

Review Article

<https://doi.org/10.20546/ijcmas.2017.611.226>

Biotechnology a Modern Tool for Fruits Production - A Review

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ABSTRACT

The problem of long juvenility, incompatibility and sterility are the biggest hindrances for the improvement of fruit crops through conventional fruit breeding. To mitigate the biotic and abiotic stresses, improvement is desired in already cultivated cultivar for one or few traits. In that regard, the biotechnological tools will prove quite useful to expedite the rate of fruit crop improvement. For production of quality planting material by tissue culture is commercialized in banana and strawberry. Biotechnological tools such as somaclonal variation and *in vitro* mutagenesis are the choicest method for varietal development in banana. GCTCV-119 and Novaria are the result of somaclonal variation and *in vitro* mutagenesis, respectively. Somatic hybridization has been explored for the rootstock and scion improvement of citrus. Somatic hybridization research of almost two decades in citrus is yielding fruit now. Embryo rescue has helped in accelerating the pace of stenospermocarpic grape improvement and 'Sweet Scarlet' and 'Thomcord' are two of the seedless table grapevines, which were released using this technique. Transgenic variety Rainbow and Sun Up carrying resistance to PRSV are being commercially cultivated in Hawaii of USA. With the introduction of concept of *cisgenics*, the genetically transformed fruits are likely to get public acceptance. Molecular marker aid great help in early diagnosis of sex of dioecious fruit plant like date palm and papaya. Presently marker linked to resistance for Dagger nematode and pierce disease at university of California in the breeding programs are routinely used for grapevine rootstock improvement. Genome sequencing has unveiled the mystery about various genes, the phylogenetic relationship and evolution history of sequenced fruit crop.

Keywords

Tissue culture, *in vitro*, Somaclonal variation, Embryo rescue, Molecular marker.

Article Info

Accepted:
15 September 2017
Available Online:
10 November 2017

Introduction

The requirement of fruits is increasing proportionally with the increasing population. Although conventional plant breeding techniques have made considerable progress in the development of improved varieties, they have not been able to keep pace with the increasing demand for fruits. Therefore an immediate need is felt to integrate biotechnology to speed up the crop improvement programmes. Biotechnological

tools have revolutionized the entire crop improvement programmes by providing new strains of plants, supply of planting material, more efficient and selective pesticides and improved fertilizers. Fruit crops are conventionally propagated through cutting, budding, grafting, layering *etc.* but these techniques are time consuming and cumbersome. Plant tissue culture provides an effective measure of such problems of fruit

crop multiplication. There is need of efficient technology which facilitates faster multiplication of propagules with potent regeneration. In recent years, synthetic seed technology using encapsulation of non-embryogenic propagules derived under *in vitro* condition has become an important asset to micropropagation. Many genetically modified fruits and vegetables are already in the market in developed countries. It is a new aspect of biological and agricultural science which provides new tools and strategies in the struggle against world's food production problem.

Biotechnological tool in fruit crop improvement

Tissue culture

It is one of the most widely used techniques for rapid asexual *in vitro* propagation. This technique is economical in time and space affords greater output and provides disease free and elite propagules.

It also facilitates safer and quarantined movements of germplasm across nations. When the traditional methods are unable to meet the demand for propagation material this technique can produce millions of uniformly flowering and yielding plants. Micropropagation technology ensures true to type, rapid and mass multiplication of plants that possesses special significance in vegetatively propagated plant species. This technology has witnessed a huge expansion globally.

Some basic techniques of tissue culture, such as anther/microspore culture, somaclonal variation, embryo culture, and somatic hybridization, are being exploited to generate useful genetic variability for obtaining incremental improvement in commercial cultivars.

Micropropagation

Micropropagation of almost all the fruit crops is possible now. Production of virus free planting material using meristem culture has been made possible in many horticultural crop Strawberry is perhaps first fruit crop in which micropropagation technique has been standardized (Sharma and Singh, 1999). *In vitro* plants are more uniform, produce higher number of runners, have better survival in the field, and the fruit yield increase in 24% than plants propagated by the traditional method (Kikas *et al.*, 2006). Banana is commercially propagated through tissue culture. The future of banana industry in the country is totally dependent upon control of BBTV disease. The ultimate choice is productions of disease-free banana plants through *in vitro* techniques to replace infected fields. There are number of fruit crop in which propagation method is sexual. Some of them are dioecious *i.e.* male and female flower borne on different flowers. So through commercial point of view it is desirable to produce more female plant which, are the ones which bear fruits, this is possible only through tissue culture techniques. The grafting a very small shoot tip excised from an elite mother tree grafted onto a decapitated rootstock seedling grown under aseptic condition. Murashige *et al.*, (1972) made the first attempt in citrus and subsequently it was refined by Navarro *et al.*, (1975).

Embryo rescue

Embryo rescue is another area, where plant breeders are able to rescue their crosses, which would otherwise abort. Culture of excised embryos of suitable stages of development can circumvent problems encountered in post zygotic incompatibility. This technique is highly significant in intractable and long duration horticultural species. Ovule culture in grapes (Singh *et al.*, 1991) aids in developing hybrids, even in

seedless grapes. Embryo rescue has helped in accelerating the pace of stenospermocarpic grape improvement and 'Sweet Scarlet' and 'Thomcord' are two of the seedless table grapevines, which were released using this technique (Anonymous, 2004).

Anther/pollen culture

Application of anther culture is obtaining haploids and homozygous diploids. It provides a short cut for obtaining pure lines. As tetraploid plants are produced in greater abundance in breeding programme, it may be feasible to culture their anther to produce useful diploids.

Regeneration of androgenic embryos in apple (*Malus x domestica* Borkh.) via anther and microspore culture (Hofer, 2005) has been succeeded.

***In vitro* mutagenesis**

Mutagens used physical (gamma rays, X-rays, UV rays) and chemical (EMS, MMS *etc.*). In fruit crops EMS and gamma rays are widely used. Notable example of *in vitro* mutagenesis in Banana variety Grand Nain explants used was shoot tips; mutagen source and dose were 80 Gy gamma rays developed improved cultivar Novaria-10 with improved trait early flowering and high keeping quality (Mac *et al.*, 1996).

Somatic hybridization

It is crossing of crop plants through fusion of somatic cells. Protoplast of two cells is fused. It breaks the barriers of cross incompatibility among plant species and makes incompatible crosses possible. Somatic hybridization has been explored for the rootstock and scion improvement of citrus. In scion improvement production of allotetraploids $2x \times 4x = 3x$ (seedless) and direct $3x$ production whereas,

in rootstock improvement, production of tetraploid rootstock for greater dwarfing effect, which is used in HDP. Gene transfer/wide somatic hybrids development of resistant rootstock through gene transfer from sexually incompatible or difficult to hybridized genera (Grosser *et al.*, 2000). Somatic hybridization research of almost two decades in citrus is yielding fruit now.

***In vitro* germplasm conservation**

In vitro conservation is of great significance in providing solutions and alternative approaches to overcoming constraints in management of genetic resources. Complete plants have been successfully regenerated from tissues cryopreserved at -196°C in liquid N_2 in several crops for several months to years (Ford *et al.*, 2000; Panis *et al.*, 2001; Halmagyi *et al.*, 2004; Gupta and Reed, 2006; Ding *et al.*, 2008). This method is now being practically used at several national and international germplasm banks.

In crops, which are propagated vegetatively and which produce recalcitrant seeds and perennial crops which are highly heterozygous seed storage is not suitable. In such crops especially, *in vitro* storage is of great practical importance. These techniques have successfully been demonstrated in a number of fruit crops and there are now various germplasm collection centers.

A portion of plants regenerated by tissue culture often exhibits phenotypic variation atypical of the original phenotype. Such variation, termed soma clonal variation may be heritable *i.e.* genetically stable and passed on to the next generation. Also, presently, synseed technology using encapsulation of non-embryogenic propagules derived under *in vitro* condition has become an important asset to micropropagation and *in vitro* conservation of planting material for long term.

Somaclonal variation

Somaclonal variation (Larkin and Scowcroft, 1988) is considered a source of new plant genotypes for breeding, and advances at the tissue culture level have opened new possibilities for applications in viticulture. The advancements made in tissue culture techniques has made it possible to regenerate various horticultural species *in vitro* as micropropagation protocols for commercial scale multiplication are available for a wide range of crops. However, plant tissue culture may generate genetic variability *i.e.*, somaclonal variations as a result of gene mutation or changes in epigenetic marks. The occurrence of subtle somaclonal variation is a drawback for both *in vitro* cloning as well as germplasm preservation. Somaclonal variation has provided a new and alternative tool to the breeders for obtaining genetic variability relatively rapidly and without sophisticated technology in horticultural crops, which are either difficult to breed or have narrow genetic base. Somaclonal variations are beneficial for creating variability and resistance to stresses (Table 1).

Molecular marker

A molecular marker is a DNA sequence that is readily detected and whose inheritance can easily be monitored. The phenotype is imperfect predictor of genotype. The phenotype is always expression of its genotype and environment interaction. Two plants having similar phenotype but genotype point of view they may be different. Application of molecular marker in fruit crops are genetic diversity assessment, estimation of genetic distance between cultivar and phylogenic analysis, detection of quantitative trait loci and marker assisted selection. Molecular marker is used in sex determination of dioecious plants at juvenile stage. Genetics of control mechanisms that

underlies sex differentiation in date palm is not known. Sex of the plants becomes known only at the time of first flowering, which takes around 5 years. In comparison, molecular diagnosis (if available/feasible) promises quick and reliable identification of sex types very early when plantlets are growing in seedbeds. To develop such an assay, genomic DNA from 45 individual plants (25 female and 20 male) belonging to different varieties of date palm was subjected to PCR amplification using 100 RAPD and 104 ISSR (Dhawan *et al.*, 2013). In papaya identification of female and hermaphrodite specific marker papaya sex determination by RAPD (Diwedi *et al.*, 2014).

Genetic diversity assessment

Assessment of diversity has traditionally been made through morphological characters, chemical composition, and cytological characters, however, they have several limitations especially in perennial crops. Morphological characters are often limited in number, have complex inheritance pattern and are vulnerable to environmental conditions, hence it is desirable to develop alternative methods, which are rapid, reliable and more or less not influenced by environment. Therefore, in the recent past, rapid use of molecular markers has complemented the classical strategies. The use of DNA markers has enabled the characterization of genotypes independent of the influences of environmental growth conditions, physiological age of the plant and the type of tissue being analyzed. Molecular markers have been looked upon as tools with a large number of applications including localization of a gene and improvement of plant varieties by marker-assisted selection. Molecular markers thus, are excellent tools for analysis of genetic diversity and relationship (Table 2). Polymerase chain reaction (PCR) based random amplified polymorphic DNA (RAPD)

markers have been extensively used in DNA finger printing. These DNA markers are generated by PCR amplification of random genomic segments with single primers (usually 10 nucleotides long) of arbitrary sequence (Williams *et al.*, 1990). These markers are mostly dominant and detect

variation in both coding and non-coding region of genome. RAPD analysis is technically simple and provides an approach to characterize different genotypes, thus to estimate genetic diversity which will further be useful in improvement of breeders.

Table.1 Stability of some somaclones to biotic stresses under field condition

Fruit crop /cultivar	Somaclonal variant	Resistant/tolerant to	Field performance	Reference
<i>Prunus persica</i>	S19-1 and S-156 (Sunhigh) S122-1 (Redhaven) S122-1	Bacterial leaf spot <i>Pseudomonas syringae</i>	*HR after 3 yrs in field HR resistance compared to Redheaven	Hammerschlag <i>et al.</i> , 1994
Apple cv. Green Sleeves	-	Fire blight (<i>Erwinia amylovora</i> Strain T)	60% less symptoms as compared parent cultivar	Donovan <i>et al.</i> , 1994
<i>Citrus limon</i>	FS 01 and FS 11	Mal Secco (<i>Phoma tracheiphilla</i>)	Tolerance level comparable to Monachello (T)	Gentile <i>et al.</i> , 1998
Banana cv. William	CIEN BTA-03	Resistance to yellow and black Sigatoka	Similar performance even after 5 year of field trails	Unai <i>et al.</i> , 2004; Gimenez <i>et al.</i> , 2008

Table.2 DNA markers for genetic diversity assessment in fruit crops

Fruit	Marker type	Reference
Apple	AFLP and RAPDs	Coart <i>et al.</i> , (2003); Botez <i>et al.</i> , (2009); Sestras <i>et al.</i> , (2009)
Avocado	Mini satellite DNA	Ashworth <i>et al.</i> , (2003)
Banana	RAPDs	Brown <i>et al.</i> , (2009)
Citrus	RFLP	Durham <i>et al.</i> , (1992)
Grapes	RFLP and SSRs	Bourquin <i>et al.</i> , (1993)
Mango	CpSSR and RAPDs	He <i>et al.</i> , (2007) Marcela <i>et al.</i> , (2009)
Pistachio	Mini satellite marker	Riaz Ahmad <i>et al.</i> , (2003)
Cashew	RAPD and ISSR	Thimmappaiah <i>et al.</i> , (2009)
Pear	SSRs and AFLP	Sisko <i>et al.</i> , (2009)

Table.3 Markers associated to main polygenic traits in fruit crops

Fruit	Trait	Marker type	References
Apple	Fire blight resistance	SCAR, SSR	Sylwia <i>et al.</i> , (2009)
Citrus	Citrus leprosis virus resistance	AFLP and RAPD	Bastianel <i>et al.</i> ,(2009)
Pear	Incompatibility	AFLP and SSR	Sun <i>et al.</i> , (2009)
Banana	Sugar content	RFLP	Ming <i>et al.</i> , (2001)
Grapes	Seedlessness, Berry Size, and Ripening Date	AFLP, SSR,RAPDs, ISSRs and SCARs	Mejía <i>et al.</i> , (2007)
Strawberry	Day-neutrality	AFLP	Weebadde <i>et al.</i> , (2008)
Apricot	Plum Pox Virus	SSR	Soriano <i>et al.</i> , (2007)

Identification of QTLs

Many important heritable characters are a consequence of the joint action of several genes. Such characters are often referred to as polygenic or quantitative. Several characters of plant species, among which are traits of agronomic importance, are inherited quantitatively. Yield, maturity date and drought tolerance are examples of such characters. The genetic loci for such characters have been referred to as quantitative trait loci (QTLs). The essential feature which makes feasible the finding and characterization of a QTL is its linkage with a known marker locus segregating with Mendelian ratios. DNA markers provide this opportunity by making it feasible to identify, map and measure the effects of genes underlying quantitative trait. In grape QTLs were used for features such as like critical photoperiod, growth cessation, or dormancy, bud break (BB) and winter hardiness (Table 3). Approximate positions of 28 major genes were mapped in different populations of peach (orange background), almond (yellow

background) and Myrobalan plum (green background) on the framework of the *Prunus* reference map. Gene abbreviations correspond to: *Y*, peach flesh colour; *Sharka*, plum pox virus resistance; *Mi*, nematode resistance from peach; *D*, almond shell hardness; *Br*, broomy plant habit; *Dl*, double flower; *Cs*, flesh color around the stone; *Ag*, anther color; *Pcp*, polycarpel; *Fc*, flower color; *Lb*, blooming date; *F*, flesh adherence to stone; *D*, non-acid fruit in peach, *Sk*, bitter kernel; *G*, fruit skin pubescence; *Nl*, leaf shape; *Dw*, dwarf plant; *Ps*, male sterility; *Sc*, fruit skin color; *Gr*, leaf colour; *Ma*, nematode resistance from Myrobalan plum; *E*, leaf gland shape; *Sf*, resistance to powdery mildew. Genes *Dl* and *Br* are located on an unknown position of G2.

Marker assisted selection

The use of molecular markers can greatly accelerate the pace of selection in fruit crops with inherent long juvenile period and when trait under study is controlled by the recessive gene and incorporating more than one gene

for disease resistance. Presently, the markers linked to resistance to Dagger nematode (*Xiphinema index*) and pierce disease are being routinely used at University of California in the breeding programs aimed at improving grapevine rootstock.(Xu *et al.*, 2008).The resistance to most successful resistance gene (Vf) for scab has been overcome in apple by new race pathogen (Gygax *et al.*,2004).

Genetic transformation

Process of transfer, integration and expression of transgenes in the host cells is known as genetic transformation. Resistant to biotic stress for example citrus decline, CTV, greening, papaya ring spot virus resistance, plum pox virus *etc.* Higher tolerance to abiotic stress *i.e.* salinity, flood, drought *etc.*, shortening the juvenile growth phase and quality improvement such as shelf life improvement and edible vaccine.

Shelf life improvement

The compositional similarity of the transgenic papayas with delayed ripening trait with the non-transgenic control papayas. Fruit characteristics such as days to maturity, fruit weight and total soluble sugars were similar for both transgenic and control papaya trees (Cabanos, *et al.*, 2014).

Two ACC oxidase genes, CpACO1 and CpACO2 are reported and CpACO1 is associated with late stage fruit ripening and leaf senescence whereas CpACO2 is induced before colors break (Chen *et al.*, 2003). Two of the ripening-related genes MaMads-rin and MaExp2 are used for banana transformation in order to increase shelf-life and fruit quality Liu *et al.*, (2009). Juan *et al.*, (2009) have shown that PG plays a significant role in strawberry ripening. Two ripening-related PG genes, FaPG1 and FaPG2 identified for

regulation of fruit ripening. Using tobacco rattle virus-induced gene silencing, the down regulation of FaSS1 transcripts significantly delayed fruit ripening, as evidenced by the changes of firmness, and soluble sugar and anthocyanin contents, as well as the transcripts of several ripening-related genes, including PE1, PL1, XYL2, CHS, CHI and DFR. Furthermore, the mRNA expression level of FaSS1 was inhibited by abscisic acid or sucrose, but not by glucose after fruit disc incubation in vitro (Cheng *et al.*, 2017). The two banana E class (SEPALLATA3 [SEP3]) MADS box genes, MaMADS1 and MaMADS2, homologous to the tomato (*Solanum lycopersicum*) RIN-MADS ripening gene. Transgenic banana plants repressing either gene (via antisense or RNA interference [RNAi]) were created and exhibited specific ripening delay and extended shelf-life phenotypes, including delayed color development and softening (Tomer *et al.*,2016).

Disease resistance

A viral diseases papaya ring spot virus came to Hawaii in the 1940's and had wiped out papaya production on Oahu by the 1950's. Transgenicpapaya was developed through coat protein mediated gene transformation. The two transgenic varieties were developed *i.e.* Sun Up and Rainbow, those are resistant to Papaya ring spot virus, which revolutionized the papaya cultivation in Hawaii (Gonsalves, 2000).

Plum pox virus causes fruit deformation and reduced quality, premature fruit drop, leaf chlorosis, tree decline and death in severe infections or co-infections with other viruses. Honey Sweet an improved cultivar of plum was developed using mechanism of RNAi silencing, which was resistant to virus (Ravelonandro *et al.*, 2000).

Future prospect

Plant biotechnology has the potential to play a key role in the sustainable production of fruit crops. However, there is enormous potential for genetic manipulation of some vegetative propagated fruit crops in order to improve their disease and pest resistance. The use of appropriate constructs may allow the production of nematode, fungal, bacterial and virus resistant plants in a significantly shorter period of time than using conventional breeding, especially if several traits can be introduced simultaneously. It may also be possible to incorporate other characteristics such as drought tolerance, thereby extending the geographic spread of some fruit crops for production, and thus contributing substantially to enhanced food security and poverty alleviation. However, high caution is required for biosafety experiments and potential risk assessment bearing in mind that these are consumed by most humans and mainly by children.

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How to cite this article:

Anuradha, Subhash Chander and Arvind Malik. 2017. Biotechnology a Modern Tool for Fruits Production - A Review. *Int.J.Curr.Microbiol.App.Sci.* 6(11): 1902-1912.
doi: <https://doi.org/10.20546/ijcmas.2017.611.226>