Ash Gourd Genomics: Achievements, Challenges and Future Perspectives

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Abstract

Ash gourd is an important crop plant species belonging to family cucurbitaceae. This traditional vegetable is versatile in nature, which can be used as vegetable, medicine and for making post-harvest products like sweets and candies. Advanced molecular breeding strategies have been applied to improve yield, quality and disease resistance and to meet consumer and farmer requirements. The next-generation sequencing techniques have fast tracked the sequencing of all the vegetable crops. In 2019, Chinese researchers were able to sequence the ash gourd genome, which will aid researchers in determining the precise positions of corresponding genes and employing these connected markers in marker-assisted breeding procedures. Also, the sequencing methods have contributed for the development of molecular markers, gene identification and QTL mapping, transcriptomics and genome editing. This chapter highlights genome-based gene identification methodologies for numerous features and problems for future functional genomic studies, and this could provide as a theoretical foundation for current ash gourd breeding efforts.

Keywords

Ash gourd · Genome · Mapping · Transcriptomics · Sequencing

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3.1 Introduction

Cucurbits are the popular vegetable crops belonging to Cucurbitaceae family. The major cucurbitaceous crops like cucumber, muskmelon, water melon and pumpkin are grown worldwide, while bottle gourd, bitter gourd, wax gourd and luffa are prevalent in many Asian and African countries. *Benincasa hispida*, is also known as ash gourd, wax gourd, white gourd and winter melon. The winter melon is the only member of the genus *Benincasa* (Chopra and Nayar 1956; Chen et al. 2021). Ash gourd is considered as the native of the Indo-China region (Rubatazky and Yamaguchi 1999) and is extensively cultivated in old world tropical countries viz. India, China and Japan whereas it is less common in New World. Ash gourd has huge popularity in Asia because of its medicinal and nutritional properties (Al-Snafi 2013). It is well known for long storage and value-added products as it is baked, fried, boiled, pickled and candied (Robinson and Decker-Walters 1999).

During last decade, there was a rapid development in genomics research including draft genomes and high-density genetic maps in cucurbit crops. Despite high economic importance, the genomic information in ash gourd is limited, thus, retarding the translational research in its breeding. Advanced molecular breeding tactics have been used in ash gourd not only to enhance yield but also to meet out consumer demands and to bring convenience to farmers. In ash gourd, the traits like plant architecture, increased yield, resistance to biotic and abiotic stresses, quality traits, pest resistance are some of the main targets of modern breeding. The biotechnological tools like next-generation sequencing have significantly accelerated the process of genome sequencing. The published genome sequence of cucurbits including *Cucumis sativus* (Huang et al. 2009), *Cucumis melo* (Garcia-Mas et al. 2012), *Citrullus lanatus* (Guo et al. 2013), *Momordica charantia* (Urasaki et al. 2017), *Cucurbita pepo* (Montero-Pau et al. 2018), *Lagenaria siceraria* (Wu et al. 2017), *Cucurbita maxima* (Sun et al. 2017), *Cucurbita moschata* (Sun et al. (2017), *Cucurbita argyrosperma* (Barrera-Redondo et al. 2019) and *Benincasa hispida* (Xie et al. 2019) have helped researchers in determining the gene position on chromosomes. These identified linked markers can be used for marker-assisted based molecular breeding.

To examine genetic variation and evolutionary links among related crops, new generation sequencing has been used to produce molecular markers (Hu et al. 2021; Levi et al. 2010). With the advancement in sequencing technology, the application of SNPs has surpassed the other molecular markers. Earlier, the sequencing was costly and time consuming when it was performed after the PCR amplification for genomic region of interest (Edwards and Batley 2010). Nowadays, a large number of sequences are generated through NGS methods, which are less costly with higher efficiency of SNP discovery. These markers have been used to create molecular genetic and physical maps, as well as to identify the genes or quantitative trait loci that govern economically significant traits (Varshney et al. 2009). In addition, resequencing approaches based on reference genomes are employed to investigate genome-wide diversity. Bulk segregant analysis (BSA) has been proposed as the primary method for whole genome sequencing. A number of approaches based on

BSA like QTL-seq (Takagi et al. 2013), MutMap (Abe et al. 2012), bulked segregant RNA-Seq (BSRseq) (Liu et al. 2012), specific locus-amplified fragment sequencing (SLAF-seq) (Xu et al. 2014) and genome-wide association studies (GWAS) (Saidou et al. 2014) have been used in many vegetable crops for identification of the linked gene and marker development.

The sequence data obtained from transcriptomic analysis as well as their expression profiles linked to various physiological situations will aid in the identification of genes that control various traits. This will unravel the regulatory mechanisms behind different traits and help to elucidate the complete pathway. For the past five decades, there have been significant advancements in molecular biological approaches. The discovery of sequence-specific nucleases (SSNs) and (CRISPR)/CRISPR-associated protein (Cas) system has taken gene editing to the next level, resulting in vegetables with modified functions and desired traits (Abdallah et al. 2015; de Caceres et al. 2020).

In this chapter, the author will summarize studies related to genome sequencing and genome-based techniques for the identification of genes and molecular breeding, which can serve as a theoretical reference for ash gourd and Cucurbitaceae crop improvement programmes.

3.2 The Ash Gourd Genome

The genome of the ash gourd was discovered to be larger than that of cucumber, melon and watermelon (Xie et al. 2019). Authors completed the sequencing of genome in 2019 by using ash gourd line B227 with large (80 cm length) dark green fruits, using Illumina and single-molecule real-time (SMRT) sequencing technologies. Based on k-mer analysis the estimated 1.03 Gb genome comprises 55.4 Gb high-quality cleaned sequences with ~50-fold coverage of the genome. De novo assembly of Illumina and PacBio sequences resulted in a 913 Mb long-draft genome. The number of scaffolds was 2197 with a scaffold N50 of 3.4 Mb, N90 scaffold of 0.9 Mb and longest scaffold of 14.5 Mb (Xie et al. 2019). Using the high-density genetic map, 859 Mb (94.1%), comprising 397 scaffolds, could be attached to the 12 linkage groups. The number of predicted coding genes was 27,467 in number with average gene length of 3962 bp. According to a comparative examination of the genomes of all the cucurbit species, the genome of wax gourd is the most ancestral karyotype studied so far.

3.3 Beyond Ash Gourd to Family Cucurbitaceae

The cucumber genome published in 2009 was the first ever published genome among the cucurbit species. The reference genome of cucumber '9930' was sequenced with a genomic coverage of 72.2-fold with a genome size of 367 Mb (Huang et al. 2009), subsequently genome of semi-wild cultivar 'GY14' and wild cultivar 'PI 183967' were sequenced (Qi et al. 2013). The genome size of melon

(450 Mb) is larger than its close relative cucumber (367 Mb), and this increased size may be due to the transposon amplification which may have led to duplications in the melon lineage. Moreover, the variations in phenotypic and quality traits and other stress-related genes in melon may also have occurred due to transposon amplification (Garcia-Mas et al. 2012). Guo et al. (2013) sequenced the genome of watermelon by using an inbred line '97103' using Illumina sequencing which resulted in a genome size of 353.5 Mb. The resequencing of watermelon genome in 2019 using inbred line '97103' have led to the genome size of 365.1 Mb, and authors indicated the role of selection during domestication and improvement of watermelon fruit size and fixation of a non-bitter allele in *C. lanatus* (Guo et al. 2019). A more confident gene set of bitter gourd genome (Cui et al. 2020) of line Dali-11(300 Mb) compared to line OHB3-1 (Urasaki et al. 2017) provided valuable insights into domestication of bitter gourd in Southern Asia. The evidence for allotetraploidization event in *Cucurbita* was provided by studying the high-quality genome sequences of *C. maxima* and *C. moschata* (Sun et al. 2017). The first high-quality genome of *Cucurbita argyrosperma* wild relatives (Barrera-Redondo et al. 2021) provided insight into domestication genes having their role in hormonal regulation, defence mechanisms, seed germination and development. The occurrence of shared alleles among *C. argyrosperma* and *C. moschata* suggested the introgression during domestication between both the taxa. The genome sequence of bottle gourd (313.4 Mb) provided understanding of the history of genomic evolution of the family and identified chromosome-level syntenic relationships between bottle gourd and other cucurbits (Wu et al. 2017). Snake gourd (*Trichosanthes dioca*) and chayote (*Sechium edule*) diverged from sponge gourd and have one of the largest genome sizes of 919.8 Mb (Ma et al. 2020) and 606.42 Mb (Fu et al. 2021), respectively, among all the cucurbit species. The Illumina HiSeq 4000 platform was used to sequence the whole chloroplast genome of wax gourd (156,758 bp). Based on the entire chloroplast genome and 72 genes, phylogenetic analysis revealed sibling ties between *B. hispida* and *Citrullus*, *Lagenaria* and *Cucumis* (Song et al. 2022). In 2019, The Cucurbit Genomics Database (CuGenDB; http://cucurbitgenomics.org) was created to utilize the large-scale datasets and to provide an essential gateway for the cucurbit research. The database provides information about all the available cucurbit genomes and genomic information with respect to expressed sequence tag (EST) sequences, genetic maps, transcriptome profile synteny blocks etc. (Zheng et al. 2019).

3.4 Molecular Markers in Ash Gourd Breeding

Molecular markers are the vital tools which have been used to study genetic diversity and variability, phylogenetic relationships, purity of F_1 hybrids and mapping the location of qualitative and quantitative traits. Randomly amplified polymorphic DNAs (RAPD) owe the qualities like simplicity, speed and comparatively less cost (Rafalski and Tingey 1993) due to which they are generally used for genetic diversity studies in ash gourd (Parkash et al. 2000; Singh 2002; Verma et al. 2007;

Pandey et al. 2008; Sikdar et al. 2010). The factors like number of molecular markers and per cent polymorphism are an important factor for DNA marker-based studies. Jiang et al. (2013) identified 6242 SSRs in ash gourd, and out of the 200 synthesized primer pairs, 28.8% markers showed polymorphism. SSR markers have played an important role in comparative genomics as they are conserved among closely related species and genus (Ghebretinsae et al. 2007; Yang et al. 2012; Guo et al. 2013). In a cross-species transformation study, ash gourd was found closely related to watermelon as the transferable SSR markers were more from watermelon than melon, pumpkin and cucumber (Hu et al. 2021). Pandey et al. (2021) checked the validity of 70 SSR markers developed from cucumber genome in 16 different species and reported 49.21% polymorphism in ash gourd, and these cross-transferable markers can be used for marker-based studies like molecular mapping and marker-assisted breeding. In another phylogenetic study using EST-SSR and EST-PCR primers, *B. hispida* was reported closest to *P. fistulosus* (Levi et al. 2010).

Pericarp colour, controlled by single gene, is an important trait as it represents the quality of a fruit. A total of 140 F_2 plants were used for SLAF sequencing, detecting 142,653 SLAF sequences with 22,151 (15.42%) polymorphic sequences (Jiang et al. 2015). In another study using BSA-seq data and distribution and density of the SNP physical sites, 14 KASP markers were identified linked with peel colour (Ma et al. 2021). Zhu et al. (2016) reported a primer ISSR-855 linked with the fruit colour trait at a genetic distance of 9.04 cM. Some winter melon germplasm has a pleasant aroma, which adds to its value-added potential. The 'pandan' like aroma found in rice and soybean due to 2-acetyl-1-pyrroline (2AP) was also reported in some of the landraces in ash gourd. A PCR-based marker AroGourd was identified to detect the 804 bp deletion in the aromatic ash gourd (Ruangnam et al. 2017). First female flower node is an important trait in all the cucurbitaceous crops as it leads to early harvest of the fruit, and markers linked with this trait were identified (Cheng et al. 2010).

3.5 Gene and QTL Mapping

Development of novel varieties by way of traditional breeding methods is labour intensive and time consuming. This becomes more difficult when the genes-controlling desirable traits are to be introduced from a donor source. Also, the negative correlation between desired traits obstructs the breeder's ability to select and breed effectively (Nicholson 1960). Introducing contemporary biotechnological technologies utilizing molecular markers in breeding programmes can alleviate these types of challenges. In cucurbits, numerous molecular markers linked with qualitative and quantitative traits have been identified and mapped on various chromosomes (Miao et al. 2011; Qi et al. 2013; Yang et al. 2013, 2019; Nimmakayala et al. 2014; Wei et al. 2014; Cui et al. 2015).

In ash gourd, nine QTLs related to fruit morphological traits namely fruit weight, length, diameter and flesh thickness were identified on chromosome 3, 4, 5, 6, 9, 10 and 11. Among these nine, four QTLs were showing large effect and were

responsible for 10.0% phenotypic variance (Liu et al. 2018). Fruit shape in wax gourd is controlled by a single-candidate gene *Bch02G016830* designated as BFS. The sequencing of a F_2 population by using BSASeq leads to the identification of the candidate gene located in the 17.18 Mb region on chromosome 2, and it was reduced to 19.6 Kb region by using a kompetitive allele-specific polymerase chain reaction (KASP) marker (Cheng et al. 2021). The QTLs controlling first female flower trait, viz. *fn1*, *fn2* and *fn3* were located on linkage group 1 (fn1) and linkage group 6 (fn2 and fn3). The length of map was 1651.9 cM with average distance of 11.47 between two markers. These QTLs viz., *fn1*, *fn2* and *fn3* accounted for 62.54%, 0.2% and 37.39% of the phenotypic variance, respectively (Cheng et al. 2010).

SLAf-seq technique is an important technique especially in those species where the sequenced genome is not available. Use of SLAF-seq leads to the identification of a gene-controlling pericarp colour on chromosome 5, map covered 2172.86 cM, with an average distance of 0.49 cM between neighbouring markers (Jiang et al. 2015). Similarly, bulk segregant analysis sequencing (BSA-seq) was also employed to uncover the candidate gene *Bch05G003950* (*BhAPRR2*), which encodes the *Arabidopsis* pseudo-response regulator2 (APRR2) protein that controls peel colour modulation. The coding sequence of *BhAPRR2* in green-skinned wax gourd had two bases (GA) that were missing in white-skinned wax gourd (Ma et al. 2021). The primary gene that gives winter melon its "pandan-like" scent is *BhAMADH*. This aroma is imparted by an aminoaldehyde dehydrogenase (AMADH), which is encoded by *BhAMADH*. When the aromatic and non-aromatic accessions of *BhAMADH* were compared, an 804-bp deletion-covering exon 11–13 was discovered in the aromatic accession. The genes and QTLs identified so far in ash gourd are presented in Table 3.1.

3.6 Transcriptomics in Ash Gourd

Transcriptome sequencing of different resistant species is proving to be an effective method for discovering linked resistant genes and identifying biological processes implicated in multiple stresses (Jiang et al. 2011; Pan et al. 2012; Garg et al. 2016). RNA sequencing has been widely employed in cucurbitaceous crops to study significant traits such as fruit growth, parthenocarpy, sex expression and responses of plants to biotic and abiotic challenges. (Grassi et al. 2013; Behera et al. 2016; Pawełkowicz et al. 2016; Guo et al. 2018; Wang et al. 2019). Tissue-specific expression of certain miRNAs was detected in a comparative analysis of five tissues, with considerably higher expression in the wax gourd fruit. Fruit had the highest level of MiR164-x expression, suggesting that miR164 may play a role in wax gourd fruit growth by making the miR164-NAC module (Yan et al. 2021). The transcriptome responses of heat-tolerant and heat-sensitive genotypes in chieh-qua genotypes (*Benincasa hispida* Cogn. var. Chieh-qua How) revealed significant change in differentially expressed genes (DEGs). DEGs related to heat shock proteins (HSPs), ubiquitin-protein ligase, transcriptional factors and pentatricopeptide repeat-containing proteins were significantly changed after heat

Table 3.1 Genes and QTLs Identified So Far in Ash Gourd

Trait	Approach	Population	Identified gene/ QTL	Compound/protein involved	Details	Reference
'pandan-like' aroma	Sanger sequencing	237, F_2 progenies	*BhAMADH*	2-acetyl-1-pyrroline (2AP)	804-bp deletion encompass exons in aromatic accession	Ruangnam et al. (2017)
Fruit shape	BSA-seq mapping	6461 F_2 individuals	*Bch02G016830* (BFS)	IQD protein	BFS expression was substantially higher in circular fruits than long cylindrical fruits.	Cheng et al. (2021)
Peel colour	BSA-seq mapping	6244 F_2 individuals	*Bch05G003950* (*BhAPRR2*)	*Arabidopsis* pseudo-response regulator2 (APRR2)	Significantly higher chlorophyll content and *BhAPRR2* expression	Ma et al. (2021)
First pistillate flower node	QTL mapping	115, F_2 individuals	*fn1*, *fn2*, *fn3*	–	Accounted for 62.54%, 0.2% and 37.39% of the phenotypic variance	Cheng et al. (2010)
Fruit related traits	QTL mapping	140 F_2 individuals	FW: *fw3.1*, *fw 6.1* FL: *fl4.4*, *fl10.1* FD*: fd3.1, fd11.1* FT*: ft3.1, ft5.1, ft9.1*	–	Four of the nine identified QTLs had a significant impact	Liu et al. (2018)
Pericarp colour	SLAF-seq	140 F_2 individuals	Single locus on chromosome 5	–	The map covered 2172.86 cM, with an average distance of 0.49 cM between neighbouring markers	Jiang et al. (2015)

stress. This will aid in the discovery of useful genes in heat stress and the study of the mechanisms involved in high-temperature tolerance. At the ovary development stage, gene expression analyses of the gene-determining fruit shape (BFS) in wax gourd revealed that BFS expression was significantly higher in circular fruits than the fruits with cylindrical shape. The BFS gene was thought to alter Ca2+ CaM signalling, cell division and the cytoskeleton, resulting in changes in fruit shape. The protein produced by the BFS gene was supposed to be a member of the IQD protein family, which has been reported to affect cell shape and number in arabidopsis and rice (Duan et al. 2017; Sugiyama et al. 2017), as well as the slenderness of tomato fruit (Wu et al. 2011). A gene called *BhAPRR2* regulates the colour of the fruit's peel in wax gourd. Green-skinned fruit had much higher chlorophyll content and *BhAPRR2* expression than white-skinned wax gourd fruit (Ma et al. 2021).

3.7 Role of Genomics in Detection of Viral Diseases

Diseases cause significant production and quality losses in a wide range of cultivated crops. Similarly, cucurbits are affected with a variety of diseases, but viral diseases are the most serious, since they are causing havoc on these crops. At least 59 distinct plant viruses affect cucurbitaceous crops (Lecoq and Desbiez 2012), majority of which are vector-borne (mostly aphids and whiteflies). The detection and identification of the virus is an important aspect for developing an effective management strategy. The use of molecular tools like RT-PCR has enhanced the rapidity and accuracy of viral diagnosis. Multiple virus detection methods have been developed, and (NGS) is now a major focus of this field since it allows for impartial and hypothesis-free assessment of plant samples in ash gourd, the viruses like *Cucumber mosaic virus* (CMV), *Cucurbit aphid-borne yellows virus* (CABYV), *Papaya ringspot virus* (PRSV), *Zucchini yellow mosaic virus* (ZYMV), Watermelon mosaic virus (WMV), *Cucumber mosaic virus* (CMV), *Cucurbit aphid-borne yellows virus* (CABYV), *Papaya ringspot virus* (PRSV), Watermelon mosaic virus (WMV), *Watermelon silver mottle orthotospovirus* (WSMoV) and *Cucumber green mottle mosaic virus* (CGMMV) contain RNA as a genetic material. The detection of these viruses is done following RT-PCR using a specific set of primers. The cloned and sequenced representative PCR products are submitted to GenBank, followed by the identification by comparing its sequence to that of other isolates. On the other hand, begomoviruses infecting ash gourd which contain DNA as their genetic material namely, *Squash leaf curl China virus* (SLCCNV), *Squash leaf curl Philippines virus* (SLCuPV), *Tomato leaf curl New Delhi virus* (ToLCNDV) and *Tomato leaf curl virus* (ToLCV) are identified by PCR using virus specific primers. The full-length genome is isolated by rolling circle amplification, followed by restriction enzyme digestion, vector ligation and vector transformation. The sequences are entered into a database of nucleotide sequences, and the sequence identities of different isolates are compared. The viruses infecting ash gourd along with their method detection are presented in detail in Table 3.2.

Table 3.2 Viral Diseases Infecting Ash Gourd at Various Locations

Virus	Genus	Family/ Species	Detection	Transmission	Location	Reference
CGMMV	Tobamovirus	*Virgaviridae*	–	Mechanical sap-inoculation	India	Kumar et al. (2017)
CMV	Cucumovirus	*Bromoviridae*	ELISA	Aphid	Western Samoa	Pearson and Liayanage (1997)
CABYV	Polerovirus	*Luteoviridae*	RT-PCR, Sequencing	Aphid, *Myzus persicae*, *M. euphorbiae*, and *Aphis gossypii*	China	Xiang et al. (2008)
CABYV	Polerovirus	*Luteoviridae*	RT-PCR, Sequencing	Aphid, *Myzus persicae*, *M. euphorbiae*, and *Aphis gossypii*	Taiwan	Knierim et al. (2010)
MYSV	Orthotospovirus	*Tospoviridae*	RT-PCR, Sequencing	*Thrips palmi*	Japan	Okuda et al. (2002)
PRSV	Potyvirus	*Potyviridae*	Dot Immuno Binding Assay, DIBA, RT-PCR, Sequencing	Aphid vectors, mechanical sap inoculation	India	Nagendran et al. (2017)
SLCCNV	Begomovirus	*Geminiviridae*	PCR with geminivirus-specific primers, sequencing	White fly (*Bemisia tabaci*)	Thailand	Sawangjit (2009)
SLCCNV	Begomovirus	*Geminiviridae*	PCR with geminivirus-specific primers, sequencing	White fly (*Bemisia tabaci*)	India	Mohammed-Riyaz et al. (2013)
SLCuPV	Begomovirus	*Geminiviridae*	PCR with geminivirus-specific primers, sequencing	White fly (*Bemisia tabaci*)	Taiwan	Liao et al. (2007)
ToLCNDV	Begomovirus	*Geminiviridae*	PCR with geminivirus-specific primers, sequencing	White fly (*Bemisia tabaci*)	India	Roy et al. (2013)
ToLCV	Begomovirus	*Geminiviridae*	PCR with geminivirus-specific primers, sequencing	White fly (*Bemisia tabaci*), Graft transmission	Thailand	Samretwanich et al. (2000)
WMV	Potyvirus	*Potyviridae*	–	Aphid vectors, *Myzus persicae* and *Aphis gossypii*, by mechanical sap-inoculation	India	Bhargava and Bhargava (1977)

(continued)

Table 3.2 (continued)

Virus	Genus	Family/ Species	Detection	Transmission	Location	Reference
WSMoV	Orthotospovirus	*Tospoviridae*	ELISA and Western blotting with polyclonal antibodies	Thrips vector, *Thrips palmi*, mechanical sap-inoculation and grafting	Taiwan Japan	Chen et al. (1995) Okuda et al. (2002)
ZYMV	Potyvirus	*Potyviridae*	–	Aphid and mechanical sap-inoculation	Japan	Fukumoto et al. (1993)

3.8 Summary and Future Perspectives

The genome sequencing of ash gourd has significantly accelerated molecular breeding practices. To find key qualitative and quantitative traits, tools such as next-generation sequencing, QTL mapping, gene mapping, genotyping and other newly identified technologies have been created and used. A lot of molecular markers directly connected to specific features have been identified and produced for marker aided breeding. The breeding methods for ash gourd, on the other hand, still need to be improved. For example, gene-editing technology has opened up new possibilities for development in a variety of vegetable crops, although no studies on this technique have been published in this crop yet. Approaches like GWAS, QTL-seq, SLAF-seq, BSA-seq, MutMap+ and MutMap-Gap have yet to be successfully implemented. These approaches have been proven to be reliable in other cucurbitaceous crops; thus, there is a great scope of utilizing them in ash gourd to discover target genes and associated molecular markers. For the identification of SNPs, considerable resequencing is required. The high cost of sequencing is the major hurdle. However, in recent years, the price has reduced dramatically and is expected to continue to do so in coming time. The techniques like microarray and RNA-seq can be utilized to monitor the gene expression of different physiological processes which can provide information regarding various functions of the gene. Before attempting gene transfer, however, thorough characterization is required. Pleiotropic side effects must also be taken into account (Salgotra et al. 2014). By using this knowledge to ash gourd breeding, crop variants with superior quality, resilience to biotic and abiotic stresses and increased yield could be developed.

References

Abdallah NA, Prakash CS, McHughen AG (2015) Genome editing for crop improvement: challenges and opportunities. GM Crops Food 6:183–205. https://doi.org/10.1080/21645698.2015.1129937

Abe A, Kosugi S, Yoshida K, Natsume S, Takagi H, Kanzaki H et al (2012) Genome sequencing reveals agronomically important loci in rice using MutMap. Nat Biotechnol 30:174–178

Al-Snafi AE (2013) The pharmacological importance of *Benincasa hispida*. A review. Int J Pharm Sci Res 4(12):165–170

Barrera-Redondo J, Sanchez-de La Vega G, Aguirre-Liguori JA, Castellanos-Morales G, Gutiérrez-Guerrero YT, Aguirre-Dugua X, Aguirre-Planter E, Tenaillon MI, Lira-Saade R, Eguiarte LE (2021) The domestication of *Cucurbita argyrosperma* as revealed by the genome of its wild relative. Horticul Res 8:109

Barrera-Redondo J, Ibarra-Laclette E, Vázquez-Lobo A, Gutiérrez-Guerrero YT, de la Vega GS, Piñero D, Montes-Hernández S, Lira-Saade R, Eguiarte LE (2019) The genome of *Cucurbita argyrosperma* (silver-seed gourd) reveals faster rates of proteincoding gene and long noncoding RNA turnover and neofunctionalization within *Cucurbita*. Mol Plant 12(4):506–520

Behera TK, Rao AR, Amarnath R, Kumar RR (2016) Comparative transcriptome analysis of female andhermaphrodite flower buds in bitter gourd (*Momordica charantia* L.) by RNA sequencing. J Pomol Hortic Sci 91:250–257

Bhargava B, Bhargava KS (1977) Cucurbit mosaic viruses in in Gorakhpur. Indian J Agric Sci 47: 1–5

Chen CC, Ho HM, Chang TF, Chao CH, Yeh SD (1995) Characterization of a tospovirus-like virus isolated from waxgourd. Plant Protect Bull (Taipei) 37:117–131

Chen X, Xu F, Jiang H, Xu Z, Wang H (2021) Evolution of *Benincasa hispida* in the Cucurbitaceae family and phylogenetic relationships of the phenylalanine ammonia-lyase gene family in six Cucurbitaceae species. Plant Growth Regul 95(2):157–167

Cheng Z, Chen Q, Peng Q, Zhang H, Wang R (2010) Construction of a molecular genetic linkage map and QTL analysis of the first pistillate flower node trait in Chieh-qua. Sci Agric Sin 43(7): 1508–1515

Cheng Z, Liu Z, Xu Y, Ma L, Chen J, Gou J, Su L, Wu W, Chen Y, Yu W, Wang P (2021) Fine mapping and identification of the candidate gene BFS for fruit shape in wax gourd (*Benincasa hispida*). Theor Appl Genet 134(12):3983–3995

Chopra RN, Nayar SL (1956) Glossary of Indian medicinal plants. Council of Scientific and Industrial Research, New Delhi

Cui JJ, Cheng JW, Wang GP, Tang X, Wu ZM, Lin MB, Li LF, Hu KL (2015) QTL analysis of three flower-related traits based on an interspecific genetic map of Luffa. Euphytica 202(1): 45–54

Cui J, Yang Y, Luo S et al (2020) Whole-genome sequencing provides insights into the genetic diversity and domestication of bitter gourd (*Momordica* spp.). Hortic Res 7:85. https://doi.org/10.1038/s41438-020-0305-5

de Caceres N, Gonzalez FF, De la Mora FD (2020) In: Bhattacharya A, Parkhi V, Char B (eds) "Vegetable crop improvement using CRISPR/Cas9" in CRISPR/Cas genome editing: strategies and potential for crop improvement. Springer International Publishing, Cham, pp 119–129

Duan P, Xu J, Zeng D, Zhang B, Geng M, Zhang G, Huang K, Huang L, Xu R, Ge S, Qian Q, Li Y (2017) Natural variation in the promoter of GSE5 contributes to grain size diversity in rice. Mol Plant 10:685–694

Edwards D, Batley J (2010) Plant genome sequencing: applications for crop improvement. Plant Biotechnol J 8(1):2–9

Fu A, Wang Q, Mu J, Ma L, Wen C, Zhao X, Gao L, Li J, Shi K, Wang Y, Zhang X (2021) Combined genomic, transcriptomic, and metabolomic analyses provide insights into chayote (*Sechium edule*) evolution and fruit development. Horticul Res 8:35

Fukumoto F, Terami F, Ishii M (1993) Zucchini yellow mosaic virus isolated from wax gourd (*Benincasa hispida* Cogn.) and balsam pear (*Momordica charantia* L.) (in Japanese). Proc Kanto Plant Protect Soc 40:101–103

Garcia-Mas J, Benjak A, Sanseverino W, Bourgeois M, Mir G, González VM, Hénaff E, Câmara F, Cozzuto L, Lowy E, Alioto T (2012) The genome of melon (*Cucumis melo* L.). Proc Natl Acad Sci 109(29):11872–11877

Garg R, Shankar R, Thakkar B, Kudapa H, Krishnamurthy L, Mantri N, Varshney RK, Bhatia S, Jain M (2016) Transcriptome analyses reveal genotype-and developmental stage-specific molecular responses to drought and salinity stresses in chickpea. Sci Rep 6(1):1–15

Ghebretinsae AG, Thulin M, Barber JC (2007) Relationships of cucumbers and melons unraveled: molecular phylogenetics of *Cucumis* and related genera (Benincaseae, Cucurbitaceae). Am J Bot 94(7):1256–1266

Grassi S, Piro G, Lee JM, Zheng Y, Fei Z, Dalessandro G, Giovannoni JJ, Lenucci MS (2013) Comparative genomics reveals candidate carotenoid pathway regulators of ripening watermelon fruit. BMC Genomics 14:781

Guo S, Zhao S, Sun H, Wang X, Wu S, Lin T, Ren Y, Gao L, Deng Y, Zhang J, Lu X (2019) Resequencing of 414 cultivated and wild watermelon accessions identifies selection for fruit quality traits. Nat Genet 51(11):1616–1623

Guo WL, Chen BH, Chen XJ, Guo YY, Yang HL, Li XZ, Wang GY (2018) Transcriptome profiling of pumpkin (*Cucurbita moschata* Duch.) leaves infected with powdery mildew. PLoS One 13: e0190175

Guo S, Zhang J, Sun H, Salse J, Lucas WJ, Zhang H, Zheng Y, Mao L, Ren Y, Wang Z et al (2013) The draft genome of watermelon (*Citrullus lanatus*) and resequencing of 20 diverse accessions. Nat Genet 45:51–58

Hu Q, Wang H, Jiang B, Zhu H, He X, Song P, Song J, Yang S, Shen J, Li Z, Jianbin H (2021) Genome wide SSR development and their application in genetic diversity analysis in wax gourd. https://doi.org/10.21203/rs.3.rs-147921/v1

Huang S, Li R, Zhang Z, Li L, Gu X, Fan W, Lucas WJ, Wang X, Xie B, Ni P et al (2009) The genome of the cucumber, *Cucumis sativus* L. Nat Genet 41:1275–1281

Jiang B, Xie DS, Liu WR, Peng QW, He XM (2013) De Novo assembly and characterization of the transcriptome, and development of SSR markers inwax gourd (*Benicasa hispida*). PLoS One 8 (8):e71054

Jiang B, Liu W, Xie D, Peng Q, He X, Lin YE, Liang Z (2015) High-density genetic map construction and gene mapping of pericarp color in wax gourd using specific-locus amplified fragment (SLAF) sequencing. BMC Genomics 16(1):1–10

Jiang F, Wang F, Wu Z, Li Y, Shi G, Hu J, Hou X (2011) Components of the Arabidopsis CBF cold-response pathway are conserved in non-heading Chinese cabbage. Plant Mol Biol Rep 29: 525–532

Knierim D, Deng TC, Tsai WS, Green SK, Kenyon L (2010) Molecular identification of three distinct Polerovirus species and a recombinant Cucurbit aphid-borne yellows virus strain infecting cucurbit crops in Taiwan. Plant Pathol 59:991–1002

Kumar A, Jailani AAK, Roy A, Mandal B (2017) The occurrence, biology and genomic properties of tobamoviruses infecting crop plants in India. In: Mandal B, Rao GP, Baranwal VK, Jain RK (eds) A century of plant virology in India. Springer Publishers, Singapore, pp 429–443

Lecoq H, Desbiez C (2012) Viruses of cucurbit crops in the Mediterranean region: an ever-changing picture. In Advances in virus research, vol 84. Academic Press, pp 67–126

Levi A, Harris KR, Wechter WP, Kousik CS, Thies JA (2010) DNA markers and pollen morphology reveal that Praecitrullus fistulosus is more closely related to *Benincasa hispida* than to Citrullus spp. Genet Resour Crop Evol 57(8):1191–1205

Liao J-Y, Hu C-C, Lin T-K, Chang C-A, Deng T-C (2007) Identification of Squash leaf curl Philippines virus on *Benincasa hispida* in Taiwan. Plant Pathol Bull 16:11–18

Liu S, Yeh CT, Tang HM, Nettleton D, Schnable PS (2012) Gene mapping via bulked segregant RNA-Seq (BSR-Seq). PLoS One 7:e36406

Liu W, Jiang B, Peng Q, He X, Lin YE, Wang M, Liang Z, Xie D, Hu K (2018) Genetic analysis and QTL mapping of fruitrelated traits in wax gourd (*Benincasa hispida*). Euphytica 214(8):1–8

Ma L, Liu Z, Cheng Z, Gou J, Chen J, Yu W, Wang P (2021) Identification and application of *BhAPRR2* controlling peel colour in wax gourd (*Benincasa hispida*). Front Plant Sci 12:716772

Ma L, Wang Q, Mu J, Fu A, Wen C, Zhao X, Gao L, Li J, Shi K, Wang Y, Zhang X (2020) The genome and transcriptome analysis of snake gourd provide insights into its evolution and fruit development and ripening. Horticulture research, p 7

Miao H, Gu XF, Zhang SP, Zhang ZH, Huang SW, Wang Y, Cheng ZC, Zhang RW, Mu SQ, Li M, Zhang ZX, Fang ZY (2011) Mapping QTLs for fruit-associated traits in *Cucumis sativus* L. Sci Agric Sin 44(24):5031–5040

Mohammed-Riyaz SU, Deepan S, Dharanivasan G, Jesse MI, Muthuramalingam R, Kathiravan K (2013) First report on avariant of Squash leaf curl China virus (SLCCNV) infecting Benincasa hispida in India. New Dis Rep 28:20

Montero-Pau J, Blanca J, Bombarely A, Ziarsolo P, Esteras C, Martí-Gómez C, Ferriol M, Gómez P, Jamilena M, Mueller L, Picó B (2018) De novo assembly of the zucchini genome reveals a whole-genome duplication associated with the origin of the *Cucurbita* genus. Plant Biotechnol J 16(6):1161–1171

Nagendran K, Mohankumar S, Aravintharaj R, Balaji CG, Manoranjitham SK, Singh AK, Rai AB, Singh B, KarthikeyanG (2017) The occurrence and distribution of major viruses infecting cucurbits in Tamil Nadu state, India. Crop Prot 99: 10–16

Nicholson GE (1960) The production, history, uses and relationships of cotton (*Gossypium* spp.) in Ethiopia. Econ Bot 14:3–36. https://doi.org/10.1007/BF02859364

Nimmakayala P, Abburi VL, Bhandary A, Abburi L, Vajja VG, Reddy R et al (2014) Use of VeraCode 384-plex assays for watermelon diversity analysis and integrated genetic map of watermelon with single nucleotide polymorphisms and simple sequence repeats. Mol Breed 34: 537–548

Okuda M, Takeuchi S, Taba S, Kato K, Hanada K (2002) Melon yellow spot virus and Watermelon silver mottle virus: outbreak of cucurbit infecting tospovirus in Japan. Acta Hortic 588:143–148

Pan Y, Seymour GB, Lu C, Hu Z, Chen X, Chen G (2012) An ethylene response factor (ERF5) promoting adaptation to drought and salt tolerance in tomato. Plant Cell Rep 31:349–360

Pandey S, Kumar S, Mishra U, Rai A, Singh M, Rai M (2008) Genetic diversity in Indian ash gourd (*Benincasa hispida*) accessions as revealed by quantitative traits and RAPD markers. Sci Hortic 118(1):80–86

Pandey S, Yadav PS, Ansari WA, Pandey M, Yang L, Singh B, Dubey RK, Singh PM, Singh J (2021) Development of high conserved cross-species microsatellite markers from cucumber genome and their applicability in genetic diversity and comparative mapping. Sci Hortic 288: 110408

Parkash C, Singh KP, Kalloo G (2000) Variability analysis and cause and effect relationship in ash gourd [*Benincasa hispida* (Thunb.) Cogn.] Indian. J Plant Genet Res 13:298–301

Pawełkowicz M, Zielińskia K, Zielińskia D, Plader W, Yagi K, Wojcieszek M, Siedlecka E, Bartoszewski G, Skarzynska A, Przybecki Z (2016) Next generation sequencing and omics in cucumber (*Cucumis sativus* L.) breeding directed research. Plant Sci Int J Exp Plant Biol 242:77–88

Pearson MN, Liayanage AS (1997) Records of cucurbit viruses infecting vegetable crops in Western Samoa. Aust Plant Pathol 28:188–191

Qi JJ, Liu X, Shen D, Miao H, Xie BY, Li XX, Zeng P, Wang SH, Shang Y, Gu XF et al (2013) A genomic variation map provides insights into the genetic basis of cucumber domestication and diversity. Nat Genet 45(12):1510–1515

Rafalski JA, Tingey SV (1993) Genetic diagnosis in plant breeding: RAPDs microsatellites and machines. Trends Genet 9:275–280

Robinson RW, Decker-Walters DS (1999) Cucurbits. CAB International, Wallingford, Oxford, UK

Roy A, Spoorthi P, Panwar G, Kumar Bag M, Prasad TV, Kumar G, Gangopadhyay KK, Dutta M (2013) Molecular evidence for occurrence of Tomato leaf curl New Delhi virus in ash gourd (*Benincasa hispida*) germplasm showing a severe yellow stunt disease in India. Indian J Virol 24(1):74–77

Ruangnam S, Wanchana S, Phoka N, Saeansuk C, Mahatheeranont S, de Hoop SJ, Toojinda T, Vanavichit A, Arikit S (2017) A deletion of the gene encoding amino aldehyde dehydrogenase enhances the "pandan-like" aroma of winter melon (*Benincasa hispida*) and is a functional marker for the development of the aroma. Theor Appl Genet 130(12):2557–2565

Rubatazky VE, Yamaguchi M (1999) World Vegetables. Chapman and Hall, New York, p 843

Saidou AA, Thuillet AC, Couderc M, Mariac C, Vigouroux Y (2014) Association studies including genotype by environment interactions: prospects and limits. BMC Genet 15:3

Salgotra RK, Gupta BB, Stewart CN Jr (2014) From genomics to functional markers in the era of next-generation sequencing. Biotechnol Lett 36(3):417–426

Samretwanich K, Chiemsombat P, Kittipakorn K, Ikegami M (2000) Yellow leaf disease of cantaloupe and wax gourd from Thailand caused by Tomato leaf curl virus. Plant Dis 84:200

Sawangjit S (2009) The complete nucleotide sequence of Squash leaf curl China virus-(wax gourd) and its phylogenetic relationship to other geminiviruses. Sci Asia 35:131–136

Sikdar B, Bhattacharya M, Mukherjee A, Banerjee A, Ghosh E, Ghosh B, Roy SC (2010) Genetic diversity in important members of Cucurbitaceae using isozyme, RAPD and ISSR markers. Biologia Plantarum 54(1):135–140

Singh DK (2002) Genetic analysis of yield and its components in ash gourd [*Benincasa hispida* (Thunb.) Cogn.]. Ph.D Thesis. UP College, Varanasi

Song W, Chen Z, He L, Feng Q, Zhang H, Du G, Shi C, Wang S (2022) Comparative chloroplast genome analysis of wax gourd (*Benincasa hispida*) with three Benincaseae species, revealing evolutionary dynamic patterns and phylogenetic implications. Gene 13(3):461

Sugiyama Y, Wakazaki M, Toyooka K, Fukuda H, Oda Y (2017) A novel plasma membrane-anchored protein regulates xylem cell-wall deposition through microtubule-dependent lateral inhibition of Rho GTPase domains. Curr Biol 27:2522–2528

Sun H, Wu S, Zhang G, Jiao C, Guo S, Ren Y, Zhang J, Zhang H, Gong G, Jia Z, Zhang F, Tian J, Lucas WJ, Doyle JJ, Li H, Fei Z, Xu Y (2017) Karyotype stability and unbiased fractionation in the paleo allotetraploid cucurbita genomes. Mol Plant 10:1293–1306

Takagi H, Abe A, Yoshida K, Kosugi S, Natsume S, Mitsuoka C et al (2013) QTL-seq: rapid mapping of quantitative trait loci in rice by whole genome resequencing of DNA from two bulked populations. Plant J 74:174–183

Urasaki N et al (2017) Draft genome sequence of bitter gourd (*Momordica charantia*), a vegetable and medicinal plant in tropical and subtropical regions. DNA Res 24:51–58

Varshney RK, Nayak SN, May GD, Jackson SA (2009) Next-generation sequencing technologies and their implications for crop genetics and breeding. Trends Biotechnol 27(9):522–530

Verma VK, Behera TK, Munshi AD, Parida SK, Mohapatra T (2007) Genetic diversity of ash gourd [*Benincasa hispida* (Thunb.) Cogn.] inbred lines based on RAPD and ISSR markers and their hybrid performance. Sci Hortic 113(3):231–237

Wang M, Jiang B, Liu W, Lin YE, Liang Z, He X, Peng Q (2019) Transcriptome analyses provide novel Insights into heat stress responses in Chieh-Qua (*Benincasa hispida* Cogn. var. Chieh-Qua How). Int J Mol Sci 20(4):883

Wei QZ, Wang YZ, Qin XD, Zhang YX, Zhagn ZT, Wang J et al (2014) An SNP-based saturated genetic map and QTL analysis of fruit-related traits in cucumber using specific length amplified fragment (SLAF) sequencing. BMC Genomics 15:1158

Wu S, Shamimuzzaman M, Sun H, Salse J, Sui X, Wilder A, Wu Z, Levi A, Xu Y, Ling KS, Fei Z (2017) The bottle gourd genome provides insights into Cucurbitaceae evolution and facilitates mapping of a Papaya ring-spot virus resistance locus. Plant J 92:963–975

Wu S, Xiao H, Cabrera A, Meulia T, van der Knaap E (2011) SUN regulates vegetative and reproductive organ shape by changing cell division patterns. Plant Physiol 157:1175–1186

Xiang HY, Shang QX, Han CG, LiDW YJL (2008) First report on the occurrence of Cucurbit aphid-borne yellows virus on nine cucurbitaceous species in China. Plant Pathol 57:390

Xie D, Xu Y, Wang J, Liu W, Zhou Q, Luo S, Huang W, He X, Li Q, Peng Q, Yang X (2019) The wax gourd genomes offer insights into the genetic diversity and ancestral cucurbit karyotype. Nat Commun 10(1):1–12

Xu X, Xu R, Zhu B, Yu T, Qu W, Lu L et al (2014) A high-density genetic map of cucumber derived from Specific Length Amplified Fragment sequencing (SLAF-seq). Front Plant Sci 5: 768

Yan J, Wang M, Liu W, Xie D, He X, Peng Q, Jiang B (2021) Identification and characterization of known and novel microRNAs in five tissues of wax gourd (*Benincasa hispida*) based on high-throughput sequencing. Appl Sci 11(21):10068

Yang J, Zhang J, Han R, Zhang F, Mao A, Luo J et al (2019) Target SSR Seq: a novel SSR genotyping technology associate with perfect SSRs in genetic analysis of cucumber varieties. Front Plant Sci 10:531

Yang L, Koo DH, Li Y, Zhang X, Luan F, Havey MJ, Jiang J, Weng Y (2012) Chromosome rearrangements during domestication of cucumber as revealed by high-density genetic mapping and draft genome assembly. Plant J 71(6):895–906

Yang X, Li Y, Zhang W, He H, Pan J, Cai R (2013) Fine mapping of the uniform immature fruit color gene u in cucumber (*Cucumis sativus* L.). Euphytica 196:341–348

Zheng Y, Wu S, Bai Y, Sun H, Jiao C, Guo S, Zhao K, Blanca J, Zhang Z, Huang S, Xu Y (2019) Cucurbit Genomics Database (CuGenDB): a central portal for comparative and functional genomics of cucurbit crops. Nucleic Acids Res 47(D1):D1128–D1136

Zhu D, Liu Z, Bao Z, Jiao X, Wang X (2016) Analysis of ISSR markers linked to the fruit color trait gene in Chieh-qua. Genomics Appl Biol 35(10):2781–2787