



Review Retrospective Genetic Analysis of Qualitative and Quantitative Traits in Sweet Watermelon (*Citrullus lanatus* var. *lanatus*): A Review

Jacob Mashilo ^{1,2,*}, Hussein Shimelis ², Dorcus Maja ¹, and Richard Mantlo Ngwepe ^{1,2}

- ¹ Towoomba Research Centre, Limpopo Department of Agriculture and Rural Development, Agriculture Regulatory and Technology Development, Crop Science Directorate, Private Bag X1615, Bela-Bela 0480, South Africa; dorcusmaja69@gmail.com (D.M.); ngwepem@gmail.com (R.M.N.)
- ² African Centre for Crop Improvement (ACCI), College of Agriculture, Engineering and Science (CAES), University of KwaZulu-Natal, Private Bag X01, Scottsville, Pietermaritzburg 3209, South Africa; shimelish@ukzn.ac.za
- * Correspondence: jacobmashilo@yahoo.com

Abstract: Understanding the genetic basis of a crop's qualitative and quantitative traits is vital to designing market preferred varieties. The aim of this review is to present a retrospective genetic analysis of qualitative and quantitative phenotypic traits in sweet watermelon as a guide for trait integration and the development of novel varieties with yield potential and desirable horticultural attributes. The first section outlines genes conditioning the inheritance of plant architecture (e.g., leaf attributes and plant architecture), floral characters (flowering rate, sex expression, and male sterility), fruit traits (shape, colour, rind colour and stripe patterns and flesh colour) and seed morphology (seed length, width, size and coat colour). In the second section, developments in molecular markers and quantitative trait loci (QTL) to aid marker-assisted breeding are discussed. Further, the review highlights the clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) gene-editing technology and its scope in gene manipulations and new variety development. The information presented in this review is useful for optimised and demand-led breeding to develop new variets to serve growers, consumers and the sweet watermelon industry.

Keywords: gene-editing technology; transcriptome analysis; quantitative trait loci; retrospective genetic analysis; sweet watermelon

1. Introduction

Sweet watermelon [*Citrullus lanatus* (Thunb.) Matsum. and Nakai var. *lanatus*; 2n = 2x = 22] is an important cucurbit crop belonging to the family Cucurbitaceae of the genus *Citrullus* [1]. Of the six species within the genus namely: *C. lanatus* var. *citroides*, *C. mucosospermus*, *C. colocynthis*, *C. ecirrhosus*, *C. rehmii*, and *C. naudinianus* [1]. Sweet watermelon is the favourite and an extensively cultivated and consumed fruit crop. The fruit comprises of essential nutrients (i.e., N, P, K, Ca, Mg, Fe, Mn, Cu and Zn), and phytochemical compounds such as sugars (fructose, sucrose and glucose), amino acids (citrulline and arginine) and organic acids (citric and malic), and carotenoids (lycopene, phytoene, prolycopene, violaxanthin, neoxanthin, lutein and β -carotene) [2–12].

Consumer preferences for fruit and seed traits drive the purchasing and consumption patterns of watermelon in the marketplace. As a result, watermelon breeders are constantly faced with the task of developing ideal varieties that are desired by the market. Key aspects in the product profiles in a watermelon variety include desirable plant architecture, high leaf biomass and fruit yield, fruit quality (high sugar and lycopene contents), optimum fruit size, external fruit features such as rind stripe patterns and colour, fruit flesh colour, seedlessness, and acceptable seed coat colours. The targeted selection of qualitative and



Citation: Mashilo, J.; Shimelis, H.; Maja, D.; Ngwepe, R.M. Retrospective Genetic Analysis of Qualitative and Quantitative Traits in Sweet Watermelon (*Citrullus lanatus* var. *lanatus*): A Review. *Agronomy* 2022, *12*, 1633. https://doi.org/ 10.3390/agronomy12071633

Academic Editors: Jinfeng Chen, Xiaqing Yu and Biao Jiang

Received: 9 June 2022 Accepted: 7 July 2022 Published: 8 July 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). quantitative phenotypic traits can aid in breeding watermelon varieties that meet a range of requirements by consumers and growers.

Understanding the genetic control of economic phenotypic traits could inform the required selection criteria, genetic advancement, and use of complementary molecular breeding strategies. Essential qualitative and quantitative phenotypic traits in watermelon breeding are broadly grouped into plant, flower, fruit, and seed attributes. Plant traits include (leaf biomass and tenderness, plant height, number of primary and secondary branches per plant), flower traits (flowering rate and time, number of male and female flowers, a ratio of male to female flowers), fruit (length, width, weight and rind thickness) and seed (length, width, colour, and weight). Hence, knowledge of the genetic basis of the crop's qualitative and quantitative phenotypic traits is vital to design market-preferred varieties.

Functional genes conditioning qualitative and quantitative phenotypic traits in watermelon have been identified through comparative genetic analysis [11–17]. Qualitative phenotypic traits in watermelon are conditioned by major genes [13,14,18–22]. On the contrary, multiple minor genes are involved in the regulation of quantitative phenotypic traits in watermelon [23–30]. Quantitative trait loci (QTL) analysis in watermelon identified several genomic regions linked to important traits for molecular breeding. The development of various molecular marker systems linked to qualitative and quantitative phenotypic traits allowed for genetic analysis and marker-assisted breeding in watermelon [24,29,31–34]. Genomic resources will further facilitate the breeding of novel watermelon cultivars. The QTL mapping of genes controlling the key traits will facilitate the application of geneediting technology to accelerate the breeding process and allow for the timeous release of desirable watermelon varieties. The clustered, regularly interspaced, short palindromic repeats (CRISPR/Cas9) gene-editing technology has been integrated in a few breeding programs and led to the development of watermelon progenies with excellent qualitative and quantitative attributes [35–38]. The conventional breeding of consumer-and-industry preferred watermelon varieties will benefit from the application CRISPR/Cas9 gene-editing technology. This requires a detailed understanding of genetic and genomic resources and a genetic analysis of qualitative and quantitative phenotypic traits in watermelon. The aim of this review is to present a retrospective genetic analysis on qualitative and quantitative phenotypic traits in sweet watermelon as a guide for traits' integration, selection and the development of novel varieties with yield potential and desirable horticultural attributes. The first section outlines the major and minor genes conditioning the inheritance of plant architecture, floral characters, fruit traits and seed attributes. The second section discusses developments in molecular markers and QTL analysis to aid in marker-assisted breeding. Further, the review highlights CRISPR/Cas9 gene-editing technology and its scope in genetic dissection and manipulation in watermelon. The information presented in this review is useful for optimised and demand-led breeding to develop new varieties that can serve growers, consumers and the sweet watermelon industry.

2. Methodology

The following databases were accessed to retrieve relevant peer-review publications for the present study, namely:

- (1) Web of Science TM (www.webofknowledge.com (accessed on 3 February 2022);
- (2) Science Direct (www.sciencedirect.com (accessed on 3 February 2022);
- (3) Scopus (www.scopus.com (accessed on 13 February 2022);
- (4) Google Scholar (scholar.google.com(accessed on 16 February 2022).

The primary terms used for the search were "*C. lanatus* spp.", "genetic analysis", "plant traits", "flower traits", "fruit traits", "seed traits", "QTL" "molecular markers" and "gene editing". The following secondary or expanded search words were used for qualitative phenotypic traits: "genetic analysis of leaf shape in watermelon", "genetic analysis of plant architecture in watermelon", "genetic analysis of sex expression in watermelon", "genetic analysis of fruit shape in watermelon", "genetic analysis of fruit colour in watermelon", "genetic analysis of rind stripe patterns in watermelon", "genetic analysis of fruit rind colour in watermelon", "genetic analysis of fruit flesh colour in watermelon", and "genetic analysis of seed coat colour of watermelon". For quantitative phenotypic traits, the following expanded search words were employed: "genetic analysis of fruit yield and component traits in watermelon", and "genetic analysis of seed yield and component traits in watermelon". To retrieve studies on genomic resources linked to qualitative and quantitative phenotypic traits, the search terms "QTL for qualitative and quantitative traits in watermelon", "molecular markers for qualitative and quantitative traits in watermelon" were used. The most relevant publications from the search, irrespective of the publication year, were used for the present study.

3. Genetic Regulation of Qualitative Phenotypic Traits in Sweet Watermelon

Watermelon genotypes show extensive variation in the qualitative phenotypic traits that can be selected for breeding market-preferred varieties. The following sections present the hitherto identified genes that govern variation in the qualitative traits of watermelon.

3.1. Leaf Attributes

3.1.1. Leaf Shape

Leaf shape in sweet watermelon is categorized into lobed and non-lobed leaf phenotypes [39–41]. The degree of leaf lobation varies from tri-lobate to penta-lobate, with wide and round lobes [39,40]. The lobes vary in size, which determines the overall leaf size and area. In Africa where watermelon leaves are consumed as a leafy vegetable, the shape and size of the leaf is an important trait for breeding consumer-desired varieties. The majority of cultivated watermelon varieties possess the lobed leaf phenotype, which is controlled by a single dominant gene, designated as *ClLL1* [20]. Two genes, such as *ORF18* and *ORF22* (encoding a homeobox-leucine zipper-like protein), are thought to confer the lobed-leaf phenotype of watermelon [20]. There is need for molecular marker development of leaf phenotypes to facilitate the breeding of new varieties with desirable leaf attributes, including high leaf biomass production.

3.1.2. Leaf Bitterness

The tender cooked leaves of watermelon are widely consumed in sub-Saharan Africa (SSA). The leaves are sources of essential nutrients and phytochemical compounds [8,9,42]. The predominant phytochemical compound in the leaves is cucurbitacins, which results in bitterness [42]. Cucurbitacins possess various pharmacological and pharmaceutical values [43,44]. The following cucurbitacins, namely, B, D, E and E-glucoside, are reported to accumulate in the leaves of watermelon [42]. There are limited studies on the genetic analysis of bitterness in watermelon leaves. In the fruit, bitterness is reportedly conditioned by a single dominate gene [45,46]. *Cla007077, Cla007078, Cla007079* and *Cla007080* genes regulate the biosynthesis of cucurbitacins B and E in watermelon fruit [47]. The gene *ClBt* (gene ID: *Cla011508*) regulate fruit-specific cucurbitacins biosynthesis in watermelon fruit [46]. Additionally, genes, namely, *CcCDS1, CcCDS2* and *ClCDS1*, regulate the cucurbitacins biosynthesis pathway via the catabolism of cucurbitadienol synthase [48]. Whether the genes causing fruit bitterness are the same as those in leaves has yet to be determined.

4. Flower Characteristics

4.1. Sex Expression

Sex expression is an important trait, which determines fruit set and yield development in watermelon. The following sex phenotypes are present in watermelon: monoecious (producing male and female flowers on the same plant), andromonoecious (male and hermaphrodite flowers on the same plant), partially andromonoecious (male, female, hermaphrodite and bisexual flowers on the same plant) and gynoecious (female flowers only on the same plant) [13,21]. Monoecious sex expression is a desirable trait for selfing and seed multiplication within the same genotype. Monoecious and andromonoecious sex expression are undesirable for hybrid breeding, because there is a need to emasculate male flowers for pollination and hybrid breeding [21]. Gynoecious sex phenotype does not require the removal of male flowers and is ideal for cultivar development. Dioecy (separate male and female plants) is a desirable sex phenotype for hybrid breeding, though not common in watermelon.

Genetic analysis between parents of gynoecious \times monoecious flowers revealed the ratios of three monoecious: 1 gynoecious flower in the F_2 populations, and 1 monoecious and 1 gynoecious ratios in the backcross population, indicating a that single gene controls monoecy in watermelon [13]. The ethylene biosynthesis gene *CitACS4*, which encodes for a flower-specific ACS enzyme, regulates monoecy in watermelon, favouring more male flowers than female flowers [49,50]. Natural mutations of the *CitACS4* gene are responsible for converting female flowers into hermaphrodite flowers, monoecy into partial and romonoecy or andromonoecy in watermelon [51,52]. A recessive gene, pa (gene ID: ClCG01G020800), controls the occurrence of bisexual and hermaphrodite flowers in watermelon [52]. The following genes are reportedly involved in ethylene biosynthesis and signalling, flower development and sex determination in watermelon: ClCG01G020030, ClCG01G020040, CICG01G020060, CICG01G020080, CICG01G020260, CICG01G020430, CICG01G020700, ClCG01G020770, ClCG01G020780, ClCG01G020790 and ClCG01G020800 [52]. Starch and sucrose metabolism genes such as Cla021762, Cla004462, Cla015099, Cla009288, Cla011403, Cla017383 and Cla005857, phenylpropanoid biosynthesis genes including Cla005785, Cla020908, Cla009234, Cla015297, Cla015296 and Cla012598, pentose and glucuronate interconversions genes, and nitrogen metabolism genes Cla017784, Cla017687, Cla010086, Cla005080 and Cla002787 are involved in the development of male flowers in watermelon [53]. Of these, a pollen-specific gene, *Cla001608*, plays a key role in the development of male flowers. The multiple genes involved in flower development indicate the presence of a complex metabolic pathway in sex expression in watermelon.

4.2. Male Sterility

Watermelon hybrid cultivar development involves the recombination of desirable contrasting parental genotypes. The complex sex phenotypes, including monoecy, limit hybrid breeding due to laborious procedures in genotype emasculation, isolation and pollination. Male sterility provides an alternative approach to the rapid and efficient breeding of watermelon hybrid varieties. Quantitative genetic analysis between male sterile and fertile watermelon genotypes revealed a 3:1 segregation ratio in the F_2 populations [22], indicating a single dominant gene confer male sterility in watermelon. Rhee et al. (54) identified 1259 differentially expressed genes associated with male sterility through comparative transcriptome analysis. These genes are involved in various physiological processes, including stamen and pollen development and pollen tube elongation [54]. Some of the reported genes included Cla021983, Cla015362, Cla006728, Cla016924, Cla022958, Cla022957, Cla022600, Cla013638, Cla015385 and Cla006729 [54]. Of the stated genes, Cla006625, which encodes a pollen-specific leucine-rich repeat protein (*ClaPEX1*), resulted in sterile male flowers [22]. Cla009410, Cla007521, Cla006625, Cla006738, Cla006737 and Cla009382, reportedly up-regulated male-sterillity in watermelon [22]. The gene Citrullus lanatus Abnormal *Tapetum 1* (*ClATM1*), which encodes a basic helix-loop-helix (*bHLH*) transcription factor, regulates flower development in watermelon [55]. The disruption of *ClATM1* results in male sterility in watermelon [55]. Recently, CER1, FAR, LOX2S, HPL, OPR, CHS and F3H, were identified as regulating male sterility in watermelon, being involved in anther cuticle and pollen wall development [56]. The identified male sterility genes will facilitate hybrid breeding and deliver the desired watermelon cultivars.

5. Fruit Attributes

5.1. Fruit Colour

Fruit colour is an important trait that influences watermelon market and consumer choice. The external fruit colour of watermelon varies from light green, to dark-green and yellow (Figure 1). Segregation analysis involving green and yellow fruit colours revealed

 F_2 population ratios of 3 yellow and 1 green fruit, indicative of a single dominant gene conferring yellow fruit colour in watermelon [14]. The gene *Cla002755*, which is highly expressed in dark green and yellow rind phenotypes, reportedly conferred both the yellow and dark-green rind colour of watermelon [14]. Genes conditioning other fruit colours in watermelon have yet to be discovered.



Figure 1. Fruit shape, colour and rind stripe patterns of sweet watermelon. (**A**); Round fruit with no distinctive stripes; (**B**), Round fruit with medium light-green rind stripes; (**C**), Light-green oblong fruit with no distintive rind stripes; (**D**), Moon and star trait on fruit (source: http://www.starkeayres.co.za (accessed on 15 April 2022); (**E**), Light-green elongated fruit; (**F**), Round fruit with dark-green and thin rind stripes; (**G**), Dark-green fruit with light-green rind stripes; (**H**), Medium-green elongated fruit with no stripes; (**I**,**J**), Medium-green oblong fruit with dark-green rind stripes (Photographs except (**G**) by J. Mashilo).

5.2. Fruit Shape

Fruit shape is an important trait for the selection and breeding of market-preferred varieties. Fruit shape in watermelon can be round, oblong and elongated (Figure 1A,C,E). *ClSUN25-26-27a* (gene ID: *Cla011257*) conditions an elongated fruit shape in watermelon [15,16]. Maragal et al. [57] reported that *Cla011257* regulates fruit shape. Fruit shape is defined based on the fruit shape index (FSI), which is the ratio of the fruit length (FL) to the fruit

diameter (FD). Various QTLs controlling FSI have been identified, which are associated with QTL for FL and FD [15,23,25,58]. Hence, fruit shape in watermelon is a complex trait, controlled by multiple genes.

5.3. Fruit Rind Stripe Patterns

Rind patterns of watermelon fruit are a vital trait that influence the markets and consumer choice (Figure 1B,F,G). Rind stripes are characterized by stripe width (i.e., narrow, medium, or wide), colour, and background colour (i.e., dark green, medium green or light green) [18,59,60]. Three loci, namely, *S*, *D*, and *Dgo*, reportedly control rind stripe patterns [61]. The dark-green rind stripe pattern is controlled by a single dominant gene, designated as *Cla019205* [34]. Yue et al. [62] identified a single dominant gene, denoted as *ClSP*, controlling the stripe pattern of the fruit.

Transcriptome analysis revealed a total of 356 differentially expressed genes (DEGs) between watermelon genotypes with dark- and light-green stripes. Genes involved in chloroplast development and photosynthesis were downregulated in the light-green striped genotype. Some 38 DEGs were up-regulated in dark-green striped phenotype [62]. Guo et al. [63] identified the gene designated as *Cla97C06G126710* (which encodes a WD40-repeat protein) regulating stripe rind presence in watermelon. Genomic analysis revealed two genes, namely, *Cla97C09G175170* and *Cla97C09G175150*, controlling the main rind stripe patterns and the inter-stripe colour in watermelon [64]. A unique trait termed 'moon and star' (Figure 1D), associated with yellow spots on the watermelon fruit rind, is reportedly controlled by two candidate genes, *SP1* and *SP2* [65].

5.4. Fruit Rind Colour

Fruit rind colour determines the visual appearance of cut watermelon fruit. Thus, consumer purchasing preferences are dependent on rind colour properties. The rind colour of watermelon fruit varies from light green to dark green and yellow [18,41]. A single dominant gene, denoted as D, conditioned a dark-green colour masking the lightgreen rind colour [66]. The gene ClCG08G017810 (ClCGMenG) controls the development of dark-green rind colour [66], whereas Clyr controls yellow rind colour development in watermelon [67]. The gene *Cla022573* is involved in pigment binding, and six other genes, namely Cla022675, Cla022530, Cla022517, Cla022495, Cla022718, and Cla022725, are involved in anthocyanin metabolism. Further, two genes Cla022543 and Cla022670 involved in porphyrin and chlorophyll metabolism, while Cla022574 involved in carotenoid biosynthesis, which all contribute to the rind colour development in watermelon fruit [26]. Yang et al. [68] identified five genes, namely, Cla002942, Cla004992, Cla009181, Cla017341 and Cla018352, which contribute to the formation of the yellow and green rind colour in watermelon fruit. These genes conditioned various physiological processes, including phytohormone signalling, phenylpropanoid biosynthesis, photosynthesis, and starch and sucrose metabolism. Genetic analysis involving dark-green and light-green fruit rind colour watermelon morphotypes revealed the presence of duplicate and dominant epistasis genes conditioning these traits. Homozygous recessive genes denominated g-1 and g-2 control the light-green fruit rind colour of watermelon [69]. These suggested the involvement of several genes conditioning fruit rind colour in watermelon. The intensity of rind fruit colour is dependent on the number of genes and mode of gene actions. For instance, incomplete dominance genes reportedly conditioned dark green fruit rind colour [70], while dominance genes resulted in medium-green fruit rind colour [69,71]. From the foregone genetic variations, the number of genes and their actions affecting watermelon fruit rind colour is yet to be precisely determined.

5.5. Fruit Flesh Colour

A wide range of flesh colour exist in watermelon fruit, including white, yellow, orange, pink and red [18,41,72,73]. Furthermore, red-flesh fruits are classified into coral and scarlet red [19], whereas orange-fleshed watermelon types are classified as canary yellow and salmon-yellow [18]. Genes conferring the flesh colour of watermelon were recently reviewed by Mashilo et al. [74]; readers are referred to this publication for further reading. In summary, the coordination of physiological and molecular processes during the watermelon fruit-ripening stage is subject to multiple genes affecting fruit flesh colour development.

6. Seed Attributes

Seed Coat Colour

Seed coat color is an important trait associated with fresh or dry seed quality. The flesh appearance of cut watermelon fruit is affected by seed colour. Seed coat colour varies from cream/white, dark brown, green, black, and red/tan (Figure 2). Black seed coat (Figure 2A,B) is controlled by a single dominant gene whereas light-yellow seed coat colour (Figure 2E,F) is conditioned by a recessive gene [75]. A segregation analysis involving F_2 progenies derived from the crosses between dotted black × red seeds revealed that the dotted black seed coat is dominant over the red seed coat colour [76]. The dotted black seed coat colour is dominant over the green seed coat colour [76]. Li et al. [27] identified 30 genes associated with seed coat colour development in watermelon. These genes regulate the biosynthesis of flavonols, phenolics and proanthocyanidin. The genes *Cla019482*, *Cla019483*, and *Cla019481* are involved in polyphenol oxidase metabolism [27]. The gene *ClCS1* (gene ID: *Cla019481*), which encodes for a polyphenol oxidase, is involved in the oxidation step of melanin biosynthesis leading to the development of a black seed coat colour in watermelon [75].



Figure 2. Seed coat colours differences among sweet watermelon accessions. (**A**), Black seed coat colour of sweet watermelon cultivar "Charleston Grey"; (**B**), Black seed coat colour of sweet watermelon "Crimson Sweet"; (**C**), "All Sweet" watermelon variety with black and brown seed coat colour; (**D**), "Sugar Baby" sweet watermelon variety with black seed coat colour; (**E**–**L**), Sweet watermelon landrace accessions collected from Moletjie Ga-Mphela village in the Limpopo Province of South Africa displaying varied seed coat colours ranging from light-brown/yellow with red eyes (**E**) and black seed eyes (**F**), dark-brown and black seeds (**G**), dark-brown seeds (**H**), dark-brown seed with white seed margins (**I**), black seeds with brown seed margins (**J**), brown seeds (**K**) and maroon seeds (**L**) (Photographs by J. Mashilo).

7. Genetic Regulation of Quantitative Traits in Sweet Watermelon

Morphological diversity analysis in sweet watermelon revealed extensive variation in quantitative phenotypic traits, including plant architecture, flowering time and rates, fruit and seed yield, and fruit- and seed-related traits [41,77–79]. The section below outlines the genetic analysis of various quantitative traits.

7.1. Leaf Biomass Yield and Its Components

High leaf biomass yield in watermelon is a desired trait for use as a leaf vegetable. High leaf biomass production is also vital to enhancing photosynthetic efficiency to promote high fruit production and yield. The number of leaves per plant, which is influenced by plant height and branching capacity, as well as the length, width and size of individual leaves, are important traits that influence the overall leaf biomass yield in watermelon. A genetic analysis of leaf traits has not been adequately studied in watermelon. There are no molecular markers or QTL mapping of leaf traits for efficient selection and markerassisted breeding. Some accessions of white-fleshed citron watermelon (*Citrullus lanatus* var. *citroides*) exhibit a reduced leaf size compared to most commercially cultivated sweet watermelons. These germplasms may play a key role in understanding the genetic architecture of leaf yield and component traits in watermelon.

7.2. Plant Height

Plant height is an important trait that influences flower development and fruit yield potential in watermelon. The candidate gene Cla010726 is associated with reduced plant height in watermelon [80]. The expression levels of *Cla010726* are significantly lower in short plants [80]. The genes designated as *Cla015405* and *Cla015406* are associated with a short phenotype in watermelon [81]. Recently, Cla015407, named Citrullus lanatus dwarfism (*Cldf*), has been thought to control short plant stature in watermelon [82]. A point mutation resulting in a 13 bp deletion in the coding sequence of *Cldf* led to a GA-deficient short phenotype [83]. The gene Cla010726 encodes for gibberellin 20-oxidase-like protein, whereas *Cla015407* gene encodes gibberellic acid 3β -hydroxylase proteins, which are associated with the gibberellic acid metabolism, resulting in growth arrest and reduced plant height [82]. The gene designated as *Cla010337*, which encodes an ATP-binding cassette transporter (ABC transporter), reportedly conditioned dwarf plant height in watermelon [84]. The deletion of a single nucleotide of the gene Cla010337 causes the development of shorter watermelon plants [84]. Quantitative analysis revealed segregation ratios of 3:1 and 1:1 in the F_2 and backcross populations, suggesting that reduced plant height is controlled by a single recessive or dominant gene [82,85,86]. Cho et al. [86] identified the gene CICG09G018320, which encodes an ABC transporter, determining shorter watermelon plants in progenies derived between dwarf (Bush Sugar Baby) and normal (PCL-J1) watermelon cultivars. The ABC transporter gene results in shorter watermelon plants due to physiological changes in the levels of auxin, the phytohormone [86]. Internode length is a secondary trait that influences plant height in watermelon. Segregation ratios of 3:1 and 1:1 in the F_2 and backcross populations, respectively, were detected, suggesting that a single dominant gene controls the expression of short internode length in watermelon [29]. The gibberellin 3β -hydroxylase (GA 3β -hydroxylase) gene *Cla015407* is associated with the short internode phenotype in watermelon [29]. GA 3β-hydroxylase is an important enzyme regulating GA biosynthesis by catalyzing the inactive precursors of GA₉, GA₂₀, and GA₅ into bioactive forms, namely, GA₄, GA₁, and GA₃, respectively [29].

7.3. Branching Capacity

Branching capacity is an important trait influencing leaf biomass production, flowering potential, vine and fruit yield in watermelon. In SSA, the dried branches of the crop are used as fodder for livestock. The branches are a good source of essential macroand micro-nutrients [8]. Watermelon produces multiple lateral branches from the primary branches. A single recessive gene, *Clbl* (i.e., *Citrullus lanatus branchless*), causes branchlessness [87]. Bulked segregant sequencing (BSA-seq) analysis revealed a candidate gene, *Cla018392*, which encodes a *TERMINAL FLOWER 1* protein associated with branchlessness in watermelon [87]. This gene reduces the formation and development of axillary and apical buds, thus limiting lateral branching in watermelon [87]. Genetic analysis of lateral branch development in watermelon is key for marker-assisted selection and QTL mapping. This enables the breeding of branchless watermelon cultivars for closed and protected production or open field environments. However, the branchless trait is not required in watermelon grown for high leaf biomass for food feed. Therefore, understanding the genetic regulation of profuse branching ability is essential for breeding of watermelon genotypes with contrasting branching capacities to obtain insight into and elucidate the molecular mechanisms regulating this trait for breeding.

7.4. Flowering Time

Flowering time is another important trait influencing yield expression and potential in watermelon. Male and female flowers in watermelon are located separately on different nodes of the same plant. Male flowers appear first, followed by female flowers. The number of days before the appearance of the first male and female flowers extensively vary in watermelon. For example, McGregor and Waters [88] reported that the days to first male flower varied from 8 to 22 days after transplanting (DAT), and between 20 and 30 DAT to the first female flower among watermelon pollen parents. Stone et al. [89] reported days to first male flower varied between 44 and 60 days after planting (DAP), whereas days to first female flower varied between 52 and 70 DAP. Gimode et al. [32] reported that days to first female flower ranged from 16 to 37 DAP in watermelon. Flowering time in watermelon is subject to genotype, environment and genotype-by-environment interactions. The following genes: Cla009504 and Cla000855 [24] and Cla002795 (i.e., phosphatidylinositol-4phosphate 5-kinase (PIP-kinase) [32] regulate flowering time in watermelon. The identified genes provide opportunities for breeding watermelon varieties with desired flowering times for different production environments, and in the development of molecular markers to ensure efficient selection for earliness.

7.5. Fruit Yield and Its Components

Fruit yield is an economic trait in watermelon, and varies considerably among the diverse varieties. Fruit yield ranging from 40.5 to 84 tons/ha has been reported in watermelon [90]. Stone et al. [89] reported fruit yield varying from 2.8 to 5.7 tons per hectare. Fruit yield in watermelon is determined by fruit weight, length and width. Fruit weight vary considerably in watermelon. Stone et al. [89] reported a single fruit weight of watermelon varying from ~ 3 to 12 kg, whereas Singh et al. [72] reported fruit weight varying from 0.10 to 3.21 kg. A fruit weight ranging from 0.58 to 8.2 kg has been reported in a diverse panel of watermelon varieties [41]. Fruit length and width also vary considerably between 21 and 40 cm, and from 20 to 25 cm, respectively [89], and from 10.9 to 20.9 cm and 9.20 to 34.6 cm [41]. Other secondary traits including plant height, the number of primary, secondary and tertiary branches, the number of male and female flowers, and the number of fruits produced per plant from successfully fertilized female flowers indirectly contribute to fruit yield in watermelon. As a result, fruit yield is influenced by several yield components [23,30,58]. Multiple QTLs associated with yield component traits have been reported in watermelon [23,24,28,30,58]. The multiple QTLs conditioning yield component traits are useful for the strategic breeding of watermelon for high fruit yield potential.

7.6. Seed Yield and Its Components

Triploid seedless watermelons are preferred for fresh consumption. Triploid watermelons produce non-viable pollen and require a diploid (seeded) watermelon as a pollen parent [91–93]. The production and breeding of seeded watermelons has declined in recent years in favour of seedless watermelons. Elsewhere, seeded watermelons are preferred for seed consumption as snack and for developing value-added by-products. In such circumstances, breeding watermelon varieties with a high seed yield is an important objective. Seed yield potential is determined by the number of seeds per fruit, seed, length, width, weight and size, which are highly variable in watermelon [94,95]. Small seed sizes are preferred for fresh fruit consumption, whereas large seeds are preferred for planting and cooking. Seed size in watermelon is categorized as tomato, small, medium, and large [96]. Two candidate genes, namely, *Cla97C05G104360* and *Cla97C05G104380*, and three other genes, namely, *Cla97C05G104340*, *Cla97C05G104350* and *Cla97C05G104390* [97], conditioned seed size through their involvement in abscisic acid metabolism. The genes *Cla009290*, *Cla009291* and *Cla009310* were reportedly involved in seed size development [27]. Seed size is determined by seed length and width, which are conditioned by several QTLs [25,27,30]. Various QTLs are reported to control seed component traits in watermelon [25,27,30]. The mapped QTLs for seed component traits offer strategic breeding of watermelon varieties, targeting high seed yield potential.

8. Molecular Marker and Quantitative Trait Loci (QTL) Analyses for Qualitative and Quantitative Phenotypic Traits in Sweet Watermelon

Molecular markers linked to functional genes controlling qualitative and quantitative phenotypic traits have wider applications, including in genetic diversity analysis, parental selection, heterotic grouping and marker-assisted breeding. QTL mapping for qualitative and quantitative phenotypic traits is vital for marker-assisted and genomic selection and the development of watermelon varieties with desired agronomic and horticultural traits. The following section presents the progress made in the development of molecular marker systems and a QTL analysis of qualitative and quantitative phenotypic traits in watermelon.

8.1. Molecular Markers

Cleaved Amplified Polymorphic Sequence (CAPS) markers (i.e., Clcyb.600 and Lcyb) were developed to discriminate red-fleshed and canary-yellow or orange-fleshed watermelon varieties [98,99]. CAPS markers wsb3-24 and wsb3-9 are linked to the fruit shape index of watermelon for effective selection [25]. The CAPS markers wsb2-13 and wsb2-52 are tightly linked to seed size in watermelon to aid in early generation selection [25]. CAPS markers WII04E07-33 and WII04E07-40 are useful for the targeted selection and improvement of lycopene content in watermelon [31]. A Sequence Characterized Amplified Region (SCAR) marker wsbin6-11 was developed for the marker-assisted selection of dark-green, medium width and sharp margin stripes of watermelon [25]. Simple Sequence Repeat (SSR) markers MCPI_05 and MCPI_16, linked to fruit rind stripe patterns, were developed for the selection of fruit external attributes [100]. Wang et al. [101] developed a KASP marker chr06_7040350 to discriminate between white- and pale-yellow-fleshed watermelon varieties. Subburaj et al. [102] developed 19 CAPS markers for the selection of low- and high-lycopene-containing watermelon varieties. KASP™ markers UGA3_5820134 and UGA5_4591722 were developed for the marker-assisted selection of tan- or red-seed and dotted black-seed coat colours [76]. The KASP marker NW0248748 is linked to days to first female flower [32]. The CAPS markers CAPS90 and CAPS91 were applied for the marker-assisted breeding of short-height watermelon varieties [29]. Kompetitive Allele Specific Polymorphism (KASP) markers, namely, ClSUN-1 and ClSUN-2, have been developed for the effective selection of elongated fruit shape in watermelon [16]. KASPTM markers UGA3_10738714, UGA3_10795402, and UGA3_11016809, associated with the *Qdff3-1* loci conferring early maturity in watermelon, have been developed for breeding [32]. CAPS markers CIR6-M1 and CIR6-M4 are linked to *trans*-lycopene content [103]. Pei et al. [104] developed a KASP marker to identify pale green coloured watermelon flesh. Single-nucleotide polymorphism (SNPs) markers, namely, 32250307, 32250454, 32256177 and 32260870, are tightly linked to seed size in watermelon, which is useful for the breeding of watermelon cultivars with a desired seed size [97]. These developed markers are useful genomic resources for application in breeding programs to aid in the selection and breeding

of watermelon varieties. However, there have been limited attempts to develop molecular markers linked to other important quantitative traits in watermelon, including leaf yield component traits (e.g., leaf shape, length, width and size), plant architecture (i.e., plant height, male sterility and branching capacity). Diagnostic molecular markers for qualitative traits, including fruit rind colour, have yet to be developed.

8.2. Quantitative Trait Loci

Genome-wide sequencing of the watermelon genome has aided in the identification of QTL associated with qualitative and quantitative phenotypic traits (Table 1). Two major QTL conditioning the red flesh colour are positioned on chromosomes 2 and 8, explaining over 30% of the phenotypic variation, whereas a major QTL ($R^2 = 55.0\%$) for yellow flesh colour is positioned on chromosome 2 of watermelon [105]. QTLs fl9.1, fl9.2 and fl11.1 condition fruit length, fwd9.1, fwd9.2 and fwd11.1 condition fruit diameter, QTLs fsi9.1, fsi10.1 and fsi11.1 condition fruit shape index and QTL fwt6.1, fwt6.2, fwt9.1, fwt9.2 and fwt11.1 condition fruit weight in watermelon [23]. Of these, fl11.1, fwd9.2, fwt9.2, fsi11.1 and *rth9.1* are major QTLs, controlling fruit length, width, weight, shape index and rind thickness, in that order [23]. QTLs associated with fruit length, width and shape index were co-localized in the same genomic region [23]. Major-effect QTLs for hundred-seed weight are mapped on chromosomes 2 and 9, for seed length on chromosome 2, and for seed width on chromosomes 2 and 9 of watermelon [106]. Major QTLs associated with sex expression have been mapped on chromosome 11 of watermelon and account for >30% of the phenotypic variation [106]. QTLs controlling seed traits (i.e., seed length and width, and hundred seed weight) were mapped on chromosomes 5 and 6 of watermelon [107]. Major-effect QTLs for hundred-seed weight and seed length explained over 60% of the phenotypic variation and were mapped on chromosome 6 of watermelon [107]. Qdmf3-1, Qdff3-1 and Qfmi3 are associated with days to first male flower, days to first female flower and male–female interval [24]. LCYB4.1, located on chromosome 4, is associated with lycopene content, developing a red flesh colour and explaining 81.45% of the phenotypic variation [31]. These agreed with the genome-wide association study (GWAS) by Wu et al. [63], which revealed that chromosome 4 harbours the LCYB gene, which regulates the lycopene content of ripened watermelon fruit. QTLs linked to genes controlling foreground stripe pattern, depth of rind colour, and background rind colour (i.e., S, D, and Dgo) are located on chromosomes 6, 8 and 4 of the watermelon genome [108]. GWAS revealed chromosome 6 possesses QTLs conditioning rind stripe pattern [63], agreeing with previous studies. Cheng et al. [58] identified major-effect QTL for fruit length (FL5.15) and width (FW8.18) on chromosomes 5 and 6 of watermelon, respectively. Minor-effect QTL, namely FSH2.1 2, FSH5.1 5, FSH7.1 7 and FSH9.1 9, were positioned on chromosomes 2, 5, 7 and 9 of watermelon [58]. The QTL FSH5.1, related to fruit length, is co-localized with QTL FL5.1, related to fruit width [58]. Chromosomes 2, 3 and 6 were identified to possess QTL controlling fruit shape, with chromosome 2 harboring the gene CIFS1, which regulates elongated fruit shape [63]. Two major QTL, namely qrsp6.2, located on chromosomes 6 and RS8.1 on 8, control the rind stripe patterns of watermelon fruit [45]. A major-effect QTL named *RS8.1*, located on chromosome 8, is linked to the rind stripe width of watermelon [45]. Loci for rind colour, and seed coat colour were identified on chromosomes 1, and 3 of the watermelon genome [27]. The QTL qSS6 is linked to seed size, and was co-localized with QTL linked to thousand-seed weight, seed length and seed width in watermelon [27]. The QTL controlling the yellow rind colour (i.e., Clyr) is located on chromosome 1 of watermelon [67]. The gene Cla015407, controlling internode length in watermelon, was mapped on chromosome 9 of watermelon [29]. Two genes conferring fruit hardness, namely, FH2.1 and FH8.1, were mapped on chromosomes 2 and 8 of watermelon [109]. QTL regulating the 'moon and star' trait are located on chromosomes 1 and 8 of watermelon [65]. RH9 and RH10 are associated with rind-hardness, RTO10 with rind toughness, RTH2 with rind thickness and FW9 with fruit weight in watermelon [33]. These QTL are located on three distinct chromosomes, namely, 2, 9, and 10, of watermelon [33]. Fruit flesh bitterness is

mapped on chromosome 1 of watermelon [46]. The QTL linked to the gene controlling dark-green stripe (i.e., *ClGS*) is located on chromosome 6 of watermelon [34].

Table 1. Quantitative trait loci (QTL) associated with qualitative and quantitative phenotypic traits in watermelon.

Qualitative and Quantitative Traits	QTL Name	Crosses	PVE (%)	AdE	DomE	Chr	POC (cM)	References
Days to first male flower	Qdmf3-1	$\text{CLL} \times \text{CLL}$	48-62%	2.93-4.51	-	3	8.21-9.21	[24]
-	Qdmf2	$\text{CLL} \times \text{CLL}$	4	-0.86	-	2	75.71	[24]
	Qdmf3-2	$\text{CLL} \times \text{CLL}$	3.8	0.81	-	3	65.61	[24]
Days to first female flower	Qdff3-1	$\text{CLL}\times\text{CLL}$	39–45%	2.45-2.75	-	3	-	[24]
	Qdff3-2	$\text{CLL} \times \text{CLL}$	5.8	0.87	-	3	79.91	[24]
	Qdff11	$\text{CLL} \times \text{CLL}$	6.9	-1.15	-	11	106.21	[24]
Fruit rind stripe patterns	qrsp1.1	$\text{CLL} \times \text{CLL}$	4.6	-	-	2	-	[30]
	qrsp1.2	$\text{CLL} \times \text{CLL}$	3.6	-	-	2	-	[30]
	qrsp2.1	$\text{CLL} \times \text{CLL}$	7.6	-	-	2	-	[30]
	qrsp2.2	$\text{CLL} \times \text{CLL}$	8	-	-	6	-	[30]
	qrsp6.1	$\text{CLL} \times \text{CLL}$	7.2	-	-	6	-	[30]
	qrsp6.2	$\text{CLL} \times \text{CLL}$	33.5-37.7	-	-	6	-	[30]
	R\$8.1	$\text{CLL} \times \text{CLL}$	49.85	0.04	0.75	8	13	[45]
Fruit flesh colour	qffc4.1	$\text{CLL} \times \text{CLL}$	10.1-47.3	-	-	4	-	[30]
	qffc2.1	$\text{CLL} \times \text{CLL}$	10.4	-	-	2	-	[30]
	qffc5.2	$\text{CLL} \times \text{CLL}$	4.1	-	-	5	-	[30]
	qffc4.2	$\text{CLL} \times \text{CLL}$	3.9	-	-	4	-	[30]
	qffc5.1	$\text{CLL} \times \text{CLL}$	-	-	-	5	-	[30]
Fruit rind colour	qrc-c8-1	$\text{CLL} \times \text{CLL}$	49.94	-0.36		-	142.73-154.74	[26]
	grc6.1	$\text{CLL} \times \text{CLL}$	11.1	-		-	-	[30]
	grc8.1	$\text{CLL} \times \text{CLL}$	11.5–15.5	-		-	-	[30]
Fruit weight	fwt6.2	$\text{CLL} \times \text{CLL}$	5.2	-1.08	-	2	162.2	[23]
C C	fwt9.2	$\text{CLL} \times \text{CLL}$	45.7	3.25	-	2	75.7	[23]
	fwt6.1	$CLL \times Egusi$	8.2	0.67	-0.44	1	63.5	[23]
	fwt9.1	$CLL \times Egusi$	15.6	0.98	0.15	1	-	[23]
	fwt9.2	$CLL \times Egusi$	11.4	-1.04	-0.29	2	98.1	[23]
	fwt9.1	$CLL \times CLC$	20.1	0.54	-0.25	9	60.5	[23]
	fwt11.1	$CLL \times CLC$	13.2	0.31	0.49	11	50.9	[23]
	FW8.1 8	$\text{CLL} \times \text{CLC}$	20.93	1.35	-1.25	8	18.51	[58]
	qfw6.1	$\text{CLL} \times \text{CLL}$	15.1	-	-	6	-	[30]
	qfw8.1	$\text{CLL} \times \text{CLL}$	14	-	-	8	-	[30]
	qfw2.1	$\text{CLL} \times \text{CLL}$	8.6	-	-	2	-	[30]
	qfw3.1	$\text{CLL} \times \text{CLL}$	-	-	-	3	-	[30]
	qfw3.2	$CLL \times CLL$	9.4	-	-	3	-	[30]
Fruit length	fl9.2	$\text{CLL} \times \text{CLC}$	10.4	1.86	-	9	78.7	[23]
	fl11.1	$CLL \times CLC$	40.8	3.65	-	11	13.3	[23]
	qfl6.1	$CLL \times CLL$	13.2	-	-	6	-	[30]
	qfl8.1	$CLL \times CLL$	15.2	-	-	8	-	[30]
	qfl2.1	$\text{CLL} \times \text{CLL}$	7.2–9.6	-	-	2	-	[30]
	qfl5.1	$CLL \times CLL$	5.5 - 7.0	-	-	5	-	[30]
	FL8.1	$\text{CLL} \times \text{CLL}$	5.16	-1.06	0.27	8	-	[31]
	FL11.1	$\text{CLL} \times \text{CLL}$	7.31	0.66	1.47	11	-	[31]
	FL11.2	$\text{CLL} \times \text{CLL}$	11.33	0.11	-2.22	11	-	[31]
	qfl3.1	$\text{CLL} \times \text{CLL}$	19.7	-	-	3	-	[30]

Qualitative and Quantitative Traits	QTL Name	Crosses	PVE (%)	AdE	DomE Chr	POC (cM)	References
Fruit width	qfd2.1	$\text{CLL} \times \text{CLL}$	7.7	-	- 2	-	[30]
	qfd2.2	$\text{CLL} \times \text{CLL}$	15.5	-	- 2	-	[30]
	qfd3.1	$\text{CLL} \times \text{CLL}$	15	-	- 3	-	[30]
	qfd6.1	$\text{CLL} \times \text{CLL}$	6.5	-	- 6	-	[30]
	qfd8.1	$\text{CLL} \times \text{CLL}$	7.2	-	- 8	-	[30]
	qfd9.1	$\text{CLL} \times \text{CLL}$	11.2	-	- 9	-	[30]
	fwd9.2	$\text{CLL} \times \text{CLL}$	43.7-50.0	1.92 - 2.40	- 9	79.7	[23]
	fwd11.1	$\text{CLL} \times \text{CLL}$	8.7-9.0	-1.87	- 11	11.0-12.3	[23]
	fwd9.1	CLL imes Egusi	14.1	1.34	0.49 9	0	[23]
	fwd9.2	CLL imes Egusi	14.6	-1.71	-0.57 9	99.1	[23]
	fwd5.1	$CLL \times CLC$	9.2	-1.21	0.37 5	18.9	[23]
	fwd9.2	$\text{CLL} \times \text{CLC}$	16	1.2	0.88 9	101.6	[23]
	FW8.1	$CLL \times CLC$	13.47	-1.28	0.25 8	-	[31]
	FW8.1 8	$\text{CLL} \times \text{CLL}$	20.93	1.35	-1.25 8	18.51	[58]
Fruit shape index	qfsi2.1	$\text{CLL} \times \text{CLL}$	9.8	-	- 2	-	[30]
	ąfsi2.2	$\text{CLL} \times \text{CLL}$	4.2	-	- 2	-	[30]
	ąfsi3.1	$\text{CLL} \times \text{CLL}$	67.6	-	- 3	-	[30]
	wsbin3-9	$\text{CLL} \times \text{CLL}$	59.7	-0.25	-0.15 3	57.14	[25]
	fsi3.1	$\text{CLL} \times \text{CLL}$	79.7	-0.32	-0.13 3	64.14	[25]
	wsb3-24	$\text{CLL} \times \text{CLL}$	69.1	-0.29	-0.11 3	66.05	[25]
	fsi10.1	$\text{CLL} \times \text{CLL}$	3	-0.07	- 10	33.1	[23]
	fsi11.1	$\text{CLL} \times \text{CLL}$	21.5-56.6	0.29-0.31	- 11	15.3	[23]
	fsi11.1	$CLL \times CLC$	31.8	0.35	0.31 11	20.4	[23]
	FSH2.1 2	$CLL \times CLC$	7.72	0.07	- 2	23.61	[58]
	FSH5.1 5	$CLL \times CLC$	7.75	-0.02	0.08 5	83.21	[58]
	FSH7.1 7	$CLL \times CLC$	9.85	-0.07	- 7	97.91	[58]
	FSH9.1 9	$CLL \times CLC$	6.58	-0.01	0.08 9	4.01	[58]
Fruit rind thickness	rth9.1	$\text{CLL} \times \text{CLL}$	29.3	3.11	- 9	78.7	[23]
	rth6.1	$\text{CLL} \times \text{CLL}$	6.4	-1.168	- 6	188.7	[23]
	rth9.1	$CLL \times CLL$	45	3.26	- 9	75.7	[23]
	rth2.1	$CLL \times Egusi$	17.7	2.345	-0.432 2	42.6	[23]
	rth9.1	$CLL \times Egusi$	8.4	-1.775	-0.811 9	97.1	[23]
Seed coat colour	asc-c3-1	$CLL \times CLL$	58.69	-0.63	-	31.705-32.505	[27]
Seed length	asl5.1	$CLL \times CLL$	-	-	- 5	-	[30]
8	as15.2	$CLL \times CLL$	-	-	- 5	-	[30]
	, asl7.1	$\text{CLL} \times \text{CLL}$		-	- 7	-	[30]
	SL4	$CLL \times CLL$	8.73	-	- 4	-	[27]
	SL6	$CLL \times CLL$	94.11	-	- 6	-	[27]
Seed width	asw3.1	$CLL \times CLL$	-	-	- 3	-	[30]
	asw10.1	$CLL \times CLL$	-	-	- 10	-	[30]
	SW4	$CLL \times CLL$	10.03	-	- 4	-	[27]
	SW6	$CLL \times CLL$	95.26	-	- 6	-	[27]
Seed weight	a20swt2.1	$CLL \times CLL$	50.1	-	- 2	-	[30]
	a20swt10.1	$CLL \times CLL$	9	-	- 10	-	[30]
	TSW4	$CLL \times CLL$	8.87	-	- 4	-	[27]
	TSW6	$CLL \times CLL$	93	_	- 6	-	[27]
Seed thickness	ST6	$CLL \times CLL$	37.92	-	- 6	-	[27]
Seed size	wsh2-52	$CLL \times CLL$	83.7	0.45	0.36 2	-	[25]
occu bize	ss? 1	$CLL \times CLL$	92.3	0.45	0.39 2	-	[25]
	wsb2-13	$CLL \times CLL$	86.4	0.45	0.37 2	-	[25]

Table 1. Cont.

CLL, *citrullus lanatus* var. *lanatus*; CLC, *citrullus lanatus* var. *citroides*; PVE, phenotypic variation explained; AdE, additive effects; DomE, dominance effect; Chr, chromosome; POC, position of chromosome; cM, centimorgan, - not available.

Similarly *qsw2.1*, *qswl2.1*, and *q20swt2.1*, located on chromosome 2, reportedly regulate seed size in watermelon [30]. Some previous and recent studies indicated that major QTL controlling seed traits are located on chromosomes 2 and 6 [25,27,30], suggesting that these

regions harbour genes conditioning seed component traits in watermelon. A QTL analysis for leaf morphology (e.g., leaf shape, length, width and size), plant architecture (e.g., plant height and branching capacity), and some floral characters such as male sterility has yet to be undertaken in watermelon. A QTL analysis of qualitative and quantitative phenotypic traits will facilitate the successful application of gene-editing technologies to develop new watermelon varieties.

9. Clustered, Regularly Interspaced, Short Palindromic Repeats (CRISPR/Cas9) Gene-Editing Technology for Breeding in Watermelon

Conventional breeding approaches have aided in the release of watermelon cultivars with excellent qualitative and quantitative phenotypic traits [10,110,111]. However, accelerated breeding and the release of market-preferred watermelon varieties will require the application of both conventional and non-conventional breeding approaches.

Gene-editing technologies complement conventional breeding approaches to the targeted manipulation of genes and speed breeding. The CRISPR/Cas9 is a widely used gene-editing platform for efficient breeding in crop-improvement programs. The potential application of CRISPR/Cas9 gene-editing for breeding watermelon varieties has been proposed for fruit-quality traits [74]. In the present study, we propose CRISPR/Cas9-mediated breeding targeting other important economic traits. The application of CRISPR/Cas9 for the targeted editing of genes controlling leaf morphology, floral traits (plant architecture, external fruit traits and seed coat colours has yet to be explored in watermelon. Chromosome 3 harboured major QTLs linked to flowering traits [24,32]. This QTL is reportedly a key genomic region for CRISPR/Cas9-mediated gene-editing. Chromosomes 1, 2 and 6 harbour major QTLs for rind stripe patterns, while chromosomes 1, 3 and 4 harbour major QTLs linked to fruit flesh colour, chromosome 8 contains QTLs affecting rind colour, and chromosome 3 harbours QTLs for seed coat colour (Table 1). These QTL are candidate genomic regions for CRISPR/Cas9-mediated gene dissection and introgression. Major QTL controlling rind hardness are located on chromosomes 4 and 10 of watermelon [33,105], for variety development with an extended post-harvest shelf life and cracking resistance. Major QTL conditioning fruit-yield-related traits (i.e., fruit weight, length, and width and shape index) were located on chromosomes 2, 3, 9 and 11, which could be subjected to CRISPR/Cas9 gene-editing. Major QTL linked to seed yield-related traits (i.e., seed length, width and size) were located on chromosomes 2 and 6 of the watermelon genome (Table 1), which are suited to precision-editing using CRISPR/Cas9. The integration of the CRISPR-Cas9 gene-editing technology in watermelon improvement programs will increase breeding efficiency and aid in the accelerated development of market-preferred varieties.

10. Conclusions and Perspectives

Qualitative and quantitative phenotypic traits are key attributes for breeding marketpreferred watermelon varieties. This review presented the progress made in gene discovery and the genetic analysis of qualitative and quantitative phenotypic traits in sweet watermelon to facilitate trait integration, phenotypic and genomic selection and the development of novel varieties with yield potential and desirable agronomic and horticultural attributes. Major and minor genes conditioning the inheritance of plant architecture, floral characters, fruit traits and seed morphology were presented. The developed molecular markers and QTL analysis associated with qualitative and quantitative traits were presented to aid in marker-assisted breeding and genomic selection. The potential application of the CRISPR/Cas9 gene-editing technology for breeding new varieties with the desired agronomic and horticultural attributes was highlighted. The information presented in this review is useful in the breeding of new varieties to serve growers, consumers and the sweet watermelon industry. There is a need for further comparative transcriptome analysis to unravel the genetic regulation of leaf morphology (e.g., leaf shape, length, width and size), plant architecture such as plant height and branching capacity, and floral characters and male sterility systems. This will aid in the marker-assisted and genomic breeding of novel sweet watermelon varieties with market-preferred traits.

Author Contributions: J.M., R.M.N. and D.M. conceived the idea. J.M., H.S., R.M.N. and D.M. wrote the manuscript. H.S. reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the University of KwaZulu-Natal.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: This research was supported by the Limpopo Department of Agriculture and Rural Development and the African Centre for Crop Improvement of the University of KwaZulu-Natal.

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

References

- Chomicki, G.; Renner, S.S. Watermelon origin solved with molecular phylogenetics including Linnaean Material: Another example of Museomics. *New Phytol.* 2015, 205, 526–532. [CrossRef]
- Lewinsohn, E.; Sitrit, Y.; Bar, E.; Azulay, Y.; Meir, A.; Zamir, D.; Tadmor, Y. Carotenoid pigmentation affects the volatile composition of tomato and watermelon fruits, as revealed by comparative genetic analyses. J. Agric. Food Chem. 2005, 53, 3142–3148. [CrossRef]
- 3. Tadmor, Y.; King, S.; Levi, A.; Davis, A.; Meir, A.; Wasserman, B.; Hirschberg, J.; Lewinsohn, E. Comparative fruit colouration in watermelon and tomato. *Food Res. Int.* 2005, *38*, 837–841. [CrossRef]
- Perkins-Veazie, P.; Collins, J.K.; Davis, A.R.; Roberts, W. Carotenoid Content of 50 Watermelon Cultivars. J. Agric. Food Chem. 2006, 54, 2593–2597. [CrossRef]
- Liu, C.; Zhang, H.; Dai, Z.; Liu, X.; Liu, Y.; Deng, X.; Chen, F.; Xu, J. Volatile chemical and carotenoid profiles in watermelons [*Citrullus vulgaris* (Thunb.) Schrad (Cucurbitaceae)] with different flesh colors. *Food Sci. Biotechnol.* 2012, 21, 531–541. [CrossRef]
- Yoo, K.S.; Bang, H.; Lee, E.J.; Crosby, K.M.; Patil, B. Variation of carotenoid, sugar, and ascorbic acid concentrations in watermelon genotypes and genetic analysis. *Hortic. Environ. Biotechnol.* 2012, 53, 552–560. [CrossRef]
- Ren, Y.; McGregor, C.; Zhang, Y.; Gong, G.; Zhang, H.; Guo, S.; Sun, H.; Cai, W.; Zhang, J.; Xu, Y. An integrated genetic map based on four mapping populations and quantitative trait loci associated with economically important traits in watermelon (*Citrullus lanatus*). *BMC Plant Biol.* 2014, 14, 33. [CrossRef]
- 8. Huang, Y.; Zhao, L.; Kong, Q.; Cheng, F.; Niu, M.; Xie, J.; Nawaz, M.A.; Bie, Z. Comprehensive mineral nutrition analysis of watermelon grafted onto two different rootstocks. *Hortic. Plant J.* **2016**, *2*, 105–113. [CrossRef]
- 9. Tabiri, B.; Agbenorhevi, J.K.; Wireko-Manu, F.D.; Ompouma, E.I. Watermelon seeds as food: Nutrient composition, phytochemicals and antioxidant Activity. *Int. J. Nutr. Food Sci.* **2016**, *5*, 139. [CrossRef]
- 10. Gao, L.; Zhao, S.; Lu, X.; He, N.; Liu, W. 'SW', A new watermelon cultivar with a sweet and sour flavour. *HortScience* 2018, 53, 895–896. [CrossRef]
- Jin, B.; Lee, J.; Kweon, S.; Cho, Y.; Choi, Y.; Lee, S.J.; Park, Y. Analysis of flesh color-related carotenoids and development of a CRTISO gene-based DNA marker for prolycopene accumulation in watermelon. *Hortic. Environ. Biotechnol.* 2019, 60, 399–410. [CrossRef]
- Jawad, U.M.; Gao, L.; Gebremeskel, H.; Bin Safdar, L.; Yuan, P.; Zhao, S.; Xuqiang, L.; Nan, H.; Hongju, Z.; Liu, W. Expression pattern of sugars and organic acids regulatory genes during watermelon fruit development. *Sci. Hortic.* 2020, 265, 109102. [CrossRef]
- 13. Ji, G.; Zhang, J.; Gong, G.; Shi, J.; Zhang, H.; Ren, Y.; Guo, S.; Gao, J.; Shen, H.; Xu, Y. Inheritance of sex forms in watermelon (*Citrullus lanatus*). Sci. Hortic. 2015, 193, 367–373. [CrossRef]
- 14. Dou, J.; Lu, X.; Ali, A.; Zhao, S.; Zhang, L.; He, N.; Liu, W. Genetic mapping reveals a marker for yellow skin in watermelon (*Citrullus lanatus* L.). *PLoS ONE* **2018**, *13*, e0200617. [CrossRef]
- 15. Dou, J.; Zhao, S.; Lu, X.; He, N.; Zhang, L.; Ali, A.; Kuang, H.; Liu, W. Genetic mapping reveals a candidate gene (*ClFS1*) for fruit shape in watermelon (*Citrullus lanatus* L.). *Theor. Appl. Genet.* **2018**, *131*, 947–958. [CrossRef]
- 16. Legendre, R.; Kuzy, J.; McGregor, C. Markers for selection of three alleles of *ClSUN25-26-27a* (*Cla011257*) associated with fruit shape in watermelon. *Mol. Breed.* **2020**, *40*, 19. [CrossRef]
- 17. Liu, S.; Gao, Z.; Wang, X.; Luan, F.; Dai, Z.; Yang, Z.; Zhang, Q. Nucleotide variation in the phytoene synthase (*ClPsy1*) gene contributes to golden flesh in watermelon (*Citrullus lanatus* L.). *Theor. Appl. Genet.* **2021**, *135*, 185–200. [CrossRef] [PubMed]
- 18. Guner, N.; Wehner, T.C. The Genes of Watermelon. HortScience 2004, 39, 1175–1182. [CrossRef]
- Gusmini, G.; Wehner, T.C. Genes Determining Rind Pattern Inheritance in Watermelon: A Review. *HortScience* 2005, 40, 1928–1930. [CrossRef]

- 20. Wei, C.; Chen, X.; Wang, Z.; Liu, Q.; Li, H.; Zhang, Y.; Ma, J.; Yang, J.; Zhang, X. Genetic mapping of the *LOBED LEAF 1* (*CILL1*) gene to a 127.6-kb region in watermelon (*Citrullus lanatus* L.). *PLoS ONE* **2017**, *12*, e0180741. [CrossRef]
- Prothro, J.; Abdel-Haleem, H.; Bachlava, E.; White, V.; Knapp, S.; McGregor, C. Quantitative trait loci associated with sex expression in an inter-subspecific watermelon population. J. Am. Soc. Hortic. Sci. 2013, 138, 125–130. [CrossRef]
- Dong, W.; Wu, D.; Yan, C.; Wu, D. Mapping and analysis of a novel genic male sterility gene in watermelon (*Citrullus lanatus*). Front. Plant Sci. 2021, 12, 611. [CrossRef] [PubMed]
- Sandlin, K.; Prothro, J.; Heesacker, A.; Khalilian, N.; Okashah, R.; Xiang, W.; Bachlava, E.; Caldwell, D.G.; Taylor, C.A.; Seymour, D.K.; et al. Comparative mapping in watermelon (*Citrullus lanatus* (Thunb.) Matsum. et Nakai). *Theor. Appl. Genet.* 2012, 125, 1603–1618. [CrossRef] [PubMed]
- 24. McGregor, C.; Waters, V.; Vashisth, T.; Abdel-Haleem, H. Flowering time in watermelon is associated with a major quantitative trait locus on chromosome 3. *J. Am. Soc. Hortic. Sci.* **2014**, 139, 48–53. [CrossRef]
- Kim, K.-H.; Hwang, J.-H.; Han, D.-Y.; Park, M.; Kim, S.; Choi, D.; Kim, Y.; Lee, G.P.; Kim, S.T.; Park, Y.-H. Major quantitative trait loci and putative candidate genes for powdery mildew resistance and fruit-related traits revealed by an intraspecific genetic map for watermelon (*Citrullus lanatus* var. *lanatus*). *PLoS ONE* 2015, *10*, e0145665. [CrossRef]
- Li, B.; Lu, X.; Dou, J.; Aslam, A.; Gao, L.; Zhao, S.; He, N.; Liu, W. Construction of a high-density genetic map and mapping of fruit traits in watermelon (*Citrullus lanatus* L.) based on whole-genome resequencing. *Int. J. Mol. Sci.* 2018, 19, 3268. [CrossRef] [PubMed]
- 27. Li, N.; Shang, J.; Wang, J.; Zhou, D.; Li, N.; Ma, S. Fine mapping and discovery of candidate genes for seed size in watermelon by genome survey sequencing. *Sci. Rep.* **2018**, *8*, 17843. [CrossRef]
- 28. Fall, L.A.; Perkins-Veazie, P.; Ma, G.; McGregor, C. QTLs associated with flesh quality traits in an elite × elite watermelon population. *Euphytica* **2019**, *215*, 30. [CrossRef]
- 29. Gebremeskel, H.; Dou, J.; Li, B.; Zhao, S.; Muhammad, U.; Lu, X.; He, N.; Liu, W. Molecular mapping and candidate gene analysis for GA₃ responsive short internode in watermelon (*Citrullus lanatus*). *Int. J. Mol. Sci.* **2019**, *21*, 290. [CrossRef]
- 30. Liang, X.; Gao, M.; Amanullah, S.; Guo, Y.; Liu, X.; Xu, H.; Liu, J.; Gao, Y.; Yuan, C.; Luan, F. Identification of QTLs linked with watermelon fruit and seed traits using GBS-based high-resolution genetic mapping. *Sci. Hortic.* **2022**, *303*, 111237. [CrossRef]
- 31. Liu, S.; Gao, P.; Wang, X.; Davis, A.R.; Baloch, A.M.; Luan, F. Mapping of quantitative trait loci for lycopene content and fruit traits in *Citrullus lanatus*. *Euphytica* 2014, 202, 411–426. [CrossRef]
- 32. Gimode, W.; Clevenger, J.; McGregor, C. Fine-mapping of a major quantitative trait locus Qdff3-1 controlling flowering time in watermelon. *Mol. Breed.* **2019**, *40*, 3. [CrossRef]
- Yang, T.; Amanullah, S.; Pan, J.; Chen, G.; Liu, S.; Ma, S.; Wang, J.; Gao, P.; Wang, X. Identification of putative genetic regions for watermelon rind hardness and related traits by BSA-seq and QTL mapping. *Euphytica* 2021, 217, 1–18. [CrossRef]
- Wang, D.; Zhang, M.; Xu, N.; Yang, S.; Dou, J.; Liu, D.; Zhu, L.; Zhu, H.; Hu, J.; Ma, C.; et al. Fine mapping a ClGS gene controlling dark-green stripe rind in watermelon. Sci. Hortic. 2021, 291, 110583. [CrossRef]
- 35. Tian, S.; Jiang, L.; Gao, Q.; Zhang, J.; Zong, M.; Zhang, H.; Ren, Y.; Guo, S.; Gong, G.; Liu, F.; et al. Efficient CRISPR/Cas9-based gene knockout in watermelon. *Plant Cell Rep.* **2017**, *36*, 399–406. [CrossRef] [PubMed]
- 36. Tian, S.; Jiang, L.; Cui, X.; Zhang, J.; Guo, S.; Li, M.; Zhang, H.; Ren, Y.; Gong, G.; Zong, M.; et al. Engineering herbicide-resistant watermelon variety through CRISPR/Cas9-mediated base-editing. *Plant Cell Rep.* **2018**, *37*, 1353–1356. [CrossRef] [PubMed]
- 37. Zhang, J.; Guo, S.; Ji, G.; Zhao, H.; Sun, H.; Ren, Y.; Tian, S.; Li, M.; Gong, G.; Zhang, H.; et al. A unique chromosome translocation disrupting *ClWIP1* leads to gynoecy in watermelon. *Plant J.* **2019**, *101*, 265–277. [CrossRef]
- Wang, Y.; Wang, J.; Guo, S.; Tian, S.; Zhang, J.; Ren, Y.; Li, M.; Gong, G.; Zhang, H.; Xu, Y. CRISPR/Cas9-mediated mutagenesis of CIBG1 decreased seed size and promoted seed germination in watermelon. *Hortic. Res.* 2021, *8*, 70. [CrossRef]
- Levi, A.; Thies, J.A.; Wechter, W.P.; Harrison, H.F.; Simmons, A.M.; Reddy, U.K.; Nimmakayala, P.; Fei, Z. High frequency oligonucleotides: Targeting active gene (HFO-TAG) markers revealed wide genetic diversity among *Citrullus* spp. accessions useful for enhancing disease or pest resistance in watermelon cultivars. *Genet. Resour. Crop Evol.* 2012, 60, 427–440. [CrossRef]
- 40. Achigan-Dako, E.G.; Avohou, E.S.; Linsoussi, C.; Ahanchede, A.; Vodouhe, R.S.; Blattner, F.R. Phenetic characterization of *Citrullus spp*. (*Cucurbitaceae*) and differentiation of egusi-type (*C. mucosospermus*). *Genet. Resour. Crop Evol.* **2015**, *62*, 1159–1179. [CrossRef]
- Yang, X.; Ren, R.; Ray, R.; Xu, J.; Li, P.; Zhang, M.; Liu, G.; Yao, X.; Kilian, A. Genetic diversity and population structure of core watermelon (*Citrullus lanatus*) genotypes using DArTseq-based SNPs. *Plant Genet. Resour.* 2016, 14, 226–233. [CrossRef]
- Kim, Y.-C.; Choi, D.; Zhang, C.; Liu, H.-F.; Lee, S. Profiling cucurbitacins from diverse watermelons (*Citrullus* spp.). *Hortic.* Environ. Biotechnol. 2018, 59, 557–566. [CrossRef]
- Tannin-Spitz, T.; Bergman, M.; Grossman, S. Cucurbitacin glycosides: Antioxidant and free-radical scavenging activities. *Biochem. Biophys. Res. Commun.* 2007, 364, 181–186. [CrossRef]
- 44. Duangmano, S.; Sae-Lim, P.; Suksamrarn, A.; E Domann, F.; Patmasiriwat, P. Cucurbitacin B inhibits human breast cancer cell proliferation through disruption of microtubule polymerization and nucleophosmin/B23 translocation. *BMC Complement. Altern. Med.* **2012**, *12*, 185. [CrossRef] [PubMed]
- 45. Zhang, Z.; Zhang, Y.; Sun, L.; Qiu, G.; Sun, Y.; Zhu, Z.; Luan, F.; Wang, X. Construction of a genetic map for *Citrullus lanatus* based on CAPS markers and mapping of three qualitative traits. *Sci. Hortic.* **2018**, *233*, 532–538. [CrossRef]
- Gong, C.; Li, B.; Anees, M.; Zhu, H.; Zhao, S.; He, N.; Lu, X.; Liu, W. Fine-mapping reveals that the *bHLH* gene *Cla011508* regulates the bitterness of watermelon fruit. *Sci. Hortic.* 2021, 292, 110626. [CrossRef]

- Zhou, Y.; Ma, Y.; Zeng, J.; Duan, L.; Xue, X.; Wang, H.; Lin, T.; Liu, Z.; Zeng, K.; Zhong, Y.; et al. Convergence and divergence of bitterness biosynthesis and regulation in Cucurbitaceae. *Nat. Plants* 2016, *2*, 16183. [CrossRef]
- Davidovich-Rikanati, R.; Shalev, L.; Baranes, N.; Meir, A.; Itkin, M.; Cohen, S.; Zimbler, K.; Portnoy, V.; Ebizuka, Y.; Shibuya, M.; et al. Recombinant yeast as a functional tool for understanding bitterness and cucurbitacin biosynthesis in watermelon (*Citrullus* spp). Yeast 2015, 32, 103–114.
- Manzano, S.; Martínez, C.; García, J.M.; Megías, Z.; Jamilena, M. Involvement of ethylene in sex expression and female flower development in watermelon (*Citrullus lanatus*). *Plant Physiol. Biochem.* 2014, 85, 96–104. [CrossRef]
- Aguado, E.; García, A.; Manzano, S.; Valenzuela, J.L.; Cuevas, J.; Pinillos, V.; Jamilena, M. The sex-determining gene *CitACS4* is a pleiotropic regulator of flower and fruit development in watermelon (*Citrullus lanatus*). *Plant Reprod.* 2018, 31, 411–426. [CrossRef]
- 51. Manzano, S.; Aguado, E.; Martínez, C.; Megías, Z.; García, A.; Jamilena, M. The ethylene biosynthesis gene *CitACS4* regulates monoecy/andromonoecy in watermelon (*Citrullus lanatus*). *PLoS ONE* **2016**, *11*, e0154362. [CrossRef]
- Aguado, E.; García, A.; Iglesias-Moya, J.; Romero, J.; Wehner, T.C.; Gómez-Guillamón, M.L.; Pico, M.B.; Garces-Claver, A.; Martínez, C.; Jamilena, M. Mapping a partial andromonoecy locus in *Citrullus lanatus* using BSA-seq and GWAS approaches. *Front. Plant Sci.* 2020, 11, 1243. [CrossRef] [PubMed]
- 53. Zhu, Y.C.; Yuan, G.P.; Jia, S.F.; An, G.L.; Li, W.H.; Sun, D.X.; Liu, J.P. Transcriptomic profiling of watermelon (*Citrullus lanatus*) provides insights into male flowers development. *J. Integr. Agric* 2022, 21, 407–421. [CrossRef]
- 54. Rhee, S.-J.; Seo, M.; Jang, Y.-J.; Cho, S.; Lee, G.P. Transcriptome profiling of differentially expressed genes in floral buds and flowers of male sterile and fertile lines in watermelon. *BMC Genom.* **2015**, *16*, 914. [CrossRef] [PubMed]
- 55. Zhang, R.; Chang, J.; Li, J.; Lan, G.; Xuan, C.; Li, H.; Ma, J.; Zhang, Y.; Yang, J.; Tian, S.; et al. Disruption of the bHLH transcription factor Abnormal Tapetum 1 causes male sterility in watermelon. *Hortic. Res.* **2021**, *8*, 258. [CrossRef]
- 56. Yi, L.; Wang, Y.; Wang, F.; Song, Z.; Li, J.; Gong, Y.; Dai, Z. Comparative transcriptome analysis reveals the molecular mechanisms underlying male sterility in autotetraploid watermelon. *J. Plant Growth Regul.* **2022**. [CrossRef]
- 57. Maragal, S.; Rao, E.S.; Reddy, D.C.L. Genetic analysis of fruit quality traits in prebred lines of watermelon derived from a wild accession of *Citrullus amarus*. *Euphytica* **2019**, 215, 199. [CrossRef]
- Cheng, Y.; Luan, F.; Wang, X.; Gao, P.; Zhu, Z.; Liu, S.; Baloch, A.M.; Zhang, Y. Construction of a genetic linkage map of watermelon (*Citrullus lanatus*) using CAPS and SSR markers and QTL analysis for fruit quality traits. *Sci. Hortic.* 2016, 202, 25–31. [CrossRef]
- Kim, H.; Han, D.; Kang, J.; Choi, Y.; Levi, A.; Lee, G.P.; Park, Y. Sequence-characterized amplified polymorphism markers for selecting rind stripe pattern in watermelon (*Citrullus lanatus* L.). *Hortic. Environ. Biotechnol.* 2015, 56, 341–349. [CrossRef]
- 60. Lou, L.; Wehner, T.C. Qualitative inheritance of external fruit traits in watermelon. HortScience 2016, 51, 487–496. [CrossRef]
- 61. Yang, H.-B.; Park, S.-W.; Park, Y.; Lee, G.P.; Kang, S.-C.; Kim, Y.K. Linkage analysis of the three loci determining rind color and stripe pattern in watermelon. *Korean J. Hortic. Sci. Technol.* **2015**, *33*, 559–565. [CrossRef]
- 62. Yue, Z.; Ma, R.; Cheng, D.; Yan, X.; He, Y.; Wang, C.; Pan, X.; Yin, L.; Zhang, X.; Wei, C. Candidate gene analysis of watermelon stripe pattern locus CISP ongoing recombination suppression. *Theor. Appl. Genet.* **2021**, *134*, 3263–3277. [CrossRef] [PubMed]
- 63. Guo, S.; Zhao, S.; Sun, H.; Wang, X.; Wu, S.; Lin, T.; Ren, Y.; Gao, L.; Deng, Y.; Zhang, J.; et al. Resequencing of 414 cultivated and wild watermelon accessions identifies selection for fruit quality traits. *Nat. Genet.* **2019**, *51*, 1616–1623. [CrossRef] [PubMed]
- 64. Maragal, S.; Nagesh, G.C.; Reddy, D.C.L.; Rao, E.S. QTL mapping identifies novel loci and putative candidate genes for rind traits in watermelon. *3 Biotech* 2022, *12*, 46. [CrossRef] [PubMed]
- 65. Liu, D.; Sun, D.; Liang, J.; Dou, J.; Yang, S.; Zhu, H.; Hu, J.; Sun, S.; Yang, L. Characterization and bulk segregant analysis of 'moon and star' appearance in watermelon. *Sci. Hortic.* **2021**, *285*, 110140. [CrossRef]
- Li, B.; Zhao, S.; Dou, J.; Ali, A.; Gebremeskel, H.; Gao, L.; He, N.; Lu, X.; Liu, W. Genetic mapping and development of molecular markers for a candidate gene locus controlling rind color in watermelon. *Theor. Appl. Genet.* 2019, 132, 2741–2753. [CrossRef] [PubMed]
- Liu, D.; Yang, H.; Yuan, Y.; Zhu, H.; Zhang, M.; Wei, X.; Sun, D.; Wang, X.; Yang, S.; Yang, L. Comparative Transcriptome Analysis Provides Insights Into Yellow Rind Formation and Preliminary Mapping of the Clyr (Yellow Rind) Gene in Watermelon. *Front. Plant Sci.* 2020, *11*, 192. [CrossRef]
- Yang, K.-K.; Liang, Z.-H.; Wu, C.-J. Analysis on differentially expressed genes in watermelon rind color based on RNA–Seq. J. Cent. South Univ. 2016, 23, 2818–2826. [CrossRef]
- Kumar, R.; Wehner, T.C. Discovery of Second Gene for Solid Dark Green versus Light Green Rind Pattern in Watermelon. J. Hered. 2011, 102, 489–493. [CrossRef]
- 70. Poole, C.F.; Grimball, P.C.; Porter, D.R. Inheritance of seed characters in watermelon. J. Agric. Res. 1941, 63, 433-456.
- 71. Wehner, T.C. Watermelons. In *Handbook of Plant Breeding. Vegetables I: Asteraceae, Brassicaceae, Chenopodiaceae, and Cucurbitaceae;* Prohens, J., Nuez, F., Eds.; Springer Science Business LLC: New York, NY, USA, 2008; pp. 381–418.
- 72. Singh, D.; Singh, R.; Sandhu, J.S.; Chunneja, P. Morphological and genetic diversity analysis of *Citrullus landraces* from India and their genetic inter relationship with continental watermelons. *Sci. Hortic.* **2017**, *218*, 240–248. [CrossRef]
- 73. Assefa, A.D.; Hur, O.S.; Ro, N.Y.; Lee, J.E.; Hwang, A.J.; Kim, B.S.; Rhee, J.H.; Yi, J.Y.; Kim, J.H.; Lee, H.S.; et al. Fruit morphology, citrulline, and arginine levels in diverse watermelon (*Citrullus lanatus*) germplasm collections. *Plants* 2020, 9, 1054. [CrossRef] [PubMed]

- 74. Mashilo, J.; Shimelis, H.; Ngwepe, R.M.; Thungo, Z. Genetic analysis of fruit quality traits in sweet watermelon (*Citrullus lanatus* var. *lanatus*): *A review. Front. Plant Sci.* **2022**, *13*, 834696. [CrossRef] [PubMed]
- Li, B.; Lu, X.; Gebremeskel, H.; Zhao, S.; He, N.; Yuan, P.; Gong, C.; Mohammed, U.; Liu, W. Genetic mapping and discovery of the candidate gene for black seed coat color in watermelon (*Citrullus lanatus*). Front. Plant Sci. 2020, 10, 1689. [CrossRef] [PubMed]
- Paudel, L.; Clevenger, J.; McGregor, C. Chromosomal locations and interactions of four loci associated with seed coat color in watermelon. *Front. Plant Sci.* 2019, 10, 788. [CrossRef]
- 77. Solmaz, I.; Sarı, N. Characterization of watermelon (*Citrullus lanatus*) accessions collected from Turkey for morphological traits. *Genet. Resour. Crop Evol.* 2009, 56, 173–188. [CrossRef]
- Szamosi, C.; Solmaz, I.; Sari, N.; Bársony, C. Morphological characterization of Hungarian and Turkish watermelon (*Citrullus lanatus* (Thunb.) Matsum. et Nakai) genetic resources. *Genet. Resour. Crop Evol.* 2009, 56, 1091–1105. [CrossRef]
- 79. Elbekkay, M.; Hamza, H.; Neily, M.H.; Djebali, N.; Ferchichi, A. Characterization of watermelon local cultivars from Southern Tunisia using morphological traits and molecular markers. *Euphytica* **2021**, 217, 74–89. [CrossRef]
- Dong, W.; Wu, D.; Li, G.; Wu, D.; Wang, Z. Next-generation sequencing from bulked segregant analysis identifies a dwarfism gene in watermelon. *Sci. Rep.* 2018, *8*, 2908. [CrossRef]
- Jang, Y.J.; Yun, H.S.; Rhee, S.-J.; Seo, M.; Kim, Y.; Lee, G.P. Exploring molecular markers and candidate genes responsible for watermelon dwarfism. *Hortic. Environ. Biotechnol.* 2020, 61, 173–182. [CrossRef]
- 82. Zhang, T.; Liu, J.; Amanullah, S.; Ding, Z.; Cui, H.; Luan, F.; Gao, P. Fine mapping of *Cla015407* controlling plant height in watermelon. *J. Am. Soc. Hortic. Sci.* 2021, 146, 196–205. [CrossRef]
- Wei, C.; Zhu, C.; Yang, L.; Zhao, W.; Ma, R.; Li, H.; Zhang, Y.; Ma, J.; Yang, J.; Zhang, X. A point mutation resulting in a 13 bp deletion in the coding sequence of Cldf leads to a GA-deficient dwarf phenotype in watermelon. *Hortic. Res.* 2019, *6*, 132. [CrossRef] [PubMed]
- 84. Zhu, H.; Zhang, M.; Sun, S.; Yang, S.; Li, J.; Li, H.; Yang, H.; Zhang, K.; Hu, J.; Liu, D.; et al. A single nucleotide deletion in an ABC transporter gene leads to a dwarf phenotype in watermelon. *Front. Plant Sci.* **2019**, *10*, 1399. [CrossRef] [PubMed]
- 85. Li, Y.; Xu, A.; Dong, W.; Li, Z.; Li, G. Genetic analysis of a dwarf vine and small fruit watermelon mutant. *Hortic. Plant J.* **2016**, *2*, 224–228. [CrossRef]
- Cho, Y.; Lee, S.; Park, J.; Kwon, S.; Park, G.; Kim, H.; Park, Y. Identification of a candidate gene controlling semi-dwarfism in watermelon, *Citrullus lanatus*, using a combination of genetic linkage mapping and QTL-seq. *Hortic. Environ. Biotechnol.* 2021, 62, 447–459. [CrossRef]
- Dou, J.; Yang, H.; Sun, D.; Yang, S.; Sun, S.; Zhao, S.; Lu, X.; Zhu, H.; Liu, D.; Ma, C.; et al. The branchless gene *Clbl* in watermelon encoding a *TERMINAL FLOWER 1* protein regulates the number of lateral branches. *Theor. Appl. Genet.* 2021, 135, 65–79. [CrossRef]
- McGregor, C.; Waters, V. Flowering patterns of pollenizer and triploid watermelon cultivars. *HortScience* 2014, 49, 714–721. [CrossRef]
- 89. Stone, S.; Boyhan, G.; McGregor, C. Inter- and intracultivar variation of heirloom and open-pollinated watermelon cultivars. *HortScience* **2019**, *54*, 212–220. [CrossRef]
- 90. Karaca, F.; Yetişir, H.; Solmaz, I.; Çandir, E.; Kurt, Ş.; Sari, N.; Güler, Z. Rootstock potential of Turkish *Lagenaria siceraria* germplasm for watermelon: Plant growth, yield and quality. *Turk. J. Agric. For.* **2012**, *36*, 167–177.
- 91. Freeman, J.H.; Miller, G.; Olson, S.; Stall, W. Diploid watermelon pollenizer cultivars differ with respect to triploid watermelon yield. *HortTechnology* **2007**, *17*, 518–522. [CrossRef]
- Dittmar, P.J.; Monks, D.W.; Schultheis, J.R. Maximum potential vegetative and floral production and fruit characteristics of watermelon pollenizers. *HortScience* 2009, 44, 59–63. [CrossRef]
- 93. Dittmar, P.J.; Monks, D.W.; Schultheis, J.R. Use of commercially available pollenizers for optimizing triploid watermelon production. *HortScience* 2010, 45, 541–545. [CrossRef]
- Garantonakis, N.; Varikou, K.; Birouraki, A.; Edwards, M.; Kalliakaki, V.; Andrinopoulos, F. Comparing the pollination services of honey bees and wild bees in a watermelon field. *Sci. Hortic.* 2016, 204, 138–144. [CrossRef]
- 95. Kombo, M.D.; Sari, N. Rootstock effects on seed yield and quality in watermelon. *Hortic. Environ. Biotechnol.* **2019**, *60*, 303–312. [CrossRef]
- 96. Dia, M.; Wehner, T.C.; Hassell, R.; Price, D.S.; Boyhan, G.E.; Olson, S.; King, S.; Davis, A.R.; Tolla, G.E. Genotype × environment interaction and stability analysis for watermelon fruit yield in the United States. *Crop Sci.* **2016**, *56*, 1645–1661. [CrossRef]
- 97. Gong, C.; Zhao, S.; Yang, D.; Lu, X.; Anees, M.; He, N.; Zhu, H.; Zhao, Y.; Liu, W. Genome-wide association analysis provides molecular insights into natural variation in watermelon seed size. *Hortic. Res.* **2022**, *9*, 74. [CrossRef]
- 98. Bang, H.; Kim, S.; Leskovar, D.; King, S. Development of a codominant CAPS marker for allelic selection between canary yellow and red watermelon based on SNP in lycopene β-cyclase (*LCYB*) gene. *Mol. Breed.* 2007, 20, 63–72. [CrossRef]
- Bang, H.; Yi, G.; Kim, S.; Leskovar, D.; Patil, B.S. Watermelon lycopene β-cyclase: Promoter characterization leads to the development of a PCR marker for allelic selection. *Euphytica* 2014, 200, 363–378. [CrossRef]
- 100. Gama, R.; Santos, C.; Dias, R.; Alves, J.; Nogueira, T. Microsatellite markers linked to the locus of the watermelon fruit stripe pattern. *Genet. Mol. Res.* 2015, 14, 269–276. [CrossRef]

- 101. Wang, C.; Qiao, A.; Fang, X.; Sun, L.; Gao, P.; Davis, A.R.; Liu, S.; Luan, F. Fine Mapping of lycopene content and flesh color related gene and development of molecular marker–assisted selection for flesh color in watermelon (*Citrullus lanatus*). Front. Plant Sci. 2019, 10, 1240. [CrossRef]
- 102. Subburaj, S.; Lee, K.; Jeon, Y.; Tu, L.; Son, G.; Choi, S.; Lim, Y.-P.; McGregor, C.; Lee, G.J. Whole genome resequencing of watermelons to identify single nucleotide polymorphisms related to flesh color and lycopene content. *PLoS ONE* **2019**, *14*, e0223441. [CrossRef]
- 103. Lee, S.; Park, G.; Choi, Y.; Park, S.; Kim, H.; Lee, O.; Kim, T.; Park, Y. Whole-genome resequencing of near-isogenic lines reveals a genomic region associated with high *trans*-lycopene contents in watermelon. *Plants* **2021**, *11*, 8. [CrossRef] [PubMed]
- 104. Pei, S.; Liu, Z.; Wang, X.; Luan, F.; Dai, Z.; Yang, Z.; Zhang, Q.; Liu, S. Quantitative trait loci and candidate genes responsible for pale green flesh colour in watermelon (*Citrullus lanatus*). *Plant Breed.* 2021, 140, 349–359. [CrossRef]
- 105. Hashizume, T.; Shimamoto, I.; Hirai, M. Construction of a linkage map and QTL analysis of horticultural traits for watermelon [*Citrullus lanatus* (THUNB.) MATSUM & NAKAI] using RAPD, RFLP and ISSR markers. *Theor. Appl. Genet.* 2003, 106, 779–785. [CrossRef] [PubMed]
- 106. Prothro, J.; Sandlin, K.; Abdel-Haleem, H.; Bachlava, E.; White, V.; Knapp, S.; McGregor, C. Main and epistatic quantitative trait loci associated with seed size in watermelon. *J. Am. Soc. Hortic. Sci.* **2012**, *137*, 452–457. [CrossRef]
- 107. Meru, G.; McGregor, C. Quantitative trait loci and candidate genes associated with fatty acid content of watermelon seed. *J. Am. Soc. Hortic. Sci.* 2014, 139, 433–441. [CrossRef]
- Park, S.W.; Kim, K.T.; Kang, S.C.; Yang, H.B. Rapid and practical molecular marker development for rind traits in watermelon. *Hortic. Environ. Biotechnol.* 2016, 57, 385–391. [CrossRef]
- 109. Sun, L.; Zhang, Y.; Cui, H.; Zhang, L.; Sha, T.; Wang, C.; Fan, C.; Luan, F.; Wang, X. Linkage mapping and comparative transcriptome analysis of firmness in watermelon (*Citrullus lanatus*). *Front. Plant Sci.* **2020**, *11*, 931. [CrossRef]
- Rivera-Burgos, L.A.; Silverman, E.; Sari, N.; Wehner, T.C. Evaluation of resistance to Gummy stem blight in a population of recombinant inbred lines of watermelon × citron. *Hortscience* 2021, *56*, 380–388. [CrossRef]
- Rivera-Burgos, L.A.; Silverman, E.J.; Wehner, T.C. NC-GSB-524W, NC-GSB-527W, NC-GSB-528W, NC-GSB-530W, NC-GSB-531W, and NC-GSB-532W watermelon lines with Gummy stem blight resistance and good fruit quality. *HortScience* 2021, 56, 1599–1604. [CrossRef]