



RESEARCH ARTICLE

Persistent phenotypic segregation and genotype-level random amplified polymorphic DNA diversity in the first selfed generation of farmer-collected melon (*Cucumis melo* L.)

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Abstract

Farmer-collected melon (*Cucumis melo* L.) germplasm is widely cultivated but often exhibits high genetic heterogeneity, which complicates early-generation selection in breeding programmes. This study evaluated the persistence of phenotypic segregation at the individual plant level and genetic heterogeneity at the genotype level in the first selfed (S1) generation derived from heterogeneous farmer-maintained populations. Ten S1 genotypes were grown under uniform greenhouse conditions during a single evaluation season conducted in 2025. Qualitative and quantitative morphological traits were recorded for individual S1 plants and converted into binary variables using predefined biological thresholds. Genetic relationships among S1 genotypes were assessed using random amplified polymorphic DNA (RAPD) markers, scored as band presence (1) or absence (0), from pooled DNA samples for each genotype. Phenotypic similarity among individual plants was calculated using Jaccard's similarity coefficient and hierarchical clustering was performed using the unweighted pair group method with arithmetic mean (UPGMA) based on qualitative, quantitative and combined binary phenotypic datasets. Genotype-level genetic relationships were inferred using Jaccard's similarity and UPGMA clustering of RAPD data. Phenotypic clustering revealed pronounced individual-level segregation with plants originating from the same S1 genotype rarely formed cohesive clusters, indicating substantial within-genotype variation after a single selfing cycle. The RAPD analyses revealed marked multilocus heterogeneity among S1 genotypes, with only partial grouping in the combined analysis. These results demonstrate that extensive phenotypic segregation persists in S1 plants, while RAPD-based evidence indicates substantial genetic heterogeneity among genotypes, suggesting that reliable selection for uniformity should be deferred to later selfed generations.

Keywords: *Cucumis melo*; genetic diversity; phenotypic segregation; random amplified polymorphic DNA markers

Introduction

Melon (*Cucumis melo* L.) is a high-value horticultural crop ($2n = 2x = 24$) (1), whose commercial success is strongly determined by fruit morphology, internal quality and visual uniformity, traits that directly shape consumer preference and market acceptance (2). In many production systems, melon cultivation continues to rely on heterogeneous planting materials, including farmer-collected cultivars and recycled seeds (3). Because melon is predominantly cross-pollinated under open-field conditions, largely mediated by insect pollinators (4), consequently, such farmer-maintained populations are typically highly heterozygous and genetically heterogeneous (5–7). Although this genetic heterogeneity may represent a valuable reservoir of diversity, it simultaneously generates extensive phenotypic variation (8), thereby hindering systematic breeding, line fixation and cultivar improvement (9). These challenges become particularly pronounced when genetic variation originating from open-pollinated germplasm is not characterised at the earliest stages of inbreeding, when critical decisions regarding selection intensity and line advancement are initially formulated.

The development of genetically stable inbred lines through successive self-pollination, therefore, represents a central objective in melon breeding programmes (10), as such lines form the parental foundation for the production of uniform and high-performing hybrid cultivars (11). The first selfed generation (S1) constitutes the initial step in this genetic purification process, during which heterozygosity is theoretically reduced by 50 % relative to the parental population (12–14). However, when S1 populations are derived from genetically heterogeneous, open-pollinated farmer germplasm, a substantial proportion of genetic variation is expected to persist. Consequently, segregation of both qualitative and quantitative traits may remain pronounced, resulting in high phenotypic heterogeneity within S1 populations (15). Despite this generation's pivotal role in breeding pipelines, the extent to which phenotypic segregation persists after a single selfing cycle remains insufficiently documented, particularly for materials from farmer-maintained populations. Morphological characterisation remains a fundamental approach for assessing variability in melon germplasm (16, 17). Qualitative traits, such as fruit shape, rind texture and flesh colour, are often governed by major genes and tend to exhibit

discrete phenotypic classes even in segregating populations, rendering them particularly informative at early stages of inbreeding (18). In contrast, quantitative traits, including fruit size, flesh thickness and soluble solids content, are polygenic in nature and strongly influenced by environmental conditions, which may obscure underlying genetic differences and complicate early-generation selection decisions (19). Accordingly, evaluation of S1 populations commonly adopts a parent-progeny framework, in which vegetative traits were assessed directly on S1 plants, whereas fruit traits were evaluated using fruits produced after self-pollination of the corresponding S1 plants, representing the subsequent selfed progeny (S2) generation (20).

Because phenotypic assessment alone is often insufficient to resolve genetic relationships among closely related genotypes, particularly in early-generation populations with limited replication, molecular marker analysis provides a complementary means of assessing genetic diversity at the DNA level (21, 22). Random amplified polymorphic DNA (RAPD) markers have been widely applied in melon to reveal genetic differentiation and clustering among closely related genotypes based on presence-absence banding patterns (23–25). Although RAPD analysis is typically conducted at the genotype level and does not capture within-genotype segregation when DNA samples are pooled, it remains informative for evaluating residual genetic diversity and overall genetic structure following early selfing cycles (26). Therefore, the present study aimed to evaluate the persistence of phenotypic segregation at the individual plant level and RAPD-based genetic diversity at the genotype level in the first selfed generation of melon derived from farmer-collected germplasm. Specifically, qualitative and quantitative morphological traits were assessed to determine the extent of within-genotype phenotypic variation among S1 plants, whereas pooled-DNA RAPD analysis was used to evaluate residual genetic heterogeneity among S1 genotypes. These analyses were conducted to clarify segregation patterns at the S1 stage and to support more effective early-generation selection strategies in melon breeding programmes.

Materials and Methods

Experimental design, plant materials and cultivation procedures

Ten melon (*Cucumis melo* L.) genotypes representing the first selfed generation, S1 SR, S1 FJ, S1 AR, S1 GA, S1 EL, S1 DM, S1 GG, S1 HD, S1 BD and S1 AL, were developed through a single cycle of self-pollination from farmer-collected, open-pollinated S0 plants and used as experimental materials. The experiment was conducted during May–August 2025 growing season under greenhouse conditions at SMK Moenadi, Ungaran, Central Java, Indonesia, using a completely randomised design with genotype as the single factor and three biological replicates, each consisting of one plant. Because the present study was intended as an initial exploratory evaluation of segregation persistence and genotype-level heterogeneity in farmer-derived S1 materials, the results should be interpreted as indicative patterns rather than definitive estimates of segregation frequency within each genotype. The S1 plants were subsequently self-pollinated to generate fruits and seeds corresponding to the S2 generation. Vegetative morphological traits were evaluated directly on S1 plants, whereas fruit traits were assessed using fruits derived from their selfed progeny (S2) following a parent-progeny evaluation framework. The RAPD-based molecular analyses were performed using young leaf tissue

collected from S1 plants. Prior to planting, the greenhouse was cleaned and planting medium was prepared in polybags containing a mixed substrate supplemented with *Trichoderma* spp. and basal nitrogen, phosphorus and potassium (NPK) fertilisation. Seeds were germinated under controlled conditions and transplanted at the 2–3 true-leaf stage. Plants were maintained under standard greenhouse cultivation practices, including irrigation, fertilisation, vertical training, pruning and manual self-pollination. Developing fruits were thinned to retain a single fruit per plant and fruits were harvested at physiological maturity.

Morphological characterisation

Morphological characterisation was conducted using the descriptors of the International Plant Genetic Resources Institute (IPGRI) (27). Observations were limited to traits evaluated directly on S1 plants, while fruit traits were assessed on fruits derived from their selfed progeny, following a parent-progeny evaluation framework. Fruit traits were evaluated on fruit produced after self-pollination of individual S1 plants as part of a parent-progeny evaluation framework. No independent S2 population was established or analysed in this study. Qualitative traits were recorded using predefined discrete phenotypic categories. Vegetative qualitative traits, including leaf shape and leaf colour, were assessed on fully expanded leaves at 44 days after transplanting (DAT). Fruit qualitative traits, comprising fruit shape, rind colour, rind surface texture and flesh colour, were evaluated on fruits harvested at physiological maturity. Leaf, rind and flesh colours were determined using the Royal Horticultural Society (RHS) colour chart and all qualitative traits were scored according to standard descriptor categories. Quantitative traits were measured using standardised quantitative measurement procedures. Leaf area was measured at 44 DAT on fully expanded leaves located between nodes 12 and 15 and leaf images were captured and analysed using ImageJ (<https://imagej.net/ij/>) following image calibration and threshold-based segmentation. Flowering time was recorded as the number of days from transplanting to the appearance of the first male flower and the first female flower respectively. At harvest, fruit-related quantitative traits were measured, including fruit length (measured from the proximal, peduncle end to the distal end), fruit diameter (measured at the widest transverse section), fruit weight (determined using a digital balance), flesh thickness (measured on longitudinally sectioned fruits as the distance from the inner rind surface to the seed cavity) and soluble solids content, which was determined using a handheld refractometer and expressed as degrees Brix (°Brix).

DNA extraction and random amplified polymorphic DNA analysis

Genomic DNA was extracted from young, healthy leaf tissue collected from S1 plants at 21 DAT using a modified cetyltrimethylammonium bromide (CTAB) method following previous related studies (28). Approximately 2 g of leaf tissue was ground in CTAB extraction buffer, incubated at 65 °C and purified using chloroform-isoamyl alcohol (24:1). The DNA was precipitated with cold absolute ethanol, washed with 70 % ethanol, air-dried and resuspended in nuclease-free water. The DNA samples were stored at -20 °C until further analysis. Further, DNA quality was assessed by electrophoresis on 1 % (w/v) agarose gel prepared in Tris-Borate-ethylenediaminetetraacetic acid (TBE) buffer, while DNA concentration and purity were determined using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA) based on A260/A280 and A260/A230 absorbance ratios. Only DNA samples with adequate concentration and acceptable purity were used for subsequent polymerase chain reaction (PCR) amplification. For each

S1 genotype, equal amounts of DNA obtained from the three biological replicate plants were pooled prior to RAPD amplification to generate a genotype-level DNA representation. The pooled DNA approach was adopted because the molecular component of this study was intended to evaluate residual multilocus genetic heterogeneity and overall genetic structure among S1 genotypes derived from heterogeneous farmer-maintained populations after a single selfing cycle, rather than to resolve segregation among individual plants within each genotype. Because RAPD markers generate dominant multilocus banding profiles, pooled DNA samples were considered appropriate for detecting broad genotype-level genetic relationships and residual diversity while minimising stochastic variation associated with individual RAPD reactions. Although pooled DNA analysis does not capture within-genotype segregation at the individual plant level, such segregation was independently evaluated through individual-based phenotypic clustering analyses using qualitative and quantitative morphological traits. The RAPD analysis was performed using five single primers (OPA-02, OPA-09, OPA-12, OPA-14 and OPA-15 procured from Widya Life Science, Republic of Indonesia). These primers were selected based on previous studies reporting high amplification reproducibility and substantial polymorphism for genetic diversity analyses. The PCR amplification was carried out in a total reaction volume of 12.5 μ L containing 2 $\times$ PCR master mix (i-Taq, iNtRON Biotechnology, Republic of Korea), primer, template DNA and nuclease-free water. The PCR program consisted of an initial denaturation at 94 $^{\circ}$ C for 5 min, followed by 35 cycles of denaturation at 94 $^{\circ}$ C for 30 sec, annealing at 38 $^{\circ}$ C for 30 sec, and extension at 72 $^{\circ}$ C for 1 min, with a final extension at 72 $^{\circ}$ C for 5 min (Applied Biosystem SimpliAmp Thermal Cycler, Thermo Fisher Scientific). Amplification products were separated by electrophoresis on 1 % agarose gel, stained with RedSafe nucleic acid staining solution (iNtRON Biotechnology) and visualised using a Gel Doc EZ Imager (Bio-Rad).

Data analysis

Qualitative morphological traits were scored according to International Plant Genetic Resources Institute (IPGRI) descriptors, whereas quantitative morphological traits were measured using standard procedures and subsequently converted into binary variables based on predefined biological thresholds for qualitative and quantitative traits (Table S1-S4). Binary qualitative and quantitative traits were combined into a single phenotypic data matrix suitable for similarity-based analysis. Phenotypic similarity among individual plants was calculated using Jaccard's similarity coefficient and hierarchical clustering was performed using the unweighted pair group method with arithmetic mean (UPGMA) to visualise patterns of phenotypic segregation within and among S1 genotypes. The RAPD amplification products were scored as present or absent. Polymorphism information content (PIC) values were calculated for each RAPD primer based on band presence (1) and absence (0) using the formula $PIC = 2p(1-p)$, where p represents the frequency of band presence. Because RAPD analysis was performed using pooled DNA samples from the three biological replicate plants within each S1 genotype, molecular analysis represented pooled genotype-level profiles rather than individual plant-level variation. Genetic similarity among S1 genotypes was estimated using Jaccard's similarity coefficient, followed by UPGMA clustering to generate a RAPD-based dendrogram representing genetic diversity among S1 genotypes. All analyses were conducted using R version

4.3.1 (<https://www.r-project.org/>) with standard multivariate procedures.

Results

Hierarchical clustering based on binary-coded qualitative morphological traits separated S1 melon individuals into multiple clusters that comprised individuals originating from different S1 genotypes (Fig. 1A). Two plants, S1 GA2 and S1 BD3, failed to survive prior to phenotypic evaluation and were therefore excluded from all qualitative clustering analyses. Their missing data are documented in the qualitative binary phenotypic matrix (Table S2) and the quantitative binary phenotypic matrix (Table S4). Among the remaining individuals, a prominent upper cluster grouped S1 HD2 with S1 DM3, as well as a closely related subcluster comprising S1 DM2 and S1 DM1, forming a compact assemblage defined by short branch lengths. The S1 BD1 branched separately from this assemblage, indicating phenotypic differentiation despite its proximity to the DM-HD cluster. In contrast, S1 HD1 formed an independent branch and did not cluster with S1 SR individuals, whereas S1 SR3 and S1 SR1 constituted a distinct clade. A separate mixed cluster included GG and EL individuals, in which S1 BD2 clustered with S1 GG3. Additional subclades grouped S1 GG2 with S1 EL2 and S1 GG1, while S1 EL3 and S1 EL1 formed a closely related pair on a neighbouring branch. Further downstream, S1 AR3, S1 AR1 and S1 HD3 clustered together, followed by a mixed clade comprising S1 GA1, S1 AR2, S1 AL1 and S1 AL2, whereas S1 AL3 occupied a separate branch. At the basal part of the dendrogram, S1 FJ1, S1 SR2, S1 FJ2, and S1 FJ3 formed a terminal cluster with relatively long branch lengths and S1 GA3 was positioned as the most divergent individual. The binary coding scheme for qualitative traits and the complete individual-level data matrix underlying this analysis are provided in Tables S1 and S2. Clustering based on standardised quantitative morphological traits produced a highly heterogeneous dendrogram structure (Fig. 1B), characterised by limited formation of shared clades. Only a small number of individuals clustered at identical nodes, including S1 HD2 with S1 AR2, S1 AL3 with S1 HD1, and S1 GG1 with S1 FJ2. In contrast, many individuals, including S1 SR1, S1 DM3, S1 GA3, S1 EL2, S1 EL3, S1 GA1, S1 BD2, S1 DM1, S1 GG2, S1 AL1, S1 AR3 and S1 GG3, occupied independent branches, indicating extensive dispersion of quantitative trait variation and the absence of large, cohesive clusters. The binary threshold criteria and quantitative phenotypic matrix used for this analysis are provided in Tables S3 and S4. When qualitative and quantitative traits were combined into a single phenotypic dataset, the resulting dendrogram revealed partial reinforcement of local pairwise clades rather than the formation of large cohesive clusters (Fig. 1C). Several individuals clustered within the same higher-level clades in the combined qualitative-quantitative dendrogram (Fig. 1C). These clusters included a group comprising S1 AL2 and S1 AL1, a cluster containing S1 GA1, S1 AR2 and S1 GA3, a cluster of S1 EL3 and S1 EL2 that was closely associated with S1 EL1 and S1 FJ1. A grouping of S1 BD2 with S1 AR1, a cluster including S1 SR3 and S1 SR1 with S1 FJ3, a cluster formed by S1 HD2, S1 DM3 and S1 BD1. A grouping of S1 DM1 and S1 DM2, a cluster containing S1 AL3, S1 GG2 and S1 HD1 and a basal cluster comprising S1 HD3 and S1 AR3, which was separated from the GG group formed by S1 GG3 and S1 GG1. The qualitative, quantitative and combined phenotypic clustering analyses demonstrated that substantial phenotypic segregation persisted within the S1

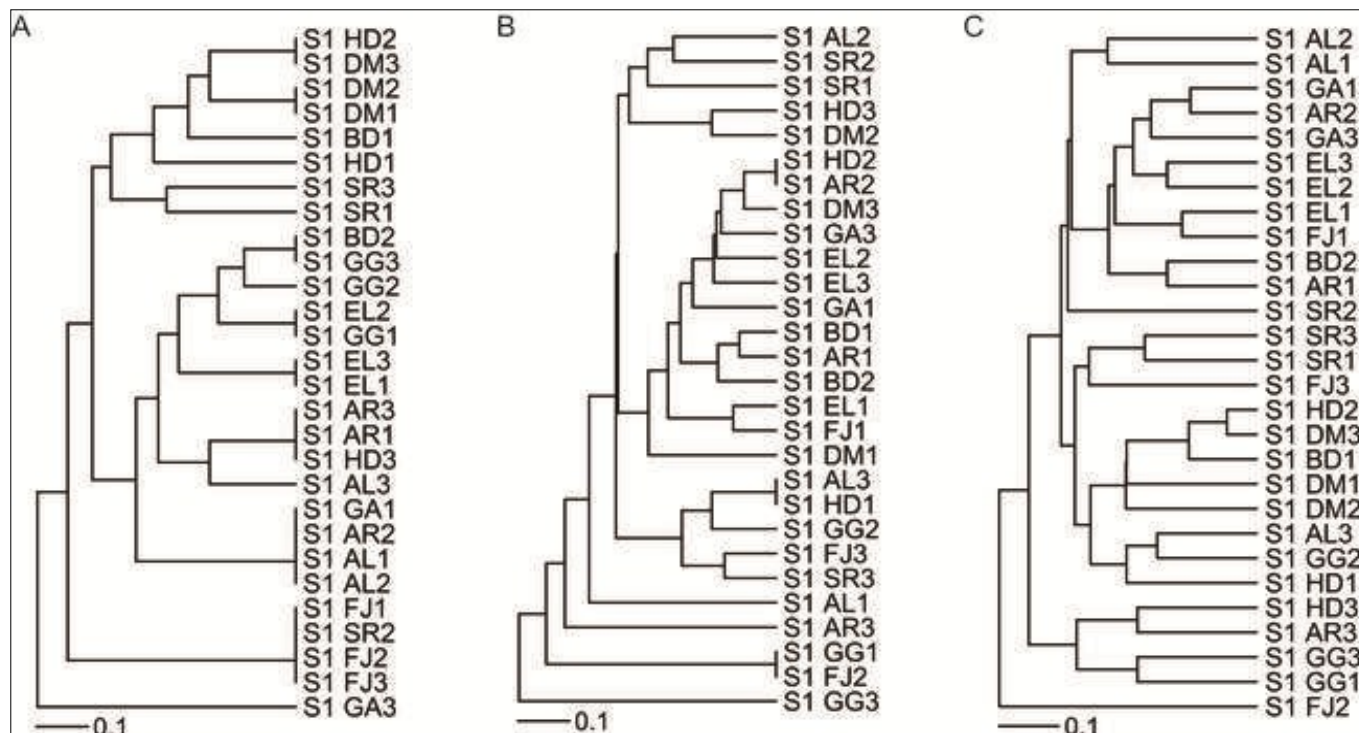


Fig. 1. Hierarchical clustering of first-selfed melon individuals based on qualitative, quantitative and combined phenotypic datasets. A: binary-coded qualitative morphological traits; B: standardised quantitative morphological traits; C: combined qualitative and quantitative phenotypic dataset. Clustering was performed using Jaccard's similarity coefficient and the unweighted pair group method with arithmetic mean (UPGMA). Scale bar = 0.1.

generation, as individuals derived from the same S1 genotype rarely formed cohesive clusters and were frequently distributed across multiple branches of the dendrograms. Furthermore, variation in fruit morphology among fruits produced by individual S1 plants further supported the persistence of phenotypic heterogeneity within the evaluated S1 materials. These findings confirm that a single cycle of self-pollination was insufficient to achieve phenotypic uniformity in farmer-collected melon germplasm.

Hierarchical clustering based on RAPD markers generated primer-dependent clustering patterns among S1 melon genotypes (Fig. 2). Using primer OPA-02, the dendrogram separated the S1 genotypes into 4 distinct clusters (Fig. 2A). The first cluster comprised S1 HD and S1 AR, which grouped closely with short branch lengths. A second cluster grouped S1 EL with S1 FJ, whereas a third cluster consisted of S1 GA and S1 SR, forming a separate paired grouping. The remaining genotypes, S1 GG, S1 DM, S1 BD and S1 AL, formed a fourth cluster that was clearly separated from the other three clusters by longer branch lengths. The RAPD band presence-absence matrix underlying the OPA-02 clustering is provided in Table S5 and representative amplification profiles are shown in Fig. S2A. Clustering based on primer OPA-09 produced a distinct dendrogram topology (Fig. 2B). Four genotypes, S1 DM, S1 SR, S1 AR and S1 AL, were grouped within a single clade. This clade was separated from the remaining genotypes by longer branch lengths. Downstream, S1 BD occupied an intermediate position as an independent branch. A separate cluster grouped S1 HD and S1 GG with relatively short branch lengths, whereas S1 EL and S1 FJ formed another closely associated pair. In this dendrogram, S1 GA was positioned as a distinct terminal branch, separated from all other genotypes. The complete RAPD scoring matrix for primer OPA-09 and the corresponding gel image are presented in Table S6 and Fig. S2B respectively. Primer OPA-12 resolved the S1 genotypes into two principal lineages in the RAPD-based dendrogram (Fig. 2C). The first lineage comprised S1 BD and S1 HD as a closely associated pair,

which was further joined by S1 EL at a higher hierarchical level. The second lineage grouped S1 FJ and S1 SR as a paired cluster, together with S1 AL and S1 DM as successive branches within the same lineage. In contrast, S1 AR, S1 GG and S1 GA were separated on a distinct basal branch characterised by longer branch lengths relative to the two main lineages. The RAPD banding data underlying the OPA-12 clustering are summarised in Table S7 and representative amplification patterns are shown in Fig. S2C.

The dendrogram derived from primer OPA-14 revealed a relatively compact clustering structure among the S1 melon genotypes (Fig. 2D). In this analysis, S1 HD and S1 GG formed a closely associated pair with short branch lengths, which was subsequently connected to a cluster comprising S1 EL and S1 DM. A single clade included S1 GA together with the paired genotypes S1 FJ and S1 BD. Downstream, S1 AL formed a separate cluster, whereas S1 AR and S1 SR were positioned on longer, more basal branches, indicating increased genetic divergence relative to the remaining genotypes. The RAPD band presence-absence matrix and representative gel image for OPA-14 are provided in Table S8 and Fig. S2D respectively. Clustering based on primer OPA-15 generated a structured dendrogram with several well-defined groupings among the S1 melon genotypes (Fig. 2E). The S1 HD and S1 AR formed a closely associated pair, which was subsequently connected to S1 EL and then to S1 FJ and S1 GA as successive branches within the same upper lineage. A separate cluster comprised S1 AL and S1 BD as a paired group, which was further linked to S1 DM. In contrast, S1 GG and S1 SR were positioned on long, basal branches, indicating greater genetic divergence from the remaining genotypes. The RAPD band presence-absence matrix and representative amplification profile for primer OPA-15 are provided in Table S9 and Fig. S2E respectively. When RAPD data from all five primers were combined, the resulting dendrogram resolved two broad clusters at the genotype level (Fig. 2F). The PIC values of the RAPD primers ranged from 0.118 to 0.324, indicating low to

moderate levels of marker informativeness for detecting genetic variation among S1 melon genotypes. The first cluster comprised S1 AL and S1 BD as a closely associated pair, which was subsequently joined by S1 DM within the same lineage. The second major cluster included two distinct sub-clades, one grouping S1 EL with S1 FJ and the other pairing S1 HD with S1 AR, indicating parallel but separate associations within this cluster. Outside these two main clusters, S1 GG and S1 SR were positioned on more peripheral branches, whereas S1 GA occupied the most basal position, representing the

greatest genetic divergence among the analysed genotypes. The combined RAPD presence-absence matrix used for this analysis is provided in Table S9. Representative mature fruit phenotypes of the ten S1 melon genotypes are shown in Fig. 3 to provide a visual overview of the phenotypic diversity observed among genotypes. Clear differences were evident in external fruit traits, including fruit shape, rind colour and rind surface characteristics, as well as in internal traits such as flesh colour and seed cavity morphology. These visual contrasts complement the individual-level phenotypic

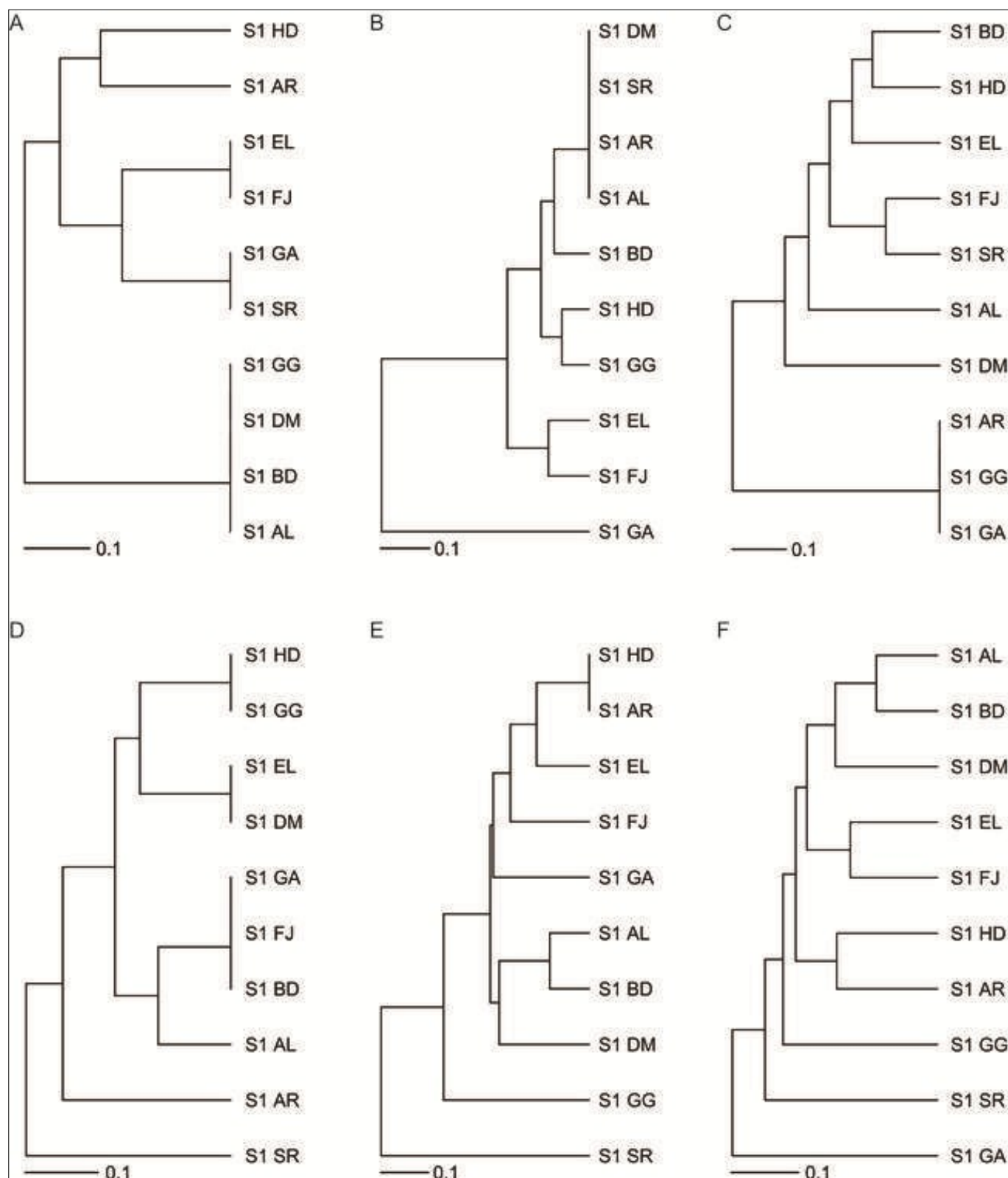


Fig. 2. Hierarchical clustering of first-selfed melon genotypes based on RAPD markers amplified using different primers. A: OPA-02; B: OPA-09; C: OPA-12; D: OPA-14; E: OPA-15; F: the combined RAPD dataset. Clustering was performed using Jaccard's similarity coefficient and UPGMA. Scale bar = 0.1. OP = Operon Primer.

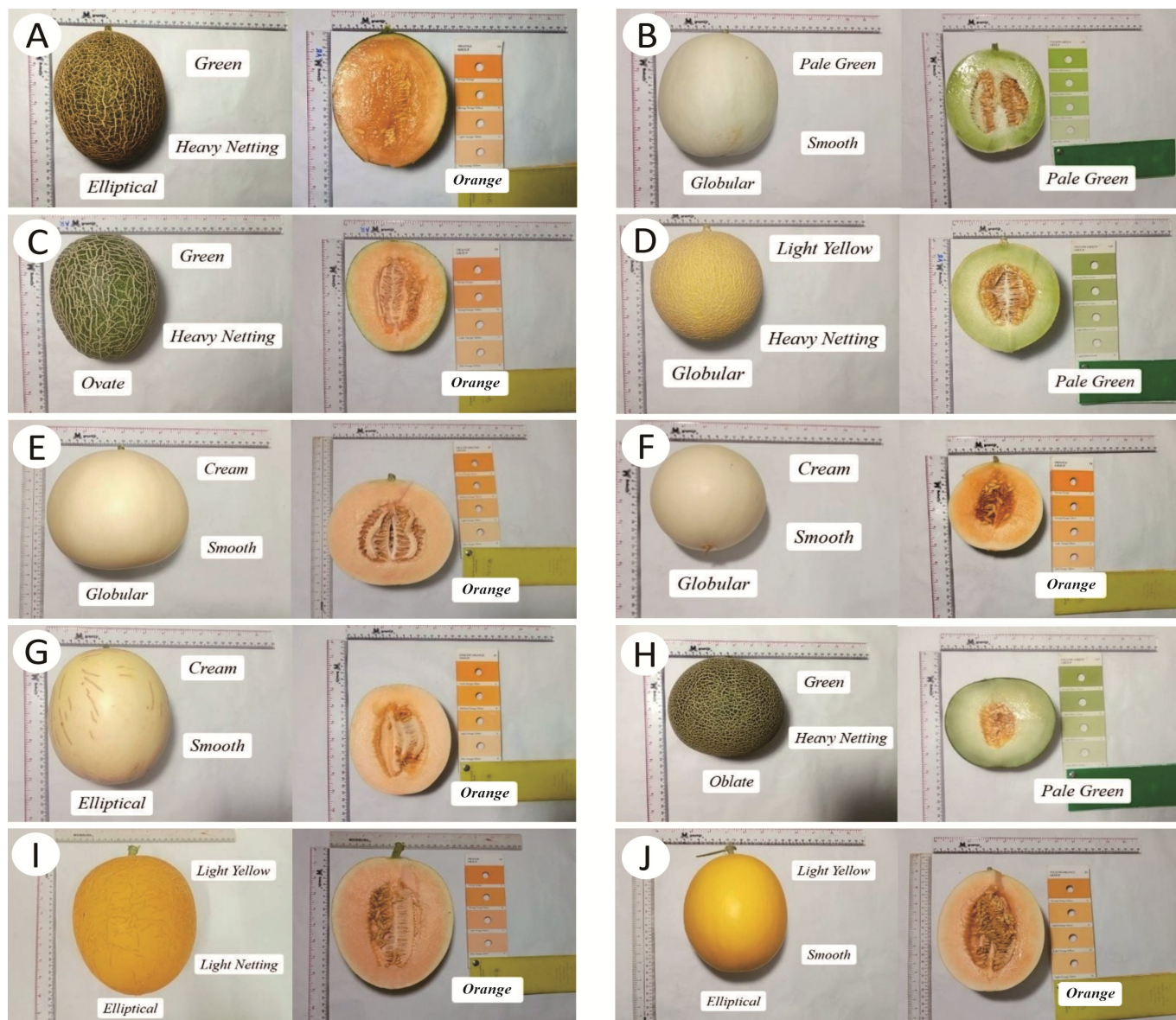


Fig. 3. Representative mature fruit phenotypes of first-selfed melon, showing ten S1 genotypes. A: S1 SR; B: S1 FJ; C: S1 AR; D: S1 GA; E: S1 EL; F: S1 DM; G: S1 GG; H: S1 HD; I: S1 BD; J: S1 AL. For each genotype, whole fruits (left) and longitudinal fruit sections (right) are shown, highlighting variation in fruit shape, rind colour, rind surface (netting intensity), flesh colour and seed cavity characteristics. Colour references were determined using the Royal Horticultural Society (RHS) colour chart. A ruler in cm is included in each panel for size reference.

clustering analyses by illustrating the breadth of fruit morphological diversity retained after the first selfing of farmer-collected germplasm, without implying within-genotype uniformity.

Discussion

This study provides clear evidence that extensive segregation persists in the first selfed (S1) generation of farmer-collected melon germplasm. Across analyses, clustering patterns consistently showed high heterogeneity at both the individual and genotype levels. These findings confirm that a single cycle of selfing is insufficient to generate genetically or phenotypically uniform material when the parental population is heterogeneous and predominantly open-pollinated (29). The qualitative morphological dendrogram revealed pronounced individual-level segregation, with individuals derived from the same S1 genotype frequently distributed across multiple clusters. This pattern aligns with classical expectations of early inbreeding generations, in which heterozygosity is only partially reduced and segregation remains extensive (30). Similar segregation patterns have been reported in

other cross-pollinated and highly heterozygous crops, where early selfed generations remain highly variable despite initial inbreeding efforts. Studies in cassava, diploid potato and other segregating breeding populations have shown that early-generation materials often retain substantial within-family variation, limiting the effectiveness of strict phenotypic selection at the S1 stage (20, 30). Although qualitative traits are often treated as discrete, the observed clustering patterns suggest that their expression in S1 material remains segregating, consistent with expectations for early selfed generations derived from genetically heterogeneous populations (31). Segregation was even more pronounced when clustering was based on quantitative morphological traits. Most individuals occupied independent branches and only a limited number of shared nodes were observed. This extensive dispersion reflects the polygenic inheritance of quantitative traits and their sensitivity to residual heterozygosity and environmental variation, even under controlled conditions (32). Similar difficulties in early-generation selection for quantitative traits have also been documented in cucurbit breeding programmes, where residual segregation and genotype $\times$ environment interactions reduce the reliability of

selection before later selfing generations are reached (19). The absence of large, cohesive quantitative clusters indicates that early-generation selection based solely on quantitative traits is unlikely to be reliable in S1 populations derived from heterogeneous germplasm. When qualitative and quantitative traits were combined into a single phenotypic dataset, partial reinforcement of local clustering patterns was observed, but large genotype-wide cohesive clusters still failed to emerge. This outcome suggests that integrating multiple trait categories increases the resolution of similarity-based analyses but does not supersede the underlying genetic heterogeneity retained after a single selfing cycle. Similar outcomes have been reported in early-generation segregation studies, where integrative phenotypic approaches improve discrimination among individuals without eliminating within-family variation (33).

The RAPD-based analyses revealed substantial genetic heterogeneity among S1 genotypes, as reflected by clustering patterns that varied markedly among primers. This primer-dependent topology is consistent with the random genomic distribution of RAPD markers and their differential sensitivity to distinct genomic regions, resulting in variable resolution of genotype-level genetic relationships. While some primers resolved compact genotype pairs, others positioned the same genotypes as divergent or basal, highlighting the locus-specific nature of RAPD patterns derived from pooled DNA. The lack of congruence among individual primers cautions against overinterpretation of genetic relationships inferred from single-marker datasets. In contrast, the combined RAPD analysis across all five primers produced a more stable genotype-level structure, resolving two broad clusters while consistently placing certain genotypes at peripheral or basal positions, indicating improved robustness through the integration of multiple markers that sample a wider portion of the genome (34). The observed PIC values indicated that the RAPD primers used in this study possessed sufficient discriminatory capacity for genetic diversity assessment. Primers with relatively higher PIC values contributed more effectively to genotype differentiation, supporting the usefulness of RAPD markers for detecting residual genetic heterogeneity in early selfed melon populations. Nevertheless, several genotypes remained highly divergent even in the combined analysis, demonstrating that genetic heterogeneity among S1 genotypes persists after a single selfing cycle. Importantly, the concordance between extensive phenotypic segregation observed at the individual level and RAPD-based genetic heterogeneity at the genotype level supports the interpretation that the observed morphological variation reflects genuine underlying genetic differences rather than experimental noise. These findings indicate that S1 melon material derived from farmer-collected germplasm should be regarded as segregating populations rather than fixed genetic entities. From a breeding perspective, selection at the S1 stage should therefore emphasise population structure and segregation behaviour, rather than apparent phenotypic performance alone (35). In practical terms, breeders should prioritise S1 genotypes that exhibit relatively cohesive clustering patterns, shorter branch lengths among individuals and partial consistency between phenotypic and RAPD-based groupings, as these characteristics may indicate narrower ranges of residual heterogeneity. Conversely, genotypes displaying extensive

dispersion of individuals across multiple phenotypic clusters, long branch lengths and unstable positioning across RAPD dendrograms should be considered highly heterogeneous and subjected to additional selfing cycles before intensive selection is applied. Selection decisions at the S1 stage should therefore focus on identifying populations with reduced internal variability and more stable trait associations, rather than selecting superior individual plants solely based on phenotypic performance. Genotypes that consistently form compact phenotypic clusters and show relatively stable associations across pooled RAPD analyses may represent more advanced trajectories toward genetic fixation and can therefore be prioritised for advancement to later selfed generations, where selection for uniformity and stable trait expression becomes more reliable.

Conclusion

This study demonstrates that extensive phenotypic segregation persists at the individual plant level in the first selfed (S1) generation of farmer-collected melon germplasm, accompanied by pronounced random amplified polymorphic DNA (RAPD)-based genetic heterogeneity at the genotype level. Qualitative, quantitative and combined phenotypic analyses revealed substantial within-genotype variation among individual S1 plants, whereas RAPD markers derived from pooled DNA samples showed primer-dependent genetic structuring and persistent divergence among S1 genotypes. These findings indicate that a single selfing cycle is insufficient to achieve phenotypic uniformity or to resolve genetic heterogeneity in heterogeneous melon populations and that S1 families should therefore be classified as segregating populations rather than stable genetic entities. Importantly, genotypes that consistently form compact phenotypic clusters and show relatively stable associations across pooled RAPD analyses may represent narrower ranges of genetic heterogeneity and can be prioritised for advancement to later selfed generations, where selection for uniformity and stable trait expression becomes more reliable.

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Authors' contributions

BH, FK, SA and MS conceived and designed the study. BH performed data analysis, interpreted the results and drafted the manuscript. FK and SA provided conceptual guidance and critically reviewed the manuscript. AR conducted the experiments and collected the morphological and random amplified polymorphic DNA (RAPD) data. All authors read and approved the final manuscript.

Compliance with ethical standards

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

Ethical issues: None

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

During the preparation of this work, the authors used Grammarly to assist with language editing and grammatical refinement. The authors reviewed and edited the content as needed and took full responsibility for the publication's content.

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