

Development and application of functional markers based on the CmAPRR2 gene for peel color improvement in Muskmelon (*Cucumis melo*)

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Research Article

Keywords: melon, QTL, peel colour, foreground marker, background marker

Posted Date: May 8th, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-6524420/v1>

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Version of Record: A version of this preprint was published at Molecular Breeding on October 20th, 2025.
See the published version at <https://doi.org/10.1007/s11032-025-01604-x>.

Abstract

Peel is a crucial trait affecting consumer preference and an important focus in melon breeding. Muskmelons display extensive genetic diversity in peel color, primarily yellow, green, and white, with additional variations in blended hues and shades. To enhance the efficiency of peel color improvement, this study constructed F_2 and recombinant inbred line (RIL) populations from a cross between 'VZX' (green peel) and 'HP' (yellow peel) for genetic analysis and QTL mapping. In the F_2 ($n = 414$) and RIL ($n = 116$) populations grown under protected cultivation, the green-to-yellow peel ratios were 3:1 and 1:1, respectively. Bulk Segregant Analysis (BSA) identified a significant QTL associated with peel color at the start of chromosome 4. Additionally, a high-density genetic map based on whole-genome resequencing data of the RIL population was constructed, which revealed a significant peel color-related QTL of 77 kb located at 499.6–576.5 kb of chromosome 4 with a LOD score of 14.85, explaining 41.76% of phenotypic variation. This locus overlapped with the BSA identified interval that contains 13 annotated genes, including *EVM0008709*, which encodes the *APRR2* transcription factor known to regulate chlorophyll metabolism and pigment accumulation in various crops. Based on the *EVM0008709*, three KASP markers (RC1, RC2, and RC3) were developed and successfully guided screening of peel color phenotype in the F_2 , RIL, and 94 inbred lines. 141 KASP markers for background selection were developed based on resequencing data of the parental lines 'VZX' and 'HP'. Using 'HP' as the donor and 'VZX' as the recipient, we performed two consecutive backcrosses followed by one selfing generation. Through foreground and background selection and traditional breeding, we obtained a novel germplasm resource ('YVZX') exhibiting yellow peel with flesh quality similar to 'VZX'. This study identified effective molecular markers and demonstrated a successful strategy for supporting melon peel color breeding.

1. Introduction

Muskmelon is highly favored by consumers for its exceptional flavor quality [1, 2]. Its market value is largely determined by fruit quality traits such as peel color [3], fruit size [4], fruit shape [5], flesh color [6], and overall taste and flavor [7–9]. Among these traits, peel color, along with fruit size and shape, is one of the most visually prominent features that affect consumer choice and has therefore become a key breeding focus [10–14]. Understanding the genetic basis and key regulatory genes underlying muskmelon peel color is essential for breeders aiming to efficiently modify this trait through molecular breeding approaches.

Previous studies have investigated the genetic basis of peel color in muskmelon using various populations. In a muskmelon F_2 population derived from MS-723 (green peel) and B432 (yellow peel), peel color was mapped to chromosome 4 and revealed green to be dominant over non-green. The gene *MELO3C003375* (*CmAPRR2*) was proposed as a candidate gene [15]. Similarly, a RIL population was created from DUL (dark peel) and TAD (light peel), along with F_3 and $F_{3:4}$ families from DUL (dark peel) and NA (light peel), consistently mapped the gene controlling dark peel in young muskmelon fruits to a 290 kb region on chromosome 4. This region contained 33 annotated candidate genes, including

MELO3C003375 (*CmAPRR2*) [16]. In watermelon, QTL mapping of peel color was performed using an F_2 population and $F_{2:3}$ families derived from NY0016 (light peel) and EMB (dark peel). By integrating genetic linkage mapping and extreme pool sequencing, the gene determining dark peel at maturity was localized to a 900 kb region on chromosome 9, which encompasses 30 candidate genes, including *CICG09G012330*, a homolog of *MELO3C003375* (*CmAPRR2*) in muskmelon [16]. Beyond cucurbits, the *APRR2* gene has been proven to affect plastid development in other species. Its overexpression increases chlorophyll content in young fruits and carotenoid content in ripe fruits of tomatoes and peppers, suggesting a conserved function of *APRR2* across plant species. [17]. However, Pereira *et al.* constructed a RIL population by crossing 'Védrantais' and 'Pielde Sapo' and found that light peel in young fruits is a dominant trait controlled by a locus on chromosome 7 [18]. Similarly, Li *et al.* used F_1 , F_2 , BC_1 , and RIL populations derived from 9904 (dark peel) and Handel (light peel) to demonstrate that dark peel is dominant and controlled by *CICG08G017810* on chromosome 8 [19]. These findings indicate that other regulatory genes and mechanisms, beyond *APRR2*, are involved in peel color determination in both muskmelon and watermelon. Another key gene, *CmKFB* (*MELO3C011980*), located on chromosome 10, is almost undetectable in yellow peel lines but highly expressed in white peel lines. It acts as a negative regulator of naringenin chalcone synthesis, thus determining yellow versus white peel color. An Indel marker developed from *CmKFB* effectively distinguishes between yellow and white peels [20]. GWAS analysis of a natural population consisting of 145 white peel and 236 yellow peel accessions and extreme pool sequencing on an F_2 population (MS-723×B432) both confirmed the presence of the *CmKFB* locus on chromosome 10 [19]. Together, *CmAPRR2* and *CmKFB* are considered the two major genes responsible for green, yellow, and white peel color formation in muskmelon.

The use of molecular markers in assisted selection and breeding has become increasingly sophisticated and widely adopted [21, 22]. Among third-generation molecular markers, single-nucleotide polymorphism (SNP) markers stand out for their broad genomic distribution, codominant inheritance, high-throughput automation, and cost-effectiveness [23, 24]. These advantages have positioned SNP markers as powerful tools in functional gene mining, germplasm identification, novel germplasm development, and the exploitation of heterosis, underscoring their strong potential for practical application [25]. Kompetitive Allele Specific PCR (KASP) is a SNP genotyping technology based on allele-specific primer binding at the 3' end [26]. Due to its high reliability, accuracy, and reproducibility, KASP has become a global standard in genetic mapping and molecular breeding research [27].

In this study, the muskmelon cultivars 'VZX' (green peel) and 'HP' (yellow peel) were used as parents to construct F_2 and RIL populations for genetic analysis and QTL mapping of peel color at maturity. KASP molecular markers were developed for both foreground selection within candidate genes and background selection based on parental genotypes. By integrating molecular marker-assisted selection with conventional breeding, we successfully obtained a new yellow peel germplasm that retains the desirable flesh quality of 'VZX' after two backcross generations followed by one generation of selfing. This study provides both effective molecular markers and a successful case for functional gene analysis and targeted improvement of peel color in muskmelon.

2. Materials and Methods

2.1. Plant materials, growth, and peel color classification

'V Zui xian' (VZX) and 'HP' are high-generation inbred lines cultivated by the Hami Melon Research Center of Xinjiang Uygur Autonomous Region Academy of Agricultural Sciences. 'VZX' features oval shape, green peel, white flesh, and excellent taste quality, while 'HP' has yellow peel, white flesh, and an average flavor quality but an attractive appearance. In this work, they were used as parents to produce F_1 hybrids, which were then self-crossed to generate F_2 seeds. Then the F_2S_6 recombinant inbred lines (RILs) were developed using the single-seed descent method. The parental, F_1 , and F_2 seeds were sown and transplanted at the Turpan Experimental Station Base of Xinjiang Uygur Autonomous Region Academy of Agricultural Sciences on March 2 and March 20, 2020, respectively. Specifically, 20 seedlings from each parent and the F_1 generation, along with 414 F_2 individuals, were transplanted for cultivation. In the winter of 2021, seeds from the same set of parents, F_1 and RIL populations, were also sown at the Hainan Sanya Breeding Station Base of Xinjiang Uygur Autonomous Region Academy of Agricultural Sciences on October 20, with 20 plants from each parent and the F_1 generation were retained for cultivation. For the RIL population, plants from 116 families with 8 individuals per family were cultivated to maturity. The growth management, including planting pattern, irrigation, and fertilization, was kept consistent across trails. Vines were trained vertically using a single-vine pruning system, and one fruit per plant was retained, set between the 8th and 10th nodes. At fruit maturity, peel color was recorded as follows: lines producing fruits with uniform yellow peel were classified as yellow peel materials, while those with predominantly yellow color but visible green spots or halos on the fruit surface were classified as green peel materials. RIL families displayed both peel types.

2.2. QTL Mapping of muskmelon peel color

During the young fruit stage, tender leaves were collected, flash-frozen in liquid nitrogen, and preserved at -80°C until use. Genomic DNA was extracted from the leaf samples using the cetyltrimethylammonium bromide (CTAB) method [28]. DNA quality and concentration were determined using an ultra-micro spectrophotometer (Model: Q3000; USA), and integrity was assessed via 1% agarose gel electrophoresis.

DNA library construction, quality inspection, and sequencing on the Illumina sequencing platform were performed by Biomarker Technologies Corporation. Raw sequencing data were processed to remove adapters and low-quality data. Clean reads were aligned to the *Cucumis melo* reference genome Payzawat (https://www.ncbi.nlm.nih.gov/assembly/GCA_009760825.1) using BWA software. Subsequent analyses included recalculating sequencing depth, genome coverage, and detecting SNP variations using the GATK software toolkit. The alignment rates to the reference genome for the two parental materials, 'VZX' and 'HP', were 99.33% and 98.77%, respectively, yielding 54,638,814 and 55,335,796 clean reads with an average sequencing depth of $14\times$. The RIL population exhibited a 98.73% alignment rate and generated 15,566,500 clean reads with an average sequencing depth of $3.7\times$.

Sequencing quality assessment showed a Q30 score exceeding 90.25%, indicating high-quality sequencing data suitable for downstream QTL analysis.

To ensure the accuracy of the genetic map, markers were filtered, and 652,160 SNPs were selected for further analysis. A sliding window scan was performed across the chromosomes using 15 SNPs per window with a step size of 1 SNP, followed by marker imputation and correction. Bin division was then carried out based on the recombination pattern of the offspring. Finally, a genetic map was constructed using these bins as markers, resulting in a total map distance of 1365.94 cM. The QTL for mature peel color was located using the composite interval mapping method, and the threshold was set by performing 1000 permutation tests to establish a LOD value corresponding to a 0.99 confidence level.

Twenty green peel and twenty yellow peel samples were selected from the 116 families in the RIL population to construct separate pools for the peel color trait. Reads from each sample were extracted at a 1× depth using seqtk V1.3 software and merged based on pool information to generate pooled sequencing files for each color. The quality of fastq files was first controlled using fastp V0.20.1 software. Clean reads were then aligned to the Payzawat reference genome using bwa V0.7.17 software. The alignment results were sorted using samtools V1.10 software, and duplicated sequences were marked using Picard software. Finally, SNP calling and filtering were performed using gatk V4.2.0.0 to generate VCF files for the peel color trait pools. QTL mapping of the mature peel color traits was carried out using deepbsa software [29], employing four methods: ED4, SmoothG, SmonthLOD, and Δ SNP index.

2.3. Development of foreground and background markers

Functional annotation of candidate genes was conducted based on the Payzawat v1 Genome. The IGV 2.9.4 software was utilized to identify nucleotide polymorphisms in target candidate genes between the parental lines 'VZX' and 'HP'. Based on these variations, KASP markers were developed for key candidate loci. These markers were then validated using F₂ and RIL populations, as well as a natural population. Based on the sequence differences between 'VZX' and 'HP' across the entire genome, 204 SNP loci were selected across the 12 chromosomes, with approximately 17 SNP loci per chromosome to ensure even distribution. KASP markers were designed for these loci, and those exhibiting clear genotyping results and uniform distribution were retained for background selection.

2.4. *Marker-assisted introgression of yellow peel color into "VZX"*

'VZX' was crossed with 'HP' to generate the F₁ generation, which was then backcrossed with 'VZX' to produce BC₁F₁ seeds. Foreground selection markers were used to identify 40 plants with heterozygous target loci (*CmAPRR2*^{G/T}) from 90 BC₁F₁ individuals. Simultaneously, 141 background selection markers were utilized to screen 20 plants with the highest background recovery rate (PRPG). These selected plants were then backcrossed with 'VZX'. Upon maturity, fruit qualities were evaluated, and single-fruit seeds from three plants exhibiting the best fruit quality and overall traits were retained, forming the BC₂F₁ population.

RC3 markers were used to screen 40 plants with heterozygous target loci (*CmAPRR2^{G/T}*) from the 90 BC₂F₁ individuals. Background selection markers were used for identification of 20 plants with the highest PRPG, which were then planted. Similarly, from these, seeds from single fruits of three individuals showing the best fruit quality and traits were collected to generate the BC₂F₂ population. From the BC₂F₂ population, 15 plants with homozygous target loci (*CmAPRR2^{T/T}*) were identified using RC3 markers and subsequently cultivated. Mature fruit evaluations confirmed the successful development of a novel germplasm line YVZX, which displayed a stable yellow fruit peel similar to that of 'VZX'. This line serves as a valuable resource for both breeding and further investigations into the molecular genetic mechanism governing peel color.

Homozygous SNP loci differing between 'VZX' and 'HP' were identified across the genome. SNP loci were selected to ensure even chromosomal distribution. Sequence variations surrounding each candidate SNP were visualized using IGV2.9.4 software. The most informative SNP loci were chosen to develop background selection KASP markers for precise improvement of the 'VZX' fruit peel color trait. The proportion of background recovery rate in the recurrent parent was evaluated using PRPG through the formula: $PRPG(g) \% = (L + X(g)) / 2L \times 100$, where L represents the total number of markers used, and X(g) represents the number of markers in the backcross progeny matching the genotype of the recurrent parent [30].

3. Results

3.1. Peel color genetic analysis of ripe muskmelon fruits

To investigate the inheritance of peel color in muskmelon, green-peel cultivar 'VZX' and yellow-peel melon 'HP' were used to generate F₁, F₂, and recombinant inbred line (RIL) population (Fig. 1A), followed by a phenotypic survey of ripe fruit peel color conducted in Turpan, Xinjiang. All 39 F₁ plants exhibited green peel. In the F₂ population (n = 414), 294 plants had green peel and 120 had yellow peel, fitting an approximately 3:1 segregation ratio (Fig. 1B). The F₂ population was subsequently self-crossed for six generations through single-seed descent to develop an RIL population consisting of 116 families. In the winter of 2021, peel colors of the RIL population were recorded for plants cultivated in Sanya, Hainan, with 59 families showing green peel and 57 families displaying yellow peel, consistent with an expected 1:1 segregation ratio (Fig. 1B). These results collectively suggest that green peel is dominant over yellow, and that ripe fruit peel color is likely governed by a single gene.

3.2 QTL Mapping and Candidate Gene Analysis

Based on the peel color phenotypes of 116 families from the RIL population, a high-density genetic linkage map was constructed to analyze peel color inheritance. The locus controlling green peel color was mapped to the chromosome 4, within a confidence interval of 77 Kb (chromosome 4: 499.6–576.5 kb). It exhibited a LOD score of 14.9 and explained 41.8% of genetic variation (Fig. 2A, 2B). Within this 77

Kb interval, 13 candidate genes were annotated (Fig. 2C). Among them, *EVM0008709* encodes the two-component response regulator protein APRR2, which has been reported in various crops to govern chlorophyll metabolism and pigment accumulation [15, 16]. To further validate the mapping results, 20 RIL families with green peel and 20 with yellow peel were selected to construct pooled samples. Multiple methods (ED4, SmoothG, SmoNthLOD, SNP) based on extreme pooling sequencing were used to locate the gene controlling peel color of mature fruits. All approaches consistently located the candidate locus at the start of chromosome 4 (Figure S1), aligning with the results from linkage mapping, which validates the accuracy of the mapping result.

3.3 Development and Validation of KASPMarkers Based on the EVM0008709 Gene

Based on previous findings, the *EVM0008709* gene has been identified as a candidate gene controlling green peel color in ripe muskmelon fruits. Using IGV2.9.4 software, we examined the sequence variations of this gene between the parental lines 'VZX' and 'HP'. Four variant sites were identified at positions 553645 bp (A/G), 555015 bp (T/C), 555803 bp (G/T), and 558147 bp (A/T). Corresponding KASP markers were developed and designated RC1, RC2, RC3, and RC4, respectively (Fig. 3A, Table S1). Initial validation of these four markers in a population of 94 RIL lines showed that RC2 and RC3 exhibited strong genotype–phenotype correlation, with complete consistency between genotype and peel color (Fig. 3B, Table S2). Further validation was performed in both an F₂ population and a natural population consisting of 94 inbred lines. In the F₂ population, RC2 and RC3 genotypes were completely consistent with their phenotypes (Fig. 3C, Table S3). In the natural population, 87 out of 94 lines showed matching genotypes and phenotypes, resulting in an accuracy rate of 92.5% (Fig. 3D, Table S4).

3.4 Targeted introgression of yellow peel trait into the 'VZX' melon

'VZX' is a high-quality inbred line developed through a multi-parent composite crossing system in the 1980s. While it is known for its good taste and internal quality, its external appearance can be suboptimal, as late harvesting often results in a yellow-green peel with uneven coloration. To improve the appeal of 'VZX' fruit color, this study aimed to develop a new germplasm line retaining the quality of 'VZX' but exhibiting a stable yellow peel. We integrated the foreground and background selection with traditional breeding. Based on genotypic differences between 'VZX' and 'HP', 204 SNP loci were selected across all 12 chromosomes (17 SNP loci per chromosome), and KASP markers were designed. 141 KASP markers demonstrating good genotyping performance and relatively even chromosomal distribution were chosen as background selection markers (Table S5). The RC3 marker (*CmAPRR2*^{G/T}) was used as the foreground selection marker (Fig. 4, Table S5). Using this approach, the yellow peel allele *CmAPRR2*^{TT} from the 'HP' was introduced into the green peel background of 'VZX' (*CmAPRR2*^{GG}).

'VZX' was crossed with 'HP' to generate the F₁ generation, which was then backcrossed with 'VZX' to produce BC₁F₁ seeds. Using the RC3 marker, 40 BC₁F₁ plants heterozygous at the CmAPRR2 locus (*CmAPRR2*^{G/T}) were selected from 90 BC₁F₁ individuals (Fig. 5A). Simultaneously, background selection was performed using the 141 genome-wide markers (Fig. 5B, 5E), and the 10 plants with the highest background recovery rate (PRPG) were chosen for further backcrossing with 'VZX' (Fig. 6). Overall qualities of mature fruits were evaluated, and seeds from the two best-performing plants were retained as the BC₂F₁ population. Again, 40 heterozygous plants (*CmAPRR2*^{G/T}) were selected from 90 BC₂F₁ plants (Fig. 5C), and RC3 selection led to the identification of 10 plants with the highest PRPG (Fig. 5D, 5E). At last, BC₂F₂ seeds were produced via self-crossing (Fig. 6). From this population, 20 homozygous individuals (*CmAPRR2*^{T/T}) were chosen from the BC₂F₂ population using RC3 and cultivated for phenotypic assessment (Table 1). A new germplasm, 'YVZX', with a pure yellow peel and fruit quality comparable to 'VZX' was successfully developed. This material is suitable for fruit color breeding and further studies on the molecular genetic mechanisms regulating peel color in melon.

Table 1
Appearance and quality of muskmelon fruit

Traits	HP (<i>CmAPRR2</i> ^{TT})	VZX (<i>CmAPRR2</i> ^{GG})	YVZX (<i>CmAPRR2</i> ^{TT})
Peel color	yellow	green	yellow
Fruit shape	round	oval or elliptical	elliptical
Flesh color	greenish white	white	white
Net pattern	fine and dense	sparse	dense and complete
Taste	soft, juicy, bland	crispy, moderate juice, sweet	crispy, moderate juice, sweet
Total sugar content	10.5 ± 0.65	16.5 ± 1.2	14.5 ± 1.1

4. Discussion

Fruit peel color is a crucial trait influencing consumer preferences in melon [31], sparking significant interest among researchers in its genetic pattern and the identification of key controlling genes [32, 33]. Ripe melon fruits exhibit diverse peel colors, primarily green, yellow, and white, with variations in mixed hues and shades [34]. In a previous study, three peel colors, including green, yellow, and white, were observed in the F₂ population derived from MS-723 and B432. Genetic analysis of the F₁ and F₂ generations supported that green peel is dominant over non-green colors and is controlled by a single gene. Further analysis of a pool of green, yellow, and white cultivars, in combination with GWAS analysis of natural populations, led to the identification of peel color-related QTLs on chromosomes 4 and 10,

harboring the *CmAPRR2* and *CmKFB* genes, respectively [15]. In our study, we used 'VZX' and 'HP' as parental lines to generate F₂ and RIL populations. Only green and yellow peel phenotypes were observed, with no white lines detected. Phenotypic segregation in the F₁, F₂, and RIL populations indicates that green peel color is dominant over yellow and is determined by a single gene. Sequence analysis of the *CmKFB* gene in 'VZX' and 'HP' reveals no polymorphisms (Figure S1). QTL mapping in the RIL population derived from 'VZX' and 'HP' identified a major locus associated with green peel color at the start of chromosome 4. This QTL spans 77 kb, exhibits a high LOD score of 14.9, and accounts for 41.8% of the phenotypic variation (Figure S2). 13 candidate genes were annotated within this QTL including *CmAPRR2*, which suggests a genetic basis for the peel color phenotypes observed in the F₁, F₂, and RIL populations of this study.

Homologs of the *CmAPRR2* gene have been reported to regulate chlorophyll metabolism and pigment accumulation in the peels of watermelon, cucumber [35, 36], cucurbita pepo [37, 38], tomato, and pepper [17], indicating the conserved function of the *APRR2* gene in fruit peel color regulation. However, a GWAS analysis of 177 natural populations failed to detect significant associations with young fruit peel color. Instead, *CmAPRR2* was identified via biparental population mapping. Further resequencing of 19 representative light peel materials revealed multiple *CmAPRR2* haplotypes, which likely explains the absence of significant GWAS signals [16]. Based on sequence differences in the *CmAPRR2* gene between 'VZX' and 'HP', four KASP markers were developed. Among them, RC2 and RC3 showed high genotyping accuracy. These markers were applied to 94 materials from the F₂ and RIL populations derived from 'VZX' and 'HP', showing complete concordance between phenotype and genotype. Further screening of 94 inbred lines identified seven lines exhibiting discordance between genotypes and phenotypes, indicating the need for additional haplotype analysis of *CmAPRR2* or the investigation of other genes potentially involved in peel coloration.

The RC3 marker was developed based on a G/T variation site in the exon of *CmAPRR2*, where a change from G to T causes premature termination of protein translation. This marker was identified as the optimal functional marker for the targeted improvement of melon peel color. Traditionally, modifying ripe fruit peel color in melon requires lengthy growth processes and phenotypic evaluation at fruit maturity [39]. However, the development of functional markers like RC3 enables accurate determination of ripe fruit peel color at the seedling stage, significantly improving the efficiency of melon peel color breeding. To facilitate the targeted modification of 'VZX's peel color and create new germplasm without reducing flesh quality, 141 KASP markers were developed based on genomic sequence polymorphisms between the recurrent parent 'VZX' and the donor parent 'HP'. These markers were evenly distributed across 12 chromosomes and used for background recovery rate screening. By combining foreground and background selection with traditional breeding methods [40], a new germplasm line was successfully developed. This line exhibits yellow peel, improved visual appearance, and flesh quality comparable to 'VZX', and was achieved within two backcrosses and one self-crossing of the F₁. Compared to traditional breeding methods, this approach significantly reduced the breeding cycle and accelerated the breeding process. By extending the application of functional gene discovery to practical variety improvement, this

work provides reliable molecular markers and a successful case for melon peel color improvement breeding in melon. It also offers a valuable reference for the application of marker-assisted selection in the targeted enhancement of other traits.

5. Conclusion

Using genetic populations derived from 'VZX' and 'HP', including F_1 , F_2 , and RIL, the gene controlling ripe fruit peel color was mapped to the start of chromosome 4. A major QTL was identified, with a LOD score of 14.9 and an explained phenotypic variation of 41.8%. Based on the target candidate gene *CmAPRR2*, two high-performing KASP markers (RC2 and RC3) were developed for foreground selection and validated across RIL, F_2 , and natural populations. Additionally, based on genomic sequence differences between 'VZX' and 'HP', 141 KASP markers for background selection were designed. By integrating foreground and background selection with traditional breeding, a new germplasm line was successfully developed, which displayed a yellow peel while retains the high flesh quality of 'VZX'. This line possesses a genetic background closely resembling the recurrent parent 'VZX'.

Declarations

Funding:

This research was funded by the Project of Key Research and Development Project of the Xinjiang Uygur Autonomous Region (2022B02002-3); the China Agriculture Research System of MOF and MARA (CARS-25-G2); the earmarked fund for XJARS (XJARS-06-04); Regional Collaborative Innovation Project of the Autonomous Region (Shanghai Cooperation Organization Science and Technology Partnership Program)-Collection and Accurate Evaluation of Germplasm Resources of Water melon and Melon in Central Asia (2022E01024); The specific research fund of The Innovation Platform for Academicians of Hainan Province (YSPTZX202141).

Data Availability Statement: All data generated or analyzed in the study are included in the article and its Supplementary Materials. Further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

Author Contributions:

Conceptualization, R.F., Y.Y.; methodology, R.F. and Y.Z.; software, M.L.; validation, R.F., C.F., and W.L.; formal analysis, R.F., C.D., and B.L.; investigation, C.F., and W.L.; resources, Y.Y. and X.Z.; data curation, R.F., M.L., and Y.Z.; writing—original draft preparation, R.F.; writing—review and editing, R.F., M.L., Y.Z., B.L., X.Z., C.D., C.F., W.L. and Y.Y.; visualization, R.F. and Y.Y.; supervision, R.F.; project administration, Y.Y.; funding acquisition, R.F. and Y.Y.; All authors have read and agreed to the published version of the manuscript.

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Figures

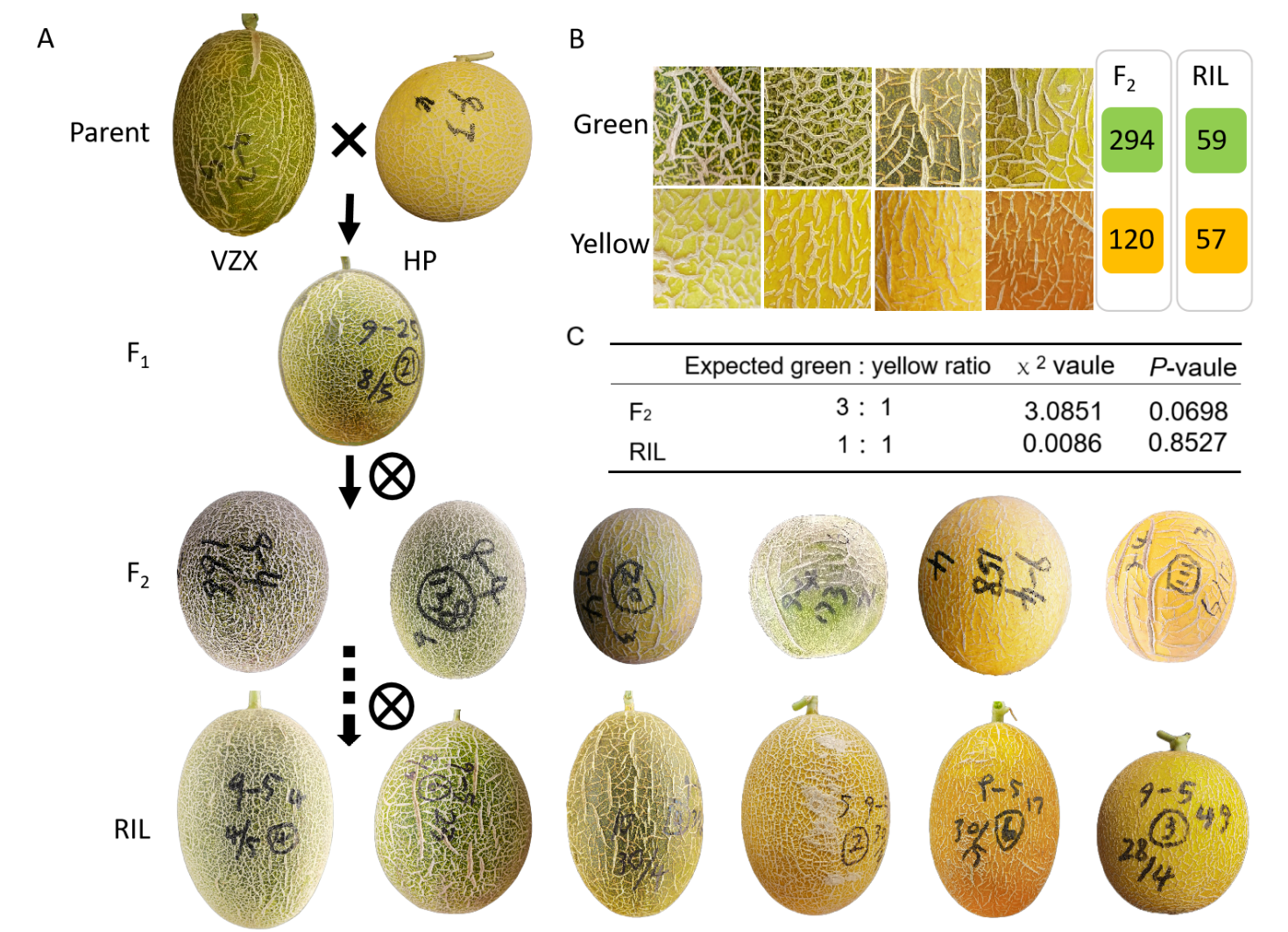


Figure 1

Phenotypic variation in ripe fruit peel color within F₂ and RIL populations. (A) Schematic of the development of F₂ and RIL populations derived from a cross between 'VZX' and 'HP', along with representative peel color phenotypes at fruit maturity; (B) Classification criteria for green and yellow peel types and the distribution of each phenotype in the F₂ and RIL populations.

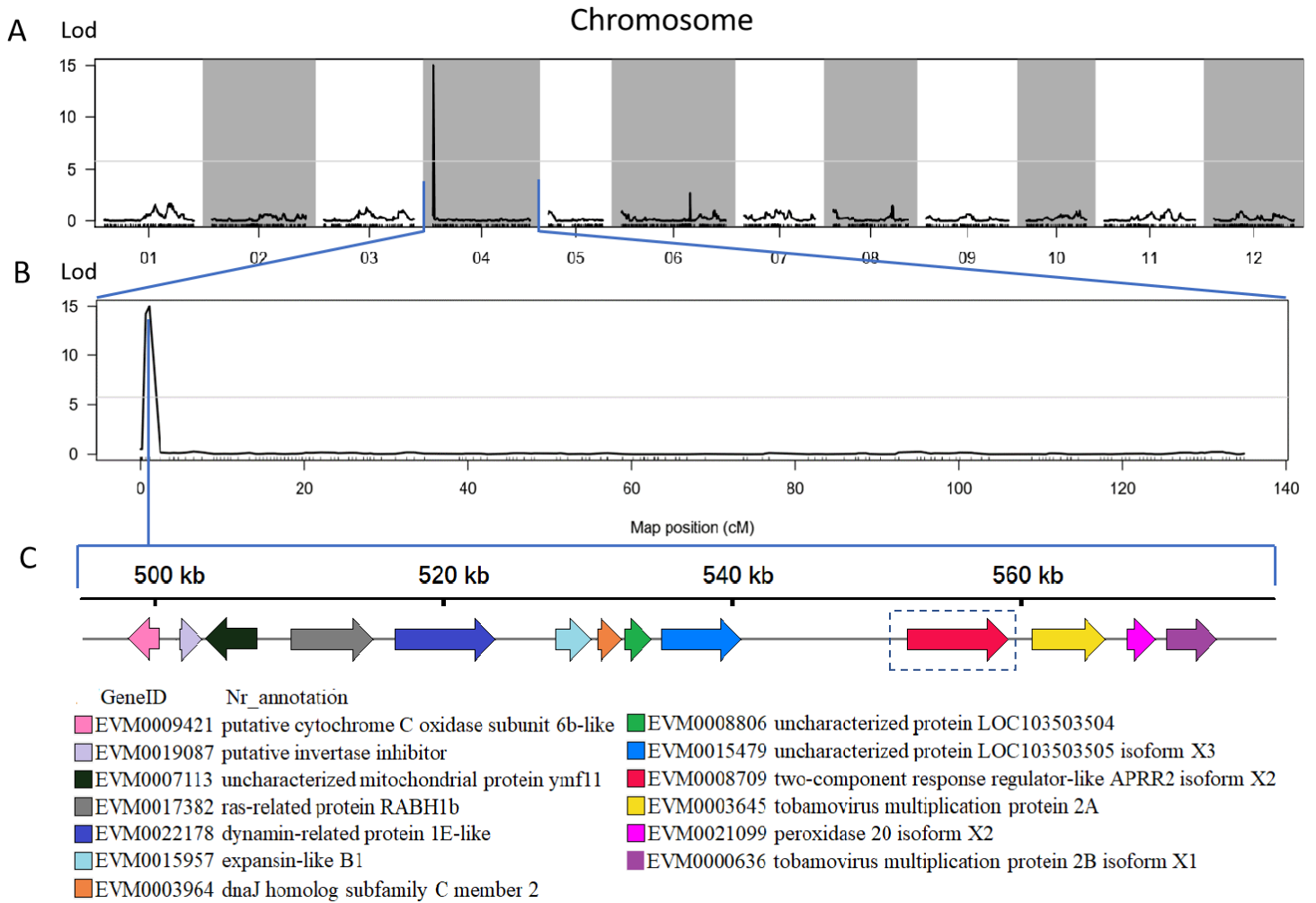


Figure 2

Genome-wide QTL mapping of peel color in the RIL population. (A) Manhattan plots showing the distribution of QTLs for peel color across the genome. (B) The significant QTL was localized to a 77 kb interval on chromosome 4 (499.6–576.5 kb). (C) A total of 13 genes were predicted within the candidate region. The LOD threshold for significance of QTL was determined via 1000 permutation tests ($P = 0.05$).

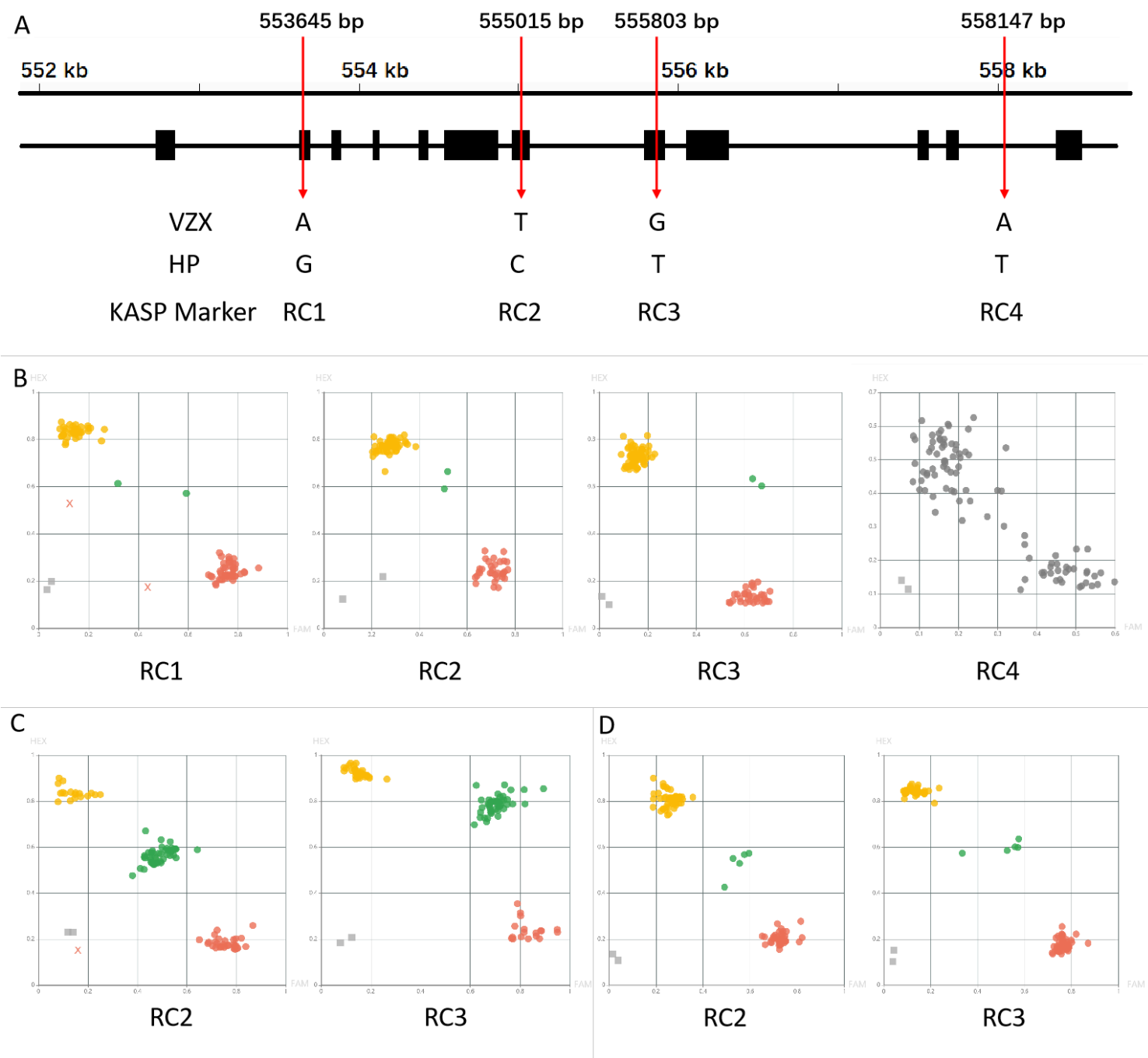


Figure 3

Genotyping results using the Kompetitive Allele Specific PCR (KASP) assay. (A) Gene structure of *EVM008709*. The black line represents the intron, rectangles represent exons, and the red arrows indicate the position of two adjacent SNPs in the parental lines 'VZX' and 'HP'; these loci were used to develop KASP markers RC1, RC2, RC3, and RC4. (B) Genotyping of RIL individuals from the 'VZX' × 'HP' mapping population. (C) Genotyping of F_2 individuals from the 'VZX' × 'HP' mapping population. (D) Genotyping of 94 natural population materials, including varieties and advanced inbred lines. Scatter plots for selected KASP assays showing clustering of varieties on the X- (FAM) and Y- (HEX) axes. The

yellow and red dots represent homozygous lines, and the green dots represent heterozygous lines from each population. The gray squares denote the non-template control (NTC).

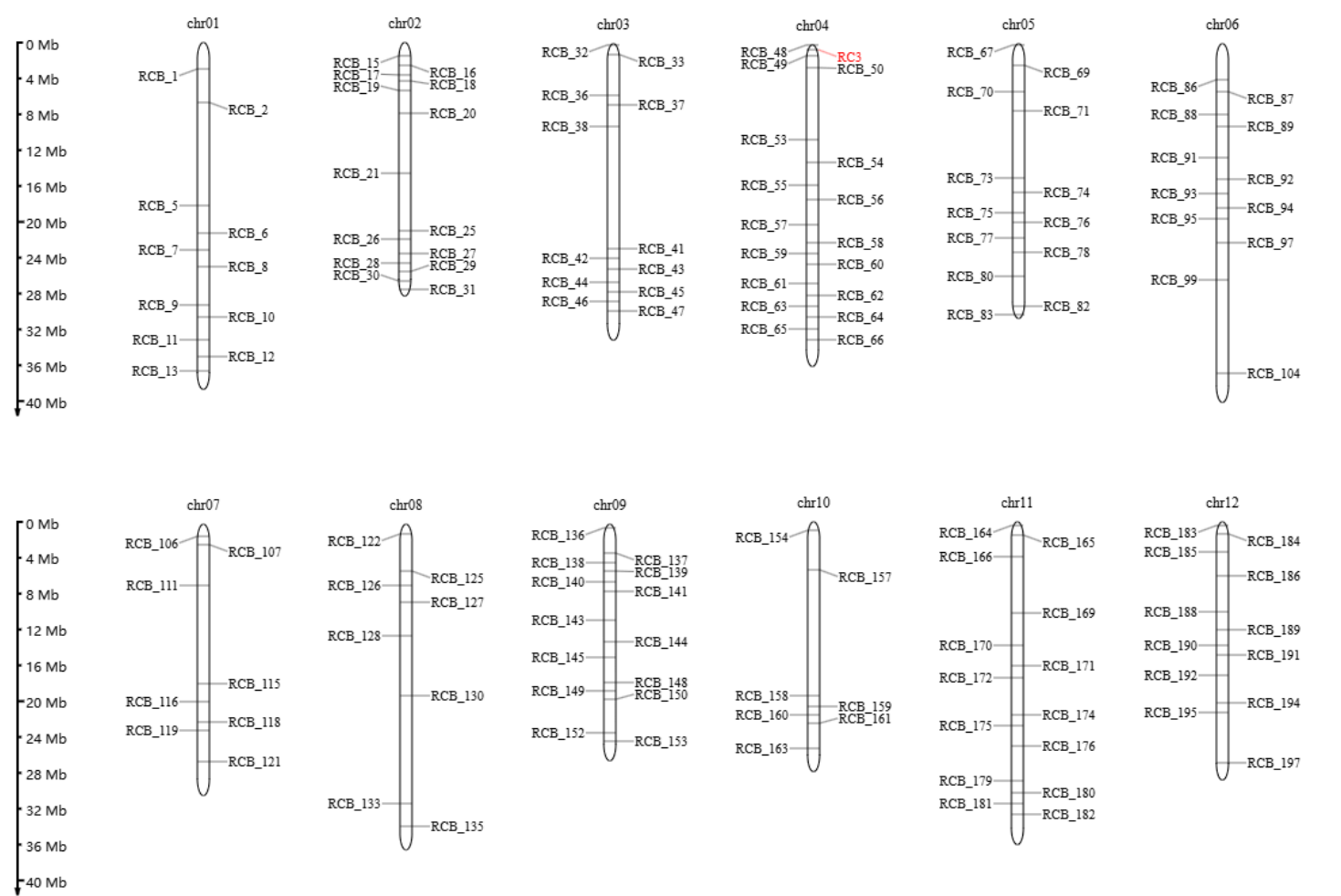


Figure 4

The physical distribution of foreground (RC3) and background (141 markers) selection markers across the 12 melon chromosomes.

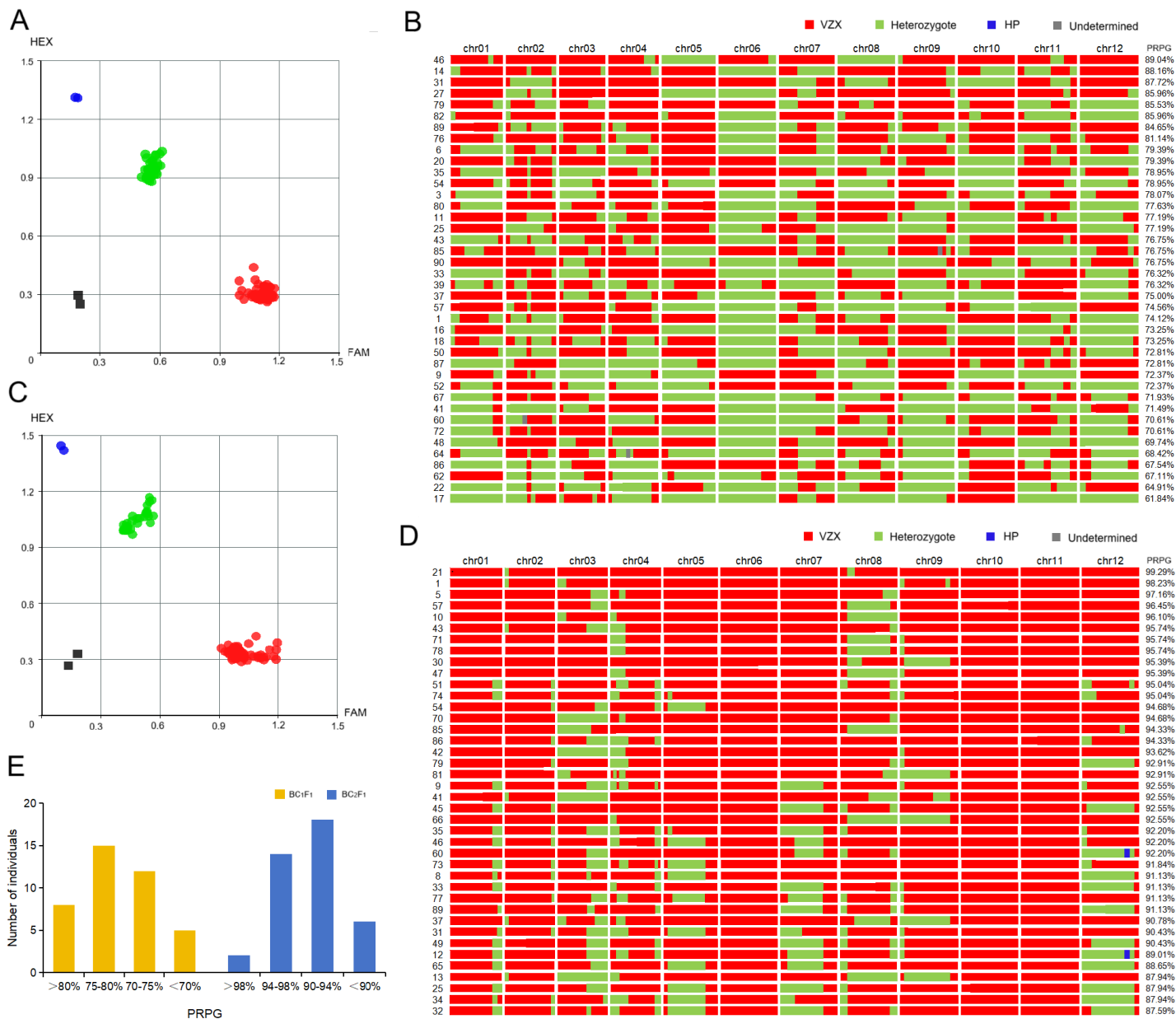


Figure 5

Genotyping and statistics for candidate individuals in BC₁F₁ and BC₂F₁ populations. (A) Schematic of foreground selection in the BC₁F₁ population using the RC3 marker. (B) The genotypic profiles of selected BC₁F₁ individuals. Red represents genomic regions from the recurrent parent VZX; blue represents regions from the donor parent HP; green represents heterozygous regions; gray represents undetermined regions. (C) Schematic of foreground selection in the BC₂F₁ population using the RC3 marker. (D) Genotypic profiles of selected BC₂F₁ individuals with color codes as described in (B). (E) Summary of the number of heterozygous individuals selected in BC₁F₁ and BC₂F₁ populations.

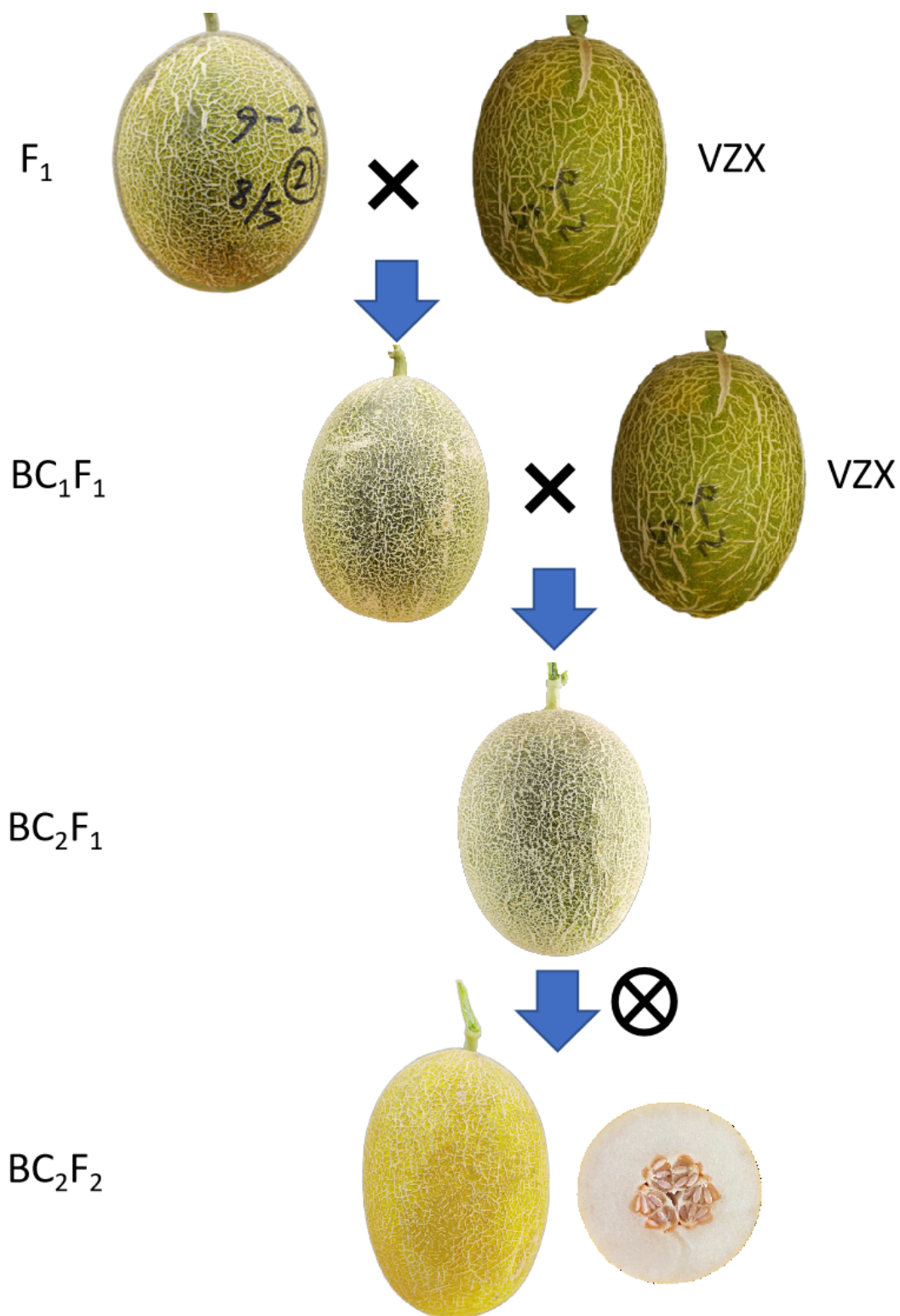


Figure 6

Improvement of peel color NILs by MAS.

Supplementary Files

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