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REVIEW

Cutting-Edge of Genotyping by Sequencing (GBS) for Improving Drought and Salinity Stress Tolerance in Watermelon (*Citrullus lanatus* L.): A Review

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Abstract

Genotyping by sequencing (GBS) is a very helpful approach and one of the most useful techniques for examining and analysing the genetic variation of various lines and varieties. GBS technology was used to identify single nucleotide polymorphisms (SNPs) and assess genetic variation in several watermelon accessions. GBS application in watermelon breeding programs has recently become a popular technique among many breeders. Watermelon (*Citrullus lanatus* L.) is a warm-season crop that is widely cultivated for its delicious fruit. And it is one of the most economically significant crops in the world. However, watermelon cultivation is frequently hampered by abiotic stressors such as drought and salinity. Recently, there has been a growing body of research on the mechanisms that allow watermelon to tolerate these stresses and improve crop yield. Generally, cucurbits are beneficial to human health, they provide necessary minerals, fibre, and nutrient components. Therefore, this review demonstrates the cutting edge of using GBS technology to identify genetic design of several features in watermelon to improve abiotic stresses (drought and saline). The application of the GBS technique has provided a distinct advantage in watermelon breeding studies. Based on GBS approach, many new candidate genes in watermelon lines control a variety of traits including saline and drought tolerance, fruit rind color, disease tolerance, nutrient components, size, and fruit shape were discovered. Modern breeding techniques are being used to develop economically viable vegetable crops that will meet customer preferences and needs. Further research is needed to enhance watermelon production.

Keywords *Cucurbitaceae* · Breeding · DNA-seq · Genetic · Watermelon

Abbreviations

BSA	Bulked segregant analysis
DM	Downy mildew
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
GBS	Genotyping by sequencing
GS	Genomic selection
GWAS	Genome Wide Association Study
MAS	Marker-assisted selection
NPGS	The United States National Plant Germplasm System
NGS	Next-generation sequencing
PM	Powdery mildew
RAPD	Random amplified polymorphic DNA
SCAR	Sequence characterized amplified region
SLAF-seq	Specific length amplified fragment sequencing
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeats
QTLs	Quantitative trait loci
WGS	Whole genome sequencing

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Introduction

The genotyping by sequencing (GBS) is a highly beneficial technique for analysis and examination of the genetic diversity of distinct genotypes and varieties (Lee et al. 2019). Several developments in plant breeding and genetics have been improved focused on the use of GBS (Kim et al. 2016). The idea of variant detection and genotyping in mapping research

has been changed, especially in plant species with complicated genomes or few public resources accessible. Genotyping by sequencing, a novel idea, combines scoring with the detection of sequence variations (specifically SNPs) in a sizable segregating or mutant population, enabling a quick and direct analysis of the diversity with a focus on mapping a trait or a desired mutation (Deschamps et al. 2012). The *Cucurbitaceae* family's most economically significant crop is watermelon (*Citrullus lanatus* L.). It is highly sensitive to cold temperatures (Rivero et al. 2002; Li et al. 2016). Watermelon as summer vegetable, it is known to be a good source of sugars, amino acids, calcium, and carotenoids and several antioxidants. Numerous health advantages of watermelon have been found in recent studies, particularly in terms of intestine and kidney safety (Aderiye et al. 2020). Its plant has long branches that bear spherical or cylindrical fruits with green, light green, or dark green skins and red centres surrounded by black seeds and white pulp. It also plays a greater role in cosmetics industry. It offers a number of high-level vitamins and minerals, antioxidants, and a small number of calories. Due to the fact that watermelon has a number of beneficial compounds, including citrulline and lycopene, several seed companies were driving the improvement of new watermelon varieties to satisfy market demand (Lee et al. 2019). Watermelon is one of the most broadly cultivated plants in world. In Asia, watermelon cultivar 97,103 (2n = 2 = 22), the high-quality draft genome sequence of the east was revealed, having 23,440 predicted protein-coding genes (Huh et al. 2014). Global production of watermelon in the year of 2020 was 101.6 M tonnes, includes 60,246,888 M tonnes of China (first producer), followed by Turkey, India, and Iran (FAOSTAT 2022). This review highlights the implementation of GBS technology for developing drought and salinity stress tolerance/resistance in watermelon plant.

Breeding involves modifying the genetic make-up of current types to improve their qualifications, efficacy, utility, and cost-effectiveness. A possible or promising development in crop breeding is the development of F1 hybrids that vary from cultivars regarding superior output, plant uniformity concerning the fruit color, quality, fruit sizes, ripening date, freshness, and resilience to abiotic and biotic challenges (Napolitano et al. 2020). Breeding with current techniques, called improved molecular breeding, has been applied to improve the quality and productivity of major crops, besides meet market preferences, and quickly provide farmers with convenient cultivars. This mechanism is thought to be the basis for the creation of inbred pure lines (Hao et al. 2020). According to conventional breeding, it takes time to provide pure lines for upcoming breeding studies of vegetable types, especially cucurbits plants. The process is anticipated to move more quickly owing to improve agro-biotechnology, genetic engineering, and genomics. Quantitative trait loci (QTLs) on a chromosome termed polygenes, which link to environmental factors, specifically affect economically significant quantity and quality in various crops (Zahid et al. 2022). The GBS has emerged as a prominent platform for breeding due to its ability to quickly genotype and determine numerous of SNPs in the population. The next-generation sequencing (NGS) accepted as an iterative process of a high throughput tool for detecting a fragment of genome's nucleotide sequence for individual. Moreover, NGS platforms, including Illumina MiSeq, Roche 454 FLX Titanium, Life Technologies 5500xl, Ion Torrent PGM, and Illumina HiSeq2500, are very useful varieties of options for applying GBS (Deschamps et al. 2012). Furthermore, in a long sequence read techniques, the PacBio high seq is one of the most fundamental resources for population genomics, comparative genomics, and functional genetics. PacBio single molecule, real-time (SMRT) sequencing creates long reads with uniform coverage and a powerful technology for de novo genome assembly. Therefore, the PacBio platform become an attractive core technology through its improvements in throughput and accompaniment reductions in cost for several genome initiatives (Kingan et al. 2019; Xie et al. 2020; Manimekalai et al. 2020). This review gives an immense summary on the status of application of GBS technology to provide some studies-based drought and salinity stresses improvements in watermelon.

Gene Editing Technologies in Agricultural Improvements of Watermelon

Gene editing (Fig. 1) is powerful approach (Tian et al. 2017). Furthermore, there are three main site-specific genome-editing nucleases with the capacity to target precise regions of the genome: zinc finger nucleases (ZFN), transcription activator-like effector nucleases (TALENs), and clustered

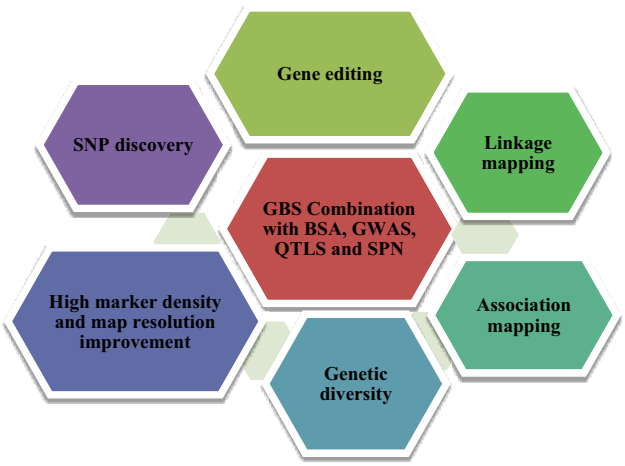


Fig. 1 Application of Genotyping By Sequencing Technology

regularly interspaced short palindromic repeats associated to nuclease Cas9 (CRISPR/Cas9). The CRISPR/Cas9 system has risen as the preeminent genome editing technique, due to its versatility, efficiency, and ease of engineering in comparison to ZFN and TALENs. In cucurbits, there are no reports describing the application of ZNF nor TALENs, as far as the authors know. Nevertheless, CRISPR/Cas9 has been applied successfully in the study of the control of cucumber fruit set cucumber (Mitra et al. 2020), the research on herbicide resistance in watermelon (Tian et al. 2018) and the control of resistance to *Fusarium* races 0 and 2, and to papaya ring spot virus in melon (Nizan et al. 2019). For instance, in watermelon, the herbicide-resistant watermelon variety through CRISPR/Cas9-mediated base-editing has been carried out (Tian et al. 2018). In the study, authors have selected the watermelon acetolactate synthase (ALS) gene as the target for base-editing. The ALS is the key enzyme for biosynthesis of branched chain amino acids, leucine, valine, and isoleucine in plants. Then, examination of ALS gene ClALS (*Cla019277*, ICuGI database) in watermelon has been indicted that conversion of cytosine (C) to thymine (T) in the codon of Pro190 (CCG) can provoke the change of amino acid which could result in a mutation for delivering herbicide resistance in watermelon (Chen et al. 2017). The successfully study has been also carried out to produce commercial hybrid seeds from elite and strain watermelon varieties by using *agrobacterium*-mediated with the high-efficient base-editing system and creation of non-GM herbicide-resistant watermelon varieties were produced. The obtained transgene-free base-edited herbicide-resistant watermelon plants were genetically identical to the bred from traditional mutagenesis there was no extra regulations applied those transgene-free base-edited mutant plants and currently they can be used for field application (Huang et al. 2016).

Advantages of Using CRISPR Gene Editing Technology

In *Cucurbitaceae* plants, the genome sequences of watermelon, cucumber, melon, pumpkin, zucchini, squash, winter squash, and bottle gourd are available. However, a*grobacterium*-transformation's protocol is still the main bottleneck to apply genome editing in this family (Hooghvorst and Nogués, 2020). For the success of a CRISPR/Cas9 application, breeders demand the sequenced genome of the target specie available, an adequate a*grobacterium*-mediated transformation protocol and an efficient binary vector containing the sequence of the Cas9 protein durable to induce target mutations. These examples have been showing that the CRISPR/Cas9-mediated base-editing system is a tremendously powerful tool for improvement of watermelon varieties (Tian et al. 2018).

Watermelon and Abiotic Stress

Currently, there has been a growing body of research on the mechanisms that allow watermelon to tolerate these stressors and improve crop yield. One of the key mechanisms that allows watermelon to tolerate abiotic stress is its ability to regulate water loss through its stomata. Stomata are microscopic apertures in the leaves that allow gases to pass between the plant and the environment (Hetherington and Woodward 2003). At the time watermelon is exposed to high temperatures or drought, the stomata close to prevent excessive water loss. This helps to maintain the water balance in the plant, allowing it to continue to grow and produce fruit even under stressful conditions (Mariani and Ferrante 2017). Additionally, a study by Mo et al. (2016a) found that watermelon plants subjected to drought stress had a higher stomatal conductance compared to the control plants, which helped them to tolerate the stress. Another key mechanism that allows watermelon to tolerate abiotic stress is its ability to tolerate high levels of salinity. The plant is able to do this by regulating the amount of salt that enters its cells through specialized transport proteins. This helps to maintain the balance of ions inside the cells and prevents damage to the plant. Furthermore, a study (Zhu et al. 2018b) found that watermelon plants treated with a salt solution had higher levels of the transport protein HKT1;5 which is responsible for salt tolerance in plants. Additionally, watermelon plants have also been found to have a high level of antioxidant enzymes which help to protect a plant from the detrimental effect of stress factors. Therefore, watermelon plants exposed to salt stress had higher levels of antioxidant enzymes such as peroxidase, superoxide dismutase, and catalase (Yanyan et al. 2018). These enzymes help to scavenge free radicals and protect the plant from oxidative damage. The watermelon can tolerate a variety of abiotic stress factors through a combination of mechanisms such as regulation of water loss through stomata, tolerance to high levels of salinity, and high levels of antioxidant enzymes. The understanding of these mechanisms can help to improve watermelon crop yield and productivity in areas where abiotic stress is a major concern (Bijalwan et al. 2021).

Background and Adoption of Genotyping by Sequencing and Related Techniques in Watermelon

Many genetic markers have been found in an organism can be analysed simultaneously with GBS approach, which makes it a potential tool. For the purpose of identifying

genetic differences and enhancing plant breeding programs, this approach is extensively adapted in variety of crops including watermelon. The foundation of GBS is the genomic DNA digestion with restriction enzymes, which followed by high-throughput sequencing of the resultant fragments. This enables the simultaneous investigation of several single nucleotide polymorphisms (SNPs), or genetic markers. The GBS can detect a greater number of genetic changes at a lower cost than more conventional techniques like restriction fragment length polymorphism (RFLP) and amplified fragment length polymorphism (AFLP). Additionally, in watermelon, GBS has been used for a diversity of applications comprising the recognition of genetic variations related with important characters such as fruit quality, tolerance/disease resistance to abiotic stress. Moreover, Yang et al. (2021) used bulked segregant analysis sequence (BSA-seq) and QTL to identify the rind hardness and related traits in watermelon. While Cheng et al. (2016) used GBS to identify SNPs associated with fruit quality characteristics as well as sugar content and fruit shape in the population of wild and cultivated watermelon. Therefore, Liang et al. (2022) applied high-resolution genetic mapping based on GBS to identify QTLs associated with watermelon fruit and seed attributes. The genotyping by sequencing platform was used to segregate the F2 population ($n = 89$; Calhoun Gray × Sugar Baby) for resistance to *Fusarium oxysporum* race 1 in order to define the GBS for SNP identification and genetic mapping (Meru and McGregor 2016). Furthermore, numerous bioinformatics utilities have been created to examine the extensive volume of data produced by GBS technology. These approaches include pipelines for the filtering, alignment, and annotation of GBS data, as well as software for the identification of genetic variations and the creation of genetic maps (Cheng et al. 2016; Fernández-Silva et al. 2008). Hence, in order to create a high-density genetic map of the crop, a study of Liang et al. (2022) developed a workflow for the processing of GBS data in watermelon. Watermelon genotypes experienced growth inhibition due to drought and salinity stress factors. By using qRT-PCR, it was possible to ascertain the relative expressions of genes linked to chlorophyll degradation, drought tolerance, and transcription factors (*WRKY70-like* and *MYB96-like*), as well as ROS scavenging systems (CAT, Cu–Zn SOD, GR, and APX) (Mo et al. 2016b).

GBS, SNP Marker, and GWS Study

Due to the low cost of nucleotide sequencing, GBS approaches have altered the criteria for genomic and genetic studies. Whole genome sequence datasets have been used to assess SNP polymorphisms for various crops using genome sequence. The GBS is an intriguing method. For meeting research demands since it concurrently performs genotyping and SNP finding without the requirement of prior genomic knowledge of the relevant species. Consequently, GBS addresses the limitations of array based SNP genotyping and classic PCR methods, meanwhile substantially reducing the time and money required to gather a critical quantity of data (Kim et al. 2016). Various cucurbit crops are currently using GBS technology; watermelon was one of these crops (Yagcioglu et al. 2016; Xiang et al. 2020), while GWAS has been used in watermelon and melon (Oren et al. 2019). This section summarized by Table 1.

Bulk Segregant Analysis Sequence in Watermelon

The bulked segregant analysis (BSA) is an effective method for discovering genetic variants linked to certain phenotypes of interest. Normally, BSA is used in biological assays for crop growth, including genetics, genomics, and genetic mapping. These investigations need a high number of samples drawn from a variety of populations. The BSA is an effective technique for sampling certain characteristics, and analysis using a variety of molecular markers is conducted after phenotyping (Zou et al. 2016; Vikram et al. 2012). The notion of BSA is the selection of a minimal number of individuals from a segregated population that belong to phenotypes opposing extremes of the desired feature. The individuals were then split into two distinct categories and fingerprinted to get genetic polymorphisms. Whenever the feature is monogenic, the number of individuals per group can be diminished to 10–20. Nevertheless, in occurrence of QTLs, the number must be increase (Pujol et al. 2019). The combination of GWAS and BSA-seq was recently applied to watermelon (Aguado et al. 2020). This method is based on the comparison of the genomic sequences of two or more bulked populations, one consisting of individuals displaying the trait of interest and the other consisting of individuals which do not display the trait. The goal of BSA is to identify genetic variations which are related with the feature of interest and that segregate differently between two populations. The BSA can be performed using various high-throughput sequencing technologies, including RNA-seq, GBS, and whole-genome sequencing (WGS). Particularly, RNA-seq, has been widely used in BSA studies due to its ability to genotype large numbers of individuals in a cost-effective way. The RNA-seq has a greater importance based on expression, for instance the identification of gene involved watermelon's fruit cracking (Jiang et al. 2019; Zhan et al. 2023) and morphological observation study performed in watermelon grafted onto pumpkin in the use of RNA-seq (Liu et al. 2017). This allows for the simultaneous analysis of many markers, recognized as SNPs. The BSA is a robust approach for identifying genetic variations linked with specific traits of interest. The method can be performed using various high

Table 1 The brief of latest advancement of genotyping by sequencing in watermelon

No:	The aim of the research	Applied Techniques	Obtention	Reference
1	Using selective genotyping and genotyping-by-sequencing for SNP discovery, a significant QTL linked with *Fusarium oxysporum* race 1 resistance was discovered in genetic of watermelon populations originating from closely related lineages	GBS, SNPs	From 2.3 to 8.4 cM on chromosome 1, a substantial quantitative trait locus (QTL) linked to resistance to Fon race 1 was discovered. Chromosomes 1, 3, 4, 5, 9, and 10 were found to have minor QTLs	(Lambel et al. 2014)
2	GBS was utilized to generate single nucleotide polymorphisms used to describe genome-wide diversity, linkage disequilibrium, and selection sweeps in cultivated watermelon	SNPs, GBS, GWAS	GBS was used to collect the 11,485 SNPs from 183 evaluated cultivated watermelons. The identified 5254 SNPs minor allele frequencies of ≥ 0.05 were distributed across the genome. The potent selection observed on chromosomes 3 and 9 and has aided in the process of domestication	(Nimmakayala et al. 2014)
3	GBS for SNP finding and genetic mapping of *Fusarium oxysporum* race 1 resistance in watermelon	SNPs, QTL	Eight resistance genes were identified QTL. As a physical map for QTL in watermelon, the resistance locus on chromosome 1 was detected	(Meru and McGregor 2016)
4	Identification of linkage map and quantitative trait loci (QTL) based on GBS-SNP linked to against *Fusarium oxysporum* f.sp. *niveum* race 2 resistance in *Citrullus lanatus* var. *citroides*, were discovered	GBS, SNPs	The Clc genetic linkage map was created using 2495 SPN obtained through GBS. The largest QTL, which was located within a 1.2-Mb region on chr9 and encompassed 44 genes, was found to account for 43% of variance for Fon race 2 resistance	(Branham et al. 2017)
5	Improvement of SNP set for marker-assisted breeding based on the GBS of 20 elite watermelon inbred lines	GBS, SNPs	The set of SNPs generated in the study possesses potential to be used for marker-assisted backcross (MABC) for various watermelon breeding resources, cultivar identification, and F1 seed purity testing	(Lee et al. 2018)
6	Genome of the main watermelon variety in America, Charleston Gray, and genetic profiling of 1365 accessions from the National Plant Germplasm System of the USA	SNP, GBS, QTLs and GWAS	The "Charleston Gray" genome served as the reference, and from the GBS data, approximately 25,000 high-quality (SNPs) were produced	(Fei et al. 2018)
7	Genetic diversity evaluation in watermelon germplasm via GBS	GBS, SNPs	A suitable screen of watermelon germplasm for watermelon development and preservation was chosen from among 68 watermelon accessions, and four clusters were assessed	(Lee et al. 2019)
8	The use of high-density GBS genetic map, to analyse the QTL of seed size of watermelon	GBS, SNPs, and QTLs	On chromosome 2, the two QTLs SW2.1 and 20SWT2.1 were discovered in the same locus. On chromosomes 2, 3, 5, 7, and 10, respectively. Nine QTLs were obtained	(Gao et al. 2019)
9	Evaluation of location and interactions of chromosomal four loci that are linked to the color of the seed coat in watermelon	GBS, QTL-seq, SNPs, and KASP™ (Kompetitive allele specific PCR)	The four loci were created to help with marker-assisted selection (MAS) for the color of the watermelon seed coat	(Paudel et al. 2019)
10	Using high-density linkage mapping based on genotyping by sequencing, QTL mapping of seed and fruit related traits in watermelon was carried out	QTLs, GBS	For 12 characteristics, QTL analysis showed 47 QTL of that 24 have been moderate or major effects and 34 were new QTL not found in other researches. The QTL for RSP was discovered and localized to a brief period on chromosome 6	(Gao et al. 2020)

Table 1 (continued)

No:	The aim of the research	Applied Techniques	Obtention	Reference
11	The improvement of SNP markers to identify the commercial Korean watermelon cultivars using fluidigm genotyping analysis	GBS, SNPs	For determining the 92 F1 watermelon cultivars that were domestically registered, a core collection of 96 SNP markers was created	(Park et al. 2022)
12	Watermelon QTL mapping for bacterial fruit blotch (BFB) by Genotyping by Sequencing (GBS)	GBS, QTLs	On chromosome 10, two BFB resistance QTLs (*ClBFB10.1* and *ClBFB10.2*) were reported	(Yeo et al. 2022)
13	Employing high-resolution genetic mapping based on GBS to identify QTLs associated with watermelon fruit and seed traits	GBS, QTLs	The RC, RSP, and FFC characteristics genetic loci were discovered on chr 8, 6, and 4. A minor QTL on chromosome 3 was consistent with fruit shape, but the novel QTL of FD and QTL of FL were deeply associated with QTL of FSI over chromosome 2. On chromosome 10, the QTL of seed width was discovered to be plotted with the 20-seed weight QTL	(Liang et al. 2022)
14	Analysis of QTL linked watermelon resistance to gummy stem blight	GBS, SNPs, and QTLs	WmGSB1.1–2 and WmGSB1.1–7, two high-resolution melting (HRM) markers, exhibit a strong positive connection with the phenotypic differences	(Hong et al. 2022)

throughput sequencing technologies as well as GBS, RNA-seq, and WGS. These technologies have been used in a variety of plant species to recognize candidate genes linked to essential agronomic features such as fruit size, shape, and drought tolerance (Garcia-Lozano et al. 2020).

Watermelon GBS and Genomic Linkage Mapping

The application of high-resolution genetic mapping was performed to map 12 horticultural features based on GBS. The segregated mapping populations (F2 and F2:3) obtained by crossing two separate inbred parents. The 6164 SNP loci and 1004 bins in the genetic linkage map covered 1261.13 cM of genetic material, with an average distance of 1.26 cM (329.31 kb) in the middle of whole-genome markers adjacent. A quantitative trait locus (QTL) is a locus that is linked to a variety of quantitative traits in the phenotypic of the population. A number of 34 QTLs discovered, 16 of which displayed major and moderate effects. However, 25 of these QTLs were brand new QTLs which has been not discovered in other studies (Liang et al. 2022). Based on the frequency of recombination, genetic linkage maps display the corresponding distances between markers across the chromosomes. Since they make association and QTL analysis faster, these maps are helpful in breeding studies. To specify the location of the interested character on chromosome, molecular markers are employed to map QTLs, which can be thought of the relationship between phenotype and genotype at various genomic locations regarding genomic interaction, positions, number, and effects of QTLs (Scheben et al. 2017). Linkage mapping and QTL mapping are powerful techniques to identify genetic variations associated with specific traits in watermelon. These methods have been widely adopted in watermelon plant breeding to improve potential agronomic characters such as tolerance to abiotic stress, fruit quality, and biotic stress resistance. Linkage mapping in watermelon is focused on the inheritance patterns of several DNA markers (Sun et al. 2020; Levi et al. 2002). These markers are applied to create genetic maps of the watermelon genome that may be employed to pinpoint sections of the genome linked with various phenotypes. For instance, in Cheng et al. (2016)'s study, QTL, CAPS, and SSR markers have been performed to analysis fruit quality features.

The QTL mapping in watermelon is used to state regions of the genome which relate to specific traits. This method involves the analysis of the genotypes of individuals from a mapping population and the phenotypes of the same individuals for a given traits (Pereira et al. 2018). QTL mapping has been used to discover areas of the genome related with essential agronomic traits in watermelon such as fruit quality, disease resistance, and tolerance to abiotic stress (Guo et al. 2019). A linkage map and QTL analysis

of horticultural features for watermelon were investigated using RAPD, RFLP, and ISSR markers. For instance, in watermelon, five QTLs have been discovered based on four agronomic traits. The QTL for rind hardness was detected in group 4. The QTL for flesh sugar concentration, measured in Brix of the juice, was found in linkage group 8. On groups 2 and 8, the QTL for red flesh color was discovered. The group 3 gene harbors the QTL for rind color (Hashizume et al. 2003). By transferring beneficial wild watermelon genes to cultivars, the current QTL and map analysis serve as a beneficial tool to breeders. Applying SSR and CAPS markers, as well as QTL analyses for traits related to fruit quality, a watermelon genetic linkage map has been created (Cheng et al. 2016; Cho et al. 2021). For instance, a watermelon genetic map was developed utilizing F2:3 segregating population of 145 individuals descended from a cross between a cultivated inbred line (garden female parent) from China and an American inbred line (LSW-177). A map includes 125 polymorphic markers, consist of 43 SSR and 82 CAPS markers. The map comprises of 11 major and three minor linkage groups covering a total length of 1244.5 cM, with an average distance between markers of 9.96 cM. All of the CAPS markers were derived from high-throughput sequencing data for the garden female parent and LSW-177 (Cheng et al. 2016).

Marker-Assisted Selection Application in Watermelon

Marker-assisted selection (MAS) is a powerful tool for improving crop breeding programs by identifying and selecting individuals with specific genetic variations associated with desired traits. In recent years, MAS has been widely adopted in watermelon breeding studies to improve distinct fruit and plant features as well as abiotic stress. A system called MAS was established to prevent and circumvent various challenges related to classical plant breeding studies that include shifting the selection criterion style from genotype to phenotype which should be in an indirect or direct (Francia et al. 2005; Kim et al. 2016; Sharma and Sharma 2018; Zhu et al. 2018a). Application of DNA markers assists in choices made throughout crop enhancement that are made in accordance with the phenotypic (morphologically) known as MAS. Whole-genome sequencing has significantly improved owing to NGS (next-generation sequencing) technique application in changing plant genotype and breeding commonly called ultra-high-throughput sequences. As the most effective and efficient MAS technology, GBS has been utilized successfully in wide-ranging plant breeding projects to undertake genomic selection, genetic diversity studies, genetic linkage analysis, GWAS, and discovery of molecular markers (He et al. 2014). Generally, this technology has been widely employed in watermelon breeding studies to determine genetic variations associated with crucial and important characters such as fruit quality, disease resistance, fruit size, shape, and for identifying tolerant watermelon varieties to abiotic stresses (Lee et al. 2018; Liu et al. 2016; Meru and McGregor 2016; Branham et al. 2017; Gao et al. 2019). However, MAS is a powerful tool for improving watermelon breeding programs by identifying and selecting individuals with specific genetic variations associated with desired traits. The implementation of MAS in watermelon breeding studies should be facilitated by the use of high-throughput genotyping technologies such as GBS, as well as the development of bioinformatics tools for the analysis of genotyped data. So far, there has been no more research about the application of GBS in watermelon tolerance to drought and saline stress. The continued development and implementation of MAS in watermelon breeding programs is expected to lead to the improvement of new varieties with developed disease resistance, fruit quality, and tolerance to abiotic stress.

Discussion and Conclusion

This overview summarized the present GBS in watermelon. In general, it is clear from the results of many studies that the GBS tool is used in diverse crops from the *Cucurbitaceae* family for a variety of goals and objectives. GBS is a possible research strategy to a various agricultural study. Yet, it yields contemporaneous genotyping and marker identification using basic molecular biology approach on effective expenses. While GBS pay a pivotal role by its capability of simultaneously find and a large number of SNPs and genotype them in watermelon genotypes. Moreover, it has recently shown to be a viable platform for genome-wide genotyping since its library can be developed internally. The GBS is expected to have major impact on mapping studies policies that rely on the intensity of markers distribution on the whole genome. This may be supported by genomic selection (GS), linkage map and QTLs, GWAS, MAS, and bulk DNA sequencing (Deschamps et al. 2012). GBS is often applied in high throughput of SNP to detect genetic diversity of plants. GBS is a feasible tool for reducing research expenditures in genetic diversity investigations (Friel et al. 2021). Several candidate genes that control fruit morphologies, textural differences, color, and disease tolerance/resistance in watermelon, cucumber, melon, or other cucurbit genotypes have been found in various researches using the GBS technology (Nyirahabimana et al. 2022). The identification of genetic variations linked to significant features has been accomplished in watermelon study with the help of GBS and related bioinformatics technologies. Using this technology, it has been possible to pinpoint genetic differences linked to features including fruit quality, disease resistance, and

abiotic stress tolerance (Hashizume et al. 2003). Moreover, advanced bioinformatics techniques were employed to investigate the extensive data provided by GBS, resulting in the creation of highly detailed genetic maps for watermelon. Further research using GBS is needed to fully understand the mechanisms underlying watermelon tolerance to abiotic stress. And the creation of strategies for enhancing crop output under stressful circumstances are highly needed.

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Informed Consent N/A

Consent for Publication All authors agreed on the final version.

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