Genomic and biotechnological interventions for crop improvement in cucurbitaceous crops: A review
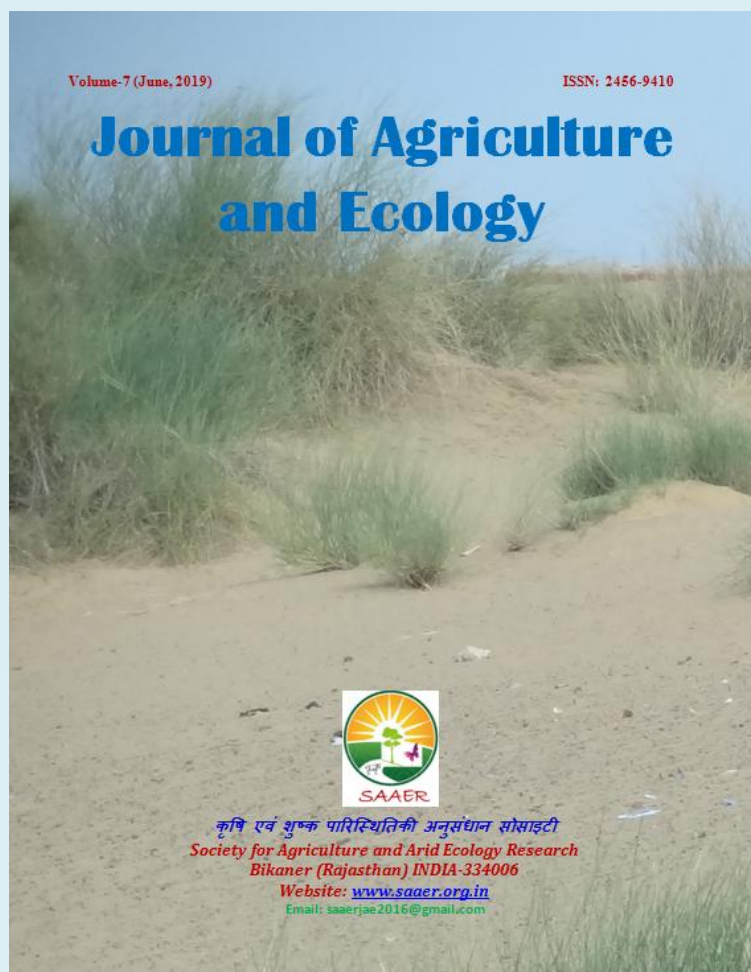
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Genomic and biotechnological interventions for crop improvement in cucurbitaceous crops: A review

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Article Info	Abstract
	The cucurbitaceous family has comprised with diverse economically important cucurbits. It primarily comprised of 118 genera and 825 species which being consumed as food worldwide since the domestication of the plants. In India, cucurbits are being grown throughout regions of the country including hot semi-arid and arid zones. With the advent of genomic breakthrough, a large number of genomic and biotechnological interventions have been developed in cucurbitaceous crops. The plenty of molecular markers are available in cucurbits and these markers were deployed to assess the genetic diversity and mapping of the QTLS/genes of interest. The success in development of genomic tools may happens by genome sequencing of mostly important cucurbitaceous crops such as watermelon, cucumber, muskmelon, bottle gourd, pumpkins. Transgenic and non-transgenic plants were developed in various cucurbitaceous crops by employing of *Agrobacterium*-mediated transformation and CRISPR/CAS9 approach, respectively. Thus cucurbitaceous crops have been considerably exploited at molecular level and biotechnological interventions were developed for crop improvement. However, a comprehensive report in cucurbitaceous crops regarding genomic and biotechnological developments is not available in public domain. Therefore, in the present review, we have collected the information related to genomics and biotechnology in cucurbits and emphasized on some successful interventions.

Introduction

The cucurbitaceous family primarily comprised of 118 genera and 825 species which being consumed as food worldwide since the domestication of the plants. In India, cucurbits are being grown throughout regions of the country including hot semi-arid and arid zones. Globally India occupied 2nd position in the area and production of the cucurbitaceous crops. Cucurbits share about 5.6 % of the total vegetable production of India and according to the FAO (FAOSTAT 2015) estimation, cucurbits are cultivated on about 4,290,000 ha with the productivity of 10.52 t/ha. The cucurbitaceous crops are very important crops for human health. These are being consumed as salad (long melon, cucumber, gherkins,

etc.), sweet (ash gourd, pumpkins, pointed gourd, etc.), pickles (gherkins), deserts (watermelon and muskmelon) and culinary purpose. Some cucurbits such as bitter gourd pose unique medicinal properties. In the commercial cropping system, cucurbitaceous crops are being cultivated as intercrops. According to an estimate, India will need to produce a large production of vegetables in the future to provide food and nutritional security at individual level and, being a large group of vegetable; cucurbits provide better scope to enhance overall productivity and production (FAOSTAT 2015). There is tremendous genetic diversity within the Cucurbitaceae family, and they can adapt in the range of climate regimes includes tropical and subtropical regions, arid deserts, and temperate regions.

The genetic diversity in cucurbitaceous crops is widen and significantly ranged in the monoploid chromosome number like 7 (*Cucumis sativus*), 11 (*Citrullus spp.*, *Momordica spp.*, *Lagenaria spp.*, *Sechium spp.*, and *Trichosanthes spp.*), 12 (*Benincasa hispida*, *Coccinia cordifolia*, *Cucumis spp.* other than *C. sativus*, and *Praecitrullus fistulosus*), 13 (*Luffa spp.*), and 20 (*Cucurbita spp.*). Among the cucurbits, cucumber, melon, watermelon, pepo and bottle guard are explored for genetic diversity assessment, development of genomic tools and genetic improvement (Bhawna et al. 2014; Park et al. 2015). The genomes of these cucurbits have been sequenced and a genomic database (http://cucurbitgenomics.org) is available for the basis of fundamental and scientific research. The genomic and biotechnological

interventions can pave the enhanced utilization of the cucurbits and it can help to identify the genomic tools for agro-economical importance. However, the admired genomic and biotechnological interventions have been carried out considerably in cucurbit crops. The present scenario deciphers the more interventions on melon genomic and biotechnology for various aspects. However other cucurbits also paid the attention regarding identification of economical genes and development of molecular markers which can enhance the germplasm for molecular breeding. Therefore, we are focusing on some cucurbitaceous crops as a case studies like cucumber, watermelon, pepo, musk melon and bottle gourd which are well described and characterized at molecular levels.

Development of EST database

The details about gene expression studies against the biotic and abiotic stresses in the cucurbit vegetables are very limited. Thus the progress to generation of ESTs is very steady except with few cases. Consequently, the identification of the genes of interest for a particular trait is directly affected and not much intervention has been formulated in this context. The development of EST database in *Cucurbita pepo* (Blanca et al. 2011), *Cucumis sativus* (Guo et al. 2010; Ando & Grumet, 2010), *C. melo* (Clepet et al. 2011), *Citrulus vulgaris* (Guo et al. 2011), etc. make the basis for identification of genes of interest and other important genomic resources. Due to easy accessibility, highly conserved in nature and belong to the functional part of the genome, the EST

database were utilized for development of gene based molecular markers (Fernandez-Silva et al. 2008; Hu et al. 2010; Xu et al. 2011; Kwon et al. 2013; Verma & Arya, 2015), designing of probes (Wechter et al. 2008), identification of SNPs (Deleu et al. 2009) and for *in silico* expression analysis of the genes in various cucurbitaceous crops (Zheng et al. 2018). Similarly, the generated EST databases were also utilized in molecular assisted breeding for enhanced utilization of cucurbitaceous germplasm.

Genome sequencing and database

In the Next-Generation Sequencing (NGS) technique, various modern, high-throughput and sophisticated sequencing technologies are being utilized for sequencing the genome of plants, microbes, etc. These recent technologies lead to sequence the genomes much more robust than the previously used classical sequencing techniques, and as such have revolutionized the study of genomics and molecular biology. In the current era of genomics, a large numbers of crops plants have been sequenced with the advent of NGS technologies. Regarding cucurbitaceous crops, some useful cucurbits have been sequenced in recently for understanding the genetic mechanisms of the useful traits (Table 1). Such genomic interventions may create the scientific opportunity to understand the complex mechanisms related to sex determination, adaptation to adverse climatic conditions, fruits qualities etc.

Cucumber is an economically important crop as well as a model system for sex determination studies and plant vascular biology in cucurbits. To understand the genetic phenomena of these important traits, the draft genome sequence of *Cucumis sativus* var. *sativus* L. has been assembled into 367 Mb with 23248 protein coding genes (Li et al. 2011). The sequenced cucumber genome made the afford insight into important traits such as its sex expression, disease resistance, biosynthesis of cucurbitacin and fresh green odor. The investigation has identified 686 gene clusters related to vascular function especially phloem function of the plant. This intervention can provide the valuable resource for developing elite cultivars and for studying the evolution and function of the plant vascular system.

Watermelon is an important cucurbit crop grown throughout the world. A high-quality draft genome sequence of the watermelon containing 23,440 predicted protein-coding genes has been reported recently (Guo et al. 2013). The genome sequences of watermelon revealed the crop diversification within the species which provided an evolutionary scenario for the origin of the 11 watermelon chromosomes derived from a 7-chromosome paleohexaploid eudicot ancestor. By virtue of sequencing data, genomic regions that were preferentially selected during domestication were identified. It was also reported that various R-genes were lost during domestication in the plant species. In addition, integrative genomic and transcriptomic analyses resulted into important aspects of phloem-based vascular signaling in watermelon and cucumber and identified genes which are crucial to play

sugar accumulation and citrulline metabolism. With the advent of genome sequencing, the genome-wide identification and detailed characterization of a large number of gene families have been carried out in watermelon (Zhang et al. 2017; Yu et al. 2017). These interventions may make the fundamental and applied research in watermelon for crop improvement against various environmental stresses. Similarly, the genome sequence of melon has been carried out and predicted 27,427 protein-coding genes, which we analyzed by reconstructing 22,218 phylogenetic trees, allowing mapping of the orthology and paralogy relationships among sequenced plant. They have observed the absence of recent whole-genome duplications in the melon lineage. The ancient eudicot triplication and transposon amplification may increase the genome size of the melon as compared to close relative cucumber. A low number of nucleotide-binding leucine-rich repeat (disease resistance genes) were annotated and suggested the existence of specific defense mechanisms in this species. Similarly, the *Cucurbita* genus contains several economically important species in the Cucurbitaceous family. The report of high-quality genome sequences of *C. maxima* and *C. moschata* provided evidence supporting an allotetraploidization event in *Cucurbita* species (Sun et al. 2017).

The genome sequencing of bottle gourd reported a high-quality 313.4 Mb genome sequence of a bottle gourd inbred line with a scaffold N50 of 8.7 Mb and the longest of 19.0 Mb (Wu el at. 2017). About 98.3% of the assembled scaffolds are anchored into the 11 pseudomolecules. The comparative genomic analysis identified chromosome-level syntenic relationships between bottle gourd and other cucurbits, as well as lineage-specific gene family expansions in bottle gourd. The sequencing data of bottle gourd were applied to identify and map a dominant monogenic locus, *Prs* (317.8-kb region on chromosome 01) which exclusively confers resistance against *Papaya ringspot virus* (*PRSV*) resistance in bottle gourd. A cleaved amplified polymorphic sequence (CAPS) marker tightly linked to the *Prs* locus has been developed and demonstrated its potential application in marker-assisted selection of PRSV resistance in bottle gourd. This study provides insights into the paleohistory of *Cucurbitaceae* genome evolution, and the high-quality genome sequence of bottle gourd provides a useful resource for plant comparative genomics studies and cucurbit improvement. Thus, the advent of genome sequences could be useful for other unexploited cucurbits for valuable traits.

Table 1. Genome sequencing of cucurbitaceous crops

Crop	Botanical Name	Chromosome numbers	Genome size (Mb)	Number of Genes	References
Cucumber (Chinese Long)	*Cucumis sativus*	7	367	23248	Li et al. 2011
Melon	*Cucumis*	13	375	27427	Ruggieri et al.

	melo				2018
Wild cucumber (PI 183967)	*Cucumis sativus*	7	-	23667	Qi et al. 2013
Watermelon	*Citrullus lanatus*	12	425	23440	Guo et al. 2013
Pumpkin	*Cucurbita maxima* cv. Rimu	21	500	32076	Sun et al. 2017
Bottle gourd	*Lagenaria siceraria*	12	313	22472	Wu et al. 2017

Molecular markers

By virtue of availability of the genomic resources such as EST database, genomic libraries, transcriptome assemblies, genome sequences, etc. a large number of molecular markers have been developed in cucurbits (Bhawna et al 2015; Park et al. 2015). The generated molecular markers being utilized for genetic diversity assessment, genetic mapping, tagging of important traits, molecular assisted breeding (Harel-Beja et al. 2010; Diaz et al. 2011; Ren et al. 2014). In some cucurbits where genomic informations are not available, the molecular markers have been generated through cross-species transferability (Table 2).

Table 2. List of molecular markers developed and utilized in cucurbits

Crop	botanical name	Molecular markers	References
Bottle gourd	*Lagenaria siceraria*	ISSR	Bhawna et al. 2014
		SSR	Bhawna et al. 2015
		RAPD	Decker-Walters et al. 2001
		SSR	Sarao et al. 2014
		RAPD	Srivastava et a., 2014
Melon	*Cucumis melo*	SSR	Bhawna et al. 2015
		EST-SSR	Fernandez-silva et al. 2008
		SSR	Patricia et al. 2004
Watermelon	*Citullus lanatus*	SCAR	Lin et al. 2009
		RAPD and SCAR	Oumouloud et al. 2008
		CAPS	Liu et val., 2016
		SSR	Lu et al. 2018
		RAPD and SSR	Mujaju et al. 2010
Cucumber	*C. sativus*	AFLP	Sigva et al. 2015
		cDNA-AFLP	Bae et al. 2006
		SSR	Fukino et al. 2008
		EST-SSR	Hu et al. 2010
		SRAP	Meng et al. 2012
		SSR	Watcharawongpaiboon et al. 2008
Pepo	*Cucurbita moschata*	RAPD	Gwanama et al. 2000

Gene tagging/MAS breeding/QTLs

Many simple sequence repeat (SSR) and sequence related amplified polymorphism (SRAP) markers have been developed in cucumber and watermelon. The genomic information has been used for the development of high density genetic maps and for whole-genome analysis, and identification of candidate genes for the important traits in cucumber (Meng et al. 2012). Several amplified fragment length polymorphism (AFLP) marker combinations were used for extensive genome analysis and identification of candidate genes in cucumber (Bae et al. 2006). Additionally, the molecular markers linked to the ZYMV resistance gene in cucumber (*C. sativus* L.) using SRAP, SSR, and AFLP markers have been developed. Fusarium wilt, caused by *Fusarium oxysporum* f.sp. *melonis*, is one of the major diseases of melons (*Cucumis melo* L.) worldwide. Resistance genes to races 0 and 2 is conferred by the linked dominant gene Fom-I. Molecular markers linked to *Fom-I* would be useful for marker-assisted selection in breeding for resistance. This population was screened for resistance to race 2 *Fusarium* wilt. Bulk segregant analysis (BSA) was used to evaluate the RAPD and AFLP markers potentially linked to *Fom-I*. BSA was performed to identify markers linked to the genes conferring ZYMV resistance (Karsies et al. 2000). In this study, SSR marker was found to be tightly linked to the *Zym*-1 gene. Similarly, NIL populations were used to identify four RAPD markers associated with CMV resistance in melons. Two of the markers were mapped into two different

linkage groups. Subsequently, the SCAR markers were developed from these associated RAPD markers to the CMV resistance in melon (Danin-Poleg et al. 2000). A high-density genetic map was developed using SNPs in pepo (*Cucurbita pepo*) which provides an important insight into the quantitative trait loci (QTL) mapping and anchoring sequence to the scaffolds (Esteras et al. 2012).

Since 1994, several kinds of markers have been used to assess the genetic diversity of cucumber accessions including RAPD, AFLP, SCAR, SRAP, SSR (Bradeen et al. 2001; Fazio et al. 2003; Sun et al. 2006; Yuan et al. 2008). However, SNP markers were not available for cucumber in the past genomic era. With the rapid development of the NGS in cucumber, Zhu et al. (2016) developed a high-density linkage map of cucumber using specific locus amplified fragment sequencing (SLAF), using 153 F2 populations. The high-density genetic map composed of 3057 SLAFs, including 4475 SNPs on seven chromosomes spanned with 1061.19 cM. On the basis of this high-density genetic map, fifteen QTLs were detected for fruit length and fruit diameter in cucumber. Additionally, various important QTLs and horticultural traits associations such as disease resistance, sexual expression, formation of bisexual flowers, lateral branch, parthenocarpy, fruit shape, fruit warty and flesh thickness of fruit have also been identified in cucumber (Pan et al. 2005; Sakata et al. 2005; Sun et al. 2006; Li et al. 2009; Yang et al. 2014; Xu et al. 2015). These research interventions have been successfully used in cucumber marker-assisted

selection breeding for tagging of agro-economically important traits including fruit yield and quality. In China, northern Chinese type cucumber with many fruit spines is very popular, whereas in US countries processing type cucumber with a few spines is mainly cultivated as vegetable consumption. However, European countries consume the cucumber with smooth surface and almost no spine. The clarification of the inheritance and identification of molecular markers for the fruit spine density gene will provide a theoretical basis for breeding of fruit quality and lay the foundation for fine mapping and gene cloning. It was reported that several genes, such as s, s-2, s-3, ss, and ns, were related to the formation of cucumber fruit spines (Fanourakis & Simon 1987). In this aspects, the tightly linked marker with trait governing of spines have been identified and a genetic map was constructed in cucumber (Zhang et al. 2016). The developed markers can be utilized for screening of the cucumber germplasm for trait of interest in future breeding programmes.

Genetic Engineering for fruit quality and yield enhancement

In the Cucurbitaceae family, transformation has been carried out in *Cucumis spp.*, *Cucurbita spp.* and *Citrullus spp.* by using co-cultivation with *Agrobacterium* (Table 3). Some improved protocols have been lately reported in melon (Bezirganoglu et al. 2014), cucumber (Wang et al. 2015) and watermelon (Liu et al. 2016). In the cucurbitaceous species, an efficient regeneration procedure via somatic embryogenesis from embryogenic calli,

developed from leaf explants, has also been recently published (Vinoth & Ravindhran, 2015). Attempts to develop *in planta* transformation methods (either via pollen tube or microinjection of the shoot apical meristem) have been made in watermelon and cucumber (Chen et al. 2016). The genetic and molecular basis of shoot regeneration helped in understanding the regeneration ability from leaf explants in cucumber (Nadolska-Orczyk & Malepszy 1989). Simultaneously, distinct expression profiles of WUSCHEL-related homeobox (WOX) genes have been studied and reported with different regeneration abilities in watermelon (Zhang et al. 2015). Even a large number of genetic transformation protocols have been developed in cucurbits. However, the current protocols of genetic transformation are not high throughput and remain less important for validation of candidate genes for particular functions. Therefore, there is need to develop high throughput genetic transformation protocols in cucurbits.

Cucumber (*Cucumis sativus* L.) is one of the most popular vegetables worldwide. The first transformation in cucumber through an *Agrobacterium*-mediated system was described two decades ago (Trulson et al. 1986). In addition to the marker and reporter genes, various types of transgenes with agronomic potential have been introduced into cucumber. The enhanced biotic resistance was observed after introduction of cucumber mosaic virus coat protein (CMV-cp) gene (Nishibayashi et al. 1996), zucchini green mottle mosaic virus coat protein (ZGMMV-cp) gene and chitinase genes (Tabei et al.

1998). Additionally, the introduction of *DHN10* gene was also associated with a slightly enhanced tolerance to abiotic stresses (Yin et al. 2005). The transformation of thaumatin II cDNA construct in cucumber enhanced the sweetness in fruits (Szwacka et al. 2002), whereas *mSOD1* gene caused higher level of superoxide dismutase (SOD) and

might be useful as a functional cosmetic material (Lee et al. 2003). The genetic transformation of *UGT* and *ACB* genes resulted in an increased yield (Salyaev et al. 2002). Similarly, introduction of *iaaM* gene led to parthenocarpic fruit production in cucumber (Yin et al. 2006).

Table 3. List of transgenic developed in cucurbits

Crop	Gene	Trait	References
Watermelon	WUSCHEL-related homeobox (WOX)	Regeneration	Zhang et al. 2015
Cucumber	CMV-cp	Virus resistance	Nishibayashi et al. 1996
Cucumber	ZGMMV-cp	Virus resistance	Lee et al. 2002
	Chitinase	Virus resistance	Tabei et al. 1998
Cucumber	Thaumatin II	Sweetening	Szwacka et al. 2002
Cucumber	mSOD1	Cosmetic materials	Lee et al. 2003
Cucumber	DHN10	abiotic stresses	Yin et al. 2005

Genome editing

The genetic modifications through breeding of crop plants have long been used to improve the yield and quality which is time consuming and labor intensive. However, precised genome editing tools could be a very useful and effective supplementary approach for improvement of crop plants by targeted gene or locus modification in the genome of an organism. This approach is mostly effective against biotic stress because the biological interaction between plant and disease causing biological agents can be disturbed at particular site. Subsequently, numerous genome editing techniques including zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) have been successfully employed in various crop horticultural crops including cucurbitaceous crops (Koltun et al. 2018; Reem & Eck 2019). In the current era of genomics and

biotechnology, clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 (CRISPR-associated protein 9) has become the choice of interest for genome editing and it has apparently utilized for crop improvement in cucurbitaceous crops (Tian et al. 2017). Whole cucurbits are severely affected by various biotic stresses including virus infection worldwide. In this context, targeted mutagenesis using CRISPR/CAS9 approach was deployed in cucumber and developed non-transgenic exhibiting resistance against cucumber vein yellowing virus (ipomovirus). This study non-transgenic virus resistance plants were produced by disruptbing *eIF4E* gene. The generated non-transgenic plants exhibited partial resistance against ipomovirus and two potyviruses such as *Papaya ring spot mosaic virus*-W *and Zucchini yellow mosaic virus*. Additionally, the phytene desaturase gene of watermelon (ClPDS) was mutated using

CRISPR/CAS9 approach very recently (Tian et al. 2017). By disturbing the function of this gene, albino phenotype was created in watermelon. Thus this CRISPR/CAs9 based foundation research in watermelon could be useful for understanding the functions of other genes of interest.

Future prospective

Since the mostly cucurbits are grown in arid and semi-arid region therefore they are very hardy against the environmental factors. For examples, the deep root system of bitter apple (*Citrullus colosynthes*) makes the basis to understand the mechanism behind the root architecture of the plant. Similarly, in the desert region various such type of crops are flourishing under the adverse condition. Additionally, in some cucurbits, anti-nutritional factor have been reported. The silencing of genes encoding the anti-nutritional product can be initiated for better quality food. In addition to this, physiological, biochemical and molecular understanding of the mechanisms of adaptive behavior of cucurbitaceous crop in very hot and dry environment is making the future thrust area in cucurbit research. The targeted mutagenesis using CRISPR/CAS9 technology is very recent breakthrough in biotechnology. Therefore, these are a huge scope of using genome editing approaches in cucurbits for crop improvement particularly for disease resistance and fruit quality parameters.

Conclusions

In nut shell, the cucurbit crops have been exploited sufficiently at molecular level to develop the genomic tools and carrying the biotechnological interventions. However, still it required more intentions to refine the study. The developed technologies so far in cucurbits may useful to enhanced utilization of the cucurbitaceous germplasms for sustainable development of the horticulture.

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