Chapter

Genomic Tools to Accelerate Improvement in Okra (*Abelmoschus esculentus*)

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Abstract

Okra (*Abelmoschus esculentus* L. Moench), is an important vegetable crop with limited studies on genomics. It is considered as an essential constituent for balanced food due to its dietary fibers, amino-acid and vitamins. It is most widely cultivated for its pods throughout Asia and Africa. Most of the okra cultivation is done exclusively in the developing countries of Asia and Africa with very poor productivity. India ranks first in the world with a production of 6.3 million MT (72% of the total world production). Cultivated okra is mostly susceptible to a large number of begomoviruses. Yellow vein mosaic disease (YVMD) caused by Yellow vein mosaic virus (YVMV) of genus Begomovirus (family Geminiviridae) results in the serious losses in okra cultivation. Symptoms of YVMD are chlorosis and yellowing of veins and veinlets at various levels, small size leaves, lesser and smaller fruits, and stunting growth. The loss in yield, due to YVMD in okra was found ranging from 30 to 100% depending on the age of the plant at the time of infection. Exploitation of biotechnological tools in okra improvement programmes is often restricted, due to the non availability of abundant polymorphic molecular markers and defined genetic maps. Moreover, okra genome is allopolyploid in nature and possess a large number of chromosomes (2n = 56–196) which makes it more complicated. Genomics tools like RNA- seq. for transcriptome analysis has emerged as a powerful tool to identify novel transcript/gene sequences in non-model plants like okra.

Keywords: Improvement, NGS, transcriptome, YVMV, bhindi, marker, orphan crop

1. Introduction

Okra (*Abelmoschus esculentus* L. Moench), which belongs to Malvaceae family, is an important fruit vegetable grown throughout the tropics and warmer parts of the temperate zone. It is cultivated in India, Nigeria, Europe, Turkey, Iran, West Africa, Afghanistan, Pakistan, Burma, Japan, Bangladesh, Brazil, China, Ethiopia, Cyprus, United States and all parts of tropics. It has 1.12 million hectare area and 8.7 million tonnes production in the world. It is one the most important traditional vegetable crops of India from production point of view, as India contributes around 73% of total worlds okra production. Okra is one of the important vegetables export from India. India produces annually over 63 lakhs metric tonnes of okra from an area of 5.24 lakh hectares which is valued at Rs. 534,037 lakhs at current market rates [1]. It is fourth most important crop after tomato, brinjal and chilli from seed industry

viewpoints in India. Share of hybrid seed is more than 70% in nearly 6000 metric tonnes seed market in India. Okra is proved to be a very remunerative crop for farmers, but due to Bhendi Yellow Mosaic and Enation Leaf Curl Virus disease its successful production has become a challenge for the farmers all over the country, as most of the previously bred varieties like Parbhani Kranti, P-7, Arka Anamika and Arka have lost the resistance to YVMV and ELCV diseases [2]. Therefore, against viruses development of varieties/hybrids should the continuous process to enhance the crop productivity.

Tender pods of okra are used as delicious vegetable. To a limited extent, it is used in canned, dehydrated and frozen form. It removes constipation when 2-3 fresh pods are eaten regularly. It is often included in weight loss diet as it is both fat and cholesterol free and rich in fibre. It is rich source of protein, calcium, potassium and iodine. Fresh pod contains around 88% water, 0.1% fat, 8% carbohydrate, 1.8% protein and 0.9% fibre. Okra mucilage has potential for use as food, non-food products and medicine. Dried stems and roots of okra are used for cleaning sugarcane juice from which molasses is prepared. The dry seeds are rich source of oil (18-20%) and crude protein (20-23%). Edible oil of okra has pleasant taste and odour and it is high in unsaturated fats such as oleic and linoleic acid. Its ripe, roasted seeds are also used as coffee additive or substitute after grinding. It has a vast potential as one of the foreign exchange earning crops and accounts for about 60% of the export of fresh vegetables excluding potato, onion and garlic. Fresh okra is exported to Middle East, U.K., Western Europe, Singapore and USA. Frozen okra is also exported to U.K. cultivated okra, *A. esculentus* (2n = 130), is natural amphidiploid from chromosome doubling of cross between *A. tuberculatus* (2n = 58) as one parent and *A. ficulneus* (2n = 72) the other probable parent.

Genomic resources are practically absent in *Abelmoschus*, only two mRNA and few coding sequences of this genus are deposited in the public domain [3]. Okra genome is allopolyploid in nature and posses a large number of chromosomes (2n = 56–196) which makes it more complex for genome sequencing. Some, of the complexities in non model genomes like okra can be bypassed by sequencing the transcriptome rather than the genome [4]. mRNA sequencing also known as RNA-seq. Has emerged as a powerful tool to identify novel transcript/gene sequences and to develop molecular marker in non-model plants like okra.

2. Begomoviruses infecting okra

There are at least 27 begomoviruses which infects okra; of which, two viruses i.e. Yellow Vein Mosaic Virus (YVMV) and Okra Enation Leaf Curl Virus (OELCV) most severely affect quality of pod and lowers production by reducing yield [5]. The diseases of begomoviruses are mainly transmitted by insect vector whitefly (*Bemisia tabaci*). Begomoviruses can also be transmitted by grafting; but, seed-transmission or transmission through mechanical inoculation has not yet been established [6].

The yellow vein mosaic disease of okra (YVMD) caused by Bhendi yellow vein mosaic virus (BYVMV) was first reported in 1924 from the erstwhile Bombay Presidency [7]. The begomoviruses native to the New World have only bipartite genomes (having DNA-A and DNA-B components) whereas, of Old World are mostly monopartite (have DNA-A homolog and lacks DNA-B). In India, bhendi-infecting monopartite begomoviruses were mostly associated along with a specific betasatellite, Bhendi yellow vein betasatellite (BYVB). These BYVBs have been reported to be pathogenicity determinant and found responsible for the characteristic yellow vein mosaic symptoms. Minimum16 types of begomoviruses and 4 types

of beta satellites are found associated with the YVMD in different combinations [8]. Begomoviruses isolated from okra throughout the world are of monopartite nature [9]. However, tomato leaf curl New Delhi virus (ToLCNDV), which is a bipartite begomovirus and bhendi yellow vein Delhi virus (BYVDeV) also a bipartite species were characterized from okra [10].

The typical symptom of YVMD is yellowing of veins with in green leaf and if infection becomes severe infected leaves turn completely yellowish. In case of early infection of YVMV there is significant reduction i.e. up to 94 to 100% in terms of yield [11]. Occurrence of infection after 50–65 days of germination reduces the damage and loss by 49–84% [12]. Moreover, the popular varieties of okra in India have become susceptible to YVMV [13], new biotypes of whitefly vectors have surfaced and vectors have become partly resistant to the insecticides [14]. All these factors lead to decrease in overall production of okra in India. Therefore, advance biotechnological and genomic tools like RNA interference (RNAi), genome editing and sequencing along with conventional methods like transfer of resistance from wild sources are required to enhance production of okra under YVMD condition.

3. Okra Enation Leaf Curl Virus

Okra Enation Leaf Curl Virus Disease (OELCD) was first identified from Bangalore during the early 1980s in India; OELCD can reduces yield up to 80– 90% in okra [15]. The typical symptoms of OELCD are curling in leaves, thick veins, and reduced leaf surface area. In addition, the disease bearing plants become severely stunted along with small and malformed fruits which make them unsuitable for consumers. This disease is going to be the future menace of okra cultivation and needs a strategic breeding program to evolve resistance against OELCV [8].

4. Molecular marker development and gene diversity studies in okra

Isolation of purified DNA is challenging in okra due to the presence of large amounts of mucilaginous acidic polysaccharides like polygalacturonic acid and polyphenols which reduces yield as well as purity of DNA [16]. Presence of impurities in the DNA hinder the downstream processing of DNA like PCR, digestion with restriction enzymes and labelling of DNA segment [17]. Molecular markers has emerged as a potential system for evaluation of genetic variations and associations at inter and intra species level [18, 19]. Most commonly used markers for genetic studies and marker assisted breeding programme are Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism and Simple Sequence Repeat (SSR) [20]. However, okra lacks information on molecular markers [21].

There are only few studies where markers were used to assess the genetic diversity in okra using general DNA markers [22, 23]. Gene diversity studies reported in okra is listed in **Table 1**. RAPD was initially used in genetic diversity studies between different accessions of okra [23, 26, 27, 32]. Sequence related amplified polymorphism (SRAP) [22] have also been used in okra for diversity analysis studies. SSR markers are an important marker tool in the application of plant genetics and breeding because of their high reproducibility, multiallelic nature, codominant inheritance and good genome coverage [33]. To develop the microsatellite markers in okra, Ravishankar et al. [34] has performed sequencing of genomic DNA employing Roche 454 Titanium pyrosequencing. A total of 979,806 bp data was generated and 61,722 reads were attained. Out of 3735 contigs

Species	No. of accessions/ genotypes	Type of markers	No. of primers	PIC	Reference
Abelmoschus escullentus	48	ISSRS	—	54.55%	[24]
Abelmoschus escullentus	24	ISSRS	22	0.531929	[19]
Abelmoschus escullentus	66	(iPBS)-retrotransposons and SSRs	83 iPBS, 9 SSRs	0.66 iPBS 0.62 SSRs	[25]
Abelmoschus caillei (50), *A. esculentus (43)*	93	RAPD	13	—	[26]
Abelmoschus spp	39	RAPD	31	—	[27]
Abelmoschus esculentus	50	AFLP	33	12	[28]
Abelmoschus esculentus	22	AFLP	8	0.26	[29]
Abelmoschus escullentus	23	SRAP	39	—	[22]
Abelmoschus esculentus, *A. moschatus*, *A. manihot*	65	SSR	19	0.49	[3]
Abelmoschus esculentus (21) *A. tuberculatus* (1), *A. moschatus* (1), *A. manihot* (1)	24	SSR	18	0.53	[30]
A. esculentus (92) *A. tuberculatus* (1), *A. moschatus* (1), *A. moschatus subspecies tuberosus* (1), and *A. manihot* (1)	96	SSR	40	0.52	[31]

Table 1.
Gene diversity studies in okra.

obtained from assembled reads, a total of 2708 contigs had microsatellites. Finally 402 microsatellites were used for selection of 50 SSR primers for amplification in okra. This is the first report on the development of genomic SSR markers in okra using next-generation sequencing technology.

5. Next generation sequencing (NGS) and transcriptomics studies in okra

The next-generation sequencing (NGS) technology has transformed the field of molecular breeding, particularly in the identification and development of SSR markers. The advantage of NGS techniques are cost efficiency and large number of SSR can be identified in shorter time [34]. There is limited literature available in okra related to studies using genomic approaches. Transcriptome analysis has appeared as a potential approach to identify the transcript/gene sequences in the crops like okra where limited or no genome sequence information is available. The first study on transcriptome assembly in okra was reported by [3]. Both leaf and pod tissues of okra were taken for RNA sequencing and short read assembly SRA accession no. SRX206126. They have identified more than 150,000 unigenes and 935 SSRs from unigenes (**Table 2**). These SSRs were used to study genetic diversity

Okra Species	Plant organ	Objective of study	Sequencing platform	Raw reads (M)	Final assembly	Marker discovery	N50	NCBI accession	Reference
(*Abelmoschus esculentus* (L.) Moench) CV. Mahnco Arka Abhhay	leaf and pod	Transcriptome assembly	Illumina HiSeq™ 2000	26,324,557 263	150,000 unigenes	935 SSRs	321 bp	SRX206126	[3]
Abelmoschus esculentus cv. Xianzhi	Roots, stems, and leaves	Transcriptome assembly	Illumina HiSeq X Ten platform	716,330,252 716	293,971 unigenes	—	1885 bp	SRP130180	[35]
Abelmoschus esculentus	Leaves	Transcriptome assembly	Illumina NextSeq 500	206.3 million	66,382 unigenes	9,578 SSRs	1,408 bp	SRX2995608, SRX2995609, SRX2995611, and SRX2995612	[36]
Abelmoschus esculentus cv. Arka Anamika	Leaves	Genome assembly	Roche 454 GS FLX Titanium	61,722	3735 contigs	402 SSRs markers		—	[37]
Abelmoschus esculentus	Leaves	miRNA identification	Illumina and Ion torrent	207,285,863	845 novel miRNAs			PRJNA352593	[38]

Table 2.
Description of transcriptomic and genomic studies published in okra.

in diverse okra germplasm by many workers and found informative for classification and understanding of okra germplasm. Ravishankar et al. [34] first reported development of genomic SSR markers in okra using Roche 454 Titanium pyrosequencing technology. A total of 61,722 reads were generated from 979,806 bp data. These reads were assembled into 3735 contigs of which 2708 had microsatellites. Primers were designed for 402 microsatellites, from which 50 randomly selected SSR primers were standardized for amplification of okra DNA.

MicroRNAs (miRNAs) are regulatory RNAs which plays a crucial role in regulating gene expressions at post-transcriptional levels in disease conditions. Vimala Kumar et al. [38] applied next generation sequencing approach for global profiling to characterize the miRNAs and their associated pre-miRNAs. Data analysis using miRPlant revealed 128 known and 845 novel miRNA candidates. They identified 57 known miRNAs of 15 families and 18 novel miRNAs. A total of 845 novel candidates were predicted when using cotton as a reference genome which is closely related to *A. esculentus.* In 2018, Zhang and co-workers used transcriptome approach to identify the transcripts involved in the synthesis of bioactive compounds like flavonoids and polysaccharides in various organs like roots, stems, leaves, flowers, and fruits. They have identified 293,971 unique unigene sequences, 931 unigenes related to enzymes of flavonoids biosynthesis were identified and quantified. 691 unigenes encoded 13 key enzymes related to fructose and mannose metabolism. The transcriptome data will be useful for the gene expression analysis study of the genes encoding bioactive compounds in okra. Priyavathi et al. [36] reported high quality leaf transcriptome of *A. esculentus* from leaf samples. 16,307 unigenes, 76 transcription factor, 9,578 potential SSRs have been identified from *A. esculentus* leaf transcriptome. The *A. esculentus* sequence information presented in this study will be a valuable resource for further molecular genetics and functional genomics studies for the improvement of this crop plant.

6. Proteomic studies in okra

Proteomics analysis is a tool to facilitate the study of global protein expression, and to provide a wealth of information on the role of individual proteins in specific biological processes. Due to the complex allopolyploid genome of okra little attention has been paid to the genetic improvement of this crop until recently. Soil salinity is one of the main abiotic stresses limiting plant growth and agricultural productivity. Understanding the mechanisms that protect plants from salt stress will help in the development of salt-stress-tolerant crop. Using TMT-based proteomic technique in 2019, Zhan and associates analyzed the differentially expressed proteins between the NaCl-treated seedlings and control. They have identified a total of 7179 proteins, there were 317 differentially expressed proteins (DEPs), of which 165 proteins were upregulated and 152 proteins downregulated in the presence of NaCl.

7. Discussion

The molecular markers can be effectively used to enhance okra breeding programme through marker-assisted selection (MAS). Marker assisted breeding allows selection of desired trait at early stage which leads to accelerated development of improved varieties. Although, molecular markers have been broadly employed for DNA fingerprinting, gene mapping and gene tagging, seed purity testing and to know the molecular basis of heterosis in various crops, but in okra its use is still

limited, therefore, it is the need of hour to use these approaches to accelerate the okra breeding programme at faster pace. The genomics and bioinformatics should also be well integrated into the programme for effective application of markers to okra breeding. A comparative genomics approach of other crop should also be applied for breeding programmes of those crops where the genome information is not available. Development of cost-effective genotyping technologies should always be the integral part of any improvement programme. There is need to use SSR and SNP based genotyping technologies as well as advanced technologies such as next generation sequencing.

Resilient resistance to begomoviruses like Yellow vein mosaic virus (YVMV) poses a serious challenge to both breeders and pathologists as these viruses are highly diverse, and constantly generate new forms via recombination. Biotechnological tools to generate resistant cultivar against Yellow vein mosaic disease (YVMD) are limited due to the lack of informative polymorphic markers, genetic maps and genome sequence information. Therefore, use of novel molecular and genomic tools will help in the accomplishing resistance against YVMV in okra. Identification of markers linked to the YVMV resistance gene/s and its pyramiding for combining multiple disease resistance genes in various backgrounds will help in okra crop improvement. In addition, genomic tools will help in elucidating the metabolic pathway governing disease resistance.

8. Conclusion

Okra is considered as a non model crop with a complex genome. Genomic studies like genome sequencing and transcriptome sequencing will help in identification of genes/transcripts for important agronomic traits like disease resistance in okra. Tools like RNAi and CRISPR/Cas9 genome editing can be employed for imparting resistance as well as functional characterization of genes. Identification of genes/transcripts and markers linked to the resistance genes will help in breeding for resistance varieties. Also, there is requirement to bred durable/stable resistance against multiple diseases. ELCV is emerging as new havoc for okra along with YVMV which may be more difficult for production of okra in future. Therefore, gene pyramiding for combating multiple disease resistance genes in various genetic backgrounds should be done. There is also need to breed varieties/hybrids tolerant to abiotic stresses like cold, moisture and salt stress in the changing climatic scenario.

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