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Genetic transformation of fruit crops: A review

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Abstract
Genetic engineering methods based on the use of transgene(s) have been successfully adopted to improve fruit crops and focused mainly on enhanced tolerance to biotic and abiotic stresses, increased fruit yield, improved post-harvest shelf life of fruit, reduced generation time and production of fruit with higher nutritional value. Usually two tissue culture methods have been used for regeneration of transgenic plants: organogenesis and somatic embryogenesis. Genes conferring insect resistance to plants have been obtained from microorganisms e.g *Bt* gene from *Bacillus thuringiensis*. Transgenic lines showed the potential for accelerating breeding cycles, reducing juvenility time, and adaptation to climate regimes. Sweetness is one of the major quality-determining factors in the fruit plants which depend mainly on the types, composition and endogenously content of sugar. Therefore, in order to maximize commercial success through wide adoption and public acceptance of GM food crops, it is desirable to avoid the use of antibiotic selection or to allow elimination of marker genes from the transformed plant**.** The acceptance of science-based approaches like cisgenesis or intragenesis or use of selection marker free transgenic will encourage confidence, and bring the benefits of GM products to consumers.

Keywords: Genetic transformation, Recombinant DNA, Transgene, Marker

Introduction
Genetic transformation also called *genetic modification* is the direct manipulation of an organism's genome using recombinant DNA technology. It involves the introduction of foreign gene (s) into an organism of interest. This is accomplished by isolating and cloning of a gene of interest using molecular cloning methods to generate a DNA sequence containing the required genetic elements for expression, and then inserting this construct into the host organism. Genetic engineering methods based on the use of transgene (s) have been successfully adopted to improve fruit crops and focused mainly on enhanced tolerance to biotic and abiotic stresses, increased fruit yield, improved post-harvest shelf life of fruit, reduced generation time and production of fruit with higher nutritional value. However, the development of transgenic fruit plants and their commercialization are hindered by many regulatory and social hurdles. Nowadays, new genetic transformation approaches i.e. cisgenesis or intragenesis receive increasing interest for genetic modification of plants. The absence of selectable marker gene in the final product and the introduced gene (s) derived from the same plant or plants sexually compatible with the target crop should increase consumer's acceptance.

I. Genetic Transformation of Fruit Crops: Mode of Regeneration and Transformation
Transgenic plants have been developed through a number of gene delivery methods. *Agrobacterium*- and particle bombardment mediated gene transfer are the most popular methods for the development of transgenic plants. In most fruit plants, both plant regeneration *under in vitro* condition and genetic transformation were slow to be developed, therefore, they are usually considered as 'recalcitrant' for *in vitro* culture and genetic transformation. Lack of an efficient regeneration system in fruit crops mainly perennial woody plants is one of the bottlenecks for applying gene transfer technologies to these plants. In addition, the difficulty in regeneration from elite or mature phase selections is another major problem of fruit trees.

Usually, success of genetic transformation highly depends on the regeneration pathway adopted by individual species, which is influenced by several factors, namely, genotype or cultivar, the source of the explant, and the degree of determination in the tissue. Therefore, plant regeneration system / protocol for targeted species must be optimized to achieve efficient rates of transformation.

Usually two tissue culture methods have been used for regeneration of transgenic plants: organogenesis and somatic embryogenesis. Organogenesis is the process in which plant regeneration occurs by organ (shoot and root) formation on explants, whereas somatic embryogenesis is the formation of bipolar embryos from a somatic cell. Somatic embryogenesis appears to have many advantages over organogenesis, including its potentially high multiplication rates, potential for scale-up via bioreactor and delivery through synthetic seeds. Therefore, somatic embryogenesis has been emphasized as a suitable target for gene transfer.

II. Potential application in fruit crop improvement

1. Disease resistance

In the last two decades, considerable efforts have been made on the development of transgenic fruit plants utilizing a broad range of genes to enhance disease resistance against fungal, bacterial and viral pathogens. However, transgenic disease resistance in fruit plant is currently implemented commercially only for viruses and this represents a small proportion of all transgenic plants grown commercially worldwide. Papaya cv 'Sun Up' the first virus resistant transgenic fruit authorized for field cultivation and commercialization. In another case, after extensive testing and risk assessment in laboratory, greenhouse and in the field for over 20 years, 'Honey Sweet' plum, a plum pox virus (PPV) resistant transgenic plum, has now been validated for cultivation in the USA. Several studies on genetic engineering of fruit plants with *CP* gene suggest that transgene conferred viral resistance is mediated by RNA via post-transcriptional gene silencing (PTGS). Different RNA mediated strategies like antisense, small hairpin RNA, intron spliced hairpin RNA or self-complementary inverted repeats (IR) were employed for probable resistance to viruses. For fungal and bacterial diseases, the following four strategies have been applied to make disease resistant transgenic plant: transgenic disease-resistant plants used (1) genes encoding pathogenesis-related proteins (PR proteins), antimicrobial peptides or antimicrobial metabolites (2) genes that encode detoxification mechanisms (3) genes that have a role in pathogen recognition and (4) genes that regulate defense mechanisms (Collinge *et al*., 2010) [1].

The most widely used transgenic approach to enhance resistance against fungal diseases has been based on the over-expression of PR proteins. The production of hydrolytic enzymes like chitinase and glucanase, best characterized class of PR proteins, capable of degrading the cell wall of invading pathogenic fungi is an important component of the defense response in plants against fungal pathogens. Recently *chitinase* gene has been transferred in apple rootstocks. The regenerated transgenic plants have shown the integration and of *chitinase* gene into the genome of apple rootstocks MM106 and M7 (Mathur, 2010) [9].

2. Insect Resistance

Progress in engineering insect resistance in transgenic plants has been achieved through the use of insect control protein genes of *Bacillus thuringiensis.* Today insect-resistance transgenes, whether of plant, bacterial or other origin, can be introduced in to plants to increase the level of insect resistance. Approximately 40 different genes conferring insect resistance have been incorporated into crops. Genes conferring insect resistance to plants have been obtained from microorganisms e.g *Bt* gene from *Bacillus thuringiensis*. The incorporation of insecticidal activity of proteins made by *Bacillus thuringiensis* would be an effective strategy to control codling moth, one of the major pests of apple and effective against Lepidopteran, Dipteran, and Coleopteran insects (Hofte and Whiteley, 1989) [7].

3. Reduction of the generation time

The long juvenile phase of trees is a limiting factor to their genetic improvement and preventing full domestication of most of the trees species (Pena and Seguin 2001) [12]. Flachowsky *et al*., (2007) [4] succeeded in shortening the juvenile phase in apple by overexpressing the *BpMADS4* gene. Early flowering transgenic apple line T1190 overexpressing the *BpMADS4* gene was characterized at the molecular and phenotypic level and further selected for its use in a fast breeding program (Flachowsky *et al*., 2011) [5]. Transgenic lines showed the potential for accelerating breeding cycles, reducing juvenility time, and adaptation to climate regimes.

4. Improved rooting ability, fruit quality and post-harvest shelf life of fruit

Transformation with genes that mediate horticulturally important traits like improved fruit quality, long shelf storage life of fruit, fruit softening and ripening, plant growth characteristics is a truly innovative approach for improving fruit species. The levels of plant hormones in plants were modified by over-expression of some specific genes i.e. *rol A, B* or *C* genes leading to morphological changes in transgenic plants (Pena and Seguin 2001) [12]. During the last decade, many fruit plants have been transformed with *rol A, B* or *C* genes to improve rooting ability or dwarfism. Transgenic lines showed twofold increase in ascorbic acid content over a non-transgenic control. Colour is a key quality trait of fruits and flowers and is often associated with anthocyanin, water soluble pigments that belong to the flavonoid compound family (Gambino and Gribaudo, 2012) [6]. Espley *et al*., (2007) [3] reported that the transformation of apple with the transcription factor *MdMYB10* results in the higher accumulation of anthocyanin. Sweetness is one of the major quality-determining factors in the fruit plants which depend mainly on the types, composition and endogenously content of sugar. Park *et al*. (2006) [11] reported the fruit specific suppression of AG Pase in strawberry fruit by transformation with the antisense sequence of the coding gene (*Fagp S*). The result of this study showed decreased fruit starch content to 27–47% and increased total soluble sugar content, 16–37%. Many taste-modifying sweet proteins have potential application for developing transgenic plants to improve the sweetness and quality of fruits. Short post-harvest shelf life is one of the major horticultural problems of many climacteric fruit (*e.g.* apple, avocado, banana, mango, peach, pear etc.).

5. Abiotic stress tolerance

The plant growth and productivity of many crops are adversely affected by several abiotic stresses including salinity, drought, heat, flood, frost and mineral toxicities. Salinity, drought, high temperature and other abiotic stresses, are often interconnected with oxidative stress, trigger a series of biochemical, physiological and molecular changes in plants, and may induce similar cellular damage (Rai *et al*., 2011) [13]. Plant responds to drought and/or salinity in mainly two phases, primarily as osmotic stress, resulting in the disruption of homeostasis and ion distribution in the cell and oxidative stress which may cause denaturation of functional and structural proteins (Munns and Tester, 2008) [10]. In recent years, a number of genes with diverse function and mechanism were employed for the development of transgenic temperate fruit plants to improve resistance/tolerance to different abiotic stresses.

III. Selection marker-free transgenic fruit plants

In the recent years, transgenic approaches have had many controversies and most of the European countries are not allowing the release of transgenic plants carrying antibiotic resistant gene. Therefore, in order to maximize commercial success through wide adoption and public acceptance of GM food crops, it is desirable to avoid the use of antibiotic selection or to allow elimination of marker genes from the transformed plant (Manimaran *et al*., 2011) [8]. Darbani *et al*., (2007) [2] described the different strategies for the development of transgenic plants that have substitute marker genes of non-bacterial origin. In recent years, regeneration of marker-free transgenic plants using binary vectors devoid of selectable marker genes has been reported in a number of fruit crops.

IV. Conclusion

Transgenes capable of altering many agronomic and horticulturally important traits such as biotic and abiotic stress tolerance, improved fruit quality, long shelf storage life of fruit, fruit softening and ripening, plant growth characteristics are available for a number of fruit crops. However, the development of GM fruit plants and their commercialization are hindered by many regulatory and social hurdles. From the biosafety and consumer point views, the presence of selectable marker genes, which are essential for the initial selection of transgenic plants, is undesirable. Therefore, the production of 'clean' marker-free transgenic fruit plants is now an essential requisite for their commercial exploitation. The acceptance of science-based approaches like cisgenesis or intragenesis or use of selection marker free transgenic will encourage confidence, and bring the benefits of GM products to consumers. In addition, there is a great need for global coordination of regulations to remove artificial trade barriers, promoting technology transfer, and protecting developing countries from exploitation.

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