Recent Biotechnological trends in Okra

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Abstract

Despite as Okra is one of the most important vegetable crop all over the world, very few attention has been paid to assess the genetic diversity of okra at a molecular level. DNA isolation in okra is very difficult due to the presence of mucilaginous substance in the pods. The exploitation of marker assisted breeding in okra is often limited due to the availability of a few molecular markers, the absence of molecular genetic-map(s) and other molecular tools. Chromosome linkage-groups were not yet constructed for this crop and reports on marker development are very scanty and mostly hovering around cultivar characterization. But with the advancement of various biotechnological tools like chromosome engineering, marker assisted selection, nanobiotechnology can provide a tremendous way for okra improvement.

Keywords: Okra, genetic improvement, tool, biotechnology, gene transfer

Introduction

Okra (*Abelmoschus esculentus* (L.) Moench), also known as bhindi or lady's finger belongs to the family *Malvaceae* is one of the most important commercial growing warm season vegetable crop grown in tropical, subtropical and sub-temperate parts of the world including low and mid hills of H.P. Nutritionally, okra green fruits are rich in vitamins (C, A and B) and minerals (Ca, P, Mg and Fe). It also contains iodine and is, therefore, recommended for the treatment of goitre disease. India is the largest producer of okra in the world. Besides the significance of okra, now-a-days emphasis has been given for the genetic improvement of okra in which biotechnology plays a key and dyanamic role in overall improvement of certain traits like quality, yield and major problems such as yellow vein mosaic virus disease which was reported to cause 70-80% yield loss, root rot; pest namely fruit and shoot borer which leads to almost of 30-40% yield loss. So, biotechnological tools like genetic engineering,

chromosome engineering and molecular breeding etc. has proven to be efficient in mitigating these biotic and abiotic stresses.

Need for genetic improvement of okra

- Higher yield
- Better nutritional and processing quality
- To develop new varieties and hybrids
- To develop transgenic plants
- Resistance to biotic and abiotic stresses

Biotechnological strategies

- Tissue culture
- Genetic engineering
- Chromosome engineering
- Molecular breeding
- Marker assisted selection (MAS)
- Genome wide selection (GWS)
- RNA interference (RNAi)
- Molecular mapping
- Next generation sequencing (NGS)
- Targeted gene replacement (TGR)
- Nanobiotechnology
- Nucleotide sequencing
- Transcriptome profiling
- Transgenics

Considering the latest literatures, it signifies the combined utilization of both conventional and modern breeding techniques, which can further lead to the more advancements in the field of biotechnology in okra.

Recent Interventions

Rizwan et al. (2018) at College of Horticulture, Fujian Agriculture and Forestry University, China developed in-vitro high-frequency multiple shoot regeneration protocol using cotyledonary node explants of okra var. Xiang Fu. Influence of silver nitrate ($AgNO_3$) in combination with cytokinins to regenerate multiple shoots from cotyledonary node explants of okra were observed.

- Cotyledonary node explants were excised from 10-days old okra *in vitro* seedlings and incubated on Murishage and Skoog (MS) medium supplemented with different concentration and the combinations of BAP alone and in combination with kinetin.
- The highest percentage of shoot bud regeneration (75%) was achieved on MS medium supplemented with 2.0 mgL^{-1} BAP while highest percentage of shoot multiplication and proliferation (85%) with 6.75 shoots per ex-plant having 3.75 cm length was obtained on MS medium containing 1.5 mgL^{-1} BAP + 1.5 mgL^{-1} kinetin + 3.0 mgL^{-1} AgNO3.

Ravishankar *et al.* (2017) at IIHR, Banglore, identified the novel microsatellite markers in okra through next-generation sequencing and their utilization in analysis of genetic relatedness studies and cross- species transferability. The advantage of next generation sequencing is its cost effectiveness, time saving and large number of SSR (Single Sequence repeats) marker can be identified.

- Large number of microsatellite markers was developed by using next generation sequencing data from okra.
- The microsatellite markers developed are a useful tool for the genetic diversity studies, crop improvement programmes and also in gene discovery.

Distribution of microsatellite types in okra genome

	Repeats	**Percentage**
Monorepeats	572	21.10
Direpeats	607	22.40
Trirepeats	345	12.70
Tetrarepeats	462	17.06
Pentarepeats	294	10.80

Hexarepeats	149	05.50
Complex repeats	279	10.30

Irshad *et al.* (2017) conducted experiment for controlling phenolic compound secretion by using anti-browning additives and growth regulators for in-vitro regeneration of okra cv. Wufu at College of Horticulture, Fujian Agriculture and Forestry University, **China.** In this study, various factors that affect the secretion of phenolic compounds, callus induction and subsequent regeneration in okra was optimized.

Results:

- Anti-browning additives, 200 mg L^{-1} activated charcoal (AC) and 10 mg L^{-1} citric acid (CA) + ascorbic acid (AA) in 1:1 ratio were found to be best in controlling phenolic secretion from hypocotyls and cotyledon explants in callus induction medium.
- Improved shoot regeneration was achieved with 2mg L^{-1} BAP and 0.1 mg L^{-1} indole butyric acid (IBA).
- The maximum number of healthy and strong roots was obtained on media that included 2 mg L^{-1} IBA and 200 mg L^{-1} activated charcoal (AC).
- Rooted plantlets were successfully able to adapt themselves in earthen pots and showed normal morphology and growth characteristics.

At Bangladesh Agricultural Research Institute (BARI), **Kabir et al. (2016)** worked **on organogenesis in okra**. Seedling-derived cotyledonary nodes and hypocotyl explants of variety BARI Dherosh-1 were cultured *in vitro* on MS medium, which is supplemented with different concentrations and combinations of several plant growth hormones.

Results:

- 100% shooting response with callus was observed from cotyledonary nodes on thidiazuron (TDZ).
- The cotyledonary nodes of BARI Dherosh-1 were cultured on various concentrations of TDZ for regeneration. The highest percentage (64.0) with maximum number of shoots per explants (6.8) were observed in 0.044 μM TDZ in 8.4 days.
- The highest percentage (83.3) and minimum days (9.7) required for root induction were recorded in 2.46 μM IBA.

- The rooted plantlets were transferred to the soil and hardened in the plastic pots under green house conditions and the rooted shoots also grow normally under field conditions by acclimatization.

Zhu *et al*. (2015) in Australia emphasized on integrated mapping and characterization of the gene underlying the okra leaf trait in cotton. The okra leaf gene identified, ***GhOKRA*** and the transcript levels of *GhOKRA* in shoot apices were positively correlated with the phenotypic expression of the okra leaf trait. Results revealed that, both transcription and protein activity of *GhOKRA* may be involved in regulating leaf shape. Fine mapping accurately localized the region harbouring the okra leaf shape gene in the cotton genome. Non-reciprocal gene conversion or homoeologous recombination may play a vital role in the origin of the okra leaf allele.

Manickavasagam (2015) developed a tissue culture-independent genetic transformation system for okra (Arka Anamika) using seed as an explants. This technique is called as **in planta transformation** protocol, which is applicable to transform the okra plants with disease-resistant traits, and can be generated within 60 days.

Protocol

- Surface sterilization of freshly harvested healthy okra seeds with 70 % ethanol and 0.1 % aqueous mercuric chloride.
- Pre-culture the surface sterilized okra seeds in liquid MS basal medium for 24 hours.
- Inoculate the pre-cultured okra seeds into *Agrobacterium tumefaciens* 105 containing pCAMBIA 1301–*bar* suspension containing 100 μM acetosyringone.
- Sonicate the seeds for 30 minutes in the presence of *Agrobacterium tumefaciens* 105 containing 100 μM acetosyringone.
- Vacuum in filter the sonicated seeds in *Agrobacterium* suspension for 3 minutes at 750 mm of mercury.
- Co-cultivate the infected okra seeds on solid MS basal medium containing 100 μM acetosyringone for 3 days under complete darkness.
- Wash the co-cultivated seeds with liquid MS medium containing 500 mg l^{-1} cefotaxime.

- Inoculate the seeds onto solid MS basal medium containing 250 mg l^{-1} cefotaxime and 15 mg l^{-1} BASTA (non-selective herbicide) and incubate for 20 days under a 16 hours photoperiod.
- Transfer the putatively transformed plantlets into paper cups containing a sterile potting mixture and harden the plantlets in the growth chamber.
- Transfer the hardened plantlets into plastic pots containing a sterile potting mixture and subject them for acclimatization in the greenhouse.
- Select the putatively transformed plants by spraying 200 mg l^{-1} BASTA on one-month-old greenhouse grown plants.
- Analyze the transgene (*bar* gene) inheritance to progeny plants (T_1) by PCR.

At Centre of Agricultural Biochemistry and Biotechnology (CABB)**, Pakistan, Ahmed *et al.* (2013)** extract high-quality intact DNA from okra leaves despite of their high content of mucilaginous acidic polysaccharides, which cause obstruction later in restriction digestion and amplification of PCR. So a simple, efficient and reliable protocol was developed to extract intact and high quality DNA from the highly mucilaginous leaves of okra and the resulted isolated intact DNA was free from various contaminating agents like polyphenol, protein and polysaccharides, which affect DNA purity and DNA quality subsequenty. The quality and yield of extracted DNA was satisfactory and the protocol can be followed with accurate results.

Materials required:

- Extraction buffer (Tris-HCl pH maintained at 7.5, 200 mmol/L sodium chloride, Ethylene diamine tetra acetic acid (25 mm mol/L), Sodium deodecyl sulfate (0.5%)
- Isopropanol
- 5 mmol/L NaCl
- Ethanol 75%
- Tris EDTA: (10 mm mol/L Tris-HCL (pH 8.0), 0.1 mm mol/L Ethylene diamine tetra acetic acid (pH 8.0)] 10 mg/ml
- RNase A
- Phenol-chloroform
- Chloroform: isoamyl alcohol
- Absolute ethanol
- 3 mol/L sodium acetate (pH 5.2)

Protocol

- In okra, small quanity of leaf tissue are ample for DNA extraction and large number of samples can be used at the same time. The protocol was standardized for 100 mg fresh leaves, which can be handled in a 1.5 mL disposable eppendorf tube.
- Take100 mg of vacuum dry okra leaves in a 1.5 mL screw cap tube and grind for 20 minutes at 3000 rpm in multi-beads shocker.
- Add 400 µL extraction buffer and mix it in vortex and leave the mixture for some time.
- Centrifuge at 10,000 xg. Take the supernatant and add 300 µL chilled isopropanol along with 100 µL 5 mol/L NaCl and invert gently to mix the ingredients. Incubate at 20^0C for approximately 1 hour.
- Transfer the aqueous phase gently to a new vial, without disturbing the polysaccharides and then centrifuge at 10,000 xg for 10 minutes at room temperature.
- Wash the pellet with 75% analytical grade ethanol. Dry the pellet and dissolve in 100 µL TE buffer.
- Add 1 µL (10 mg/ml) RNase and incubate at 37℃.
- Add equal volume of phenol-chloroform (1:1) and centrifuge at 10,000 xg for 5 minutes at room temperature.
- Transfer the supernatant to new tube and add one volume of chloroform isoamyl alcohol (24:1). Centrifuge at 10,000 xg for 5 minutes at room temperature and transfer the supernatant to a 1.5 mL eppendorf tube. Add 0.1 volume of 3 mol/L sodium acetate and 2.5 volumes absolute ethanol. Leave at -2℃ for 20 minutes.
- Centrifuge at 10,000 xg for 10 minutes at 4℃ and discard the supernatant then wash pellet with 75% ethanol.
- Dry the pellet for 30 minutes and dissolve in 50 µL TE buffer.

Narendran *et al.* (2013) described the procedure for agro-bacterium mediated transformation and tissue culture based plant regeneration for efficient genetic transformation of okra using embryo explants.

- Twenty one transgenic okra lines expressing the *Bacillus thuringiensis* gene *Cry1Ac* were generated from five transformation experiment.

- Molecular analysis (PCR and Southern blotting) confirmed the presence of the transgene and double-antibody ELISA analysis revealed *Cry1Ac* protein expression in the transgenic plants. All 21 transgenic plants were phenotypically normal and fertile.
- T_1 generation plants from these lines were used in segregation analysis of the transgene.

Dhande *et al.* (2012) conducted an experiment for the evaluation of the most suitable concentration of plant growth regulators for regeneration of okra *(Abelmoschus esculentus* (L.) Monech*) via* apical shoot culture system. Okra **line N-550** evolved at Research and Development, Nirmal Seeds Pvt. Ltd. was used as basic material and different auxin and cytokinins hormone combinations and concentrations.

- Nine to ten days old germinating seedlings were used for isolation of shoot tip explants.
- Isolated shoot tips were cultured on regeneration medium, Murashige and Skoog (MS) medium supplemented with indole-3-butyric acid (IBA) at various concentrations of 0.25, 0.5, 1.00, 1.5, 2.0 and 2.5 mg/L and IBA 1.0 mg/L in combination with naphthalene acetic acid (NAA) @ 0.25, 0.5, 1.00, 1.5, 2.0 and 2.5 mg/L.
- After regeneration, cultures were inoculated on elongation medium containing MS medium supplemented with kinetin (0.25, 0.5, 1.00, 1.5, 2.0 and 2.5 mg/L).
- Then the elongated shoots were transferred to rooting medium containing MS medium supplemented with indole-3-butyric acid (IAA) @ 0.25, 0.5, 1.00, 1.5, 2.0 and 2.5 mg/L.

  Results: Combination of 1.0 mg/L IBA and 0.5 mg/L NAA were found to be most effective for plant regeneration from apical shoot and best shoot elongation observed in MS medium supplemented with kinetin 0.5 mg/L.

Conclusion

Certain biotechnological interventions in okra leads to a tremendous increase in the status of genetic improvement as it improves nutritional quality and helps to combat various biotic and abiotic stresses. But in future, research needs to be conducted on incorporating genes for yellow vein mosaic virus disease and developing varieties for fruit and shoot borer resistance in okra by transferring the gene *Cry 1AC* from *Bacillus thuringiensis* (Bt) as these two are the major problems in okra and diminishes the productivity drastically.

Disclosure statement:

No potential conflict of interest was reported by the authors.

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