceroy and Versicolor Pinwheel; Fragilis from *H. fragilis*; Oiseau Blue from *H.* cannabinus; Spider from *H. schizopetalus* and Storckii from *H. storckii*), were collected from All India Co-ordinated Research Project on Floriculture, Bidhan Chandra Krishi Viswavidyalaya (BCKV), West Bengal, India, geographically located at 22°562 42.883 N, 88°322 0.863 E.

Characterization

The experiment was conducted at Horticulture Research Station, Mondouri Farm, BCKV, West Bengal during 2021 to 2023 by observing 23 morphological qualitative characters based on plant growth (growth habit and plant height), branching (branching pattern, branch attitude and branch colour), leaf (length and variegation of leaf blade, shape of leaf blade, shape of leaf base, shape of leaf apex, lobing pattern of leaf and type of incisions of margin), flower (type of flower, flower diameter, flower colour, eye zone presence, size of eye zone, colour of eye zone, petal length, petal width, petal type, arrangement of basal petals, number of colours on inner side excluding eye zone). For vegetative traits, numerical scoring was done as per DUS guidelines of *Hibiscus* given by PPV&FRA, New Delhi, India. The DUS characterization of the *Hibiscus* germplasm was done on the basis of some selected qualitative parameters and their traits according to the guidelines provided by International Union for the protection of new varieties of plants.

Statistical analysis

The experiment was laid out in completely randomized design with 19 treatments (Hibiscus germplasm) and ten replications. Morphological variables (numerical scores) that exhibited uniformity across all genotypes were excluded from dissimilarity calculation, hierarchical cluster analysis, linear discriminant analysis, network plot analysis and principal component analysis (PCA). The pairwise dissimilarity (distances) matrix and matrix plot were computed based on the numerical scores, likewise the network plot analysis and hierarchical clustering of germplasm was performed to find the association and grouping among the germplasm using PAST software® (Ver. 4.0) (Hammer & Harper, 2001). Eventually, PCA was employed to discern multidimensional contribution from various descriptors, aiding in classification of genotypes into different groups.

RESULTS AND DISCUSSION

Characterization *Hibiscus* germplasm

Utility of the *Hibiscus* DUS traits during categorization of the *Hibiscus* germplasm were critically proven since the morphological descriptors effectively classified the 19 germplasms into multiple groups. These morphological traits were plant growth habit, spreading tendency, branch colour, leaflet shape and size, leaf serration, flower shape and size, petal arrangement, and eye zone. The spectrum of plant growth habit spans from upright to strongly spreading, catering to various purposes. Varieties such as Versicolor Pinwheel and Celia, classified under *H. rosa-sinensis* (Fig. 1), exemplify upright growth. Similarly, Brilliant, Celia, and Oiseau Blue showcase an upright branching attitude. Leaf shape, a significant characteristic, manifests in four types: cordate, elliptical, ovate, and circular (Fig. 1d-g). Notably, Brilliant, Cinnamon Girl, Double Peach, and Eureka exhibit a cordate leaf type. Utilization of specific varieties is highlighted; Celia is suitable for tall growth, while dense spreading types like Brilliant and medium-spreading varieties like Fragilis can be

Table 2 : DUS characters of plant, branch and leaf for 19 germplasm of *Hibiscus*

Germplasm	Plant			Branch		Leaf blade							
	Growth habit	Height	Branching	Attitude	Colour	Length	Variegation	Shape	Shape of base	Shape of apex	Lobing	Intensity of lobing	Type of incision of margin
Brilliant	Bushy	Medium	Dense	Upright	Brown	Medium	Absent	Cordate	Truncate	Obtuse	Absent		Crenate
Celia	Upright	Tall	Dense	Upright	Greenish brown	Medium	Absent	Ovate	Truncate	Acute	Absent		Serrate
Cinnamon girl	Upright	Medium	Sparse	Semi upright	Greenish brown	Long	Absent	Cordate	Cordate	Obtuse	Absent		Dentate
Double Peach	Bushy	Short	Sparse	Horizontal	Greenish brown	Medium	Absent	Cordate	Cordate	Obtuse	Present	Shallow	Dentate
Elephant Ear	Bushy	Short	Sparse	Horizontal	Greenish brown	Long	Absent	Elliptic	Obtuse	Rounded	Absent		Dentate
Eureka	Bushy	Short	Sparse	Horizontal	Greenish brown	Long	Absent	Cordate	Cordate	Obtuse	Absent		Crenate
Fragilis	Upright	Medium	Medium	Semi upright	Green	Medium	Absent	Ovate	Cuneate	Acute	Absent		Serrate
Golden Doublon	Upright	Medium	Medium	Semi upright	Brown	Medium	Absent	Cordate	Cordate	Obtuse	Absent		Crenate
Holly's Pride	Bushy	Short	Sparse	Semi upright	Brown	Medium	Absent	Cordate	Cordate	Acute	Absent		Serrate
Isobel Beard	Upright	Short	Sparse	Semi upright	Brown	Medium	Absent	Cordate	Cordate	Obtuse	Absent		Dentate
June's Joy	Upright	Medium	Sparse	Semi upright	Greenish brown	Long	Absent	Ovate	Cordate	Obtuse	Absent		Crenate
Oiseau Blue	Upright	Tall	Dense	Upright	Green	Medium	Absent	Ovate	Cuneate	Acute	Present	Medium	Crenate
Red Dragon	Bushy	Short	Sparse	Semi upright	Brown	Medium	Absent	Circular	Truncate	Rounded	Absent		Crenate
Spider	Upright	Tall	Medium	Upright	Greenish brown	Medium	Absent	Ovate	Cuneate	Acute	Absent		Crenate
Storckii	Bushy	Medium	Medium	Semi upright	Greenish brown	Short	Absent	Cordate	Cordate	Obtuse	Absent		Crenate
Sudarshan Chakra	Upright	Tall	Medium	Semi upright	Greenish brown	Medium	Absent	Cordate	Cordate	Rounded	Absent		Crenate
Valentine's Day	Bushy	Short	Sparse	Horizontal	Greenish brown	Long	Absent	Cordate/ entire	Cordate	Rounded	Absent		Crenate
Viceroy	Bushy	Medium	Dense	Upright	Green	Medium	Absent	Obovate	Cuneate	Acute	Absent		Crenate/entire
Versicolor Pinwheel	Upright	Tall	Medium	Upright	Brown	Medium	Absent	Cordate	Cordate	Acute	Absent		Serrate

Table 3 : DUS characters of flower and petal for 19 germplasm of *Hibiscus*

Germplasm	Flower						Petal			
	Type	Diameter	Main colour	Eye zone	Size of eye zone	Colour of eye zone	Length	Width	Arrangement of basal petals	No. of colours
Brilliant	Single	Medium	Orange	Present	Medium	Dark Pink RHS N 57D	Medium	Medium	Slightly overlapping	One
Celia	Cluster	Medium	Dark red	Absent			Medium	Narrow	Touching	One
Cinnamon Girl	Single	Medium	Brown	Present	Medium	Brown Org RHS 171B	Medium	Broad	Strongly overlapping	Two
Double Peach	Semi double	Medium	Pink	Absent			Medium	Medium	Slightly overlapping	One
Elephant Ear	Double	Large	Near white	Absent			Long	Medium	Slightly overlapping	One
Eureka	Double	Large	Yellow	Absent			Long	Medium	Strongly overlapping	One
Fragilis	Single	Medium	Pink	Absent			Medium	Medium	Slightly overlapping	One
Golden Doublon	Single	Large	Yellow	Present	Small	Red RHS 47A	Long	Broad	Slightly overlapping	One
Holly's Pride	double	Medium	Light red	Absent			Medium	Medium	Slightly overlapping	One
Isobel Beard	Semi double	Medium	Purple	Present	Medium	Dark Purple Red RHS 53A	Medium	Medium	Slightly overlapping	One
June's Joy	Single	Large	Yellow	Present	Large	Red RHS 47A	Long	Broad	Slightly overlapping	Two
Oiseau Blue	Single	Medium	Purple	Present	Small	Dark Pink Red RHS 53A	Medium	Medium	Strongly overlapping	One
Red Dragon	Double	Medium	Medium red	Absent			Medium	Medium	Slightly overlapping	One
Spider	Single	Medium	Dark red	Absent			Medium	Narrow	Free	One
Storckii	Single	Medium	Orange	Present	Medium	Dark Pink Red RHS 52A	Short	Medium	Slightly overlapping	Two
Sudarshan Chakra	Single	Large	Yellow	Present	Medium	Purple Brown RHS 179A	Long	Broad	Strongly overlapping	One
Valentine's Day	Single	Large	Pink	Present	Large	Red RHS 45A	Long	Broad	Strongly overlapping	Two
Viceroy	Single	Small	Pink	Absent			Short	Narrow	Slightly overlapping	One
Versicolor Pinwheel	single	Large	Light red	Present	Small	Red RHS 47A	Long	Medium	Slightly overlapping	One

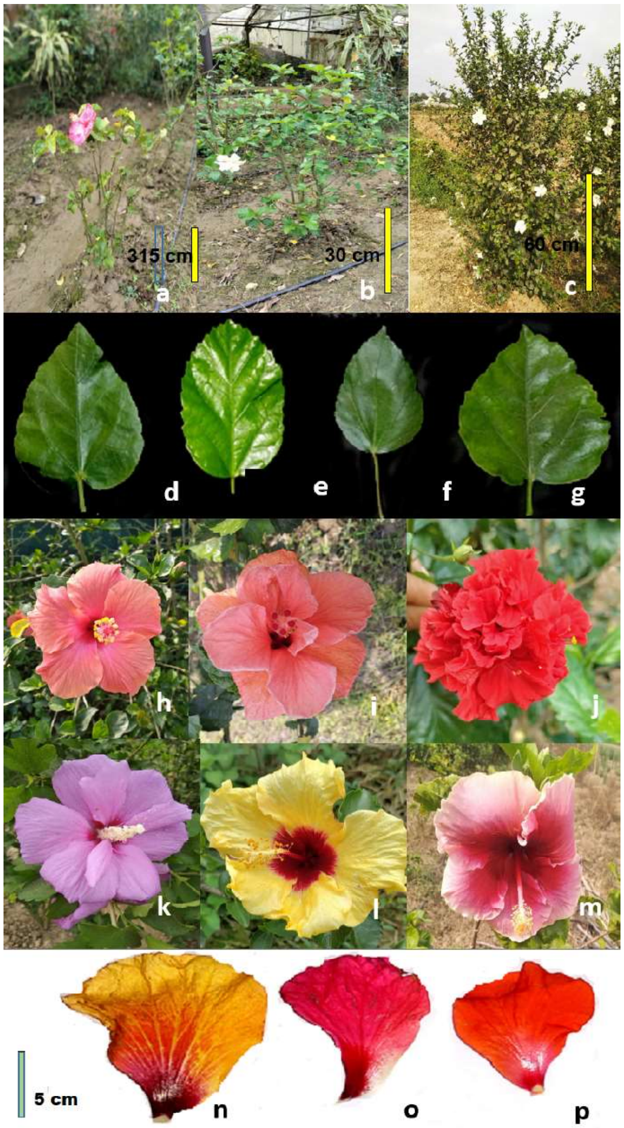

Fig. 1 : Variability on morphological traits based on DUS traits

(a-c) plant height: (a) short, (b) medium, (c) tall; (d-g) leaf shape: (d) cordate, (e) elliptic, (f) ovate, (g) circular; (h-j): type of flower: (h) single, (i) semi-double, (j) double; (k-m): size of flower eye zone: (k) small, (l) medium, (m) large; (n-p): petal length, (n) long, (o) medium, (p) short.

employed in the breeding of climber-type cultivars (Naik et al., 2022). A secondary important trait involves anthocyanin coloration of young shoots and its hue. Recognizing the well-established role of anthocyanin as an antioxidant, providing protection against abiotic stresses and delaying leaf senescence (Landi et al., 2015), varieties with intense anthocyanin coloration are deemed effective sources of resistance against abiotic stress. Varieties such as Brilliant, Golden Doublon, and Holly's Pride have been documented for their robust anthocyanin coloration, suggesting their potential utility in transferring this trait to enhance resistance (Gaurav et al., 2018).

Matrix plot analysis

A matrix plot (Fig. 2) was generated based on the variation among various DUS characters across the germplasm. In this representation, dark red indicates a higher probability of the characters, whereas, a lighter shade signifies its presence with reduced likelihood. Conversely, dark blue denotes the absence of the specific character. Notably, the character 'leaf variegation' was not present in all the germplasm under study, contributing to the similarity among them. On the other hand, characters like flower colour, eye zone colour, and leaf shape were distinctly present in all germplasm, suggesting a uniformity that highlights polymorphism in DUS characters. Since matrix plot simplifies complex numerical data into understandable visualizations (Subrahmanyeswari et al., 2022) it can offer a visual depiction of the distribution and variability of DUS characters among the studied germplasm. The variation in 21 DUS parameters were also reported in 42 genotypes of China aster (Bhargav et al., 2018 & 2023).

Network plot analysis

From the analysis of the network plot (Fig. 3), it was evident that germplasm exhibiting morphological diversity were not connected through lines to other germplasm. Notable examples include Brilliant, Fragilis, Sudarshan Chakra, and Golden Doublon. On the other hand, Eureka, Holly's Pride, Elephant Ear, and Versicolor Pinwheel formed a cohesive cluster. Additionally, varieties like Oiseau Blue and June's Joy demonstrated similarity but were entirely distinct from other germplasm. Drawing parallels with previous studies on orchid and potato (Subrahmanyeswari et al., 2022; Char et al., 2023), the utilization of network plot analysis to discern association or disparities among multiple treatments has proven to be successful. Interestingly, this study marks the pioneering application of network plot analysis in the context of *Hibiscus*, making it the first of its kind in this domain.

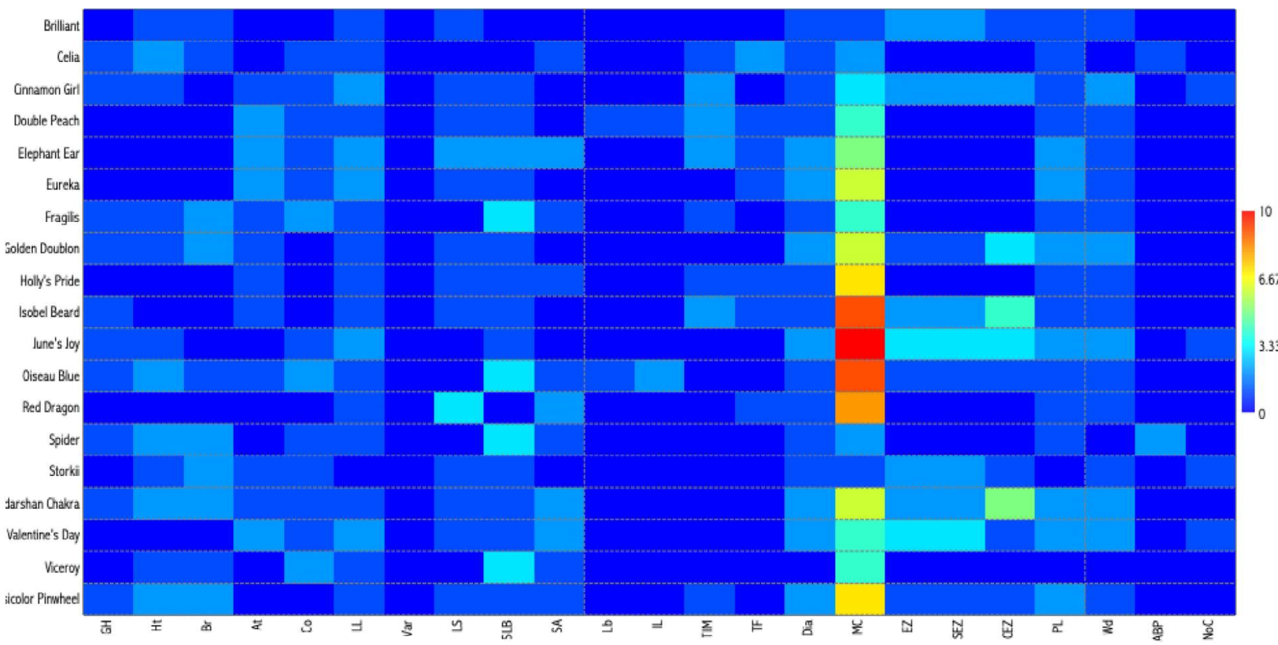

Fig. 2 : Matrix plot representing presence or absence of DUS characters of *Hibiscus* germplasm

GH: growth habit, Ht: height; Br: branching; At: attitude; Co; colour of branch; LL: leaf length; Var: leaf variegation; LS: leaf shape; SLB: shape of leaf base; SA: shape of apex; Lb: leaf lobing Il: intensity of lobing; TIM: type of incision of margin; TF: type of flower; Dia: petal diameter; MC: petal main colour; EZ: eye zone; SEZ: size of eye zone; CEZ: colour of eye zone; PL: petal length; Wd: petal width; ABP: arrangement of basal petals; NoC: number of colours on petal's inner sides

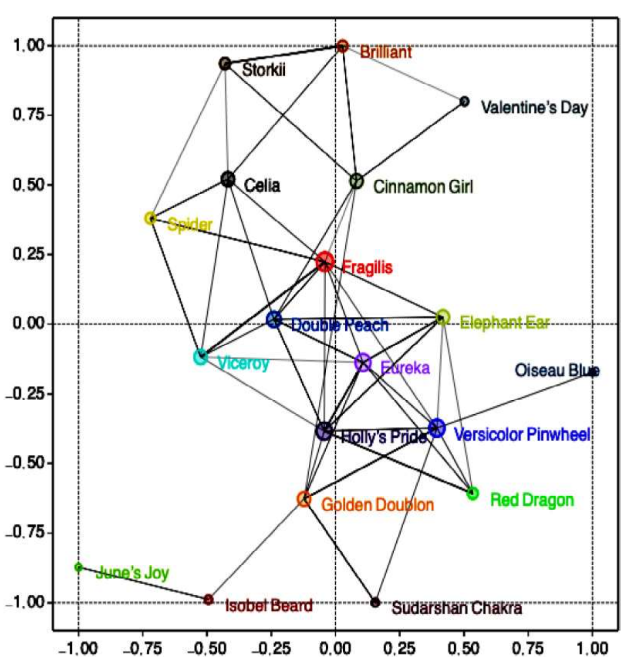

Fig. 3 : Network plot analysis of *Hibiscus* germplasm based on DUS traits

Cluster analysis

The generated UPGMA dendrogram unveiled genetic relationships among the 19 *Hibiscus* germplasm. Through this analysis, the germplasms were organized into four different clusters, with Cluster IV containing the highest number of germplasms (6), followed by clusters I, II and III, each featuring similar varieties within them (Fig. 4). The cluster analysis based on plant growth habit, branching pattern, leaf variation, and floral characters exhibited that the germplasm of semidouble flowered type (such as Double Peach, Elephant Ear, Eureka, Holly's Pride, and Red Dragon) were grouped into a single cluster. The other distinct cluster was comprised of the germplasm without an eye zone, including Fragilis, Spider, Viceroy, and Celia, which also possessed medium leaf length. A distinct cluster was exhibited for the germplasm with red and yellow-coloured petals, prioritizing flower colour in adherence to the DUS test guidelines. This separation reflected a higher emphasis on the flower colour trait. It's noteworthy that the classification based on these horticultural traits proves more beneficial for selecting cultivars for landscape use rather than indicating genetic relatedness. Similar study was conducted by Gaurav et al. (2018) in rose and found anthocyanin as a major character to distinguish germplasm. This clustering provides valuable insights into the genetic associations and diversity among the studied *Hibiscus* germplasm, aiding in better selection and utilization for specific horticultural purposes.

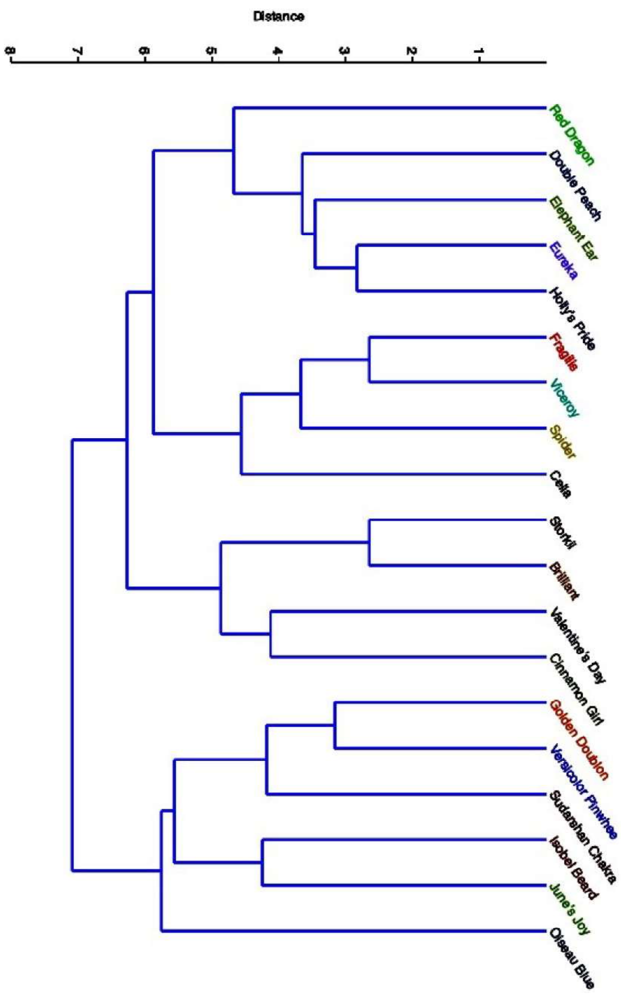

Fig. 4 : Dendrogram based on the hierarchical clustering of *Hibiscus* germplasm

Principal component analysis

PCA was employed to discern multidimensional relationships among various traits and group species. This statistical method extracts essential variables, represented as different components, from a comprehensive dataset, presenting them in a more interpretable format. The principal components collectively accounted for 100% of the cumulative variables. PCA, conducted on 23 traits of *Hibiscus* across all species, revealed that the main colour of the petal explained 41.6% of the variability in the principal component. As depicted in Fig. 5, the principal component effectively classified *Hibiscus* germplasm into three prominent clusters. Notably, June's Joy stood out distinctly from all other germplasms. The outcomes of the PCA align with the hierarchical cluster analysis, indicating a near-parallel pattern. Germplasm sharing a common cluster in the cluster analysis are positioned closer to each other in PCA (Fig. 5). This integrated approach of PCA and hierarchical clustering provides a comprehensive understanding of the relationship and similarities among different traits and species within the studied dataset. Similar observation was reported by Pornsuriya et al. (2011) in bitter melon and Rai et al. (2015) in rose.

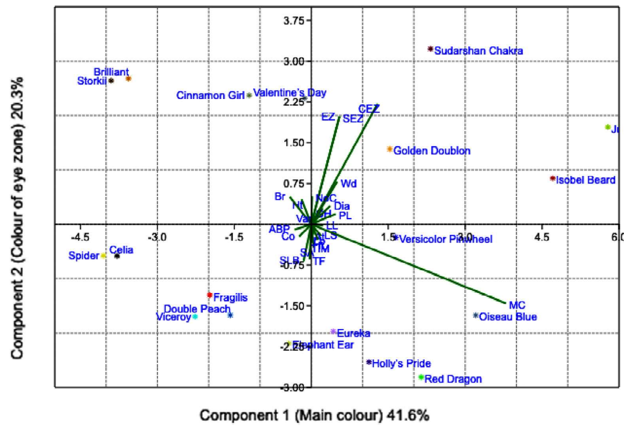

Fig. 5 : Principal component analysis of *Hibiscus* germplasm on the basis of DUS traits

GH: growth habit; Ht: height; Br: branching; At: attitude; Co: colour of branch; LL: leaf length; Var: leaf variegation; LS: leaf shape; SLB: shape of leaf base; SA: shape of apex; Lb: leaf lobing; Il: intensity of lobing; TIM: type of incision of margin; TF: type of flower; Dia: petal diameter; MC: petal main colour; EZ: eye zone; SEZ: size of eye zone; CEZ: colour of eye zone; PL: petal length; Wd: petal width; ABP: arrangement of basal petals; NoC: number of colours on petal's inner side

CONCLUSION

The *Hibiscus* genus encompasses highly variable species that readily hybridize, leading to varying interpretation of species classification. Given the absence of a singular morphological characteristic for species differentiation, morphological characterization remains essential, serving as crucial support for molecular data. Therefore, a comprehensive collaborative study is warranted, incorporating advanced molecular markers alongside a morphological database. Notably, June's Joy has been identified as the sole bicoloured variety among the examined ones. This highlights the need for a holistic approach that combines both morphological and molecular insights to enhance our understanding of the complex variability within the studied *Hibiscus* germplasm.

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