ADVANCES AND FUTURE PERSPECTIVES IN FRUIT TREE TRANSFORMATION

in

- Genetically Engineered Trees [1]

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May, 2005

Conventional breeding of temperate fruit trees is constrained by their extensive reproductive cycle with long juvenile periods, complex reproductive biology, and high degree of heterozygosity. As the commercial production of transgenic annual crops becomes a reality in many parts of the world, the question remains whether genetically engineering fruit trees will find commercial application.

Gene transfer for fruit tree improvement has several inherent advantages. Once a useful transformant is isolated, vegetative propagation, which is the normal method of multiplying fruit trees, provides unlimited production of the desired transgenic line. Fixation through the sexual cycle is unnecessary and inconvenient if commercially-accepted cultivars are transformed. Since production of most fruit tree species is based on a few cultivars, the impact of transforming one of them would be significant. Currently, however, the only transgenic fruit tree commercially produced is papaya (Carica papaya L.) resistant to PRSV (papaya ringspot virus). (Further information on commercialized transgenic crops can be found at http://www.agbios.com/[2]).

Transformation methods

Microprojectile bombardment has been used to transform papaya1. The DNA most commonly transferred to fruit trees is from disarmed and genetically engineered Agrobacterium strains, which drive foreign DNA into plant cells. Together with the gene of interest, the genes required for transformation are transferred, including marker genes that allow selection of transformed cells. Among the most commonly used selection genes are the neomycin phosphotransferase gene (nptII), which confers resistance to aminoglycoside antibiotics, and the phosphinothricin acetyl transferase gene, that confers resistance to the herbicide phosphinothricin (see review2). However, given public concern with the introduction of antibiotic resistance genes into food, methods to eliminate selection genes from the transformed plants and strategies that avoid selection of transformed cells with antibiotics are being developed. However, these new methodologies have yet to be applied to the production of transformed fruit trees.

Recent Advances

Much work has been published reporting improved methods using only marker genes and integration of putative beneficial genes, but without sufficient evaluation of the effect on the transformed plants. Additionally, some advances have also been reported specifically in the transformation of fruit trees (see review3 for more details).

- Fire blight and scab resistant apples and pears have been produced by integration of different genes, and plant growth has been modified by introducing the rol B gene in apple rootstocks. Additionally, transgenic apple releases to test possible resistance to pathogenic fungi and bacteria (B/NL/02/03/ and B/DE/03/140) or the effect of cDNA from apple self-(in)compatibility alleles on pollen-pistil incompatibility (B/BE/03/V1) are included in the European Commission data base (http://gmoinfo.jrc.it [3]).

- Introduction of genes of interest subsequent to transformation of apricot seeds and plum
hypocotyls has produced plants with increased sharka virus resistance.

- Cherry rootstocks with improved rooting and Basta herbicide resistance were obtained after transforming shoots with A. rhizogenes, and peaches with increased branching and reduced rooting were engineered using a "shooty mutant," A. tumefaciens, strain.

- Different grape cultivars have been transformed with genes coding for: 1) chitinases to confer resistance to pathogenic fungi; 2) different virus coat proteins; and 3) peptides with antimicrobial activity. Grape cultivars have also been transformed with the gene DefH9-iaaM, which induces parthenocarpy in flowers of different species.

- Japanese persimmons and walnuts transformed with the cryIA(c) gene were more resistant to lepidopteran pests, and kiwifruit transformed with the rol A, B and C genes had an improved rooting ability.

- Major objectives of Citrus transformation have been resistance to citrus tristeza virus (CTV) mediated by pathogen-derived genes, resistance to Phytophthora citrophthora using antifungal proteins, and tolerance to salinity by introducing HAL2 yeast-derived genes. In addition, Arabidopsis floral genes, such as LEAFY (LFY) or APETALA1 (AP1), constitutively expressed in citrus seedlings from apomictic seeds, shortened the juvenile phase and promoted precocious flowering. Transgenic plants produced normal, fertile flowers that set fruits containing seeds. These traits were transmitted to the progeny, resulting in trees with a generation time of one year from seed to seed. Whereas LFY lines showed alterations in growth and development, AP1 plants were adult and fully normal. Citrus plants expressing bovine lysozyme and snowdrop lectin are being evaluated in the greenhouse and in the field for their resistance to citrus canker (Xanthomonas axonopodis pv. citri) and insects, respectively.

**Transformation Constraints**

Currently, transformed seedlings from the cultivars 'Rio Red', expressing lectin, and 'Carrizo', expressing lysozyme, are currently the only Citrus field tests in the USA. Citrus cultivars have very long juvenile periods, and transformation of adult material would be preferable. Transformation of adult tissues of 'Pineapple' sweet orange produced plants that flower and set fruits in 14 months, whereas sweet oranges (which account for approximately 70% of world citrus production) need up to 20 years to completely lose juvenile characteristics and commence production. However, transformation efficiencies in adult Citrus are much lower than in juveniles. For instance, transformation efficiency in adult sweet orange 'Pineapple' is one-third of that obtained with apomictic seedlings of this cultivar. Similarly, juvenile material of Prunus is more easily transformed and, in most cases, has been transformed only with marker genes in preliminary studies. In all cases of integration of "genes of interest" in Prunus, hypocotyls have been used for transformation.

Genotype is a major determinant for transformation, and procedures developed for one cultivar are often not suitable for other cultivars. This is the most serious hindrance to the application of gene transfer technologies to fruit crops. For species with cultivars that can be reliably transformed, the literature generally reveals that few genotypes of a particular species are being transformed and, in some cases, that these genotypes are not commercially important.

Meristem transformation may eliminate the need for regeneration in production of transgenic plants, allowing genetic manipulation of established cultivars. However, high explant mortality and difficulties controlling Agrobacterium growth have limited the development of this methodology. Recently, a reliable procedure for transformation of different grape cultivars has been developed. The authors generated "meristematic bulk" (MB) tissue from in vitro shoots by mechanical (dissection of the apical dome) and chemical (progressive increase in cytokinin concentration) treatments that abolish shoot apical dominance and also promote basal meristem proliferation. The MB tissue is a large aggregate of meristematic tissue with high regenerative competence, which can be transformed efficiently by Agrobacterium given the large number of dividing cells. This system seems to be easily adapted to other fruit trees; for instance, MBs have been produced from three apricot cultivars with similar regeneration efficiencies, according to data obtained in our laboratory. We are presently conducting experiments to transform MB tissue and regenerate transgenic shoots from apricot.
Selection of transformed regenerants is a critical step in any transformation procedure. Most commonly in fruit trees, antibiotics have been used as selection agents after integration of genes that confer antibiotic resistance. Concentration of the selective agent and timing of application must be optimized for each plant species.

Selection of transformed shoots is often complicated by the inactivation of the selection agent by transformed cells and persistence of Agrobacterium in the explants, which permits regeneration of non-transformed shoots (escapes), sometimes at a high frequency.

Despite the economic importance of Prunus, transformation technology is not available for most Prunus species, which may be due to difficulties posed by adventitious regeneration and/or a high sensitivity to antibiotics. Whereas in Citrus, pear, walnut, or olive, selection is provided by 100 mg/L kanamycin, in Prunus inhibitory concentrations are frequently much lower (5 to 10 mg/L in almond, for instance), and specific selection strategies are often necessary.

Future Perspectives
The future of genetic transformation as a tool for breeding fruit trees requires the development of genotype-independent procedures, based on the transformation of meristematic cells with high regeneration potential and/or the use of regeneration-promoting genes. Yet another obstacle is that European law will neither allow deliberate release of plants carrying antibiotic resistance genes after 2004 nor their commercialization after 2008 (Directive 2001/18/EEC of the European Parliament and the Council of the European Union). Therefore, development of procedures to avoid the use of antibiotic selection or to allow elimination of marker genes from the transformed plant will be a research priority in the coming years.

References

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