# Transgenic trap crops and rootstocks show potential

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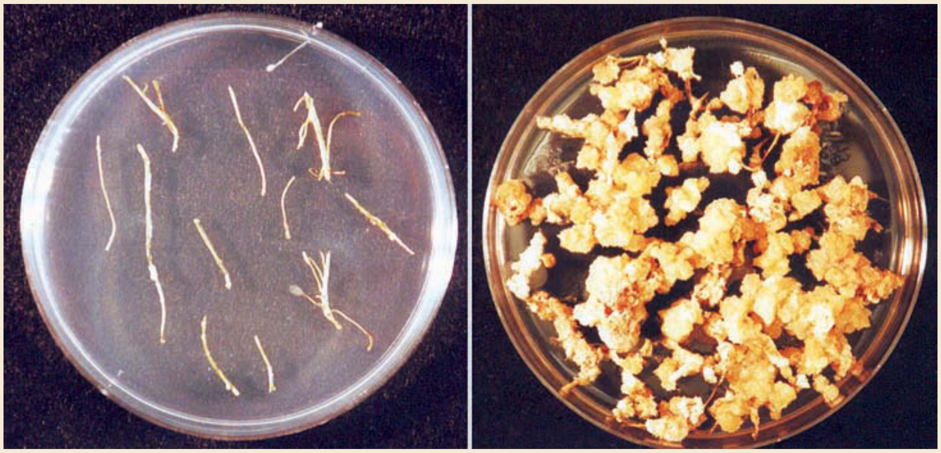

***Left,*** **apple roots engineered to silence bacterial genes are resistant to crown gall formation.** ***Right,*** **control (nontransgenic) roots infected with the same bacterial strain show extensive gall proliferation.**

Biotechnology may offer unique opportunities for pest control in perennial tree and vine crops (Dandekar et al. 2002). Trap crops — plants that an insect pest prefers to the commercial crop — have been tested in a number of agricultural settings, but in most cases have not achieved control levels high enough to completely replace chemical pesticides. Insects are attracted to the trap plant, but they multiply there and can spread to the adjacent crop. A variant on this concept is to incorporate expression of the *Bacillus thuringiensis* (Bt) insecticidal protein into the trap plant. When the insect feeds on the transgenic trap plant, it dies and the insect population is reduced, thereby protecting the nearby commercial crop.

Dry Creek Laboratories of Hughson, Calif., demonstrated this concept with codling moth *(Cydia pomonella)*, a major pest of apples, pears, walnuts and other fruits. The female moth lays eggs on the leaves or fruit, which then hatch into larvae that burrow into the fruits, making them unmarketable. Pesticide sprays and pheromone disruption are generally used to control this pest. However, the female moth prefers to lay its eggs on apple trees. Under license from Monsanto, Dry Creek Laboratories developed apple trees capable of expressing a Bt protein that was toxic to the codling moth larvae, with the intention of using these plants as trap crops in and adjacent to walnut orchards.

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A 90-acre field trial was established in 1997, and in the 4 subsequent years worm damage to the walnuts was almost completely controlled without pesticide applications, equivalent to that in the plots sprayed three times per season with pesticides. While walnuts have also been transformed to express the Bt protein directly (Dandekar et al. 2002), an attractive feature of this scheme is that the walnuts themselves are not transgenic and the method could be used to protect existing orchards by interplanting the Bt-expressing apple or crabapple trees. Broader application of this approach could result in more effective trap crops for a number of annual and perennial crops. Unfortunately, Dry Creek Laboratories is unable to move forward at this time with commercialization of the Bt apple plants due to the costs associated with the regulatory process required for biotech crops (see page 106).

Another opportunity for biotechnology in perennial crops that are normally grafted is to engineer only the rootstock for desirable traits. Commercial tree cultivars grafted onto transgenic rootstocks could benefit from increased rootstock productivity or disease resistance while producing nontransgenic pollen and fruit. For example, such applications in grapes could offer new solutions to Pierce's disease or *Phylloxera* by grafting traditional varieties onto resistant transgenic rootstocks. The feasibility of this approach was recently demonstrated for resistance to crown gall disease *(Agrobacterium tumefaciens)*. Infections by the bacterium result in the formation of a gall, an unorganized mass of plant cells that results from overproduction of two plant hormones. The bacterium has the natural ability to transfer some of its genes into the host plant's genome following infection. The transferred genes code for three specific enzymes. When the plant expresses these genes, the enzymes synthesize the two hormones that induce the plant to form the tumor, or gall, on which the bacteria live. Eventually, the galls can girdle the stems and reduce the vigor of the tree or vine.

A biotechnology tool called "gene silencing" has been used to generate resistance to crown gall. This method involves transforming plants with DNA that, when expressed, produces signals that block the expression of any genes with the same sequence as the inserted DNA. Plants transformed with these interfering versions of the three enzyme genes would be primed to block the function of the corresponding bacterial genes in infected plants. This would prevent the formation of the damaging galls without even needing to kill the bacterium itself. The feasibility of this approach was demonstrated in tomato and *Arabidopsis* plants (Escobar et al. 2001). Furthermore, both walnut (see photo; Escobar et al. 2002) and apple (see photo; J. Driver et al., un-

**Crown gall formation was suppressed in walnut plants engineered to turn off specific bacterial genes. (A) The control shoot exhibits a large, undifferentiated tumor at 5 weeks after inoculation with a virulent *A. tumefaciens* strain, while (B) a shoot engineered for resistance exhibits no tumor. Source: Escobar et al. 2002.**

published results) plants resistant to crown gall have been produced. As most crown gall infections occur in the rootstock, nontransgenic scions grafted on resistant transgenic rootstocks would be protected from the disease. Rootstock engineering holds great promise for the improvement of tree and vine crops by preserving the horticultural characteristics of existing varieties used as scions while incorporating beneficial traits into the rootstocks.

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## References

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Escobar MA, Civerolo EL, Summerfelt KR, Dandekar AM. 2001. RNAi-mediated oncogene silencing confers resistance to crown gall tumorigenesis. Proc Natl Acad Sci USA 98:13437–42.

Escobar MA, Leslie CA, McGranahan GH, Dandekar AM. 2002. Silencing crown gall disease in walnut (*Juglans regia* L.). Plant Sci 163:591–7.

the genes and enabling technologies (such as transformation protocols and promoters) required for genetically engineering plants. They are generally not interested in the smaller horticultural markets, and may not want to license their technologies, depending on the impact it could have on their other approved crops (see page 120).

**Post-commercialization.** Post-commercialization stewardship is also an increasingly important consideration to technology owners in deciding whether to license their intellectual property. In Bt crops, for example, insect-resistance management programs must be developed and monitored after commercialization. Identity preservation programs and segregation of products in the distribution channels may be required when marketing in locations where they are not approved. Herbicide applications to diverse horticultural crops have the potential to increase the Average Daily Intake (ADI) over the maximum permitted level for the pesticide active ingredient**.** (ADI is the total residues of a pesticide that a consumer can be exposed to, considering all sources; the government sets limits for each pesticide.) An agrochemical company will not approve the use of its herbicide-resistance trait in a small acreage crop if it endangers the registration of that herbicide for millions of acres of field crops.

**Regulatory requirements.** Extensive safety testing is required for regulatory approval (deregulation) of biotech crops beyond what is required for varieties bred using traditional methods (see page 106). If the trait has already been approved in other crops, the costs are lower as prior data can be used to support an application. However, for novel traits likely to be of interest for horticultural crops, the costs could be millions of dollars. For example, by some estimates it will cost $20 million to achieve deregulation of Golden Rice for humanitarian purposes in six developing countries (I. Potrykus, UC Davis seminar, Jan. 22, 2003). Since each transgenic event (each insertion of a gene in a genome) must be separately tested and approved, it is not feasible to transform multiple varieties with a given trait to amortize the research and technology investment across a given crop. Instead, a single insertion event is approved for commercialization and then must be transferred via standard backcrossing to other varieties. This is highly inefficient and often makes it difficult to regain the unique properties of all the diverse varieties. Public-private partnerships are one way to reduce the costs of commercialization (see page 116). The IR-4


Syngenta

**"Golden Rice" has been genetically engineered to produce beta-carotene, the precursor to vitamin A. However, for a variety of reasons it is not yet available to farmers in developing countries, where vitamin A deficiencies are common.**