



RESEARCH ARTICLE

Genetic diversity analysis of advanced breeding lines of watermelon (*Citrullus lanatus* (Thunb.) Mansf.)

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Abstract

Watermelon (*Citrullus lanatus* (Thunb.) Mansf.) breeding lines were subjected to 26 standardised distinctness, uniformity and stability (DUS) morphological descriptors to estimate the genetic diversity among 165 advanced breeding lines (F₄ and BC₁F₃). Descriptive statistics quantified trait variability across vegetative, floral, fruit and seed characters. The breeding lines were classified through hierarchical agglomerative clustering using Ward's method and squared Euclidean grouped into 13 clusters, revealing broad phenotypic divergence of trait combinations. Clusters of particular breeding interest included dwarf or compact genotypes with white or orange flesh, groups combining high seediness with orange flesh and compact early-growing lines or traits with direct value for parent selection. Both qualitative descriptors (flesh colour, rind pattern, sex expression) and quantitative measurements (fruit size, leaf dimensions, seed number) contributed to cluster discrimination. Statistical analyses and cluster validation were performed in SPSS and cross-validated using R (principal component analysis (PCA) and dendrogram visualisation). Cluster means, trait-wise patterns and identified outlier genotypes inform strategic parent choice for introgression, candidate lines for DUS-based varietal registration and the conversion of rare phenotypes. These results provide an actionable framework to broaden the genetic base of watermelon breeding programmes and to accelerate development of DUS-compliant cultivars with improved fruit quality and resilience.

Keywords: *Citrullus lanatus*; cluster analysis; diversity; DUS; phenotype; watermelon

Introduction

Watermelon (*Citrullus lanatus* (Thunb.) Mansf.) Matsum. and Nakai) is a major fruit vegetable grown extensively across tropical and subtropical regions because of its high-water content, pleasant sweetness and nutritional richness. Globally, it is being grown in 3.2 million ha, yielding nearly 102 million Mt (1). India stands as the 4th largest producer of watermelon and has shown a steady rise in both cultivated area and total production. The cultivation area increased from approximately 80000 ha in 2010 producing 2.04 million t, to about 110000 ha with 2.78 million t in 2023–24 (2). In addition, India's watermelon exports reached a value of USD 20.98 million (INR 174.7 crores) in 2022–23 and the crop being of international importance (3).

Breeding efforts focus on developing varieties that possess higher yield potential, better stress tolerance and superior fruit quality. The success of breeding programmes depends on the effective utilisation of genetic diversity within available germplasm. A broad genetic base allows for combining agronomic strength with consumer preferred traits (4). The genetic diversity plays a vital role in developing superior cultivars by offering new alleles that enhance resistance to both biotic and abiotic stresses and contribute to overall quality improvement (5).

Furthermore, genetic diversity forms the foundation of sustainable breeding, enabling the identification of genotypes suited to desirable agronomic traits and consumer needs (6). Similarly, it was pointed out that breeding programs relying on a narrow genetic base tend to be more susceptible to environmental changes and pest infestations, highlighting the need to preserve and effectively use genetic variability for enduring crop improvement (7).

In India, the Protection of Plant Varieties and Farmers' Rights Authority (PPV&FRA), provides a legal framework for plant variety protection (8). The authority has developed standardised distinctness, uniformity and stability (DUS) test guidelines for watermelon. These guidelines not only support varietal registration and protection but also offer a robust basis for phenotypic evaluation in breeding programs. Distinctness, uniformity and stability descriptors are especially valuable for characterising genetic variation due to their stability, heritability and ease of observation under field conditions. They enable the classification of genotypes based on vegetative, floral, fruit and seed traits, serving both regulatory and breeding purposes. Similarly, 10 standard reference varieties were evaluated for trait-based variability on the basis of 27 PPV&FRA DUS descriptors (9).

Multivariate techniques such as Ward's hierarchical clustering, when applied to DUS trait data, allow for objective genotype grouping, revealing patterns of phenotypic diversity and identifying unique trait combinations useful for breeding. Such analyses enhance the understanding of genetic structure within breeding populations and support the strategic selection of parents for varietal development. Fifty-seven watermelon genotypes were assessed for morphological diversity across 8 traits using Wards minimum variance method (10).

Principal component analysis (PCA) using phenotypic traits helps in determining total genotypic variation existing in any population along with narrow sense heritability (h^2) and genetic advance estimate. Based on PCA score for a particular trait we can identify key traits responsible for maximum variability and through K means a large population can be categorised into different clusters based on their PCA values giving specifically trait based selection.

Multivariate analysis using correlation analysis, PCA and K means clustering done from F_2 to F_5 generation revealed the extent of genetic variability in different populations and identified key traits (fruit size, shape and TSS) contributing maximum variation thereby confirming these traits as independent axes of variation dissecting the genotypes into 2 distinct breeding groups for divergent selection in watermelon (11).

Accordingly, this study evaluated 165 advanced watermelon breeding lines including F_4 and BC_1F_3 populations based on 26 DUS morphological characters to access genetic diversity. The analysis sought to recognise unique phenotypic clusters and trait combinations that could help breeders in selection decisions and varietal differentiation.

Materials and Methods

Plant materials

A total of 165 advanced breeding lines, representing F_4 and BC_1F_3 populations, included the check varieties Arka Shyama and two resistant donor parents, BIL-53, which resistant to watermelon bud necrosis virus (WBNV) and IHR-392 which is resistant to resistant to fusarium wilt.

Experimental details

The study was conducted during 2023–24 at Block 6, Division of Vegetable Crops, ICAR-Indian Institute of Horticultural Research, Hesaraghatta Lake Post, Bengaluru (13°01'31"N; 77°49'01"E; 890m AMSL). The soil at the experimental site is red sandy loam, well-drained. The experiment was laid out in an augmented block design with a single replication. Raised beds of 20cm height were prepared, maintaining 1.8m row spacing and 45cm between plants. Straight-line planting was carried out on the mulching sheaths and weed mats were covered to avoid weeds growth during the evening to minimize heat stress and promote uniform establishment.

Test of statistical analysis

Distinctness, uniformity and stability characterisation

All statistical analyses were conducted to assess the morphological diversity and cluster structure among the watermelon breeding lines. Descriptive statistics (mean, range, standard deviation) for each DUS trait were calculated to characterise trait variability. Both qualitative (categorical) and quantitative (continuous) DUS data were included in the statistical procedures. Each breeding line was characterised using 26 key morphological descriptors based on the DUS guidelines, categorised into four major trait groups: vegetative, floral, fruit and seed traits (Table 1). Vegetative parameters

Table 1. Morphological characterisation of watermelon lines based on PPV&FRA distinctness, uniformity and stability (DUS) descriptor guidelines

Trait group	Trait	Descriptor type	Standard states (Score)	Observed states in present study
Vegetative	Cotyledon shape	Qualitative	1 – Narrow elliptic, 2 – Medium elliptic, 3 – Broad elliptic	1, 2, 3
	Internode length (cm)	Quantitative (State-wise)	3 – Short (< 7 cm), 5 – Medium (7–8.5 cm), 7 – Long (> 8.5 cm)	3, 5, 7
	Leaf blade length (cm)	Quantitative (State-wise)	3 – Short (< 10 cm), 5 – Medium (10–14 cm), 7 – Long (> 14 cm)	3, 5, 7
	Leaf blade width (cm)	Quantitative (State-wise)	3 – Narrow (< 9 cm), 5 – Medium (9–12 cm), 7 – Broad (> 12 cm)	3, 5, 7
	Leaf blade colour	Qualitative	3 – Green, 5 – Dark green	3, 5
	Degree of primary lobing	Qualitative	5 – Medium, 7 – Strong	5, 7
	Degree of secondary lobing	Qualitative	5 – Medium, 7 – Strong	5, 7
	Petiole length (cm)	Quantitative (State-wise)	3 – Short (< 7 cm), 5 – Medium (7–9 cm), 7 – Long (> 9 cm)	3, 5, 7
Floral	Node of first pistillate/perfect flower	Quantitative (State-wise)	3 – Early (< 45 days)	3
	Sex expression	Qualitative	1 – Monoecious	1
	Male sterility	Qualitative	1 – Absent	1
	Ovary length (cm)	Quantitative (State-wise)	3 – Short (< 1 cm), 5 – Medium (1–2 cm), 7 – Long (> 2 cm)	3, 5, 7
	Ovary width (cm)	Quantitative (State-wise)	3 – Narrow ($\leq$ 0.8 cm), 5 – Broad (> 0.8 cm)	3, 5
	Ovary pubescence	Qualitative	1 – Sparse, 2 – Dense	1, 2
	Ovary colour	Qualitative	5 – Green	5
Fruit	Shape in longitudinal section	Qualitative	1 – Round, 2 – Flat globe, 3 – Oval, 4 – Cylindrical/Oblong, 5 – Elongated globe	1, 2, 3, 4, 5
	Fruit rind colour	Qualitative	5 – Light green, 7 – Medium green, 9 – Dark green	5, 7, 9
	Fruit grooves	Qualitative	1 – Absent, 9 – Present	1, 9
	Fruit stripes	Qualitative	3 – Weak, 5 – Diffused, 7 – Clearly defined	3, 5, 7
	Fruit length (cm)	Quantitative (State-wise)	3 – Short (< 20 cm), 5 – Medium (20–30 cm), 7 – Long (> 30 cm)	3, 5
	Fruit diameter (cm)	Quantitative (State-wise)	3 – Narrow (< 15 cm), 5 – Medium (15–25 cm)	3, 5
	Fruit weight (kg)	Quantitative (State-wise)	3 – Small (< 3 kg), 5 – Medium (3–6 kg), 7 – Large (> 6 kg)	3, 5, 7
	Flesh colour	Qualitative	1 – White, 2 – Yellow, 3 – Orange, 5 – Reddish pink, 7 – Light red, 9 – Dark red	1, 2, 3, 5, 7, 9
	Seediness (no. of seeds/fruit)	Quantitative (State-wise)	2 – Low (< 150), 3 – Medium (150–350), 4 – High (> 350)	2, 3, 4
	Seed length (cm)	Quantitative (State-wise)	5 – Medium (0.6–1.0 cm), 7 – Long (> 1.0 cm)	5, 7
Seed	Seed width (cm)	Quantitative (State-wise)	3 – Narrow (< 0.4 cm), 5 – Medium (0.4–0.7 cm), 7 – Broad (> 0.7 cm)	3, 5, 7

considered in the study included cotyledon shape, internode length, leaf blade length and width, colour of leaf blade, the degree of both primary and secondary lobing and petiole length. Floral traits comprised the appearance of the first pistillate or perfect flower, sex expression, presence or absence of male sterility, ovary length and width, ovary pubescence and ovary colour. Fruit traits encompassed shape in longitudinal section, rind colour, presence of grooves and stripes, fruit length, diameter, weight (size), flesh colour and seediness (measured as the number of seeds per fruit). Seed traits included seed length and seed width. These descriptors were further classified as either qualitative traits (e.g., fruit shape, flesh colour) with discrete, categorical states, or quantitative traits (e.g., fruit size, leaf length), which were measured on a continuous numerical scale.

Both qualitative and quantitative traits were used for evaluation and phenotypic characterisation of 138 genotypes of watermelon. Qualitative traits comprising floral traits, fruit traits and seed traits provided maximum variability through PCA and cluster analysis whereas qualitative traits turned out to be useful for DUS and morphological differentiation (12).

Similar results have been found in which maximum variability accounts for 10 varieties of watermelon across quantitative traits like leaf lobing, fruit shape, rind colour, fruit stripes, flesh colour, seed coat colour and seediness (9).

Cluster analysis

Cluster analysis and calculation of cluster means were performed using the SPSS software package (version 29.0). Supplementary analyses were also conducted using the 'stats' and 'cluster' packages in R (R Core Team, 2024) to validate results. Cluster membership and trait means were further visualised using dendrograms, circular dendrograms and PCA biplots.

Clustering of quantitative traits data were normalised and unit variance to ensure uniform contribution to the analysis, while categorical variables were numerically coded as per DUS descriptor states. Wards hierarchical clustering method, using squared Euclidean distance as a dissimilarity measure, was employed to group the 165 genotypes based on the full set of 26 DUS descriptors. This agglomerative approach minimises the increase in within-cluster variance at each step, producing compact and well-separated clusters. Agglomerative clustering technique is widely used in plant breeding and diversity studies due to its effectiveness in grouping genotypes based on multiple morphological traits (13–15).

Results and Discussion

Phenotyping of advanced breeding lines with distinctness, uniformity and stability (DUS) descriptors

Phenotypic variability was observed in filial and backcross population across 13 well defined clusters resulting across 26 DUS descriptors. Cluster means of all 13 clusters revealed significant variability among clusters providing basis for trait based selection. Cluster 1 exhibited longest and widest leaves, uniform flowering and flat globe fruit shape, with orange fleshed fruits. Cluster 2 was reported to have high seediness. Cluster 3 was associated with flat globe fruits along with orange flesh. Cluster 4 showed oval shaped yellow fleshed fruits. Cluster 5 revealed lines with uniform flowering behavior with flat globe orange fleshed fruits. Cluster 6 was characterised by compact growth habit that is shorter internodes andromonoecious, with white to reddish pink flesh. Cluster 7

accounts for longest and widest leaves with oval shaped orange fleshed fruits. Cluster 8 showed longest and widest leaves with white to reddish pink flesh. Cluster 9 possess orange fleshed cylindrical fruits with high seediness. Cluster 10 was found to have yellow fleshed fruits. Cluster 11 exhibited oval shape fruits. Cluster 12 was reported to have cylindrical fruits with narrow seeds and yellow flesh. Cluster 13 showed a range of variation for various traits and characterised by compact growth habit, cylindrical fruits with white to reddish pink flesh with high seediness and narrow seeds. Some unique trait combinations were reported among some clusters like cluster 6 and 13 was characterised by dwarf plants with white flesh fruits, cluster 5 and 9 was accountable for high seediness in fruits with orange flesh, cluster 8 was found to have distinct stripping pattern on the fruits.

Phenotypic diversity analysis plays a vital role in watermelon breeding, emphasised on the enhancements in fruit yield and quality and overall adaptability. Morphological characterisation serves as practical and economical method for identifying genotypes with favorable agronomic traits, particularly during selection stages when molecular tools are limited or not feasible (15, 16). Classification of distinct phenotypic clusters offers breeders an effective means of choosing parents, facilitating the use of heterosis and recombination potential through the selection of genetically diverse lines (15). Additionally, the visualisation of donor and check genotypes across multiple clusters enables tracking of successful introgression events and guides decisions on backcrossing or recombination strategies.

The phenotypic characterisation and their diversity analysis support the conservation of genetic resources by ensuring that rare or distinctive genotypes are conserved during selection and variety development. These unique lines may contain alleles that enhance stress tolerance or contribute in fruit characteristics that appeal to changing consumer preference (17). With the challenges posed by climate change and variable growing conditions, maintaining genetic diversity is vital for ensuring the adaptability and sustainability of watermelon production (4, 7). Altogether, highlights phenotypic variation and also offers a useful framework for identifying diverse parents, conserving valuable alleles and shaping future breeding efforts.

Cluster analysis

Cluster analysis is a powerful tool for classifying genetic resources and has been widely used in crop diversity studies. Wards hierarchical clustering grouped the 165 breeding lines into 13 well-defined clusters (Fig. 1, Table 2), based on 26 standardised DUS morphological descriptors, the clustering reflects the broad genetic base of the breeding material (4, 17) which minimises within-cluster variance while maximising inter-cluster differences. The dendrogram reveals a clear hierarchical structure, with linkage distances reflecting morphological dissimilarity among the lines, further validating the informativeness of phenotypic descriptors for diversity studies (18) (Fig. 1).

The clusters in the dendrogram offers intuitive visualisation of genetic distances, where clusters positioned opposite each other breeding lines were more divergent. This not only confirms the robustness of the clustering solution but also supports the identification of genetically distinct lines for strategic parent selection. Singleton and small clusters, in particular, may harbor unique alleles or rare trait combinations that are valuable for broadening the genetic

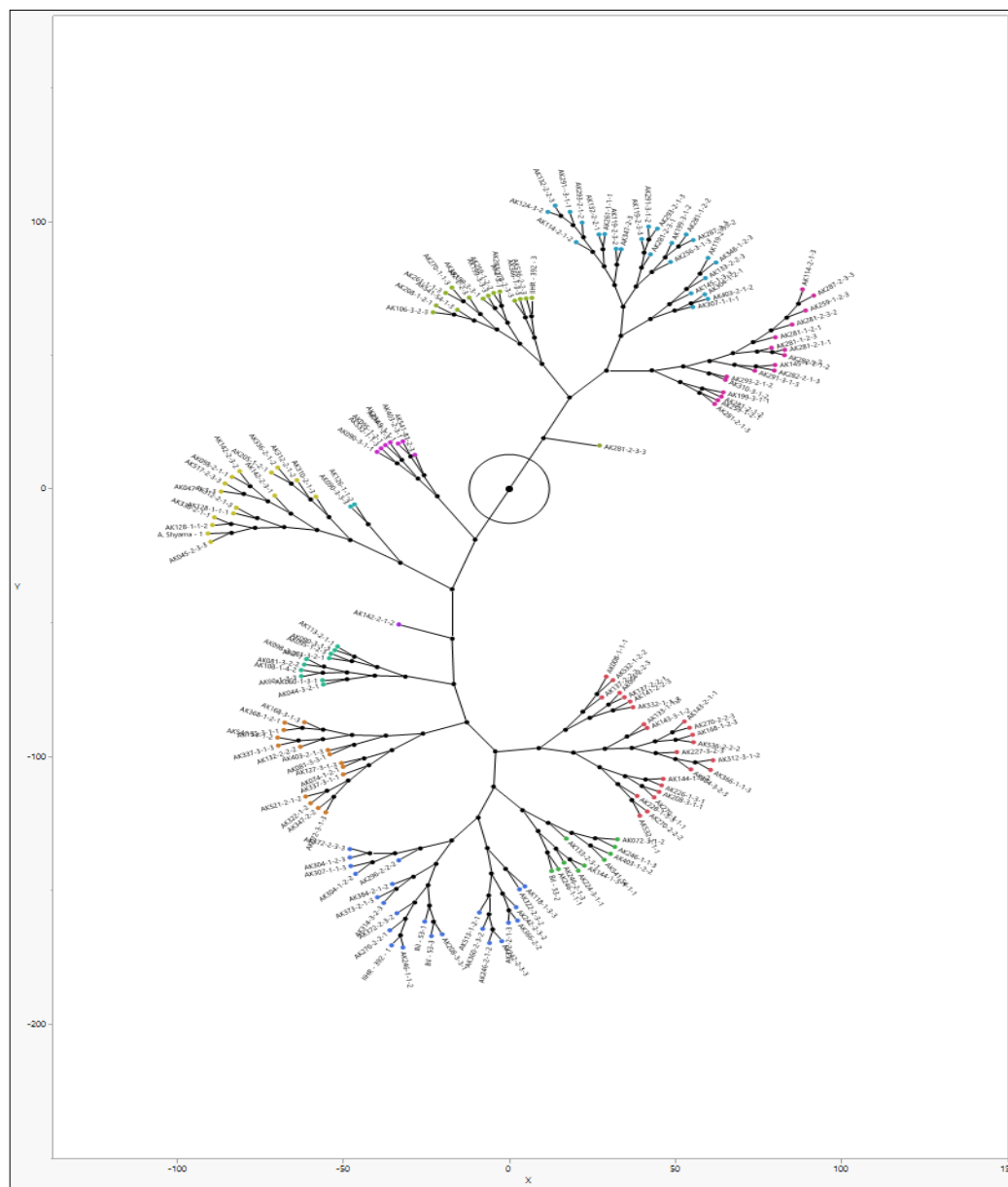


Fig. 1. Dendrogram showing the 13 clusters of watermelon breeding lines based on distinctness, uniformity and stability (DUS) descriptors supporting cluster analysis for F_4 and BC_1F_3 population.

Table 2. Clustering pattern of all breeding lines

Major clusters	List of breeding lines
1	AK008-1-1-1, AK532-1-2-2, AK137-2-2-2, AK064-2-2-3, AK137-2-2-1, AK141-2-2-3, AK532-1-1-2, AK133-1-1-3, AK143-3-1-2, AK143-2-1-1, AK270-2-2-3, AK168-1-2-3, AK536-2-2-2, AK227-3-2-3, AK312-3-1-2, AK366-1-1-3, AK384-3-2-3, AK144-1-3-2, AK226-1-3-1, AK208-3-1-1, AK270-1-1-1, AK228-1-3-3, AK270-2-2-2, AK532-1-1-1
2	AK072-3-1-2, AK246-1-1-3, AK403-1-2-2, AK541-54-1-1, AK133-2-3-1, AK144-1-3-1, AK224-3-1-1, AK246-2-1-3, AK246-1-1-1, BIL - 53 - 2
3	AK118-1-3-3, AK322-2-3-2, AK242-2-3-2, AK366-2-2, AK372-2-1-3, AK242-2-3-3, AK246-2-1-2, AK360-2-3-2, AK513-1-2-1, AK208-3-3-1, BIL 53 - 1, BIL 53-3, AK246-1-1-2, IIHR - 392 - 1, AK270-2-2-1, AK372-2-3-2, AK314-3-2-3, AK373-2-1-3, AK384-2-1-2, AK296-2-2-2, AK304-1-2-2, AK307-1-1-3, AK304-1-2-3, AK372-2-3-3
4	AK072-3-1-3, AK347-2-2, AK322-1-2, AK521-2-1-2, AK337-3-1-1, AK074-1-2-1, AK127-3-1-3, AK081-3-3-1, AK403-2-1-3, AK132-2-2, AK337-3-1-3, AK541-54-1-2, AK151-3-1-1, AK368-1-2-1, AK168-3-1-3
5	AK044-3-2-1, AK060-1-3-1, AK060-1-3-3, AK108-1-4-2, AK081-3-2-2, AK098-2-2-1, AK081-3-2-1, AK095-1-2-3, AK090-3-1-3, AK113-2-1-1
6	AK142-2-1-2
7	AK045-2-3-3, A. Shyama - 1, AK128-1-1-2, AK336-2-1-1, AK128-1-1-1, AK312-2-1-3, AK047-1-1-3, AK517-2-3-3, AK098-2-1-1, AK142-2-3-2, AK142-2-3-1, AK205-1-2-1, AK336-2-1-2, AK312-2-1-2, AK310-2-1-3
8	AK090-3-3-3, AK126-1-1-2
9	AK090-3-1-1, AK532-1-1-3, AK095-1-2-1, AK294-1-2-3, AK145-1-1-1, AK403-2-3-1 and AK541-83-2-3
10	AK106-3-2-3, AK208-1-2-1, AK541-54-1-3, AK261-1-1-3, AK270-1-1-3, AK392-2-2-3, AK199-3-3-1, AK199-3-3-3, AK259-1-2-2, AK287-2-3-1, AK278-2-3-3, AK366-1-3-3, AK536-2-2-3, IIHR - 392 - 3
11	AK114-2-1-2, AK124-3-2, AK132-2-2-3, AK291-3-1-1, AK293-2-1-2, AK132-2-2-1, AK261-1-1-1, AK119-2-3-2, AK347-2-3, AK119-2-3-3, AK291-3-1-2, AK293-2-1-3, AK119-3-1-2, AK281-1-2-2, AK287-2-3-2, AK256-3-1-3, AK119-2-3-1, AK348-1-2-3, AK133-2-2-3, AK145-1-3-3, AK304-1-2-1, AK403-2-1-2, AK307-1-1-1
12	AK114-2-1-3, AK287-2-3-3, AK259-1-2-3, AK281-2-3-2, AK281-1-2-1, AK281-1-2-3, AK281-2-1-1, AK282-2-1-2, AK145-1-1-2, AK282-2-1-3, AK291-3-1-3, AK293-2-1-2, AK310-3-1-2, AK199-3-1-1, AK281-2-1-2, AK259-1-2-1, AK281-2-1-3
13	AK281-2-3-3

base and introducing novel traits (4, 17). Such lines are of great interest for breeding programmes aimed at disease resistance, stress tolerance and fruit quality improvement (15).

The observed clustering pattern provides a practical framework for the efficient selection of parental lines from genetically distant clusters to exploit heterosis and enhance recombination potential (15) (Fig.1). Moreover, the clear separation and integrity of clusters facilitate the conservation of distinct genotypes and effective management of genetic resources, which is an essential component of sustainable breeding (7). Overall, the phenotypic cluster analysis conducted in this study reveals the extent of morphological diversity present in advanced watermelon lines and demonstrates its utility in guiding breeding decisions, trait introgression and genetic resource conservation.

Trait-wise variation and cluster association

Vegetative traits

The descriptors for the vegetative traits were assessed, including cotyledon shape, internode length, leaf blade length and width, leaf blade colour, degree of primary and secondary lobing and petiole length. Noticeable variation was observed among clusters for these traits. Cotyledon shape differed across groups, with Cluster 13 (3.00) and Cluster 3 (2.67) exhibiting a broad elliptic form, indicating distinct seedling morphology. Cotyledon shape as an International Union for the Protection of New Varieties of Plants (UPOV) descriptor was evaluated and a high variability in cotyledon shape that is narrow elliptic to broad elliptic has been reported in watermelon accessions from Turkey (19). Similarly, in line to this significant variation for cotyledon shape was also recorded among 39 Hungarian and 11 Turkish watermelon genotypes (20). Internode length was greatest in Clusters 8 (2.12) and 9 (1.95), suggesting taller and more vigorous plants, whereas Clusters 6 and 13 (0.95) displayed compact growth, favourable for high-density planting. Those results are in harmony with internodal length among Hungarian and Turkish watermelon genotypes in which considerable variability was recorded (19, 20). Leaf blade length reached its maximum in Clusters 8 (7.00), 7 (6.20) and 1 (6.00), reflecting vigorous foliage development. A similar pattern was noted for leaf blade width, with Clusters 8, 12 and 13 (7.00) producing the broadest leaves, followed by Clusters 1 and 7 with slightly narrower widths. Wide range of variation was observed for Hungarian and Turkish watermelon genotypes (19–21) recorded high variability for leaf dimensions. Leaf colour was mainly dark green (score 5.00) in most clusters, while lighter shades were observed in Cluster 5 (3.00) and Cluster 4 (4.73). Primary lobing was generally strong (score 7.00) across clusters, except in Cluster 8 (5.00), which exhibited moderate lobing. Secondary lobing was most prominent in Clusters 7 (7.00) and 8 (6.00), indicating more dissected foliage. Notable variation was recorded degree of leaf lobation among 134 Turkish watermelon accessions and it turned out to be most discriminating trait among all vegetative traits recorded for the study (19). Similar variation was observed in Hungarian and Turkish watermelon for degree of leaf lobing trait (20). Wide range of variation was reported for length of petiole in Hungarian and Turkish watermelon accessions (20).

Variability in vegetative traits such as cotyledon shape, internode length, leaf blade dimensions and lobing serves as a recognised basis for cluster differentiation in plant populations. Variability in DUS traits such as cotyledon shape, leaf blade dimensions, internode length and petiole length is reported for detecting variability among different watermelon

genotypes (9, 11, 22).

Similar patterns had been reported in wild and cultivated watermelon accessions in Kenya (23), where traits like leaf blade length and width, leaf blade colour and internodal length were proved reliable for morphological characterisation. Correspondingly, multivariate analyses in local watermelon genotypes of Turkey demonstrated that cotyledon shape, leaf dimensions, degree of leaf lobing, leaf colour significantly contribute to genetic diversity and grouping of genotypes (24). The observations were considerable for phenotypic diversity among various inbred lines of watermelon based on traits such as leaf width and internode length through analysis of variance (ANOVA) and PCA (25). Moderate to strong variability for leaf dimensions in various watermelon genotypes (9). Considerable variation for leaf length and width, degree of lobing and leaf colour was reported for *Citrullus* species (26).

Floral traits

The floral descriptors examined included the appearance of the first pistillate or perfect flower, sex expression, male sterility and ovary-related traits such as length, width, pubescence and colour. All clusters showed early flowering with a uniform mean score of 3.00, suggesting synchronised blooming behaviour. The majority of clusters were monoecious (score 1.00), whereas Cluster 6 exhibited andromonoecy (3.00), a condition that could be advantageous for hybrid breeding programmes. Male sterility was not observed in any of the clusters. Ovary length was highest in Cluster 12 (5.47), followed by Clusters 7 (5.13) and 3 (5.08), while ovary width reached its maximum in Cluster 13 (5.00), Cluster 3 (4.17) and Cluster 2 (3.80). Ovary pubescence was denser in Clusters 1, 5, 10 and 13 (score 2.00), while Clusters 6 and 8 displayed sparse pubescence (score 1.00). Ovary colour was predominantly green (score 5.00) across most clusters, though Cluster 13 exhibited a lighter yellowish-green hue (score 3.00). Notable variation for ovary related traits like ovary length was reported through phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), and PCA in F_3 and F_4 generations of watermelon (9). Genetic variability and correlation studies in various genotypes of watermelon revealed key traits like days to flowering and appearance of first pistillate flower contribute to significant variability for flowering behaviour in watermelon (22). Variation in floral traits including timing of flowering, sex expression and ovary characteristics has been widely used to distinguish between clusters. Floral morphology varies significantly among clusters, influenced by both genetic and environmental factors and the integration and decoupling of floral traits can lead to distinct cluster identities, especially with respect to sex expression and ovary morphology. Fifty-one genotypes of watermelon were accessed and considerable phenotypic variation was observed for days to first female flower (37.33–60.67 days) (27). High heritability and genetic advance was recorded for days to 50 % flowering followed by fruit weight among watermelon genotypes (28). Overall, these results confirm that floral traits such as flowering time, sex expression and ovary characteristics substantially contribute to the identification of unique genotype groups. Their evaluation provides valuable insights for germplasm characterisation, hybrid development and crop improvement initiatives in watermelon.

Fruit traits

The fruit-related traits evaluated included shape in longitudinal section, rind colour, presence of grooves and stripes, fruit length, diameter, weight (size), flesh colour and seediness, which was measured as the number of seeds per fruit (Fig 2). A wide range of



Fig. 2. The promising lines having extreme vegetative vigour with resistance to *fusarium* wilt, watermelon bud necrosis virus (WBNV) and combined resistance to *fusarium* wilt and WBNV. a) AK8-1-1-1, b) AK37-2-2-1, c) AK72-3-2-1, d) AK143-3-1-2, e) AK336-2-1-1, f) AK3312-2-1-3, g) AK90-3-3-3, h) AK106-3-2-3, i) AK208-1-2-1, j) AK242-2-3-3, k) AK90-3-1-1, l) AK532-1-1-3, m) AK44-3-2-1 n) AK114-2-1-2, o) AK124-3-2.

variation was observed among clusters for these parameters. The shape of the fruit in longitudinal section ranged from oblong (Cluster 9, mean 3.00; Cluster 4, 2.92) to round (Cluster 7, 1.00), illustrating substantial morphological diversity within the breeding lines. Rind colour was primarily green (score 5.00) but shifted towards lighter tones in some clusters, notably Cluster 10 (3.00). Most clusters exhibited little to no grooving or striping (score 1.00), though Clusters 2 and 6 displayed moderate expression (score 2.00). Fruit length was greatest in Cluster 7 (45.13 cm), Cluster 9 (44.92 cm) and Cluster 8 (44.33 cm), while Cluster 3 (34.01 cm) showed the shortest fruits. Similarly, fruit diameter reached its peak in Cluster 3 (21.51 cm), Cluster 8 (21.40 cm) and Cluster 13 (21.22 cm). Fruit weight was also highest in Cluster 8 (2.00 kg), Cluster 7 (1.95 kg) and Cluster 1 (1.93 kg), indicating that these clusters contain genotypes producing larger fruits. Flesh colour was predominantly red (score 5.00) but exhibited diversity across clusters; for instance, Cluster 13 featured white or orange flesh (score 3.00), a trait that may hold value for specialised market demands. Seediness varied considerably among clusters, with higher seed numbers in Clusters 9 (50.00 seeds per fruit) and 12 (48.73), while Clusters 13 (20.00) and 6 (24.00) recorded fewer seeds, a desirable attribute in many commercial types.

In line with this finding significant variability among exotic watermelon genotypes was reported with the help of Wards clustering, PCV, GCV, PCA and genetic advance and the key traits contributing to variability includes fruit weight, fruit length and fruit diameter (29). A previous study evaluated 138 seed purpose watermelon genotypes and reported significant variability ($p < 0.001$) for fruit shape, fruit stripe colour, fruit diameter, fruit weight and flesh colour (12). Strong genetic diversity with total variation of 43.1 % was reported in a biparental F_{23} population of watermelon genotypes and the key traits adding to variation was fruit length, fruit diameter,

fruit weight, rind colour, presence of stripes, flesh colour, ovary size and seed traits (30). Significant variability among 78 watermelon genotypes was found for fruit weight, fruit diameter and fruit length were the major contributors to genetic divergence (31). A total of 388 accessions of cucumber were evaluated for morphological variability and a considerable amount of variation was contributed for fruit length, fruit diameter, fruit shape and flesh colour (32). Landraces of cultivated pumpkin were evaluated for morphological characterisation and significant difference ($p < 0.001$) was reported for fruit weight, fruit length, fruit diameter, fruit shape, fruit colour. The extensive variation in fruit morphology and quality-related characteristics revealed through cluster analysis concurs with earlier studies, the fruit size, shape and flesh colour were major contributors to phenotypic diversity in watermelon germplasm (19).

Considerable morphological diversity was reported in *C. lanatus* in Indonesia and significant variability was contributed by fruit traits and seed traits. Fruit length, fruit diameter, fruit weight, seediness, seed length as well as seed width were found to be the major traits adding variability through dendrogram-based clustering. (33). Similarly in Indonesian melon using morphological traits significant variability was reported through fruit traits followed by vegetative traits through cluster analysis (34). In line with this, several landraces of pumpkin were evaluated for genetic variability through agro-morphological traits and the results showed that landraces exhibited notably high variability (87.86 %) and fruit diameter, fruit weight, fruit length followed by seediness were found to be the major drivers to variability providing high chances for trait-based selection (35). Significant variability was reported in *Coccinia* wild and cultivated forms through PCA, cluster analysis and discriminant analysis and fruit length and width, seediness along with seed trait contributing to genetic heritability (36). Fruit size, fruit

shape, seed size are found to be the major traits contributing to genetic variability through PCA in bottle gourd (37). A total of 171 accessions of egusi watermelon were accessed for diversity analysis, fruit size and fruit weight was found to be the key traits adding to variability through PCA (81.19 %) and cluster analysis (38). Fruit attributes (fruit shape, fruit length, fruit diameter, fruit weight, flesh colour, rind colour and rind stripe patterns) and seed traits found to be the dominant traits adding to total variability 86.38 % through PCA among 36 watermelon landraces (39). Cluster analysis of Palestinian watermelon accessions was performed and significant variation ($p > 0.05$) for fruit length, fruit diameter, fruit weight and seed traits (40). Cluster analysis and PCA results revealed considerable variability among 16 watermelon hybrids and traits associated for this genetic divergence includes fruit length, fruit diameter, fruit weight, fruit shape and rind colour (41).

The consistency between these earlier observations and the present findings underscores the importance of detailed phenotypic assessment of fruit traits in cluster differentiation, genetic resource management and the identification of promising parental lines. The clusters exhibiting specific combinations of rind colour, flesh type, seediness and fruit size offer valuable potential for breeding programmes targeting desirable market and processing traits. The broad phenotypic range observed among the evaluated lines reaffirms the utility of systematic DUS-based characterisation for both varietal improvement and the conservation of genetic resources in watermelon. The observed wide phenotypic range in fruit traits in our breeding lines demonstrates the value of systematic DUS-based characterisation for supporting both varietal improvement and genetic conservation programmes in watermelon.

Seed traits

Seed traits included seed length and width. The longest seeds were recorded in Clusters 1, 6, 8 and 13 (7.00), which may enhance germination and seedling vigour. Seed width was also highest in Cluster 1 (4.92), with Clusters 6, 8 and 13 scoring 5.00, indicating broad seeds ideal for varietal identification and stability under DUS testing. Seed length and width are critical for varietal identification and have been shown to contribute significantly to cluster differentiation. In line to this, 61.68 % of total phenotypic variability was reported and driven by seed size among 197 accessions of watermelon (42). Similarly, seed traits are found to be independent in PCA biplot analysis contributing to overall variability (30). Overall, the pronounced variability and discriminative power of seed traits in our study reinforce their practical importance for cluster differentiation, germplasm management and targeted breeding for market and nutritional traits.

Qualitative and quantitative trait contributions

Cluster means for all traits revealed clear differentiation among clusters (Table 3). Regarding vegetative traits, Clusters 1, 7 and 8 exhibited the longest and widest leaves, indicating vigorous vegetative growth. Conversely, Clusters 6 and 13 showed a compact growth habit with shorter internodes, making them distinct in plant structure. Such variability useful in breeding programmes focused on developing either vigorous types or compact genotypes suitable for dense planting systems.

Among floral traits, the early appearance of pistillate flowers and monoecious sex expression were uniformly observed in Clusters 1 and 5, indicating synchronised flowering. Cluster 6, with andromonoecious nature, offering a valuable trait for hybrid development.

Table 3. Cluster means for all 13 clusters as per 26 distinctness, uniformity and stability (DUS) descriptors for F₄ and BC₁F₃ population

Cluster	1.00	2.00	3.00	4.00	5.00	6.00	7.00	8.00	9.00	10.00	11.00	12.00	13.00
Count	24.00	10.00	24.00	15.00	10.00	1.00	15.00	2.00	7.00	14.00	24.00	17.00	1.00
Cotyledon: shape	1.92	2.50	2.67	2.00	2.10	2.00	2.60	2.50	2.00	2.29	2.21	1.88	3.00
plant: length of internodes	1.66	1.81	1.60	1.68	1.73	0.95	1.84	2.12	1.95	1.56	1.63	1.73	0.95
leaf blade: length	6.00	5.60	4.67	5.40	5.40	5.00	6.20	7.00	5.86	3.29	4.75	4.41	5.00
leaf blade: width	6.92	6.60	6.33	6.87	6.60	7.00	6.73	7.00	6.71	4.29	7.00	6.88	7.00
leaf blade: colour	5.00	5.00	4.92	4.73	3.00	5.00	5.00	5.00	4.71	5.00	5.00	5.00	5.00
leaf blade: degree of primary lobing	7.00	7.00	7.00	7.00	7.00	7.00	7.00	5.00	7.00	7.00	7.00	7.00	7.00
leaf blade: degree of secondary lobing	5.00	5.00	5.00	5.00	5.00	5.00	7.00	6.00	5.00	5.00	5.00	5.00	5.00
petiole: length	6.08	6.60	5.00	5.00	5.80	7.00	5.53	7.00	5.57	3.86	6.25	5.59	7.00
appearance of first pistillate/ perfect flower	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Sex expression (at full flowering)	1.00	1.00	1.00	1.00	1.00	3.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Male sterility	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Ovary: length	4.75	4.60	5.08	3.93	5.00	5.00	5.13	4.00	5.00	5.00	5.00	5.47	5.00
Ovary: width	3.17	3.80	4.17	3.40	3.60	3.00	3.53	4.00	3.86	3.29	3.08	3.71	5.00
Ovary: pubescence	2.00	1.50	1.88	1.13	2.00	1.00	1.93	1.00	1.86	2.00	1.88	1.94	2.00
Ovary: colour	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	3.00
Fruit: shape in longitudinal section	1.96	1.90	1.63	2.33	1.60	1.00	2.33	1.50	3.29	2.29	2.75	3.00	4.00
Fruit: colour of rind	5.25	5.40	5.25	5.80	5.80	5.00	6.07	5.00	5.00	6.43	6.33	6.88	7.00
Fruit: grooves	8.33	9.00	9.00	7.93	9.00	9.00	7.40	9.00	9.00	8.43	9.00	1.47	9.00
Fruit: stripes	4.83	4.40	3.67	4.33	4.00	3.00	4.33	7.00	3.00	4.29	5.58	4.76	5.00
Fruit: length (cm)	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	5.00	3.00	3.00	3.00	3.00
Fruit: diameter (cm)	3.83	5.00	4.25	3.40	4.00	5.00	3.40	3.00	5.00	3.00	3.08	3.12	3.00
Fruit: size (kg)	3.08	5.00	3.17	3.00	3.40	3.00	3.13	3.00	5.57	3.00	3.00	3.00	3.00
Fruit: colour of Flesh	3.17	1.80	2.13	1.53	3.30	1.00	3.93	4.00	3.14	2.00	2.58	1.35	1.00
Seediness: number of seeds/ fruit	2.96	3.70	3.04	2.60	3.50	3.00	2.93	3.00	3.86	3.21	3.04	3.41	4.00
Seed: length (cm)	7.00	6.40	6.42	6.20	6.20	7.00	5.80	7.00	5.86	5.43	5.08	5.00	7.00
Seed: width (cm)	4.92	4.60	4.50	4.47	4.40	5.00	3.67	5.00	3.86	3.43	3.25	3.00	3.00

Fruit traits exhibited remarkable variation. Clusters 1, 3, 5, 7, 9 and 11 were associated with orange flesh types; Clusters 4, 10 and 12 featured yellow-fleshed fruits, while Clusters 6, 13 and parts of Cluster 8 produced fruits with white or reddish-pink flesh. Fruit shape also varied notably, with flat-globe fruits in Clusters 1, 3 and 5; oval fruits in Clusters 4, 7 and 11; and cylindrical fruits predominantly found in Clusters 9, 12 and 13.

Seed trait analysis showed that Clusters 2, 5, 9 and 13 possessed high seediness, which is critical for seed yield. Narrow seeds were specifically observed in Clusters 12 and 13, adding another layer of differentiation in reproductive traits. Seed number and seed morphology have been recognised as important quantitative traits influencing reproductive success and have been used to distinguish clusters in cucurbits and other crops. Both qualitative and quantitative traits contributed substantially for clustering. Qualitative traits such as flesh colour, fruit shape, rind pattern and sex expression served as strong discriminators, allowing clear cluster identity based on visible descriptors.

Quantitative traits including fruit size, leaf length and width, seed number and ovary dimensions introduced a gradient of variation, enabling detection of clusters with extreme or intermediate values. The integration of qualitative and quantitative descriptors for cluster analysis, where both types of traits have been shown to maximise the detection of genetic diversity and facilitate targeted breeding.

The clusters were characterised by unique combinations of traits, for instance, dwarf plant habit combined with white flesh in Clusters 6 and 13. The co-occurrence of high seediness and orange flesh in Clusters 5 and 9. Cluster 8 was especially notable for its distinct striping pattern, making it visually and morphologically unique.

Breeding and diversity implications

The trait-based cluster differentiation observed in this study highlights the extensive genetic diversity among the advanced breeding lines evaluated. Clusters displaying novel combinations,

such as dwarf growth habit with white flesh (e.g., Clusters 6 and 13) or high seediness with orange flesh (e.g., Clusters 5 and 9), represent valuable sources for targeted breeding programmes. The identification of such unique trait combinations is crucial for broadening the genetic base and introducing new market-oriented or stress-adaptive traits into breeding pipelines. The use of diverse parental combinations from phenotypically distinct clusters increases the probability of transgressive segregation and the development of superior hybrids.

Clusters such as 1, 3 and 11 demonstrated high uniformity across multiple DUS traits, supporting their candidacy for sib lines uniformity. Outlier clusters, including 6, 8, 12 and 13, offer unique genetic resources that could be utilised for developing varieties with specialty traits.

This approach not only accelerates the breeding process but also ensures the conservation and utilisation of valuable genetic diversity.

Cluster summary

The cluster means plot illustrates phenotypic differentiation among the 13 clusters for 26 morphological traits (Fig. 3), providing a comprehensive basis for understanding genetic diversity in the watermelon breeding population. Clusters divergence for vegetative characteristics namely Clusters 1, 7 and 8 (as indicated by higher peaks for plant: length of internodes, leaf blade length and leaf blade width) exhibit vigorous, tall plants with large leaves, suggesting their potential for robust growth and higher photosynthetic capacity. Conversely, Clusters 6 and 13 had compact growth (lower means for these traits) and smaller foliage, which can be advantageous for high-density planting systems or adaptation to resource-limited environments. Variation in leaf blade colour and the degree of primary and secondary lobbing further delineates clusters, enabling the selection of lines with specific canopy architectures or aesthetic preferences. Differential expression of vegetative traits is crucial for effective breeding, as plant architecture directly impacts yield and management.

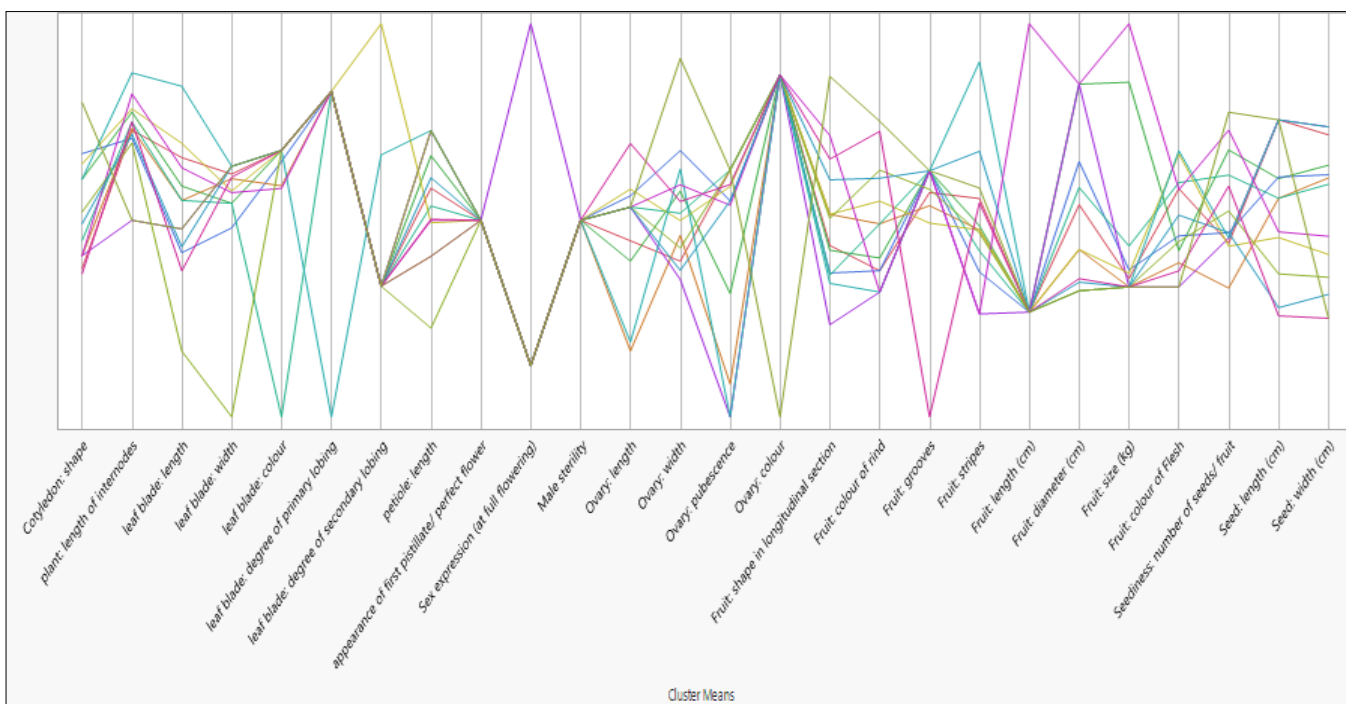


Fig. 3. Cluster summary of cluster means of 13 clusters derived from 165 advance breeding lines of F_4 and BC_1F_3 generation based on the 26 morphological distinctness, uniformity and stability (DUS) descriptors through Wards method.

Distinct patterns are also evident for floral traits. The appearance of the first pistillate or perfect flower is uniform and early across all clusters, indicating synchronised flowering behavior valuable for controlled crossing and efficient crop cycles. While most clusters are monoecious, Cluster 6 distinctly stands out as andromonoecious (higher score), a key characteristic for developing hybrid varieties by simplifying the female parent line. Importantly, male sterility is absent across all clusters, which ensures viability for pollen production. Significant variation is observed in ovary length and width; for example, Cluster 12 has high ovary length and Cluster 13 has high ovary width, which may correlate with potential fruit size. Differences in ovary pubescence and ovary colour (e.g., Cluster 13's unique yellowish-green hue) further contribute to cluster distinction.

Cluster divergence is also evident across fruit characteristics. Clusters display a wide range for fruit length (cm), fruit diameter (cm) and fruit size (kg), with some clusters clearly having larger fruits (higher peaks), which is desirable for yield-focused breeding. Beyond size, clusters differentiate by unique combinations of fruit rind colour, grooves and stripes all crucial for market differentiation and consumer appeal. The traits such as fruit shape in longitudinal section and flesh colour also exhibit cluster-specific patterns, enabling the selection of lines for particular fruit types or internal quality attributes. This extensive variation in fruit traits provides ample scope for targeted breeding, allowing the development of varieties suited to diverse market demands and processing requirements, as broadly supported by fruit trait analyses in watermelon and other cucurbits.

The cluster means plot also highlights significant differences in seed-related traits. Variation is evident in seediness (number of seeds per fruit), seed length (cm) and seed width (cm). Some clusters show higher seed numbers or larger seed dimensions, which could be prioritised for breeding programmes focusing on seed production or for specific markets where seed attributes are important. Conversely, clusters with lower seediness may be valuable for developing seedless varieties. These distinctions in seed traits are vital for optimising both commercial seed production and consumer-oriented breeding strategies, further contributing to the overall phenotypic diversity captured by the cluster analysis.

Conclusion

The study morphological characterisation of 165 advanced watermelon breeding lines (F_4 and BC_1F_3) using 26 DUS morphological descriptors and their diversity studies led by the classification of 13 distinct hierarchical clusters using Wards method, each defined by unique combinations of vegetative, reproductive, fruit and seed traits, reflecting substantial phenotypic diversity. The findings highlight clusters with desirable features such as early flowering, vigorous growth, diverse fruit shapes and flesh colors, seediness and compact growth. Outlier clusters revealed rare and unique trait expression in the advanced breeding lines, which were valuable source for future breeding innovation. These results provide a strong foundation for targeted parent selection, trait introgression and DUS-based varietal identification, contributing to the development of diverse, high-performing and market-oriented watermelon cultivars.

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Authors' contributions

SS was responsible for experimentation and drafting. MDC contributes in concept, materials, methodology, analysis, drafting and supervision. PKSG and ISN helps in concept, methodology, analysis and administration. VK done editing, formatting and finalised the manuscript. LRDC contributed in methodology, analysis and drafting. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of Interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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