# Melon (*Cucumis melo* L.): Genomics and Breeding

2

H. Chikh-Rouhou, W. Abdedayem, I. Solmaz, N. Sari, and A. Garcés-Claver

**Abstract**

Melon (*Cucumis melo* L.), belonging to the Cucurbitaceae family, is an economically important vegetable crop cultivated worldwide and highly valued for its fruit quality. Unfortunately, this crop is affected by several biotic and abiotic stresses that reduce yield and quality considerably. Melon breeding for fruit quality and disease resistance gained great achievements through Next-Generation Sequencing (NGS) technology. During the last decade, a rapid and huge development of genetic and genomics resources was achieved including draft genome assemblies, and high-density genetic maps, making it possible to accelerate translational research for melon breeding. The increasing availability of high-throughput sequencing technology has the potential to develop innovative genome-based strategies for the identification of loci involved in fruit quality and disease resistance. Advancements in genomics provide new opportunities to accelerate

H. Chikh-Rouhou (✉)
Regional Research Centre on Horticulture and Organic Agriculture (CRRHAB), LR21AGR03, University of Sousse, Sousse, Tunisia

W. Abdedayem
National Agronomic Institute of Tunisia (INAT), Wheat Septoria Precision Phenotyping Platform, Cité Mahrajène, Tunisia

I. Solmaz
Faculty of Agriculture, Department of Horticulture, Cukurova University, Adana, Turkey

N. Sari
INRAE, GAFL Unit-Genetics and Breeding of Fruit and Vegetables, Montfavet Cedex, France

A. Garcés-Claver
Department of Plant Science, Agrifood Research and Technology Centre of Aragon (CITA), Zaragoza, Spain

AgriFood Institute of Aragon – IA2 (CITA-University of Zaragoza), Zaragoza, Spain

© The Author(s), under exclusive license to Springer Nature Singapore Pte Ltd. 2023

S. Singh et al. (eds.), *Smart Plant Breeding for Vegetable Crops in Post-genomics Era*, https://doi.org/10.1007/978-981-19-5367-5_2

classical breeding programs. We report here the major findings from these investigations and future perspectives in melon-breeding programs. Genomic tools used including genome editing, improvement of the melon genome assembly, identification, and molecular mapping of important genes or quantitative trait loci for disease resistance and fruit-quality traits are summarized, and the use of such knowledge in melon breeding is reported.

**Keywords**

Genomic tools · Genome assembly · Melon · Molecular mapping · Marker-assisted selection · Reference genome · Doubled haploids

## 2.1 Introduction

Melon (*Cucumis melo* L., 2n = 2x = 24) is a cross-pollinating crop with a small diploid genome size of 450 Mb (Garcia-Mas et al. 2012). It is an important horticultural crop worldwide, belonging to the Cucurbitaceae family, and exhibits high levels of diversity in morphological, physiological, and biochemical properties (Pitrat 2016). Based on ovary pubescence, the melon was classified into two subspecies, *melo* and *agrestis* (Whitaker and Davis 1962), and then further divided into 16 horticultural groups according to morphological variations of fruit (Pitrat 2016), with the *flexuosus*, *cantalupensis*, *inodorus*, and *reticulatus* being the most economically important ones in Mediterranean countries (Chikh-Rouhou et al. 2021a; Pitrat 2016). All of these groups are intercrossable. *C. melo* subsp. *melo* is cultivated worldwide, whereas *C. melo* subsp. *agrestis* is concentrated in East Asia (Liu et al. 2004).

Until a few years ago, the study of melon genome was limited to molecular markers analysis including AFLP, RAPD, and SSR associated with some morphological and pathogen-resistance traits (Chikh-Rouhou et al. 2021a, b, c; Lakshmana Reddy et al. 2016; Garcia-Mas et al. 2000). However, genetic and genomic information for this crop has increased significantly and a broad range of genomic tools are available nowadays (Grumet et al. 2021; Ezura and Fukino 2009). These tools are generating a lot of information about genes involved in various biological processes, such as plant resistance, fruit quality, and ripening (Zhang et al. 2022, Cao et al. 2021, Tamang et al. 2021; Branham et al. 2018; Argyris et al. 2017).

Several melon accessions have been sequenced and characterized using the Illumina short-read Next-Generation Sequence (NGS) platform (Zhao et al. 2019; Pavan et al. 2017). Third-generation sequencing technologies, such PacBio and Oxford Nanopore, which can generate long reads have been also used (Yano et al. 2020). The importance of next-generation technology is increasing in melon research, allowing several applications related to understanding genetic variation and facilitating marker identification and characterization (Zhang et al. 2022; Pereira et al. 2018; Pavan et al. 2017). Indeed, given the increasing genomic data availability for breeders, genomics is playing an important role in all aspects of melon breeding,

such as Quantitative Trait Loci (QTL) mapping and Genome-Wide Association Studies (GWAS), where genomic sequencing can allow gene-level resolution of agronomic variation (Wang et al. 2021; Liu et al. 2020; Zhao et al. 2019; Pereira et al. 2018; Phan and Sim 2017; Gur et al. 2017; Zou et al. 2016; Grumet et al. 2020, 2021).

In melon improvement, conventional breeding plays an essential role to generate superior genotypes through genetic recombination. It involves growing and examining large melon populations derived from cycles of phenotypic selection and crossing which is a labor-intensive process and time consuming, as it needs several phases of crossing, selection, and testing. Thus, the emergence of doubled-haploid (DH) technology has reduced dramatically the time required to generate pure homozygous lines which can be directly released as a new variety or used as parents in breeding programs (Sari and Solmaz 2020; Fayos et al. 2015). In addition, advances in genomics have provided new opportunities to accelerate classical breeding programs. Indeed, the availability of melon genome sequence has made it possible to identify genes and genetic variants that contribute to agronomic traits (Yano et al. 2020). Likewise, the high-throughput sequencing technology is enabling the development of innovative genome-based strategies for the identification of loci involved in disease resistance and fruit quality (Branham et al. 2018, 2021; Lian et al. 2021; Liu et al. 2020). For the rapid development of new cultivars to face climate change and food scarcity, Marker-Assisted Selection (MAS), molecular Marker-Assisted Breeding (MAB), and gene-editing are needed (Marsh et al. 2021; Veillet et al. 2019). Hence, novel molecular techniques, integrated with predictions based on the genome, might provide new strategies to breed plants more efficiently as reported in several vegetable crops (Bohra et al. 2019).

For crop improvement, Varshney et al. (2020) recommended the deployment of 5 Gs (Genome assembly, Germplasm characterization, Gene(s)/marker(s) associated with breeding trait, Genomic Breeding and Gene editing). In the case of melon breeding, whole-genome assemblies have become available, melon accessions/germplasm characterization is ongoing in several countries. Similarly, gene/marker identification was accelerated due to the genomic and genetic resources availability and genotyping platforms. However, a precise phenotyping is important for the germplasm used for trait mapping (Thudi et al. 2021). Comprehensive analyses of genotyping and phenotyping data can provide genes/markers, haplotypes, genomic-estimated breeding values that can be used in genomic breeding and gene-editing approaches (Bohra et al. 2019).

The revolution in genetic and genomics research, genomic selection, computational biology and bioinformatics, genome editing, and other next-generation breeding methodologies will accelerate melon breeding. The present chapter will enumerate the latest applications of the doubled-haploid technology, genomics and genome editing, bioinformatics, and genomic resources to tackle the challenges in melon crop and its improvement in the post-genomics era.

## 2.2 Doubled-Haploid Technology

Advanced biotechnological tools are being used to develop new varieties that meet the consumers' and the producers' needs and preferences. DH technology is a biotechnological method that can be successfully applied to melon. Commercial melons are produced using open-pollinated (OP) and hybrid cultivars. Hybrid melon cultivars are mainly developed by classical hybridization technique, obtaining a stabilized line with the trait of interest is extremely labor and time-consuming (Sari and Solmaz 2020). The development of inbred pure lines is the basic step in hybrid melon breeding; however, many rounds of selfing are required. The DH technology has several advantages such as reducing the period for generating inbred pure lines and increasing breeding efficiency (Zhu et al. 2020). This technology allows the development of completely homozygous lines from heterozygous plants, which can be used either as parents in breeding programs or directly can be released as homozygous varieties (Sari and Solmaz 2020; Germanà 2011; Solmaz et al. 2011; Sari et al. 1999b).

Haploid plants contain a gametophytic chromosome number whereas DH plants are haploid plants that are subjected to spontaneous or stimulated chromosome duplication (Germanà 2011). The haploid plants provide considerable benefits to conventional breeding studies which can be achieved by three different techniques: androgenesis, gynogenesis, and parthenogenesis.

Although androgenesis is known as in vitro culture of anthers or isolated microspores, used in several plant species, no successful results have been obtained in melon. Likewise, gynogenesis, which is the in vitro culture of ovules or ovaries, has been exploited in different plants. However, it has not been routinely applied in melons and a quite few studies have been reported (Sari and Solmaz 2020; Koli and Murthy 2013; Malik et al. 2011; Ficcadenti et al. 1999). Irradiated pollen technique (parthenogenesis) is the most effective technique widely used in melon and provides successful results (Hooghvorst et al. 2020; Godbole and Murthy 2012; Solmaz et al. 2011; Sari et al. 1992, 2010a, b; Lim and Earle 2009; Lotfi et al. 2003; Abak et al. 1996).

Melon is one of the species that responds best to haploidization studies. The most effective method for haploid embryo induction in melon is the pollination of female flowers with irradiated pollen one day before anthesis. Although different methods such as ovule/ovary and anther culture have been used before, the most successful method today is the development of haploid embryos by the parthenogenesis method. The most commonly used irradiation source for this purpose is the $Co^{60}$ of gamma rays (Sari et al. 1992). However, it has been reported that Cesium (Cs137) and X-ray sources can also be used for irradiation (Dal et al. 2016).

Due to the wide diversity in melon, efficient DH protocols are variable for each botanical group and genotype (Hooghvorst et al. 2021). One of the factors affecting success in haploid plant production in melon is undoubtedly genotype selectivity. Another important issue is that melon plants to be induced with irradiated pollen should be grown in optimum environmental conditions and without stress. In the

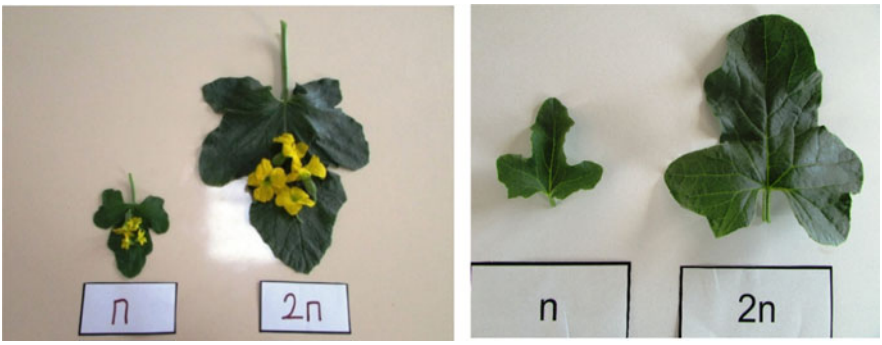


**Fig. 2.1** Leaves and flowers of haploid and doubled-haploid melons

irradiated pollen technique, male and female flowers were used the day before anthesis (Sari et al. 2010a, b).

After the first successful study by Sauton and Dumas de Vaulx (1987) on the Charentais group melon, the most appropriate irradiation dose was determined as 300 gray in summer (Galia type) and winter (Kirkagac and Yuva type) melon varieties (Sari et al. 1999a). Various methods are used to determine the level of ploidy in the obtained melons, which can be divided into either direct (chromosome counts) or indirect methods (flow cytometry, stomata size, chloroplast count, morphological observations). The most classic among these is chromosome counts in plant parts where cell growth is the fastest. Abak et al. (1996) reported that morphological observations, pollen absence/presence check, a number of chloroplasts in stomatal guard cells also were used for ploidy level determination. Haploid plants have the feature of being miniature of the same plant with smaller leaves (Fig. 2.1).

DH technology has been used in Galia-type melon breeding. Haploid plants were obtained by irradiated pollen technique and then were duplicated by colchicine treatment. New F1 DH melon cultivars belonging to *C. melo* var. *cantalupensis* were developed, resistant to race 0 and 1 of *Fusarium oxysporum* f. sp. *melonis* (*Fom*) and with high yield and quality (Sari et al. 2010a, b). After agronomic performance tests for several years, the DH melon Sari F1, Yetisir F1, Solmaz F1, Emin F1, and Yucel F1 were registered in the new varieties catalog of the Republic Turkey Ministry of Agriculture and Forestry (Sari et al. 2010a, b). Besides, melon French cv Isabelle was crossed to Italian landrace, and the resulting F1 was subjected to parthenogenesis, haploid embryo rescue, and chromosome doubling. Two DH homozygous lines, Nad-1 and Nad-2, with strong resistance to FOM race 1.2 were obtained by Ficcadenti et al. (2002).

The parthenogenetic capacity of seven genotypes of *C. melo* var. *inodorus* 'Piel de Sapo' type was evaluated to obtain DH lines which might be used in further F1 breeding studies. These lines were assessed for agronomic traits and diseases (Fusarium wilt, powdery mildew, and MNSV). DH lines with high resistance to the pathogens were produced from melon donor genotypes (six genotypes were

inbred lines and one genotype was an open-pollinated cultivar) (Hooghvorst et al. 2020).

For genomic studies, the doubled-haploid homozygous line DHL92 was used in the sequencing and assembly of the draft reference genome (Garcia-Mas et al. 2012). DHL92 line derived from the cross between the Korean accession PI 161375 (Songwhan Charmi, spp. *agrestis*) (SC) and the 'Piel de Sapo' T111 line (ssp. *inodorus*) (PS).

## 2.3 Breeding in the Genomics Era

### 2.3.1 Genomic Resources

Advanced tools in genome sequencing, assembly, and bioinformatic fields were recently and massively used (Bevan et al. 2017; Chakradhar et al. 2017; Brenton et al. 2016; Martínez-Gómez et al. 2012; Pérez-de-Castro et al. 2012; Varshney et al. 2005). A burst of publications describing the draft genomes of important crops and an important number of sources of large and complex plant genomes databases have been published to date. These sources are available for public uses in online platforms such as the Potato Genome Sequencing Consortium (PGSC) (spuddb.uga.edu), the Tomato Genetic Resource Center (tgrc.ucdavis.edu), WheatGamp which is a comprehensive platform for wheat gene mapping and genomic studies (www.wheatgmap.org), and the CuGenDB platform for several Cucurbitaceae species including melon crop (https://www.cucurbitgenomics.org/) containing assembled genomes and annotations, genetic maps, transcriptomes, Expressed Sequence Tags (ESTs), and Genotyping By Sequencing (GBS) data along with analysis and visualization tools. Two other melon crop databases were developed: the MELOGEAN (Gonzalez-Ibeas et al. 2007) and the Melonomics (www.melonomics.net) platforms for melon functional genomics and the genome assembly and annotation version of the reference genome, respectively.

Crop and wild relatives' genomic analysis helps researchers to assess and to characterize species genetic diversity and genomic evolution under natural selection and domestication (Grumet et al. 2021; Coyne et al. 2020; Preece and Peñuelas 2019). Additionally, this analysis is a crucial step facing agriculture worldwide challenges, essentially population increase and climate changes by crop improvement and breeding programs releasing more resilient crops (Shivapriya et al. 2021; Chikh-Rouhou et al. 2021a, b, c; González et al. 2020; Maleki et al. 2018). Associated with the improved and automated phenotyping tools and functional genomic studies, genomics is providing new foundations for crop-breeding and improvement systems.

Melon is an attractive model for studying valuable biological characteristics, such as fruit ripening (Pech et al. 2008), sex determination (Boualem et al. 2008), and phloem physiology (Zhang et al. 2010). Compared to other Cucurbitaceae members, the melon genome is quite larger than the genome of the watermelon and cucumber but is relatively small in comparison to other crop species (Table 2.1).

**Table 2.1** The genome size of different crops compared to melon (*Cucumis melo* L.)

| Crop/species | Genome size (megabases) and Chromosome number | Reference |
|---|---|---|
| Melon (*Cucumis melo*) | 450 Mb (x = 12) | Garcia-Mas et al. (2012) |
| Watermelon (*Citrullus lanatus*) | 425 Mb (x = 11) | Guo et al. (2019, 2013) |
| Cucumber (*Cucumis sativus*) | 367 Mb (x = 7) | Yano et al. (2020) |
| Tomato (*Solanum lycopersicum*) | 900 Mb (x = 12) | The Tomato Genome Consortium (2012) |
| Pepper (*Capsicum annum*) | 3480 Mb (x = 12) | Kim et al. (2014) |
| Wheat (*Triticum aestivum*) | 17,000 Mb (x = 7) | Brenchley et al. (2012) |

Recent research has increased the availability of genetic and genomic resources for melon, such as (1) the sequencing of ESTs (The Cucurbit Genomics (CucCAP) n.d.; Gonzalez-Ibeas et al. 2007); (2) the development of an oligonucleotide-based microarray (Mascarell-Creus et al. 2009); (2) the construction of BAC libraries (González et al. 2010a, b; Leeuwen et al. 2003; Luo et al. 2001); (4) the production of mutant collections for TILLING analyses (Dahmani-Mardas et al. 2010; Tadmor et al. 2007; Nieto et al. 2007); (5) the development of a collection of Near-Isogenic Lines (NILs) (Eduardo et al. 2005); (6) the construction of several genetic maps (The Cucurbit Genomics (CucCAP) n.d.; Harel-Beja et al. 2010, Deleu et al. 2009); and (7) the development of a genetically anchored BAC-based physical map (González et al. 2010a, b). High-density genetic maps have been also realized (Zhang et al. 2022; Pereira et al. 2018). The high-density genetic maps of melon were constructed by GBS (Oren et al. 2020; Branham et al. 2018), resequencing (Hu et al. 2018), or RNA-Seq. (Galpaz et al. 2018), which greatly improved the QTL mapping resolution for fruit-related traits and disease resistance (Sáez et al. 2022).

### 2.3.2 The Melon Genome

Since the development of next-generation sequencing technologies (NGS: 454, Illumina, SOLID), several draft genomes of important crops have been published. These genomes have been sequenced mostly using NGS technologies, sometimes complemented with Sanger sequencing. A high-quality reference genome assembly of melon was released for the first time by Garcia-Mas et al. (2012). However, EST'S, microarrays (Mascarell-Creus et al. 2009), genetic and physical mapping (Diaz et al. 2011), Bac sequencing (González et al. 2010a, b), and reverse genetic tools (González et al. 2011; Dahmani-Mardas et al. 2010) were early investigated on the melon crop.

Draft genomes of nearly a dozen cucurbit crops are now available (https://www.cucurbitgenomics.org/) and are constantly being revised using new technologies and experimental data. Garcia-Mas et al. (2012) sequenced the melon genome using the DHL92 line. The assembled genome organized in 12 chromosomes (pseudomolecules) comprised 27,427 annotated protein-coding genes, with 17% of

the genome being transposable elements (TEs) (Garcia-Mas et al. 2012). The melon reference genome v3.5.1 was obtained using 454 pyrosequencing technology. Based on a shotgun sequencing approach, several DNA fragments were produced and assembled in contigs which were grouped into larger portions (scaffolds). Shotgun sequencing involves breaking up DNA sequences into small pieces and then reassembling the sequence by looking for regions of overlap. The final sequence assembly covered 83.3% of the genome (a total of 375 Mb) in 29,865 contigs and 1594 scaffolds, with a scaffold N50 of 4.68 Mb (Garcia-Mas et al. 2012). Further, Ruggieri et al. (2018) improved the genome assembly using optical mapping to produce v3.6.1, and a new comprehensive annotation was also built composed of 29,980 protein-coding loci. However, v3.6.1 still contained 19% of gaps and more than 40 Mb unassigned sequences, probably missing complex repeat regions. Castanera et al. (2020) using PacBio single-molecule real-time (SMRT) sequencing produced an improved reference genome version v4.0. In that study, they used the hierarchical genome-assembly process 4 (HGAP4) pipeline techniques with concatenated steps to improve the assembly of the longest reads. DHL92 melon assembly v4.0 had an increase of the melon genome pseudomolecule size by 40 Mb with 90% of the v3.5.1 gaps being filled and transposable element (TE) coverage improved from 19.7% (v3.5.1) to 45.2% (v.4.0) due to the progress in TE annotation tools (Castanera et al. 2020). Specifically, 40% more full-length LTR retrotransposons, which represented the largest fraction of TE, were identified in the v4.0 assembly, mainly located in centromeric and pericentromeric regions, and a burst of these repetitive elements was found to occur less than two million years ago showing they are very young (Castanera et al. 2020). Young LTR retrotransposons have an impact on gene expression. Some of these elements are polymorphic among melon varieties and sit in the upstream regions of genes. Ito et al. (2016) evidenced that the expression of plant LTR retrotransposons (subfamilies gypsy and copia) exhibits stress-inducible transcription, i.e., up-regulated by abiotic and biotic stress (Ito et al. 2016; Grandbastien 1998). Figure 2.2 summarizes the melon reference genome assemblies and annotation analysis and the improved versions highlighting the principal outputs from each study.

The DHL92 genome reference has been utilized for supporting transcriptome analyses as well as QTL studies of important agricultural traits, including fruit ripening, fruit morphology, and disease resistance (Branham et al. 2021; Tamang et al. 2021; Lian et al. 2021; Liu et al. 2020; Argyris et al. 2017). Additionally, assembled transcriptomes have been generated through the quantitative RNA sequencing (RNA-Seq) for mapping transcribed regions, in which complementary DNA fragments are subjected to high-throughput sequencing and mapped to the genome. Assembled transcriptomes of Cucurbits species were made available on the CuGenDB database (http://cucurbitgenomics.org/rnaseq/home) allowing exploration of a Cucurbit Expression Atlas (Andolfo et al. 2021). The transcriptomes can be used as a reference for gene expression analysis in different organs and tissues and under different environmental conditions (Andolfo et al. 2016).

*C. melo* genome showed a high level of synteny with cucumber (*C. sativus*), suggesting an ancestral fusion of five melon chromosome pairs in cucumber and

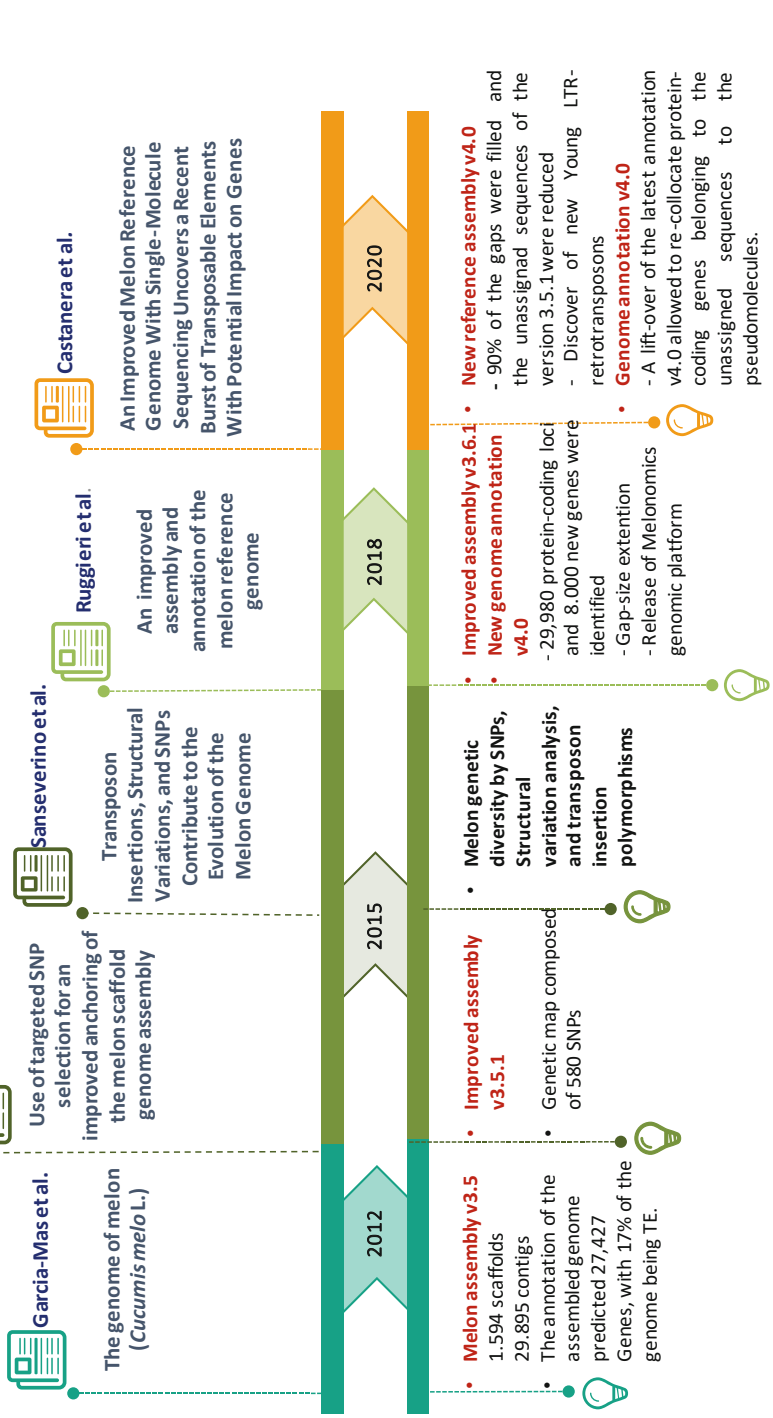


**Fig. 2.2** Reference genome assemblies and annotation of the double-haploid melon line DHL92, its improvement in time scale highlighting the outputs and improved versions

**Table 2.2** Technologies used for the sequencing of the melon DHL92 reference genome and other melon genomes

| Sequencing technology | Genotype used | Assembled genome | Reference |
|---|---|---|---|
| Shotgun strategy based on 454 pyrosequencing + Sanger reads | DHL92 | 375 Mb | Garcia-Mas et al. (2012) |
| Optical mapping approach | DHL92 | 375.36 Mb | Ruggieri et al. (2018) |
| PacBio and Illumina sequencing | DHL92 | 357.64 Mb | Castanera et al. (2020) |
| PacBio combined with the Hi-C interaction analysis | Payzawat | 386 Mb | Zhang et al. (2019) |
| Oxford nanopore | Harukei-3 | 378 Mb | Yano et al. (2020) |

several inter- and intra-chromosome rearrangements (Garcia-Mas et al. 2012). Same authors showed that the melon genome increased size, compared to its relative cucumber, may be attributed to TE amplification and that the melon genome did not have any lineage-specific whole-genome duplication as in *C. sativus* (Huang et al. 2009).

Other melon genomes have been also assembled and/or re-evaluated using newer DNA-sequencing advanced technologies (Table 2.2), such the Chinese *inodorus* melon genome, which was sequenced using a PacBio long molecule sequencing (Zhang et al. 2019) and the genome of the Japanese semi climacteric *reticulatus* cultivar using the Oxford nanopore technology (Yano et al. 2020). Information regarding the Japanese 'Harukei-3' genome assembly, annotation, and transcriptome dataset is available in the Melonet-DB database (https://melonet-db.dna.affrc.go.jp/).

Determination of the complete melon genome also includes sequencing of the chloroplast (cpDNA) and mitochondrial (mtDNA) genomes (Cui et al. 2021). Few studies were reported regarding sequence analysis of the cpDNA and mtDNA genomes (Cui et al. 2021; Rodríguez-Moreno et al. 2011). The mitochondrial genome of melon is eight times larger than other cucurbits (Rodríguez-Moreno et al. 2011). The nucleotide sequences of chloroplast and mitochondrial genomes of PIT92 melon were determined by Rodríguez-Moreno et al. (2011), showing that the chloroplast genome of 156,017 bp included 132 genes, with 98 single-copy genes and 17 duplicated genes in the inverted repeat regions (IRR). Moreover, 2.74 Mb of mitochondrial sequence, using Roche-454 sequencing technology, were assembled into five scaffolds and four additional unscaffolded contigs (Rodríguez-Moreno et al. 2011). These same authors showed that melon mitochondrial genome contained a high number of repetitive sequences and a high content of DNA of nuclear origin. Indeed, DNA transfer from organellar genomes to nuclear genome, and vice versa, seems a common phenomenon (Cui et al. 2021; Kleine et al. 2009; Martin 2003).

### 2.3.3 Genomic Tools for Melon Breeding

In recent years, efforts were dedicated to building genomic tools to be applied for breeding programs in order to obtain better melon varieties. The use of genomic resources to better understand disease resistance, fruit morphology, and quality has been facilitated by the availability of a reference genome and the rapid advances in Next-Generation Sequencing (NGS) technologies, such as Whole-Genome Sequencing (WGS), Whole-Genome Resequencing (WGR), RNA-seq, and GBS (Pereira et al. 2018). NGS has been used to extend insights in genomic research for developing molecular markers, identification of genetic variation, and gene discovery using sequencing approaches (Natarajan et al. 2016). Among these technologies, WGS allows scientists and breeders with improved analysis based on bioinformatics; therefore, discovering and identification of genes regulatory sequences, molecular markers, and quantitative trait locus controlling fruit quality, biotic and abiotic threats as well as other agronomic traits were performed (Pérez-de-Castro et al. 2012). Genome-wide SNP markers developed by sequencing enable high-density genetic maps, greatly improving the QTL mapping resolution (Gur et al. 2017) as well as the selection of core collections to capture the maximum genetic diversity with minimal redundancy (Wang et al. 2021). Likewise, WGR is widely used to discover the genetic diversity and molecular markers in a variety of plant populations and to gain a better understanding of the relationship between genotypic and phenotypic changes (Xu and Bai 2015). In addition to the identification of genetic polymorphisms such as SNP and insertion/deletion polymorphism (InDel), WGR permits the detection of Copy Number of Variation (CNV), presence/absence variation (PAV), and QTLs associated with disease resistance genes for the re-sequenced variants based on available R-genes from the reference genome (Natarajan et al. 2016). The large-scale data generated by NGS, combined with powerful computational tools enabled a major technological leap from low-resolution to high-resolution QTL mapping (Galpaz et al. 2018).

Transcriptome sequencing using RNA-seq technology allows exploring gene expression changes in melon plants during fungal and viral infections (Sáez et al. 2022; Cao et al. 2021) and fruit-related traits (Zhang et al. 2022; Galpaz et al. 2018). This tool offers a global view of expression changed during the defense response and elucidates complex resistance mechanisms in plants through comparing gene expression upon infection in susceptible and resistant genotypes (Sáez et al. 2022) and also to elucidate genetic factors that determine melon fruit-quality traits (Galpaz et al. 2018).

In addition, all these tools and resources also facilitate the melon genetic diversity studies, which are important for the management, improvement, and enhancement of germplasm.

### 2.3.4 Genomic Selection

Creating a new melon cultivar might take 10–12 years, due to several stages of crossing, selection, and testing required in the traditional production of new melon varieties. Innovative molecular tools, marker-assisted selection (MAS) including marker-assisted backcross selection, 'breeding by design,' or new strategies, like genomic selection, molecular marker-assisted breeding (MAB), and gene-editing are needed for the rapid development of new cultivars (Salgotra and Stewart 2020).

#### 2.3.4.1 MAS and FMs for Precision Breeding

MAS is a powerful genomic tool that assists phenotypic selection for the development of disease-resistant cultivars and allows breeders incorporate and pyramid resistance genes into breeding material (Zhu et al. 2020). An example is 'Carmen,' a new Yellow Canary-breeding melon line obtained by introgression of powdery mildew, CYSDV, and *A. gossypii* resistances of TGR-1551 into the genetic background of Bola de oro cultivar using molecular markers linked to *P. xanthii* races 1, 2, and 5 resistances and *Vat* gene (Palomares-Rius et al. 2018). MAS has also been extensively applied to search for the molecular markers that are linked to a specific trait during the development of disease-resistant cultivars (Teixeira et al. 2008), and it has been successfully applied to melon breeding, to improve, disease resistance and fruit quality, but these methods are not effective for detecting complex quantitative traits with small-effect QTL (Xu et al. 2012). Important genes and QTLs for disease resistance, fruit quality, and other traits in melon are listed in the Cucurbit Genetics Cooperative (https://cucurbit.info/home/gene-lists/).

Identifying genes and functional markers (FMs) that are highly associated with plant phenotypic variation is a challenge (Salgotra and Stewart 2020). Strategies to identify FMs for breeding goals include functional genomics approaches such as transcriptomics, targeting induced local lesions in genomes (TILLING), homologous recombinant (HR), association mapping, and allele mining (Salgotra and Stewart 2020). In comparison to other markers used in plant breeding, FMs had the advantage of the close genomic association with a phenotype, which may facilitate the direct selection of genes associated with phenotypic traits, and therefore, increase the selection to develop new varieties (Salgotra and Stewart 2020).

Advances in sequencing techniques enable the identification of SNPs and indels linked with various traits; FM development is, thus, enabled (Salgotra and Stewart 2020). Indels may cause phenotypic variation from extensive genomics, which are accompanied by chances of elimination from natural selection (Andersen and Lübberstedt 2003). Hence, SNP-derived FMs have advantages over indel-derived markers because of the widely distributed nature of FMs throughout the genome (Liu et al. 2012). Besides, the use of SNPs sets in high-throughput genotyping platforms is a powerful approach (due to their low cost, high genomic abundance, locus specificity, co-dominant inheritance, and low genotyping error rates (Cao et al. 2021)) for many genetic applications for breeding programs, such as germplasm characterization, quality control (QC) analysis, linkage mapping, linkage-based, and linkage disequilibrium-based QTL mapping, allele mining, marker-assisted

backcrossing (MABC), genomic selection (GS), and MAS (Salgotra and Stewart 2020; Cao et al. 2021).

#### 2.3.4.2 *R*-Genes

The large amount of generated data in melon sequencing projects can be useful to promote the *in silico* identification of important classes of genes (Andolfo et al. 2021). In recent years, the identification of genome-wide resistance (*R*) gene candidates has become a popular research aim in several species due to the development of prediction tools based on the identification of distinctive structural domains (Andolfo et al. 2013; Garcia-Mas et al. 2012). To date, more than 150 (*R*-genes) have been cloned and characterized in plants (www.prgdb.org). In melon, only a handful *R*-genes have been cloned, including *Vat*, which confers resistance to melon aphid (Dogimont et al. 2014), *Fom*-2, conferring resistance to *Fom* races 0 and 1 (Joobeur et al. 2004), and the head-to-head oriented pair of *R*-genes, *Fom*-1 and *Prv*, which confer resistance to *Fom* races 0 and 2, and the potyvirus *Papaya ring spot virus*, respectively (Brotman et al. 2013). In addition, recessive resistance genes were also identified; *nsv*, controlling resistance to the *melon necrotic spot virus*, which encodes a translation elongation factor (Nieto et al. 2007), downy mildew resistance genes encoding photorespiratory amino transferases (Taler et al. 2004), and *cmv1*, which encodes a vacuolar-sorting protein (Giner et al. 2017).

In the reference genome, 411 putative disease *R*-genes organized in clusters were identified, among them, 81 may exert their disease resistance function as cytoplasmic proteins through canonical resistance domains, such as the NBS, the LRR, and the TIR domains (Garcia-Mas et al. 2012). Besides, 15 homologs to the barley *Mlo* (Büschges et al. 1997) and 25 homologs to the tomato *Pto* (Loh and Martin 1995) genes were also identified.

To gain access to information and to facilitate the analysis of melon *R*-gene repertoires, the exploration of resources could be an important starting point (Andolfo et al. 2021). Several methodologies such as BLAST search, domain matching, sequence alignment, and phylogenetic analysis methods can be employed for R-proteins identification (Andolfo et al. 2013; Garcia-Mas et al. 2012). To search for plant resistance genes in the plant genome, DRAGO (Disease Resistance Analysis and Gene Orthology), a robust prediction tool, is available on the PRGdb platform (Andolfo et al. 2021; Osuna-Cruz et al. 2018).

To accelerate *R*-gene discovery and localization, high-resolution genetic maps can be combined with sequence data. Andolfo et al. (2021) reported that knowing the location of a given *R*-gene locus is a great advantage for mining its nucleotide sequences using both recombination analysis and protein-function prediction.

#### 2.3.4.3 Trait Mapping and Discovery of Candidate Genes

Linkage analysis has been extensively conducted to identify QTL using segregating populations derived from biparental crosses (Heffner et al. 2009). F2, backcross (BC), DH, and recombinant inbred line (RIL) populations are used as biparental mapping populations. However, Pérez-de-Castro et al. (2012) reported that low mapping resolution is provided when using biparental populations due to the

occurrence of only a few recombination events. In contrast, Pereira et al. (2018) reported that the GBS approach applied in a biparental RIL population is highly effective for QTL mapping studies highlighting that type and size of the population and map density are the main limiting factors for detecting QTLs.

The association mapping approach is a powerful method that uses historical recombination events for QTL detection in natural populations or germplasm collections via GWAS (Phan and Sim 2017; Zhu et al. 2008; Gupta et al. 2005). In comparison to linkage analysis, mapping approach is less time consuming, has a higher mapping resolution and a greater number of alleles to mine (Yu and Buckler 2006). NGS technologies facilitated the development of genome-wide molecular markers, especially SNPs, for high-throughput genotyping, providing the opportunity for association mapping (Zhu et al. 2008).

Bulked segregant analysis (BSA) is an important technique used to map QTLs and identify DNA markers. BSA provides a convenient and rapid method to identify resistance genes by generating two DNA bulks with a contrasting traits (Nie et al. 2015; Abe et al. 2012; Michelmore et al. 1991). Recently, whole-genome resequencing has been coupled with BSA to map the genes of interest that are associated with a given phenotype (Zou et al. 2016). The combined application of BSA with NGS (BSA-Seq) has accelerated the identification of tightly linked markers for gene identification and QTL mapping (Zou et al. 2016).

Linkage maps are an effective tool to study the genetic architecture of both monogenic and complex traits (Diaz et al. 2011). Recently, high-density maps (using hundreds to thousands of markers) have been constructed for QTL mapping of main traits (Pereira et al. 2018; Chang et al. 2017) demonstrating that a higher SNP density substantially increases the QTL mapping potential which affects the QTLs detection and resolution. These QTLs location in narrow genomic intervals could facilitate genes cloning and use in breeding programs by MAS (Pereira et al. 2018).

### Disease Resistance

Melon is susceptible to several pathogens. In breeding programs, identification of disease resistances and associated molecular markers is a priority. We report here the last molecular mapping of host resistances against the most important fungal and viral pathogens in melon.

For QTL mapping of the genes involved in the resistance of powdery mildew, caused by the airborne fungus *Podosphaera xanthii* (*Px*), Branham et al. (2021) used a densely genotyped RIL population and identified two major QTLs associated with resistance to *Px* race 1 in chromosomes 5 and 12 (*qPx1-5* and *qPx1-12*) and two minor QTLs (*qPx1-4* and *qPx1-10*) in chromosomes 4 and 10. For marker development across the major QTLs and functional annotation of SNPs for candidate gene analysis, the authors used the WGR of the parents. Competitive allele-specific PCR (KASP) markers were tightly linked to the QTL peaks of *qPx1-5* and *qPx1-12* in the population which will enable efficient marker-assisted introgression of Px resistance into improved germplasm. Candidate genes were identified in both major QTL

intervals that encode putative R-genes with missense mutations between the parents. These candidate genes provide targets for future breeding efforts.

Cao et al. (2021) identified, for resistance to powdery mildew (PM), a novel QTL on chromosome 12 named *qCmPMR-12*. They used an $F_2$ segregating population to map major PM resistance genes using BSA-Seq analysis. Most likely candidate genes were predicted from RNA-Seq analysis which indicated that the *MELO3C002434* gene encoding an ankyrin repeat-containing protein was the most likely candidate gene that was associated with resistance. Moreover, they successfully converted 15 polymorphic SNPs around the target area to KASP markers. KASP is a high-throughput SNP-genotyping platform, which become a global benchmark technology and has been widely used for genetic mapping and trait-specific marker development, due to its low cost and genotyping error rates, and its high reliability and reproducibility (Cao et al. 2021). So, the novel QTL and candidate gene identified provide insights into the genetic mechanism of PM resistance, and the tightly linked KASP markers developed to this disease resistance can be used for MAS in melon-breeding programs.

Natarajan et al. (2016) investigated the genetic variation of 4 melon accessions to PM. The whole-genome resequencing using the Illumina HiSeq 2000 platform was done, to characterize the genotypic variation in terms of SNPs, InDels, and structure variations (SVs). QTLs associated with PM resistance genes were detected. In addition, 112 SNPs and 45 InDels, were identified, associated with defense genes that will serve as candidate polymorphisms in the search for sources of resistance against PM and could accelerate marker-assisted breeding in melon.

Fusarium wilt in melons is caused by *Fusarium oxysporum* f. sp. *melonis* (FOM) and is considered one of the most devastating soil-borne diseases (Oumouloud et al. 2013; González et al. 2020). Two major genes *Fom*-1 and *Fom*-2 have been genetically characterized (Risser et al. 1976) and tightly linked markers to these genes are available (Oumouloud et al. 2012, 2015). *Fom-1* confers resistance to races 0 and 2, whereas *Fom-2* confers resistance to races 0 and 1 of *Fom*. Branham et al. (2018) reported that four QTLs (a major QTL co-located with the previously validated resistance gene *Fom*-2, and three minor QTLs) and an epistatic interaction were associated with resistance to FOM race 1 in a RIL population of 172 lines (MR-1 × susceptible AY).

The *Cucurbit yellow stunting disorder virus* (CYSDV) is a Crinivirus of the family Closteroviridae (Martelli et al. 2000) that severely infects melon. Pérez-de-Castro et al. (2020) reported two major QTLs to CYSDV resistance in melon line TGR-1551, both located near each other in chromosome 5. A RIL population was used, for mapping the gene/s responsible for this resistance. The RIL population was evaluated for resistance to CYSDV and genotyped in a GBS analysis. SNP markers were identified, which will be useful in MAS of CYSDV resistance introgression in elite melon cultivars. Further, Tamang et al. (2021) reported the identification of two QTLs to CYSDV resistance on chromosomes 3 and 5 for potential use in MAS. Besides, 24,673 SNP markers were identified in GBS-SNP calls in $F_{2:3}$ TM × PI313970 population. The identified QTL region that conferred resistance to CYSDV in melon line PI 313970 by Tamang et al. (2021), confirmed the QTL

regions on chromosome 5 of TGR-1551 that were previously identified by Pérez-de-Castro et al. (2020). The tightly linked markers with the CYSDV resistance QTL in TGR-1551 and PI313970 can be used to expedite the development of CYSDV-resistant elite breeding lines and cultivars.

Tomato leaf curl New Delhi virus (ToLCNDV) is a severe disease on melon. Sáez et al. (2022) performed an RNA-seq assay to identify associated genes that are differentially expressed, during ToLCNDV infection, between resistant and susceptible melon genotypes and transcript levels were also compared. Differentially expressed genes (DEGs) were classified using gene ontology (GO) terms, and genes of the categories transcription, DNA replication, and helicase activity were down-regulated in the resistant genotype but up-regulated in the susceptible, suggesting that reduced activity of these functions reduces ToLCNDV replication and intercellular spread and thereby contributes to resistance. The expression levels of selected candidate genes were validated by qRT-PCR in resistant and susceptible genotypes and SNPs with an effect on structural functionality of DEGs linked to the main QTLs for ToLCNDV resistance have been identified.

### Fruit Quality

Fruit quality is the main target for melon-breeding improvement. Morphology (external and internal color, shape, netting, sutures), aroma, nutritional content, sweetness, acidity, ripening, and post-harvest storage are complex traits that contribute to the final fruit quality in melon (Monforte et al. 2004). The availability of genomic resources in melon is contributing to the understanding of the processes that control fruit quality (Ramamurthy and Waters 2015; Monforte et al. 2004). In recent years, many loci involved in the genetic control of these traits have been described (Perpiña et al. 2016; Diaz et al. 2011; Fernandez-Silva et al. 2010). Once the genes underlying these traits are identified, the use of natural variation found in germplasm collections or induced variation through genome editing is a promising way for fruit-quality improvement.

Tomason et al. (2013) used 87 melon accessions from different geographic regions for association mapping study and identified 22 major QTLs for fruit shape, fruit length, fruit diameter, soluble solid content, and rind pressure.

Ramamurthy and Waters (2015) used an $F_2$ mapping population constructed from a cantaloupe orange-fleshed melon and a green-fleshed snake melon and identified a total of 31 QTLs associated with fruit quality and fruit morphological traits. They showed that most of the phenotypic variation for yield is explained by a small segment of LG8.

Argyris et al. (2017) reported that a valuable resource for QTL mapping is the NILs, which contains a single homozygous introgression of a donor line in the genetic background of a recipient line. NILs are a powerful tool that has advantages over other types of mapping populations in making possible the detection and estimation of QTL of small effect (Keurentjes et al. 2007). Dissection of QTL identified in NILs through the development of subNILs has been utilized to effectively map and clone QTL involved in melon fruit morphology (Fernandez-Silva et al. 2010), and fruit ripening (Rios et al. 2017). Fine-mapping of QTL involved in

sugar accumulation in melon has been reported (Argyris et al. 2017). Despite this, there is a correspondence of positions of QTL in different mapping populations, with clustering of QTL for SSC and soluble sugars identified on chromosomes 2, 3, and 5 (Diaz et al. 2011).

Sugar accumulation in melon flesh has been reported to have strong GxE interactions and low heritability (Perpiña et al. 2016) which complicates breeding of this trait. Argyris et al. (2017) identified a stable QTL, *SUCQSC5.1*, which reduced SSC and sucrose content. Through fine mapping with the subNILs, the authors accurately estimate its phenotypic effect and provide its function. Indeed, expression analysis of the candidate genes in mature fruit showed differences between the 'high' sugar and 'low' sugar phenotypes for *MELO3C014519*, encoding a putative BEL1-like homeodomain protein. The molecular markers linked to the QTL developed can be used in breeding programs with wild accessions to select against those alleles reducing SSC.

Galpaz et al. (2018), to elucidate genetic factors that determine melon quality, they used RNA-Seq-based QTL and eQTL mapping and identified *Thiol acyltransferase* (*CmThAT1*) gene, within the QTL interval of its product, the *S*-methyl-thioacetate which is a key component of melon fruit aroma, as well as a candidate major gene *CmPPR1* determining fruit white-flesh color in melon.

Zhao et al. (2019) reported a comprehensive map of the genomic variation in melon derived from the resequencing of 1175 diverse accessions. Resequencing of genomes is very useful for the genome-wide discovery of polymorphisms amenable for high-throughput genotyping platforms (Galpaz et al. 2018). Zhao et al. (2019) sheds light on the domestication history of melon suggesting that three independent domestication events occurred, two in India and one in Africa. In addition, using GWAS, 208 loci associated with fruit quality, and morphological characters were identified.

Liu et al. (2020), using GWAS, identified eight fruit size and seven flesh thickness signals overlapping with selective sweeps. *CmCLV3* was detected in most melon accessions, which has pleiotropic effects on carpel number and fruit shape. They also detected 233 and 159 potential selective signals in ssp. *agrestis* and spp. *melo*, respectively. Two alcohol acyltransferase genes (*CmAATs*) unique to the melon genome may have undergone stronger selection in ssp. *agrestis* for the characteristic aroma as compared with other cucurbits.

Amanullah et al. (2021) used an $F_2$ population and SNP-derived CAPs markers to map QTLs for seed traits (width, length, thickness, shape, and 100-seed weight), and identified three QTLs for seed width, seed length, and seed thickness on chromosomes 3 and 9. Besides, a major-effect QTL, *SW3.1*, was also detected on chromosome 3. Fine mapping or cloning of QTLs for fruit-related traits is still rarely reported in melon (Zhang et al. 2022)

The high-resolution genetic maps and QTLs analyses for fruit size described in Lian et al. (2021) provided a better understanding of the genetic basis of domestication and differentiation. Indeed, two loci for fruit size were identified on chromosomes 5 and 11. An auxin response factor and a YABBY transcription factor

were inferred to be the candidate genes for both loci. These findings could provide a valuable tool for map-based cloning and molecular marker-assisted breeding.

Zhang et al. (2022) illustrated the strength of a joint analysis combining resequencing-based genetic map for QTL mapping and a combination of KASP genotyping and RNA-seq analysis to facilitate QTL fine mapping. They reported a high-density genetic map of melon and nine major QTLs. Based on RNA-seq, *EVM0009818*, involved in cytokinin-activated signaling, was differentially expressed in the young fruits. Selective sweep analysis identified 152 sweep signals for seed size, including two seed-related QTLs and nine homologs that have been verified to regulate seed size in *Arabidopsis*.

### 2.3.5 Genome Editing

Sequencing techniques are able to provide important details on the position of functional elements of DNA, highlighting differences even of a few bases between genotypes of the same species; at the same time, great progress has been achieved in developing genomic engineering tools (Andolfo et al. 2016).

Genome-editing tools have the potential to modify genomic sequences with accuracy (Veillet et al. 2019). Some of these tools are Homologous Recombination (HR), Targeted Induced Local Lesions In the Genome (TILLING), Zinc Finger Nucleases (ZFN), Transcriptional Activator-Like Effector Nucleases (TALENs), or Clustered Regularly Interspaced Short Palindromic Repeats associated with nuclease Cas9 (CRISPR/Cas9).

Efficient gene editing in melon presents the possibility to study new gene functions for basic research, and new opportunities for melon productivity by improving biotic stress resistance, melon production, and post-harvest utilization (Bin et al. 2022; Hooghvorst et al. 2019; Dahmani-Mardas et al. 2010).

#### 2.3.5.1 Tilling

The TILLING method is useful in identifying novel alleles in genes controlling agronomic traits of interest in melon (Dahmani-Mardas et al. 2010). Indeed, a TILLING platform generated from a monoecious climacteric cantalupensis genotype and andromonoecious non-climacteric inodorus genotype has become available and has proven to be useful for improving the melon shelf life and represented a useful resource for functional studies and melon breeding (González et al. 2011).

For ethylene biosynthesis, the conversion of aminocyclopropane-1-carboxylic acid (ACC) to ethylene by the ACC oxidase (ACO) is required (Ayub et al. 1996). In melon, *CmACO1* silencing inhibits fruit ripening and extends fruit shelf life, demonstrating that ACO is involved in ripening, growth, and development (Ayub et al. 1996). Dahmani-Mardas et al. (2010) have developed a reference ethyl methanesulfonate-mutagenized (EMS) mutant population and characterized *CmACO1* TILLING mutants that inhibit fruit ripening and extend fruit storage life.

#### 2.3.5.2 CRISPR/Cas9

The new CRISPR/Cas9 genome-editing technique was developed in 2013 and has transformed genetic engineering, due to its efficiency, versatility, precision, and reduced costs (Andolfo et al. 2016; Hooghvorst et al. 2019). Precise changes are produced, at preselected genomic sites with no genetic footprints and no off-targets (Hooghvorst et al. 2019; Chandrasekaran et al. 2016). Genes function studies by knocking out genes that negatively affect fruit quality is also allowed using this technique (Tian et al. 2016).

CRISPR knockout mutants in melon have been reported for the first time in 2019 by Hooghvorst et al. using CRISPR/Cas9-mediated genome editing. In plants, the major uses of CRISPR/Cas9 have been gene knockouts to elucidate the function of a target gene–by-gene mutation and transcriptional regulation (Hooghvorst et al. 2019).

Hooghvorst et al. (2019) using CRISPR/Cas9 generated multi-allelic mutations in both genomic target sites of the phytoene desaturase gene (*CmPDS*), a key enzyme for the carotenoids production in melon. Chimeric albino phenotypes have been successfully regenerated.

Giordano et al. (2022), using CRISPR/Cas9, showed the generation of melon knockout mutants CTR1 and ROS1 for fruit ripening and reported for the first time the inheritance of the introduced mutations to the following generations. Two functionally validated genes (*CmROS1* and *CmCTR1-like*) are involved in the regulation of fruit ripening and showed the role of the DNA demethylase ROS1 in fruit ripening. The authors characterized the ETHQV6.3 QTL genomic interval, a QTL involved in climacteric ripening regulation, which allowed the identification of a negative regulator of ripening *CTR1*-like (*MELO3C024518*), and a demethylase *ROS1* (*MELO3C024516*) and evidenced the role of both genes in melon climacteric ripening. Indeed, in the CRISPR mutants, the authors reported the formation of abscission layer, aroma, and ethylene production. The *CmROS1* knockout mutant revealed that during fruit ripening, the balance of global DNA methylation/demethylation is altered, which is governed by DNA demethylases (Giordano et al. 2022).

Bin et al. (2022) provided new insight regarding CmNAC-NOR function in melon fruit ripening. Two CRISPR/Cas9-mediated mutants nor-3 and nor-1 in the climacteric Védrantais background were obtained. nor-3, containing a 3-bp deletion altering the NAC domain A, resulted in the delay of ripening without affecting fruit quality. In contrast, nor-1 containing a 1-bp deletion resulting in a fully disrupted NAC domain, completely blocked climacteric ripening (ethylene was not produced, abscission layer was not formed, and external color was not changed) suggesting it as a potential target to modulate shelf life in climacteric melon.

In summary, gene-editing technology has great potential. To date, no edited plants have been obtained for disease resistance in melon but such technology can strongly contribute to making the melon more resistant to biotic/abiotic stress and improving consequently yields. The use of CRISPR and genome-editing technologies will open new opportunities, potentially circumventing restrictions on Genetically Modified crops (Veillet et al. 2019).

## 2.4 Conclusion

The increasing of high-throughput sequencing technology has made possible huge progress through molecular, and genetic research on disease resistance, fruit development, and ripening of melon. The high-quality reference genome of melon has played a primary role in this advancement and has been used for the resequencing of diverse germplasm to explore genome-wide sequence variations, especially SNPs. Several QTLs with high mapping resolution have been discovered for disease resistance, and fruit traits in melon; which have enabled the development of useful resources, such as molecular markers for these QTLs, to improve selection efficiency in melon-breeding programs.

Genomics allow the identification of polymorphic loci responsible for variation in phenotypic traits. The release of a genome assembly and large-scale sequencing and resequencing data improved knowledge of the evolution, selection footprint, genetic architecture, and gene mapping and cloning of fruit-related traits. Besides, advancements in transcriptomics, plant defense mechanisms, and genomics will provide new opportunities to accelerate melon breeding programs. Indeed, integrating genetic and genomic data will help breeders to obtain a more durable resistance to diseases and a better fruit quality.

All the resultant data should be made available according to FAIR (findable, accessible, interoperable, and reusable) principles, and linked phenotype data should also be incorporated. Indeed, huge information from high-throughput phenotyping and genomics technologies are provided which helps researchers to guide their breeding programs to biotic and abiotic stresses. In this process, bioinformatics is fundamental to exploit and integrate these data, through association studies to detect genomic targets underlying key traits useful for melon breeders.

**Acknowledgments** The authors H.C.R and W.A are grateful to Wageningen University & Research (WUR)-Plant Breeding (Netherlands) and CRRGC-Béja (Tunisia) and also to the Orange Knowledge Programme (OKP)-Nuffic for offering the 3 months Tailor-Made Training Course 'Application of Genomic Plant Breeding for Challenging Climate Change in Tunisia'.

A.G.C is grateful to PID2020-116055RB-C22 I+D+I project funded by MCIN/AEI/10.13039/501100011003 and the A11-20R project funded by the Aragon Government.

## References

Abak K, Sari N, Paksoy M et al (1996) Genotype response to haploid embryo induction with pollination by irradiated pollens in melon, obtaining of dihaploid lines, determination of haploid and diploid plants by different techniques. Tr J Agric For 20:425–430

Abe A, Kosugi S, Yoshida K et al (2012) Genome sequencing reveals agronomically important loci in rice using MutMap. Nat Biotechnol 30:174–178. https://doi.org/10.1038/nbt.2095

Amanullah S, Gao P, Osae BA et al (2021) Genetic linkage mapping and QTLs identification for morphology and fruit quality related traits of melon by SNP based CAPS markers. Sci Hortic 278:109849. https://doi.org/10.1016/j.scienta.2020.109849

Andersen JR, Lübberstedt T (2003) Functional markers in plants. Trends Plant Sci 8:554–560. https://doi.org/10.1016/j.tplants.2003.09.010

Andolfo G, Sanseverino W, Rombauts S et al (2013) Overview of tomato (*Solanum lycopersicum*) candidate pathogen recognition genes reveals important *Solanum* R locus dynamics. New Phytol 197:223–237

Andolfo G, Iovieno P, Frusciante L, Ercolano MR (2016) Genome-editing technologies for enhancing plant disease resistance. Front Plant Sci 7:1813. https://doi.org/10.3389/fpls.2016.01813

Andolfo G, Amoroso CG, Ercolano MR (2021) Disease resistance breeding with genomic tools in zucchini (*Cucurbita pepo* L.). In: Al-Khayri JM, Jain SM, Johnson DV (eds) Advances in plant breeding strategies: vegetable crops. Springer, Cham. https://doi.org/10.1007/978-3-030-66961-4_11

Argyris JM, Ruiz-Herrera A, Madriz-Masis P et al (2015) Use of targeted SNP selection for an improved anchoring of the melon (*Cucumis melo* L.) scaffold genome assembly. BMC Genomics 16:4. https://doi.org/10.1186/s12864-014-1196-3

Argyris JM, Díaz A, Ruggieri V et al (2017) QTL analyses in multiple populations employed for the fine mapping and identification of candidate genes at a locus affecting sugar accumulation in melon (*Cucumis melo* L.). Front Plant Sci 8:1679. https://doi.org/10.3389/fpls.2017.01679

Ayub R, Guis M, Ben Amor M et al (1996) Expression of ACC oxidase antisense gene inhibits ripening of cantaloupe melon fruits. Nat Biotechnol 14:862–866

Bevan MW, Uauy C, Wulff BBH et al (2017) Genomic innovation for crop improvement. Nature 543:346–354. https://doi.org/10.1038/nature22011

Bin L, Domingo MS, Mayobre C et al (2022) Knock-out of CmNAC-NOR affects melon climacteric fruit ripening. https://doi.org/10.1101/2022.02.02.478821

Bohra A, Bharadwaj C, Radhakrishnan T et al (2019) Translational genomics and molecular breeding for enhancing precision and efficiency in crop improvement programs: some examples in legumes. Indian J Genet 79:227–240. https://doi.org/10.31742/IJGPB.79S.1.13

Boualem A, Fergany M, Fernandez R et al (2008) A conserved mutation in an ethylene biosynthesis enzyme leads to andromonoecy in melons. Science 321:836–838. https://doi.org/10.1126/science.1159023

Branham SE, Levi A, Katawczik M et al (2018) Construction of a genome-anchored, high-density genetic map for melon (*Cucumis melo* L.) and identification of *Fusarium oxysporum* f. sp. *melonis* race 1 resistance QTL. Theor Appl Genet 131:829–837. https://doi.org/10.1007/s00122-017-3039-5

Branham SE, Kousik C, Mandal MK, Wechter WP (2021) Quantitative trait loci mapping of resistance to powdery mildew race 1 in a recombinant inbred line population of melon. Plant Dis 105:12. https://doi.org/10.1094/PDIS-12-20-2643-RE

Brenchley R, Spannagl M, Pfeifer M et al (2012) Analysis of the bread wheat genome using whole-genome shotgun sequencing. Nature 491:705–710. https://doi.org/10.1038/nature11650

Brenton ZW, Cooper EA, Myers MT et al (2016) A genomic resource for the development, improvement, and exploitation of sorghum for bioenergy. Genetics 204:21–33. https://doi.org/10.1534/genetics.115.183947

Brotman Y, Normantovich M, Goldenberg Z et al (2013) Dual resistance of melon to *fusarium oxysporum* races 0 and 2 and to papaya ring-spot virus is controlled by a pair of head-to-head-oriented nb-lrr genes of unusual architecture. Mol Plant 6:235–238

Büschges R et al (1997) The barley *Mlo* gene: a novel control element of plant pathogen resistance. Cell 88:695–705

Cao Y, Diao Q, Chen Y et al (2021) Development of KASP markers and identification of a QTL underlying powdery mildew resistance in melon (*Cucumis melo* L.) by bulked segregant analysis and RNA-Seq. Front Plant Sci 11:593207. https://doi.org/10.3389/fpls.2020.593207

Castanera R, Ruggieri V, Pujol M et al (2020) An improved melon reference genome with single molecule sequencing uncovers a recent burst of transposable elements with potential impact on genes. Front Plant Sci 10:1815. https://doi.org/10.3389/fpls.2019.01815

Chakradhar T, Hindu V, Reddy PS (2017) Genomic-based-breeding tools for tropical maize improvement. Genetica 145:525–539. https://doi.org/10.1007/s10709-017-9981-y

Chandrasekaran J, Brumin M, Wolf D et al (2016) Development of broad virus resistance in nontransgenic cucumber using CRISPR/Cas9 technology. Mol Plant Pathol 9:1–14. https://doi.org/10.1111/mpp.12375

Chang C, Wang Y, Tung C (2017) Genome-wide single nucleotide polymorphism discovery and the construction of a high-density genetic map for melon (*Cucumis melo* L.) using genotyping-by-sequencing. Front Plant Sci 8:1–11

Chikh-Rouhou H, Gómez-Guillamón ML, Garcés-Claver A (2021a) Melon germplasm from Tunisia with immense breeding value. Cucurbit Genet Coop Rep 44:7–11. https://cucurbit.info/wp-content/uploads/2022/06/CGC44_3_Melon-Tunisia.pdf

Chikh-Rouhou H, Gómez-Guillamón ML, González V et al (2021b) *Cucumis melo* L. germplasm in Tunisia: unexploited sources of resistance to fusarium wilt. Horticulturae 7:208. https://doi.org/10.3390/horticulturae7080208

Chikh-Rouhou H, Mezghani N, Mnasri S et al (2021c) Assessing the genetic diversity and population structure of a tunisian melon (*Cucumis melo* L.) collection using phenotypic traits and SSR molecular markers. Agronomy 11:1121. https://doi.org/10.3390/agronomy11061121

Coyne CJ, Kumar S, Wettberg EJB et al (2020) Potential and limits of exploitation of crop wild relatives for pea, lentil, and chickpea improvement. Legume Sci 2. https://doi.org/10.1002/leg3.36

Cui H, Ding Z, Zhu Q, Wu Y et al (2021) Comparative analysis of nuclear, chloroplast,and mitochondrial genomes of watermelon and melon provides evidence of gene transfer. Sci Rep 11(1):1595. https://doi.org/10.1038/s41598-020-80149-9

Dahmani-Mardas F, Troadec C, Boualem A et al (2010) Engineering melon plants with improved fruit shelf life using the TILLING approach. PLoS One 5:e15776. https://doi.org/10.1371/journal.pone.0015776

Dal B, Sari N, Solmaz İ (2016) Effect of different irradiation sources and doses on haploid embryo induction in Altinbas (*Cucumis melo* L. var. *inodorus*) melons. Turk J Agric For 40:552–559

Deleu W, Esteras C, Roig C et al (2009) A set of EST-SNPs for map saturation and cultivar identification in melon. BMC Plant Biol 9:90. https://doi.org/10.1186/1471-2229-9-90

Diaz A, Fergany M, Formisano G et al (2011) A consensus linkage map for molecular markers and quantitative trait loci associated with economically important traits in melon (*Cucumis melo* L.). BMC Plant Biol 11:111. https://doi.org/10.1186/1471-2229-11-111

Dogimont C, Chovelon V, Pauquet J et al (2014) The *Vat* locus encodes for a CC-NBS-LRR protein that confers resistance to *Aphis gossypii* infestation and *A. gossypii*-mediated virus resistance. Plant J 80:993–1004

Eduardo I, Arus P, Monforte AJ (2005) Development of a genomic library of near isogenic lines (NILs) in melon (*Cucumis melo* L.) from the exotic accession PI161375. Theor Appl Genet 112:139–148. https://doi.org/10.1007/s00122-005-0116-y

Ezura H, Fukino N (2009) Research tools for functional genomics in melon (*Cucumis melo* L.): current status and prospects. Plant Biotechnology 26:359–368. https://doi.org/10.5511/plantbiotechnology.26.359

Fayos O, Valles MP, Garces-Claver A et al (2015) Doubled haploid production from Spanish oniongermplasm: embryogenesis induction, plant regeneration and chromosome doubling. Front Plant Sci 6A:384. https://doi.org/10.3389/fpls.2015.00384

Fernandez-Silva I, Moreno E, Essafi A et al (2010) Shaping melons: agronomic and genetic characterization of QTLs that modify melon fruit morphology. Theor Appl Genet 121:931–940. https://doi.org/10.1007/s00122-010-1361-2

Ficcadenti N, Sestili S, Annibali S et al (1999) In vitro gynogenesis to induce haploid plants in melon (*Cucumis melo* L.). J Genet Breed 53(3):255–257

Ficcadenti N, Sestili S, Annibali S et al (2002) Resistance to *Fusarium oxysporum* f. sp. *melonis* race 1.2 in muskmelon lines 'Nad-1' and 'Nad-2'. Plant Dis 86:897–900

Galpaz N, Gonda I, Shem-Tov D et al (2018) Deciphering genetic factors that determine melon fruit-quality traits using RNA-Seq-based high-resolution QTL and eQTL mapping. Plant J 94:169–191. https://doi.org/10.1111/tpj.13838

Garcia-Mas J, Seros MO, Gomez-Paniagua H et al (2000) Comparing AFLP, RAPD and RFLP markers for measuring genetic diversity in melon. Theor Appl Genet 101:860–864. https://doi.org/10.1111/tpj.13838

Garcia-Mas J, Benjak A, Sanseverino W et al (2012) The genome of melon (*Cucumis melo* L.). Proc Natl Acad Sci U S A 109:11872–11877. https://doi.org/10.1073/pnas.1205415109

Germanà MA (2011) Gametic embryogenesis and haploid technology as valuable support to plant breeding. Plant Cell Rep 30:839–857. https://doi.org/10.1007/s00299-011-1061-7

Giner A, Pascual L, Bourgeois M et al (2017) A mutation in the melon Vacuolar Protein Sorting 41 prevents systemic infection of *Cucumber mosaic virus*. Sci Rep 7:10471

Giordano A, Domingo MS, Quadrana L et al (2022) CRISPR/Cas9 gene editing uncovers the role 2 of CTR1 and ROS1 in melon fruit ripening and epigenetic regulation. https://doi.org/10.1101/2022.01.30.478227

Godbole M, Murthy HN (2012) Parthenogenetic haploid plants using gamma irradiated pollen in snapmelon (*Cucumis melo* var. momordica). Plant Cell Tissue Organ Cult 109:167–170. https://doi.org/10.1007/s11240-011-0066-9

González VM, Garcia-Mas J, Arús P, Puigdomènech P (2010a) Generation of a BAC-based physical map of the melon genome. BMC Genomics 11:339. https://doi.org/10.1186/1471-2164-11-339

González VM, Rodríguez-Moreno L, Centeno E et al (2010b) Genome-wide BAC-end sequencing of *Cucumis melo* using two BAC libraries. BMC Genomics 11:618–628. https://doi.org/10.1186/1471-2164-11-618

González M, Xu M, Esteras C et al (2011) Towards a TILLING platform for functional genomics in Piel de Sapo melons. BMC Res Notes 4:289. https://doi.org/10.1186/1756-0500-4-289

González V, Armijos E, Garcés-Claver A (2020) Fungal endophytes as biocontrol agents against the main soil-borne diseases of melon and watermelon in Spain. Agronomy 10(6):820. https://doi.org/10.3390/agronomy10060820

Gonzalez-Ibeas D, Blanca J, Roig C et al (2007) MELOGEN: an EST database for melon functional genomics. BMC Genomics 8:306. https://doi.org/10.1186/1471-2164-8-306

Grandbastien MA (1998) Activation of plant retrotransposons under stress conditions. Trends Plant Science 3:181–187

Grumet R, Fei Z, Levi A et al (2020) The CucCAP project: leveraging applied genomics to improve disease resistance in cucurbit crops. Acta Hortic 1294:91–104

Grumet R, McCreight JD, McGregor C et al (2021) Genetic resources and vulnerabilities of major cucurbit crops. Genes 12:1222. https://doi.org/10.3390/genes12081222

Guo S, Zhang J, Sun H et al (2013) The draft genome of watermelon (*Citrullus lanatus*) and resequencing of 20 diverse accessions. Nat Genet 45:51–58. https://doi.org/10.1038/ng.2470

Guo S, Zhao S, Sun H et al (2019) Resequencing of 414 cultivated and wild watermelon accessions identifies selection for fruit quality traits. Nat Genet 51:1616–1623. https://doi.org/10.1038/s41588-019-0518-4

Gupta PK, Rustgi S, Kulwal PL (2005) Linkage disequilibrium and association studies in higher plants: present status and future prospects. Plant Mol Biol 57:461–485

Gur A, Tzuri G, Meir A et al (2017) Genome-wide linkage disequilibrium mapping to the candidate gene level in melon (*Cucumis melo*). Sci Rep 7(1):9770

Harel-Beja R, Tzuri G, Portnoy V et al (2010) A genetic map of melon highly enriched with fruit quality QTLs and EST markers, including sugar and carotenoid metabolism genes. Theor Appl Genet 121:511–533. https://doi.org/10.1007/s00122-010-1327-4

Heffner EL, Sorrells ME, Jannink JL (2009) Genomic selection for crop improvement. Crop Sci 49:1–12

Hooghvorst I, López-Cristoffanini C, Nogués S (2019) Efficient knockout of phytoene desaturase gene using CRISPR/Cas9 in melon. Sci Rep 9:17077. https://doi.org/10.1038/s41598-019-53710-4

Hooghvorst I, Torrico O, Hooghvorst S, Nogués S (2020) In situ parthenogenetic doubled haploid production in melon "piel de sapo" for breeding purposes. Front Plant Sci 11:378. https://doi.org/10.3389/fpls.2020.00378

Hooghvorst I, Torrico O, Nogues S (2021) Doubled haploid parthenogenetic production of melon 'piel de sapo'. In: Segui-Simarro JM (ed) Doubled haploid technology. Methods in molecular biology, vol 2289. Humana, New York, NY. https://doi.org/10.1007/978-1-0716-1331-3_5

Hu Z, Deng G, Mou H et al (2018) A re-sequencing-based ultra-dense genetic map reveals a gummy stem blight resistance-associated gene in Cucumis melo. DNA Res 25:1–10. https://doi.org/10.1093/dnares/dsx033

Huang S, Li R, Zhang Z et al (2009) The genome of the cucumber, *Cucumis sativus* L. Nat Genet 41:1275–1281

Ito H, Kim J-M, Matsunaga W et al (2016) A stress-activated transposon in arabidopsis induces transgenerational abscisic acid insensitivity. Sci Rep 6:23181. https://doi.org/10.1038/srep23181

Joobeur T, King JJ, Nolin SJ et al (2004) The fusarium wilt resistance locus *fom*-2 of melon contains a single resistance gene with complex features. Plant J 39:283–297

Keurentjes JB, Fu J, Terpstra IR et al (2007) Regulatory network construction in *Arabidopsis* by using genome-wide gene expression quantitative trait loci. PNAS 104:1708–1713

Kim S, Park M, Yeom S-I et al (2014) Genome sequence of the hot pepper provides insights into the evolution of pungency in Capsicum species. Nat Genet 46:270–278. https://doi.org/10.1038/ng.2877

Kleine T, Maier UG, Leister D (2009) DNA transfer from organelles to the nucleus: the idiosyncratic genetics of endosymbiosis. Annu Rev Plant Biol 60:115–638. https://doi.org/10.1146/annurev.arplant.043008.092119

Koli SP, Murthy HN (2013) Haploid plant regeneration from unpollinated ovules of *Cucumis melo* L. var. *conomon* cv. Mudicode. Br Biotechnol J 3(4):605–613

Lakshmana Reddy DC, Sudurashini KV, Anand CR et al (2016) Genetic diversity and population structure of Indian melon (*Cucumis melo L.)* landraces with special reference to disease and insect resistance loci. Plant Breed 13:384–390. https://doi.org/10.1111/pbr.12356

Leeuwen VH, Monfort A, Zhang HB, Puigdomènech P (2003) Identification and characterization of a melon genomic region containing a resistance gene cluster from a constructed BAC library. Microlinearity between *Cucumis melo* and *Arabidopsis thaliana*. Plant Mol Biol 51:703–718. https://doi.org/10.1023/A:1022573230486

Lian Q, Fu Q, Xu YY et al (2021) QTLs and candidate genes analyses for fruit size under domestication and differentiation in melon (*Cucumis melo L.*) based on high resolution maps. BMC Plant Biol 21:126. https://doi.org/10.1186/s12870-021-02904-y

Lim W, Earle ED (2009) Enhanced recovery of doubled haploid lines from parthenogenetic plants of melon (*Cucumis melo* L.). Plant Cell Tissue Organ Cult 98:351–356. https://doi.org/10.1007/s11240-009-9563-5

Liu L, Kakihara F, Kato M (2004) Ethylene changes during development and ripening of fruit with reference to variety of *Cucumis melo* L. Breed Sci 54:297–300

Liu Y, He ZH, Appels R, Xia XC (2012) Functional markers in wheat: current status and future prospects. Theor Appl Genet 125:1–10. https://doi.org/10.1007/s00122-012-1829-3

Liu W, Jiang Y, Wang C et al (2020) Lignin synthesized by CmCAD2 and CmCAD3 in oriental melon (*Cucumis melo* L.) seedlings contributes to drought tolerance. Plant Mol Biol 103:689–704

Loh Y-T, Martin GB (1995) The disease-resistance gene *Pto* and the fenthion-sensitivity gene fen encode closely related functional protein kinases. Proc Natl Acad Sci U S A 92:4181–4184

Lotfi M, Alan AR, Henning MJ et al (2003) Production of haploid and doubled haploid plants of melon (*Cucumis melo* L.) for use in breeding for multiple virus resistance. Plant Cell Rep 21:1121–1128. https://doi.org/10.1007/s00299-003-0636-3

Luo M, Wang YH, Frisch D et al (2001) Melon bacterial artificial chromosome (BAC) library construction using improved methods and identification of clones linked to the locus conferring

resistance to melon Fusarium wilt (Fom-2). Genome 44:154–162. https://doi.org/10.1139/g00-117

Maleki M, Shojaeiyan A, Rashidi Monfared S (2018) Population structure, morphological and genetic diversity within and among melon (*Cucumis melo* L.) landraces in Iran. Journal of Genetic Engineering and Biotechnology 16:599–606. https://doi.org/10.1016/j.jgeb.2018.08.002

Malik AA, Li C, Shuxia Z, Jin-feng C (2011) Efficiency of SSR markers for determining the origin of melon plantlets derived through unfertilized ovary culture. Hort Sci 38:27–34. https://doi.org/10.17221/47/2010-HORTSCI

Marsh JI, Hu H, Gill M et al (2021) Crop breeding for a changing climate: integrating phenomics and genomics with bioinformatics. Theor Appl Genet 134:677–1690. https://doi.org/10.1007/s00122-021-03820-3

Martelli GP et al (2000) Family closteroviridae. In: Van Regenmortel MHV et al (eds) Virus taxonomy. Seventh report of the international committee on taxonomy of viruses. Academic Press, San Diego, CA, pp 943–952

Martin W (2003) Gene transfer from organelles to the nucleus: frequent and in big chunks. Proc Nat Acad Sci 100:8612–8614. https://doi.org/10.1073/pnas.1633606100

Martínez-Gómez P, Sánchez-Pérez R, Rubio M (2012) Clarifying omics concepts, challenges, and opportunities for *Prunus* breeding in the postgenomic era. OMICS J Integr Biol 16:268–283. https://doi.org/10.1089/omi.2011.0133

Mascarell-Creus A, Cañizares J, Vilarrasa-Blasi J et al (2009) An oligo-based microarray offers novel transcriptomic approaches for the analysis of pathogen resistance and fruit quality traits in melon (*Cucumis melo* L.). BMC Genomics 10:467. https://doi.org/10.1186/1471-2164-10-467

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci U S A 88:9828–9832. https://doi.org/10.1073/pnas.88.21.9828

Monforte AJ, Oliver M, Gonzalo MJ et al (2004) Identification of quantitative trait loci involved in fruit quality traits in melon (*Cucumis melo* L.). Theor Appl Genet 108:750–758. https://doi.org/10.1007/s00122-003-1483-x

Natarajan S, Kim H-T, Thamilarasan SK et al (2016) Whole genome re-sequencing and characterization of powdery mildew disease-associated allelic variation in melon. PLoS One 11: e0157524. https://doi.org/10.1371/journal.pone.0157524

Nie J, He H, Peng J et al (2015) Identification and fine mapping of pm5.1: a recessive gene for powdery mildew resistance in cucumber (*Cucumis sativus* L.). Mol Breed 35:7. https://doi.org/10.1007/s11032-015-0206-8

Nieto C, Piron F, Dalmais M et al (2007) EcoTILLING for the identification of alleclic variants of melon eIF4E, a factor that controls virus susceptibility. BMC Plant Biol 7:34. https://doi.org/10.1186/1471-2229-7-34

Oren E, Tzuri G, Dafna A et al (2020) High-density NGS-based map construction and genetic dissection of fruit shape and rind netting in *Cucumis melo*. Theor Appl Genet 133:1927–1945. https://doi.org/10.1007/s00122-020-03567-3

Osuna-Cruz CM, Paytuvi-Gallart A, Di Donato A et al (2018) PRGdb 3.0: a comprehensive platform for prediction and analysis of plant disease resistance genes. Nucleic Acids Res 46: D1197–D1201

Oumouloud A, Mokhtari M, Chikh-Rouhou H et al (2012) Characterization of the Fusarium wilt resistance Fom-2 gene in melon. Mol Breed 30:325–334

Oumouloud A, El-Otmani M, Chikh-Rouhou H et al (2013) Breeding melon for resistance to Fusarium wilt: recent developments. Euphytica 192(2):155–169

Oumouloud A, El Otmani M, Álvarez JMA (2015) Molecular characterization of Fom-1 gene and development of functional markers for molecular breeding of resistance to Fusarium race 2 in melon. Euphytica 205:491–501

Palomares-Rius FJ, Garcés-Claver A, Picó MB et al (2018) 'Carmen', a yellow canary melon breeding line resistant to *Podosphaera xanthii*, *Aphis gossypii*, and *Cucurbit yellow stunting disorder virus*. HortScience 53(7):1072–1075. https://doi.org/10.21273/HORTSCI13013-18

Pavan S, Marcotrigiano AR, Ciani E et al (2017) Genotyping-by-sequencing of a melon (*Cucumis melo* L.) germplasm collection from a secondary center of diversity highlights patterns of genetic variation and genomic features of different gene pools. BMC Genomics 18:59. https://doi.org/10.1186/s12864-016-3429-0

Pech JC, Bouzayen M, Latché A (2008) Climacteric fruit ripening: ethylene-dependent and independent regulation of ripening pathways in melon fruit. Plant Sci 175:114–120. https://doi.org/10.1016/j.plantsci.2008.01.003

Pereira L, Ruggieri V, Pérez S et al (2018) QTL mapping of melon fruit quality traits using a high-density GBS-based genetic map. BMC Plant Biol 18:324. https://doi.org/10.1186/s12870-018-1537-5

Pérez-de-Castro AM, Vilanova S, Canizares J et al (2012) Application of genomic tools in plant breeding. Curr Genomics 13:179–195. https://doi.org/10.2174/138920212800543084

Pérez-de-Castro A, López-Martín M, Esteras C et al (2020) Melon genome regions associated with TGR-1551-derived resistance to *Cucurbit yellow stunting disorder virus*. Intl J Mol Sci 21: 5970. https://doi.org/10.3390/ijms21175970

Perpiña G, Esteras C, Gibon Y et al (2016) A new genomic library of melon introgression lines in a cantaloupe genetic background for dissecting desirable agronomical traits. BMC Plant Biol 16 (1):154

Phan NT, Sim SC (2017) Genomic tools and their implications for vegetable breeding. Hortic Sci Technol 35:149–164. https://doi.org/10.12972/kjhst.20170018

Pitrat M (2016) Melon genetic resources: phenotypic diversity and horticultural taxonomy. In: Grumet R, Katzir N, Garcia-Mas J (eds) Genetics and genomics of cucurbitaceae. Springer International Publishing, Cham, pp 25–60

Preece C, Peñuelas J (2019) A return to the wild: root exudates and food security. Trends Plant Sci 25:14–21. https://doi.org/10.1016/j.tplants.2019.09.010

Ramamurthy RK, Waters BM (2015) Identification of fruit quality and morphology QTLs in melon (*Cucumis melo*) using a population derived from flexuosus and cantalupensis botanical groups. Euphytica 204:163–177. https://doi.org/10.1007/s10681-015-1361-z

Rios P, Argyris JM, Vegas J et al (2017) ETHQV6.3 is involved in melon climacteric fruit ripening and is encoded by a NAC domain transcription factor. Plant J 91:671–683. https://doi.org/10.1111/tpj.13596

Risser G, Banihashemi Z, Davis DW (1976) A proposed nomenclature of *Fusarium oxysporum* f. sp. *melonis* races and resistance genes in *Cucumis melo*. Phytopathology 66:1105–1106

Rodríguez-Moreno L, González VM, Benjak A et al (2011) Determination of the melon chloroplast and mitochondrial genome sequences reveals that the largest reported mitochondrial genome in plants contains a significant amount of DNA having a nuclear origin. BMC Genomics 12:424. https://doi.org/10.1186/1471-2164-12-424

Ruggieri V, Alexiou KG, Morata J et al (2018) An improved assembly and annotation of the melon (*Cucumis melo* L.) reference genome. Sci Rep 8:8088. https://doi.org/10.1038/s41598-018-26416-2

Sáez C, Flores-León A, Montero-Pau J et al (2022) RNA-seq transcriptome analysis provides candidate genes for resistance to tomato leaf curl New Delhi virus in melon. Frontiers Plant Sci 12:798858. https://doi.org/10.3389/fpls.2021.798858

Salgotra RK, Stewart CN (2020) Functional markers for precision plant breeding. Int J Mol Sci 21: 4792. https://doi.org/10.3390/ijms21134792

Sanseverino W, Hénaff E, Vives C et al (2015) Transposon insertions, structural variations, and SNPs contribute to the evolution of the melon genome. Mol Biol Evol 32:2760–2774. https://doi.org/10.1093/molbev/msv152

Sari N, Solmaz I (2020) Doubled haploid production in melon. In: Aka Kaçar Y, Yalçın Mendi Y (eds) Biotechnological approaches on horticultural crops. Akademisyen Kitabevi, pp 71–84

Sari N, Abak K, Pitrat M, Dumas de Vaulx R (1992) Induction of parthenogenetic haploid embryos and plant obtention in melon (*Cucumis melo* L.var. *inodorus* Naud and *C. melo* L.var. *reticulatus* Naud). Doğa Turkish J Agric Forestry 16:302–314

Sari N, Ekiz H, Yucel S et al (1999a) Investigation of new protected cultivation melon lines resistant to *Fusarium oxysporum* f. sp. *melonis* using dihaploidization. In: Proceedings of the Third Turkish National Horticultural Congress, September 14–17, Ankara, Turkey, pp. 498–503

Sari N, Abak K, Pitrat M (1999b) Comparison of ploidy level screening methods in watermelon: *Citrullus lanatus* (Thunb.) Matsum. and Nakai. Sci Hort 82:265–277. https://doi.org/10.1016/S0304-4238(99)00077-1

Sari N, Solmaz I, Kasapoglu S et al (2010a) Effect of different pollination dates with irradiated pollens on fruit set, haploid embryo induction and plant obtention in Turkish (Kirkagac, Yuva and Hasanbey) melons. Acta Hortic 871:639–648. https://doi.org/10.17660/ActaHortic.2010.871.88

Sari N, Solmaz I, Yetisir H et al (2010b) New fusarium wilt resistant melon (*Cucumis melo* var. *cantalupensis*) varieties developed by dihaploidization: Sari F1, Yetisir F1, Solmaz F1, Emin F1 and Yucel F1, IVth International Symposium on Cucurbits. Acta Hortic 871:267–272. https://doi.org/10.17660/ActaHortic.2010.871.35

Sauton A, Dumas de Vaulx R (1987) Production of haploid plants in melon (*Cucumis melo* L.) as a result of gynogenesis induced by irradiated pollen. Agronomie 7:141–147

Shivapriya M, Mamatha S, Umesha K et al (2021) Genetic variation in melon (*Cucumis melo L.*) landraces and wild relatives of Karnataka state of southern India. Plant Genet Res 19:419–427. https://doi.org/10.1017/S1479262121000496

Solmaz I, Sari N, Gürsoy I, Kasapoğlu S (2011) Comparison of in vivo and in vitro colchicine application for production of Dihaploid 'Kirkagac' and 'Yuva Hasanbey' melons. Afr J Biotechnol 10. https://doi.org/10.5897/AJB11.2445

Tadmor Y, Katzir N, Meir A et al (2007) Induced mutagenesis to augment the natural genetic variability of melon (*Cucumis melo* L.). Israel J Plant Sci 55:159–169. https://doi.org/10.1016/j.pbi.2019.12.004

Taler D, Galperin M, Benjamin I et al (2004) Plant R genes that encode photorespiratory enzymes confer resistance against disease. Plant Cell 16:172–184

Tamang P, Ando K, Wintermantel WM, McCreight JD (2021) QTL mapping of cucurbit yellow stunting disorder virus resistance in melon accession PI 313970. Horts 56:424–430. https://doi.org/10.21273/HORTSCI15495-20

Teixeira AP, da Silva Barreto FA, Camargo LEA (2008) An AFLP marker linked to the *Pm*-1 gene that confers resistance to *Podosphaera xanthii* race 1 in *Cucumis melo*. Genet Mol Biol 31:547–550

The Cucurbit Genomics (CucCAP) (n.d.). http://cucurbitgenomics.org/

The Tomato Genome Consortium (2012) The tomato genome sequence provides insights into fleshy fruit evolution. Nature 485:635–641. https://doi.org/10.1038/nature11119

Thudi M, Palakurthi R, Schnable JC et al (2021) Genomic resources in plant breeding for sustainable agriculture. J Plant Physiol 257:153351. https://doi.org/10.1016/j.jplph.2020.153351

Tian S, Jiang L, Gao Q et al (2016) Efficient CRISPR/Cas9-based gene knockout in watermelon. Plant Cell Rep 36:399–406. https://doi.org/10.1007/s00299-016-2089-5

Tomason Y, Nimmakayala P, Levi A, Reddy UK (2013) Map-based molecular diversity, linkage disequilibrium and association mapping of fruit traits in melon. Mol Breed 31:829–841. https://doi.org/10.1007/s11032-013-9837-9

Varshney RK, Graner A, Sorrells ME (2005) Genomics-assisted breeding for crop improvement. Trends Plant Sci 10:621–630. https://doi.org/10.1016/j.tplants.2005.10.004

Varshney RK, Sinha P, Singh VK et al (2020) 5Gs for crop genetic improvement. Curr Opin Plant Biol 56:190–196. https://doi.org/10.1016/j.pbi.2019.12.004

Veillet F, Perrot L, Chauvin L et al (2019) Transgene-free genome editing in tomato and potato plants using *Agrobacterium*-mediated delivery of a CRISPR/Cas9 cytidine base editor. Int J Mol Sci 20:1–10. https://doi.org/10.3390/ijms20020402

Wang X, Ando K, Wu S et al (2021) Genetic characterization of melon accessions in the U.-S. National Plant Germplasm System and construction of a melon core collection. Mol. Horticulture 1:11. https://doi.org/10.1186/s43897-021-00014-9

Whitaker TW, Davis GN (1962) Cucurbits: botany, cultivation and utilization. Interscience Publishers, New York, NY, p 249

Xu X, Bai G (2015) Whole-genome resequencing: changing the paradigms of SNP detection, molecular mapping and gene discovery. Mol Breed 35:33. https://doi.org/10.1007/s11032-015-0240-6

Xu X, Liu X, Ge S et al (2012) Resequencing 50 accessions of cultivated and wild rice yields markers for identifying agronomically important genes. Nat Biotechnol 30(1):105–111

Yano R, Ariizumi T, Nonaka S et al (2020) Comparative genomics of muskmelon reveals a potential role for retrotransposons in the modification of gene expression. Commun Biol 3: 432. https://doi.org/10.1038/s42003-020-01172-0

Yu J, Buckler ES (2006) Genetic association mapping and genome organization of maize. Curr Opin Biotechnol 17(2):155–160

Zhang B, Tolstikov V, Turnbull C et al (2010) Divergent metabolome and proteome suggest functional independence of dual phloem transport systems in cucurbits. Proc Natl Acad Sci U S A 107:13532–13537. https://doi.org/10.1073/pnas.0910558107

Zhang H, Li X, Yu H et al (2019) A high-quality melon genome assembly provides insights into genetic basis of fruit trait improvement. iScience 22:16–27. https://doi.org/10.1016/j.isci.2019.10.049

Zhang H, Zhang X, Li M et al (2022) Molecular mapping for fruit-related traits, and joint identification of candidate genes and selective sweeps for seed size in melon. Genomics 114: 11022–110306. https://doi.org/10.1016/j.ygeno.2022.110306

Zhao G, Lian Q, Zhang Z et al (2019) A comprehensive genome variation map of melon identifies multiple domestication events and loci influencing agronomic traits. Nat Genet 51:1607–1615. https://doi.org/10.1038/s41588-019-0522-8

Zhu C, Gore M, Buckler ES, Yu J (2008) Status and prospects of association mapping in plants. Plant Genome 1(1):5–20

Zhu Y, Sun D, Deng Y et al (2020) Comparative transcriptome analysis of the effect of different heat shock periods on the unfertilized ovule in watermelon (*Citrullus lanatus*). J Integr Agric 19: 528–540. https://doi.org/10.1016/S2095-3119(19)62777-2

Zou C, Wang P, Xu Y (2016) Bulked sample analysis in genetics, genomics and crop improvement. Plant Biotechnol J 14:1941–1955. https://doi.org/10.1111/pbi.13140