

# Retrospective Genetic Analysis in Sweet Watermelon

Subjects: Genetics & Heredity

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Understanding the genetic basis of a crop's qualitative and quantitative traits is vital to designing market preferred varieties. 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*.

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, polycopene, violaxanthin, neoxanthin, lutein and  $\beta$ -carotene) [2][3][4][5][6][7][8][9][10][11][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 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][12][13][14][15][16][17]. Qualitative phenotypic traits in watermelon are conditioned by major genes [13][14][18][19][20][21][22]. On the contrary, multiple minor genes are involved in the regulation of quantitative phenotypic traits in watermelon [23][24][25][26][27][28][29][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][32][33][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 gene-editing 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][36][37][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.

## 2. 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.

### 2.1. Leaf Attributes

#### 2.1.1. Leaf Shape

Leaf shape in sweet watermelon is categorized into lobed and non-lobed leaf phenotypes [39][40][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 *CILL1* [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.

#### 2.1.2. 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 *CIBt* (gene ID: *Cla011508*) regulate fruit-specific cucurbitacins biosynthesis in watermelon fruit [46]. Additionally, genes, namely, *CcCDS1*, *CcCDS2* and *CICDS1*, 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.

## 3. Flower Characteristics

### 3.1. 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 gynoeceious (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]. Gynoeceious 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 gynoeceious × monoecious flowers revealed the ratios of three monoecious: 1 gynoeceious flower in the  $F_2$  populations, and 1 monoecious and 1 gynoeceious 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 andromonoecy or andromonoecy in watermelon [51][52]. A recessive gene, *pa* (gene ID: *CICG01G020800*), 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:

*CICG01G020030*,  
*CICG01G020040*, *CICG01G020060*, *CICG01G020080*, *CICG01G020260*, *CICG01G020430*,  
*CICG01G020700*, *CICG01G020770*, *CICG01G020780*, *CICG01G020790* and *CICG01G020800* [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.

### 3.2. 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<sub>2</sub> 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* [4]. 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-sterility in watermelon [22]. The gene *Citrullus lanatus Abnormal Tapetum 1* (*CIATM1*), which encodes a basic helix-loop-helix (*bHLH*) transcription factor, regulates flower development in watermelon [55]. The disruption of *CIATM1* 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.

## 4. 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][57][58][59]. The section below outlines the genetic analysis of various quantitative traits.

### 4.1. 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 marker-assisted 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.

### 4.2. 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 [60]. The expression levels of *Cla010726* are significantly lower in short plants [60]. The genes designated as *Cla015405* and *Cla015406* are associated with a short phenotype in watermelon [61]. Recently, *Cla015407*, named *Citrullus lanatus dwarfism* (*Cldf*), has been thought to control short plant stature in watermelon [62]. A point mutation resulting in a 13 bp deletion in the coding sequence of *Cldf* led to a GA-deficient short phenotype [63]. The gene *Cla010726* encodes for gibberellin 20-oxidase-like protein, whereas *Cla015407* gene encodes gibberellic acid 3 $\beta$ -hydroxylase proteins, which are associated with the gibberellic acid metabolism, resulting in growth arrest and reduced plant height [62]. The gene designated as *Cla010337*, which encodes an ATP-binding cassette transporter (ABC transporter), reportedly conditioned dwarf plant height in watermelon [64]. The deletion of a single nucleotide of the gene *Cla010337* causes the development of shorter watermelon plants [64]. Quantitative analysis revealed segregation ratios of 3:1 and 1:1 in the F<sub>2</sub> and backcross populations, suggesting that reduced plant height is controlled by a single recessive or dominant gene [62][65][66]. Cho et al. [66] 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 [66]. Internode length is a secondary trait that influences plant height in watermelon. Segregation ratios of 3:1 and 1:1 in the F<sub>2</sub> and backcross populations, respectively, were detected, suggesting that a single dominant gene controls the expression of short internode length in watermelon [29]. The gibberellin 3 $\beta$ -hydroxylase (GA 3 $\beta$ -hydroxylase) gene *Cla015407* is associated with the short internode phenotype in watermelon [29]. GA 3 $\beta$ -hydroxylase is an important enzyme regulating GA biosynthesis by catalyzing the inactive precursors of GA<sub>9</sub>, GA<sub>20</sub>, and GA<sub>5</sub> into bioactive forms, namely, GA<sub>4</sub>, GA<sub>1</sub>, and GA<sub>3</sub>, respectively [29].

### 4.3. 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 macro- and micro-nutrients [8]. Watermelon produces multiple lateral branches from the primary branches. A single recessive gene, *Cbl* (i.e., *Citrullus lanatus branchless*), causes branchlessness [67]. Bulk segregant sequencing (BSA-seq) analysis revealed a candidate gene, *Cla018392*, which encodes a *TERMINAL FLOWER 1* protein associated with branchlessness in watermelon [67]. This gene reduces the formation and development of axillary and apical buds, thus limiting lateral branching in watermelon [67]. 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 vegetable- and fodder-type watermelon varieties. Researchers propose a comparative genetic analysis of watermelon genotypes with contrasting branching capacities to obtain insight into and elucidate the molecular mechanisms regulating this trait for breeding.

### 4.4. 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 [68] 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. [69] 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-4-phosphate 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.

### 4.5. 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 [70]. Stone et al. [69] 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. [69] reported a single fruit weight of watermelon varying from ~ 3 to 12 kg, whereas Singh et al. [71] 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 [69], 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][72]. Multiple QTLs associated with yield component traits have been reported in watermelon [23][24][28][30][72]. The multiple QTLs conditioning yield component traits are useful for the strategic breeding of watermelon for high fruit yield potential.

### 4.6. 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 [73][74][75]. 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 [76][77]. 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 [78]. Two candidate genes, namely, *Cla97C05G104360* and *Cla97C05G104380*, and three other genes, namely, *Cla97C05G104340*, *Cla97C05G104350* and *Cla97C05G104390* [79], 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.

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