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# Gene manipulation for gourds (Cucurbits) improvement

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**Abstract**
Gene is basic unit of heredity, sequence of nucleotides in DNA. Plant breeding means the production of new crop varieties which are far better than existing ones in all aspects and improvement in the heredity of crop plant. Conventional breeding develops new plant varieties and seeks to achieve expression of genetic material which is already present within a species, through this unable to transfer only targeted genes and gene from micro-organisms. Advance method of breeding includes transgenic breeding, molecular marker breeding, gene splicing, use of recombinant DNA, forming of the monoclonal antibodies or PCR, combining multiple disease resistance (gene pyramiding), high yielding varieties and many more which can achieve through gene manipulation (genetic engineering). So it is important to know, How…? and What…? we can improve through gene manipulation. A plant in which a foreign gene from any organism has been artificially introduced through use of modern biotechnology or genetic engineering is called a transgenic plant which has desired traits and the gene is called Transgene. Molecular breeding may be defined in a broad-sense as the use of genetic manipulation performed at DNA molecular levels to improve characters of interest in plants. It also helps to make selection decisions before phenotypes are available. GM crops have been contributing to the reduction of $CO_2$ emissions by reducing use of pesticides spray, herbicide tolerant GM crops facilitate zero or no-till which results in saving of tractor and fossile fuel use. Most of the commercial vegetable crops have narrow genetic base in cultivated species. Therefore, in order to broaden their genetic base wide hybridization following biotechnological approaches should be used to generate genetic stocks with useful traits retrieved from wild relatives which could be employed for breeding desirable varieties/hybrids. Grouping of genotype based on genetic relationship, which may further used in development of improved cultivar.$AVP_1$-28 and $AVP_1$-31 are found to be resistant towards herbicide and drought, while BGAVP-18 and BGAVP-20 found to be resistant towards salt in bottle gourd. Papaya ring spot virus resistance in bottle gourd and Tomato leaf curl New delhi virus resistance in *Luffa* spp. identified towards particular disease.

**Keywords:** DNA, GM crops, genetic engineering, marker

## Introduction
Cucurbits is the largest group of summer vegetable crops. Cucurbit's term coined by - Dr. Baily. It is one of the most genetically diverse group of plants, includes 118 genera and 825 species. In India 35 genera and 108 species are cultivated. *Trichosanthus* is largest genus in cucurbitaceous family. It's used as salad or cooking, pickling, candy or preservation. Cucurbits are mostly annuals in nature except pointed gourd, ivy gourd, chow chow (Perennial). It is mostly seed propagated; except pointed gourd, spine gourd, ivy gourd (Vegetatively propagated) and mostly monocious; except pointed gourd, spine gourd, ivy gourd (Dioecious). It have many medicinal value in different part of plant used to relive diabetes, hypertension, stomachic, laxative, emetic, entihelmintic agent, treatment of cough, respiratory disease and skin disease.

Flowering in gourds normally starts in about 40-45 days after sowing depending upon the weather condition. The sequence of flowering follows a set of pattern, namely (i) Male phase (ii) Mixed phase (iii) Female phase. Cross pollination is mainly happens by honeybees (60-80%). Anthesis time in gourds mainly up to 5:30 to 7:30 a.m. but in bottle gourd, snake gourd, pointed gourd and ridge gourd it occurs in evening hour from 5:00 to 8:00 p.m. Stigma receptivity is different to the species but, mainly before anthesis 24 hours and after anthesis 24 hours observed. Pollen Fertility is on the day of anthesis till the next morning. After 24 hours of pollination pollen tube reach to the ovary and after 2-3 days ovary become enlarged. Plant breeding means the improvement in the heredity of plant and production of new crop varieties which are far better than existing one in all aspects.

Conventional breeding develops new plant varieties and seeks to achieve expression of genetic material which is already present within a species. Gene insertion is not possible in this method of breeding.

Advanced plant breeding is techniques of molecular biology to select the variations which combines biotechnology and molecular biology. Term 'Biotechnology' was coined by Karl Ereky (1919). It control use of biological agents, such as micro-organism or cellular components, for beneficial use. In the last 30 years it has become possible to take a gene out of one organism and put it into the DNA of another organism. This process is called genetic engineering (Recombinant DNA technology). The resulting organisms are genetically modified organisms (GMOs) and the gene that has been transplanted is a transgene. There are no real interspecies barriers here. A plant in which a foreign gene from any organism has been artificially introduced through use of modern biotechnology or genetic engineering is called a transgenic plant which has desired traits and the gene is called transgene.

Markers are the heritable characters whose inheritance pattern can be followed at the morphological, biochemical and molecular level. DNA marker is a fragment of DNA sequence, which is used as a substitute for phenotypic selection. Such fragments are associated with a certain location within the genome and may be detected by means of certain molecular technology. Ideally markers should be <5 cM from a gene or QTL. It include protein profiles, isozymes and DNA markers. Molecular breeding may be defined in a broad-sense as the use of genetic manipulation performed at DNA molecular levels to improve characters of interest in plants. It helps to make selection decisions before phenotypes are available. Useful for traits that are difficult to measure, exhibit low heritability and expressed late in development. Markers have been used in diversity analysis, parentage detection, DNA fingerprinting and prediction of hybrid performance.

Gene pyramiding has been proposed and applied to enhance resistance to disease and insects by selecting for two or more than two genes at a time. Widely used for combining multiple disease resistance genes for specific of a pathogen.It is Important to develop 'durable' disease resistance against different races. All plants were not able to grow well in all the climatic conditions. Plants are unable to grow well in stress condition so there is need to spray chemicals for making adopted in all stress conditions. These plants will show residual effect and which will affect quality, create health hazard, reduce export potential. So, if we go for resistance breeding we can get our trait of interest in our cultivated variety. It can be performed on seedling material. MAS are not affected by environmental conditions. When recessive alleles determine traits of interest, recessive alleles are identified by linked markers. When multiple resistance genes are pyramided together in the same variety or breeding line the presence of one resistance gene may conceal the effect of additional genes, this problem can be overcome if markers are available for each of the resistance genes. MAS are faster than conventional phenotypic screening. Four to six backcrosses are required to convert a new line into transgenic line through conventional methods, while the number of crosses can be reduced through use of molecular markers.

**Review of literature**

Han *et al.* (2005) [2] conducted an experiment to study transformation frequency of bottle gourd transgenic line and test of transformed bottle gourd transgenic line at NHRI, South Korea. They observed that inoculated cotyledon explants with *Agrobacterium tumefaciens* strain $AGL_1$ that carries the binary vector pCAMBIA3301 containing a glufosinate ammonium-resistance (bar) gene and the beta-d-glucuronidase (GUS) reporter gene which transformed successfully. Sarao *et al.* (2013) [9] conducted an experiment to study phylogenetic tree diagram depicting genetic relationships among bottle gourd genotypes based on SSR data using the computer program DARwin 5.0 at Punjab, India. They observed that ten primers exhibited polymorphic profiles, while nine exhibited monomorphic patterns and one revealed a null allele. Unique DNA profiles of all the accessions could be created using a set of five polymorphic primers. This SSR marker-based diversity would facilitate theimplementation of marker-assisted breeding schemes for efficient introduction of the desired traits into bottle gourd. The diverse bottle gourd genotypes may be further used in the development of improved cultivars with respect to quality and quantity. Deepti *et al.* (2014) [1] conducted an experiment to study genetic relationship based on RAPD marker among ten genotypes of bottle gourd at Faizabad, India. They find outdiversity of bottle gourd genotypes, which may be further used in the development of improved cultivars with respect to quality and quantity. Park *et al.* (2014) [5] conducted an experiment to study herbicide resistance test of transgenic bottle gourd andgrowth performance of $AVP_1$ *D*-transgenic bottle gourd in soil under water deficit conditions at Suwon, South Korea. They found development of a bottle gourd with resistance to herbicide and drought by ectopic expression of the Arabidopsis $AVP_1$ gene that encodes a vacuolar $H^+$-pyrophosphatase.

Han *et al.* (2015) [2] conducted an experiment to study different responses of plants subjected to salt stress of bottle gourd at Sangju, South Korea. They observed that transgenic bottle gourd lines expressing the arabidopsis $H^+$-pyrophosphatase $AVP_1$gene and confirmed the stable integration of $AVP_1$ in the genomes of bottle gourd $T_2$homozygous lines and its transcription for salt resistance. Sanjeev *et al.* (2016) [8] conducted an experiment to study agarose gel image showing the amplification profile from genomic DNA of 54 *in vitro* cultured plants of pointed gourd at Banaras Hindu University, Varanasi. They perceived monomorphic bands confirming the genetic uniformity of the *in vitro* raised plants. *In vitro* plants and that of the mother clone were identical with respect to all the amplification products. Shan *et al.* (2017) [10] conducted an experiment to studygenomic landscape of bottle gourd andgenotypes and PRSV resistance phenotypes of $F_2$ individuals showing recombination at the *Prs* locus and the phenotypes of their $F_3$ progenies of bottle gourdat USA. They noticed that genome sequence has facilitated the mapping of a dominant monogenic locus, Prs, conferring Papaya ring-spot virus (PRSV) resistance in bottle gourd, to a 317.8-kb region on chromosome 1. They have developed cleaved amplified polymorphic sequence (CAPS) marker tightly linked to the Prs locus and demonstrated its potential application in marker-assisted selection of PRSV resistance in bottle gourd.

Rao *et al.* (2018) [6] conducted an experiment to study genetic map of putative gynoecious locus (gy-1) on linkage group-12 of bitter gourd of a cross (DBGy-201 * Pusa Do Mousami) at Division of vegetable science, ICAR, New Delhi. They observed thatgynoecious (gy-1) locus is flanked by markers TP_54865and TP_54890 on LG 12 (linkage group) useful in marker development and MAS for rapid development of various gynoecious lines with different genetic background of best combiner for development of early and high yielding hybrids in bitter gourd. Wu *et al.* (2019) [11] conducted an experiment to study the genetic model of fruit bitterness in the $F_2$ population of bottle gourd, QTLs detected in the $F_2$ population under a multiple QTL model and genomic synteny of the bitterness genes between bottle gourd, cucumber, melon and watermelon at China. They observed that fruit bitterness in the bottle gourd was controlled by a pair of complementary genes. Two non-bitter landraces "Hangzhou Gourd" and "Puxian Gourd," each of which carries a single bitterness gene. They mapped the complementary genes causing fruit bitterness. Sequence-based comparative analysis showed no syntenic relationship between *QBt.1/QBt.2* and the known bitterness genes in cucumber, melon, and watermelon, suggesting that causal genes underlying *QBt.1* and *QBt.2* were not direct orthologs of the reported cucurbit bitterness genes.
Kaur *et al.* (2021) [4] conducted an experiment to study *Tomato leaf curl New Delhi virus* infected plants of susceptible check Arka Prasan (a) and resistant genotypes IIHR-137 (b) and IIHR-Sel-1 (c) screened in the field after natural infection during the spring–summer season in a ToLCNDV-hotspot area, screening *Luffa* germplasm and advanced breeding lines for resistance to *Tomato leaf curl New Delhi virus* andfield screening of *Luffa* spp. genotypes for resistance against *Tomato leaf curl New Delhi virus* at IIHR, Karnataka. They identified thatgenotypes IIHR-137 and IIHR-138 had no symptoms, IIHR-Sel-1 had only mild symptoms, resistant inbred lines identified are good candidates for a breeding program for ToLCNDV-resistant cultivars. Rodrigo *et al.* (2021) [7] conducted an experiment to study genetic diversity parameters generated by single nucleotide polymorphic markers across 11 chromosomes of bottle gourd at San Fernando, Chile. They observed that highest PIC values were on chromosome 10.

## Conclusion

Most of the commercial vegetable crops have narrow genetic base in cultivated species. Therefore, in order to broaden their genetic base wide hybridization following biotechnological approaches should be used to generate genetic stocks with useful traits retrieved from wild relatives who could be employed for breeding desirable varieties/hybrids. Grouping of genotype is based on genetic relationship, which may further used in development of improved cultivar. $AVP_1$-28 and $AVP_1$-31 are found to be resistant towards herbicide and drought, while BGAVP-18 and BGAVP-20 found to be resistant towards salt in bottle gourd. Papaya ring spot virus resistance in bottle gourd and Tomato leaf curl New delhi virus resistance in *Luffa* spp. identified towards particular disease.

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