REVIEW

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ADVANCES IN APPLE TRANSFORMATION TECHNOLOGY TO CONFER RESISTANCE TO FUNGAL DISEASES IN APPLE CROPS: A CHILEAN PERSPECTIVE

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ABSTRACT

Apple (*Malus domestica* Borkh.) is one of the most consumed fruit in the world. Genetic transformation is a key process to sustain this demand by permitting the potential enhancement of existing cultivars as well as the development of new cultivars resistant to pests, diseases, and storage problems that occur in the major production areas. This review summarizes the advances of genetic engineering applied to the development of resistant apple cultivars to fungus disease, with particular attention in the generation of apples resistant to *Venturia inaequalis* (Cooke) G.Winter, the main phytosanitary problem that affects apple crops in Chile.

Key words: apple, fungal disease, transgenic, cisgenesis.

INTRODUCTION

Apple belongs to the Pomoideae family, subfamily Rosaceae, along with other important fruit crops such as pear (*Pyrus communis* L.), prune (*Prunus domestica* L.) and cherry (*Prunus avium* L.). Domesticated apple probably originated in the area around of the Heavenly Mountains on the border Western of China, in the former USSR and in Central Asia, and it is putatively an interspecific hybrid complex, designated *Malus domestica* Borkh. (Korban and Skirvin, 1984; Phipps *et al*., 1990). In medieval times, monasteries were responsible for selection, propagation and perpetuation of hundreds of different cultivated types. These plantations became the major sources of breeding stock and selections for making controlled crosses to improve specific traits in the 1800's (MacHardy, 1996).

During the late 19th and 20th centuries, *M. domestica* cultivars were genetically improved in Europe, Russia, North America, New Zealand, Japan and Australia and finally, apples were introduced to the rest of the world. These new introductions constituted the basis for most of the current commercial apple cultivars (Way *et al*., 1991; Janick *et al*., 1996). Nowadays, more than 7000 varieties have been described and breeders worldwide create new selections annually; nevertheless, only a few dozen cultivars are produced commercially (Janick *et al*., 1996).

In 2004, the apples were the third most cultivated fruit crop in the world (5280 M ha) and the third fruit crop in production (59 059 Mt), after *Citrus sinensis* Osbeck (orange) and *Musa paradisiaca* L. (banana) (FAOSTAT, 2004). The most recent statistical information published by the Department of Agriculture of the USA, locates Chile in the third place of the exporting countries of apple fruit in the world. However, we are behind the European Union (EU), and China, but above the USA, South Africa, New Zealand and Argentina. Chile provided about 18% of the total apple market supply and the EU represented the 22% and China a 19% (USDA, 2007).

Besides its economic importance, apple has become a woody perennial angiosperm model for genomic research due to its relatively small genome size (750 Mb/haploid), availability of genetic resources such as over 300 000 expressed sequence tags (EST), bacterial artificial chromosome (BAC) libraries, genetic maps, first-draft physical map, and a development of a robust genetic transformation system (Xu *et al*., 2001; Xu and

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Korban, 2002; Aldwinckle *et al*., 2003; Liebhard *et al*., 2003; Tatum *et al*., 2005; Newcomb *et al*., 2006; Han *et al*., 2007). Most of the cultivated apple lines are diploids ($2n$ = 2x = 34), self-incompatible, open-pollinated, and display a juvenile period that ranges from 6 to 10 years or more (Korban and Chen, 1992).

Nowadays, the market demands apple cultivars with high productivity, uniformity, and good fruit quality. In addition, resistance to diseases, pests and storage disorders are also desired. Even though, the advantage of a resistant cultivar is evident, resistant cultivars still do not dominate the market. The reason is simple, the most successful commercial apple cultivars have lost the efficacy of their resistance genes toward to the two most frequent fungal pathogens, apple scab (*Venturia inaequalis* (Cooke) G. Winter), and apple mildew (*Podosphaera leucotricha* Ellis & Everh.). Then, almost all apple cultivars need constant fungicides protection, wherever the climatic conditions favors the development of these or other pathogens. For example, on average, 10 to 15 fungicide applications are necessary to produce apple scab-free fruits during a season, which increase the cost of apple production and the concerns from consumers and environmentalists over pesticide uses (MacHardy, 1996; Gessler and Patocchi, 2007). Therefore, most current apple breeding programs are oriented toward reducing the need for pesticides, without losing the fruit quality.

Unfortunately, because of the long juvenile period, high levels of heterozygocity and time required to evaluate hybrids, the process to release a new cultivars from a conventional apple breeding programs are slow, especially if the breeder transfers genes from non adapted genotypes like wild genotypes. For instance, a cross between domesticated and wild species needs several backcrosses to eliminate the unwanted traits inherited from the wild species. Releasing a new cultivar could take 10 or more years, and almost 40 years to introduce and establish the new cultivar on the market (Korban and Chen, 1992; Brown, 1992). The application of modern DNA analysis methods such as genetic maps, identification of DNA markers linked to traits of interest, and marker-assisted selection in breeding, could help to accelerate this process, and a new cultivar with resistance to diseases and pests could be developed in a shorter period. However, this new cultivar needs to be accepted by the consumers, who are very specific on fruit quality traits requirements. Under this situation, the genetic improvement of apple through the introduction of specific gene(s) onto current commercial cultivars, in a short run, is a very attractive strategy.

The objective of this work was to review the current knowledge on apple genetic transformation to improve its fungal resistance, with emphasis on *Venturia inaequalis*.

Biotechnological approaches to genetic improvement

Genetic mapping and quantitative trait *loci* (QTL) analysis. Molecular markers have been linked to a number of monogenic traits in apple (Tartarini and Sansavini, 2003). The most work has been done on the *Vf* gene for scab resistance, where over 40 markers have been identified. Markers for the other scab resistance genes have also been developed by many groups and include *Vh* from Russian seedling R12740-7A of *M. Sieversii* (Hemmat *et al*., 2002), *Vm* (Cheng *et al*., 1998), *Va* and *Vb* (Hemmat *et al*., 2003; Erdin *et al*., 2006), *Vd* (Tartarini *et al*., 2004), *Vbj* (Gygax *et al*., 2004) and *Vg* (Durel *et al*., 2000; Calenge *et al*., 2004). Gessler *et al*. (2006) reviewed the literature in this area from type of resistance through gene pyramiding.

Vinatzer *et al*. (2004) used the inverse polymerase chain reaction and simple sequence repeats to identify BAC clones containing the apples scab resistance gene *Vf* and found the gene in scab-resistance accessions of *Malus ×micromalus* Makino and 'Golden Gem' of *M. prunifolia* (Willd.) Borkh., which were previously not known to carry this gene.

Markers have also been linked to the pest resistance genes *Sd1* for *Dysaphis devecta*, and *Er1* and *Er2* for *E. lanigerum*. A few markers have also been linked to genes regulating morfological traits including the columnar habit (*Co*), fruit color (*Rf*) and fruit acidity (*Ma*). Recently a cDNA/AFLP approach was used to identify a gene that contributes to lowering of fruit acidity (Yao *et al*., 2007).

Several groups in Europe have been especially busy mapping the QTL associated with resistance to apple scab into various linkage groups (LGs). The cross of 'Prima' x 'Fiesta' and other related F_1 progenies have been used to identify major genes associated with resistance in the DARE project (Durable Apple Resistance in Europe) (Durel *et al*., 2003). The major genes for scab resistance *Vg* were found on LG12. Several different nucleotide binding site (NBS)-type resistance gene analogues were clustered at bottom of LG5 and the top LG17 for resistance to races 1 and 6. A major non-race-specific QTL was identified near an NBS-analog cluster on linkage group LG10. Three major genes for powdery-mildew resistance were also identified by bulked segregant approaches, and ones of them on LG2 was located in the same region as scab resistance.

Five apple progenies were used in the DARE project to identify QTL with broad spectrum of resistance towards a wide rande of strains of the fungus (Durel *et al*., 2004). It was verified that four major genomic regions exist that carry resistance to multiple strains of the fungus, with a

QTL region on LG 17 carrying the widest spectrum of resistance. So, molecular markers and QTLs analyses are key for the identification of genes resistance to pathogens, and allows their application for the generation of news lines of apples genetically modified.

Genomic resources. Mining of existing apples EST information, such as the studies of Newcomb *et al.* (2006) and Park *et al.* (2006), and the use of microarrays (Pichler *et al.*, 2007) promises to expand our knowledge of many genes important in the genetic improvement of apple. The development of public databases such as the GDR (Genome Database for the Rosaceae; Jung *et al.*, 2004) and the European HIDRAS ApplesBreed (Antofie *et al.*, 2007) also offer excellent prospects for enhanced collaboration amongst breeders, bioinformatics researchers and those involved in molecular biology. In the GDR database alone, over 50 000 ESTs are available from several species, tissues and developmental stages for use in genetic tranformation.

Apple transformation. Because of the high susceptibility to fungal diseases of the most important commercial apple cultivars and rootstocks, genetic transformation has been one good method for the development of resistant cultivars.

Genetic transformation of plants is the process where a defined fragment of DNA (a gene) is introduced and integrated into the genome of the plant, avoiding sexual reproduction. Genetic engineering enlarges the readiness of genes considerably, limited in conventional breeding programs, since genes isolated from other plants, animals or microorganisms can be transferred to plants (Brasileiro and Dusi, 1999).

Apple was an early target for the emerging recombinant DNA technology. Transformation of *Malus* is nowadays a common practice in several laboratories and the protocols have been constantly improved to enhance the transformation efficiency (James *et al.*, 1993; Yepes and Aldwinckle, 1993; Yao *et al.*, 1999; De Bondt *et al.*, 1994; 1996; Norelli *et al.*, 1996; Puite and Schaart, 1996; Hammerschlag *et al.*, 1997; Liu *et al.*, 1998; Sriskandarajah and Goodwin, 1998; Bolar *et al.*, 1999).

The most widely used method for introducing foreign genes into dicotyledoneous plants is the *Agrobacterium tumefaciens* mediated transformation. In this process, *A. tumefaciens*, a disarmed Ti binary vector, and leaf fragments or callus cultures are the key component for an efficient transformation (James *et al.*, 1989). Most studies started from wounded leaf sections (Norelli *et al.*, 1996), but apical internodal explants from etiolated 'Royal Gala' apple shoots has produced a higher efficiency in producing transgenic shoots (Liu *et al.*, 1998).

Genes used in genetic transformation of apples

Transformations are mostly based on traditional cultivars and they have been carried out using genes isolated from apple (Belfanti *et al.*, 2004b; Espley *et al.*, 2007; Malnoy *et al.*, 2007a; 2008) or from other organisms (Wong *et al.*, 1999; Norelli *et al.*, 1994; 2000; Bolar *et al.*, 2000; 2001; Hanke *et al.*, 2000; Liu *et al.*, 2001; Szankowski *et al.*, 2003; Markwick *et al.*, 2003; Faize *et al.*, 2004). Genes affecting some physiological or morphological characters like growth (Holefors *et al.*, 2000), flowering (Yao *et al.*, 1999) and self-fertility (Van Nerum *et al.*, 2000) have also been incorporated into transgenic apples. Rootstock scions have also been used in transgenic assays to improve rooting rates and growth (Holefors *et al.*, 1998; Welander *et al.*, 1998; Sedira *et al.*, 2001; Pawlicki-Jullian *et al.*, 2002; Igarashi *et al.*, 2002). The function of some genes like sorbitol-6-phosphate (Kanamaru *et al.*, 2004; Cheng *et al.*, 2005), stilbene synthase (Rühmann *et al.*, 2006), polygalacturonase (Atkinson *et al.*, 2002) and from several promoters (Ko *et al.*, 2000; Gittins *et al.*, 2001; 2003; Szankowski *et al.*, 2008) has also been studied using transgenic apple (see Table 1).

Use of resistance genes. Apple scab is one of the most serious diseases affecting apple orchards causing weakness of trees and fruit damages. Six major scab resistance genes (*Vf, Vm*, *Vb*, *Vbj*, *Vr*, and *Va*) have been identified from wild small fruited *Malus* species (Williams and Kuc, 1969; Biggs, 1990). Up until now, only the *Vf* gene, originated from *Malus floribunda* 821 Siebold ex Van Houtte, has been widely introgressed into susceptible commercial apple cultivars (Crandall, 1926; Crosby *et al.*, 1992; Korban, 1998). The *Vf* gene confers resistance to five out of seven known races of *V. inaequalis* and has held up quite well in the orchards for over 80 years.

A different approach to obtain scab resistant plants was attempted by the joint team of the department of Fruit Tree and Woody Plant Sciences of the Bologna University, Italy, and the Plant Pathology group at Swiss Federal Institute of Technology (ETH) Zurich, Switzerland (Sansavini *et al.*, 2004). Starting from an European Union (EU) project which offered an excellent linkage map of apple and molecular markers mapped in the region of the scab resistance *Vf*, the positional cloning of *Vf* was initiated. A contig of BAC clones spanning the region between the two *Vf* molecular markers, M18 and AM19, one on each side of *Vf*, was constructed and allowed the identification of four genes, named *HcrVf1* to *HcrVf4*. These genes codes for receptor-like proteins that have a high homology to the *Cladosporium fulvum* (*Cf*) resistance gene family of tomato. The genes have an extracellular leucine-rich repeat domain and a transmembrane domain (Vinatzer *et al.*, 2001).

Table 1. Genes used in genetic transformation of apples.

		Gene	Donator	Results	Reference
Overexpression	Genes isolated from apple	*HcrVf2* (R-genes)	*Malus floribunda* 821	Scab-resistance	Belfanti *et al*., 2004
		HcrVf1 (R-genes)	*M. floribunda* 821	Scab-resistance	Malnoy *et al*., 2008
		MdPG1 (Polygalacturonase)	*Malus domestica* Borkh.	Changes in cell adhesion/ maduration	Atkinson *et al*., 2002
		MpNPR1(pathogenesis-related gene)	*M. domestica* Borkh.	Fungal disease resistance	Malnoy *et al*., 2007a
		MdMYB10 (MYB transcription factor)	*M. domestica* Borkh.	Induce anthocyanin accumulation/red apple fruit color	Espley *et al*., 2007
	Genes isolated from apple	*Attacin E*	*Hyalophora cecropia*	Fire blight resistance	Norelli *et al*., 2000
		Cecropin MB39	*Hyalophora cecropia*	Fire blight resistance	Liu *et al*., 2001
		ech42 (Endochitinase)	*Trichoderma harzianum*	Scab-resistance	Wong *et al*., 1999
		ech42 (Endochitinase) *Nag70* (Exochitinase)	*Trichoderma harzianum*	Scab-resistance	Bolar *et al*., 2001
		Vst1 (Stilbene synthase)	Grapevine (*Vitis vinifera* L.)	Antifungal activity	Szankowski *et al*., 2003
		PGIP (Polygalacturonase inhibitor)	Kiwi (*Actinidia deliciosa*)	Antifungal activity	Szankowski *et al*., 2003
		Avidin or Streptavidin	*Streptomyces avidinii*	Insect resistance (lightbrown apple moth)	Markwick *et al*., 2003
		RolA (Hydrolyze phytohormone glucoside A)	*Agrobacterium rhizogenes*	Root induction	Holefors *et al*., 2000
		RolB (Hydrolyze phytohormone glucoside B)	*A. rhizogenes*	Root induction	Welander *et al*., 1998
		RolC (Hydrolyze phytohormone glucoside C)	*A. rhizogenes*	Root induction	Igarashi *et al*., 2002
		PinB (puroindoline)	Wheat (*Triticum aestivum*)	Antifungal activity	Faize *et al*., 2004
Silencing		*S-RNase* gene	*M. domestica* Borkh.	Self-fertility	Van Nerum *et al*., 2000
		S6PDH (Sorbitol-6-phosphate dehydrogenase)	*M. domestica* Borkh.	Regulating partitioning between sorbitol and sucrose	Kanamaru *et al*., 2004
Promotors		*ExtA* promotor	*Brassica napus* L.	Tissue-specific distribution	Gittins *et al*., 2001
		RolC promotor	*A. rhizogenes*	Tissue-specific distribution	Gittins *et al*., 2003
		HcrVf2 promotor	*M. floribunda* 821	Scab-resistance	Szankowski *et al*., 2008

Using this information, the gene *HcrVf2* under the control of the CaMV 35S promoter was introduced into the susceptible cv. Gala using the *nptII* gene for selection. In the first step, the progression of the scab infection (penetration and stroma formation by the fungus) (Barbieri *et al*., 2003; Sansavini *et al*., 2003) was evaluated *in vitro*, followed by a greenhouse scab inoculations of the experimental lines containing a single functional copy of *HcrVf2*. These evaluations demonstrated unambiguously that the four lines carrying *HcrVf2* were at least as resistant to scab (Belfanti *et al*., 2004a; 2004b) as the conventionally bred *Vf* resistant cv. 'Florina'.

As the resistance *Vf* is known to be overcome by race 6 and 7, it was of interest to test if the introgressed gene really conferred the same type of resistance, i.e., recognition of the avirulent *V. inaequalis* genotypes and induction of the defense cascade, or through some artifact. Plants transformed with both *HcrVf2* and *nptII*, transformed with *nptII* only, and wild-type 'Gala' and 'Florina' (*Vf*) were challenged with a field inoculum (mixture of genotypes) known to have the ability to cause scab on 'Gala', but not on 'Florina', and with an

inoculum derived from *M. floribunda* 821, the original donor of *Vf*. The results indicated that the *HcrVf2* line was, as expected, resistant to the field inoculum similarly to Florina, whereas all the other plant showed typical scab lesions with abundant sporulation. Inoculation with race 7 from *M. floribunda* 821 resulted in sporulating lesions on all plants; however, the inoculum was less aggressive and the sporulation was less abundant than the field inoculum. Even more, the *HcrVf2* line still retained some resistance, it is slightly more resistant than the line transformed with only *nptII* and the original 'Gala' (Silfverberg-Dilworth *et al*., 2005a; Gessler and Patocchi, 2007). This experiment demonstrated that resistance in *HcrVf2* transformed lines carried the *Vf* gene.

A further work identified the promoter sequence of the *HcrVf1*, 2 and 4 and demonstrated their functionality (Silfverberg-Dilworth *et al*., 2005b). Currently, it has been shown that *Vfa1* (*HcrVf1*) and *Vfa2* (*HcrVf2*) under the control of their own promoter confer resistance to the *V. inaequalis* (Malnoy *et al*., 2008).

Use of defense-related gene. Currently, several research groups have demonstrated that the plants produce defense-related proteins, such as pathogenesis-related proteins (PR) and antimicrobial peptides. Constitutive expression of these molecules may enhance plant resistance. For instance, the puroindolines (*PinA* and *PinB*), antimicrobial peptides are antifungal cystein-rich proteins that are present in wheat seeds. Both genes are well characterized and they have already been used to transform rice. Transformant lines expressing these genes showed an increased resistance to major fungal pathogens in rice (Krishnamurthy *et al*., 2001). A similar experimental was set up to test the potential of *PinB* in apple. The results indicated that, the Strain 104, race 1 (which represents the common *V. inaequalis* population on the commercial cultivars), is not affected by the *PinB* at any expression level; however, the strain EU D42, race 6, is inhibited progressively with the increasing amount of *PinB*. The two strains exhibited differential tolerance to *PinB* (Faize *et al*., 2004).

On the other hand, other studies have demonstrated that constitutively high-level expression of PR proteins may protect susceptible cultivars from infection by different pathogens. Transcriptional analysis of apple susceptible and resistant to scab has demonstrated that in the resistant cv. Remo, the levels of transcripts encoding a number of proteins related to plant defense (such as β-1,3-glucanase, ribonuclease-like PR10, cysteine protease inhibitor, endochitinase, ferrochelatase, and ADP-ribosylation factor) or to detoxification in reactive oxygen species (such as superoxide dismutase) were highly up-regulated when compared their relative amounts with the susceptible cv. Elstar. Most surprising was the large number of clones derived from mRNAs for metallothioneins of type 3 found in the population of the resistant cv. Remo. However, the corresponding transcripts were only present in small amounts in young uninfected leaves of the susceptible cv. Elstar, but were up-regulated in this susceptible cultivar after inoculation with *V. inaequalis* (Degenhardt *et al*., 2005).

Other studies presented by the research group at Cornell University showed that over-expression of the apple *MpNPR1* gene confers increased disease resistance in *M. domestica*. The *NPR1* gene plays an important role in systemic acquired resistance in plants. An *NPR1* homolog, *MpNPR1*-1, was cloned from *M. domestica* and overexpressed into two important apple genotypes, Galaxy and M26. Its over-expression in apple resulted in an increased disease resistance and elevated expression of pathogenesis-related (*PR*) genes. Transformed Galaxy lines over-expressing *MpNPR1* had an increased resistance towards two important fungal pathogens of apple, *V. inaequalis* and *Gymnosporangium juniperi-virginianae*. Selected transformed lines have been propagated for evaluation in field trials for disease resistance and fruit quality (Malnoy *et al*., 2007a; 2007b).

Use of microbial genes. Since 1980s great efforts have been made in Europe, USA, and New Zealand to map the major scab resistance genes from *V. inaequalis* and mildew (*P. leucotricha*). These two fungal diseases adsorb the majority, if not the total of the fungicide treatments necessary to produce high-quality apples. However, until 1990s, once the apple transformation methodology was well established, no apple resistance gene had been cloned. Therefore, emphasis was put on foreign genes, with potentially toxic or inhibitory effects on *V. inaequalis* and mildew. This strategy includes the use of genes from other species encoding chitinases and glucanases, isolated from the fungus *Trichoderma* (a well-known biocontrol agent of fungal diseases), those encoding lytic enzymes from *Lepidoptera*, and some antimicrobial peptides (AMP) from phages.

In 1998, several studies reported that the constitutive expression of chitinolytic enzymes like endochitinase and chitobiosidase from the biological control agent *Trichoderma harzianum*, shown an antifungal activity and may increase the host resistance to scab (Wong *et al*., 1999). In this work, two out of three endochitinase (*ech42*) gene transgenic lines of 'Royal Gala' were more resistant than the untransformed 'Royal Gala' when micrografted shoots were spray-inoculated with scab. At the same time, it was also reported the transformation of the cv. McIntosh with the *ech42* and *Nag70* genes, and an exochitinase gene encoding N-acetyl-β-D-glucosaminidase, isolated from

the biocontrol agent *T. harzianum* (Bolar *et al*., 1999). Transgenic lines with both genes (low *each42* and high *Nag70* expression) had a high level of scab resistance, while retaining a good vigor (Norelli *et al*., 2000).

Other studies reported by the research group at Cornell University showed that transfer of native (attacin E) and synthetic (SB-37) genes from the saturniid moth (*Hyalophora cecropia*), lysozyme genes from hen egg white, and T4 bacteriophage, all enhanced scab resistance to scab in a variable degree (Aldwinckle *et al*., 1999). A similar approach started to use the Fruit Breeding at Dresden Pillnitz, Germany, where they developed several apple transgenic lines transformed with the lysozyme, from the bacteriophage T4, and/or attacin E, from saturniid moth. However, this program concentrated its efforts on the subsequent effects on fire blight infections (Hanke *et al*., 2000).

Types of promoters. For gene expression, in many cases researchers relied on the well characterized constitutively expressed 35S promoter from *Cauliflower mosaic virus* (CaMV 35S). Some attempts have been made to find and use other promoters. For instance, promoters linked to targeted expression patterns have been identified such as the 940 extA promoter which is active in young tissue (Gittins *et al*., 2001), RBCS3C and SRS1, suitable for the expression of transgenes in green photosynthetic tissue of apple (Gittins *et al*., 2000), a native promoter for the apple scab resistance gene *HcrVf2* (Szankowski *et al*., 2008), and others promoters with particular expression patterns that are under development and characterization (Degenhardt and Szankowski, 2006).

Limitation of the technology and new development

Although considerable improvement has been gained in the process of transformation in apple, the use of antibiotics and herbicides as selectable markers still imposes a limitation according to the consumer acceptance (Penna *et al*., 2002; Degenhardt and Szankowski, 2006). Thus, a major problem for genetically modified (GM) apple is the use of the *nptII* as a selection gene-marker. Recently, some groups started to develop new selection systems that are more acceptable to the general public that include the elimination of the selectable markers from the final product, the transgenic plants.

Currently, one of the most promising alternative to antibiotic resistance and probably a more acceptable system is the use of the gene phosphomanose isomerase (PMI). PMI-transformed cells are able to use mannose as carbon source, which the untransformed apples cells cannot do and first successes using this technology have already been reported (Flachowsky *et al*., 2004; Zhu *et al*., 2004; Degenhardt and Szankowski, 2006).

Clearly the development of a "clean vector technology for marker-free transgenic" in apples and other crops is the ultimate goal. The Plant Research International in The Netherlands recently developed and proposed the use of such technology (Schaart, 2004; Schaart *et al*., 2004; Krens *et al*., 2004). This new technology allows obtaining transgenic apple plants free from the selection marker genes.

Acceptance and public perception of genetic modification

Transgenic plants. Almost all apple transformation reports reviewed, with few exceptions, relied on the selection of a foreign gene *nptII*, with a non-apple gene promoters (CaMV 35S, among others) and the utilization of *A. tumefaciens*. However, for experimental purposes, genes not influencing the target trait have been also tested, such as the gene producing β-glucuronidase (GUS).

To our knowledge, no environmental risk studies specific to transformed apple have been published. Probably researchers are still concerned with producing acceptable GM apple cultivars with commercial interest and having environmental benefits, such as reduction of pesticides use (James *et al*., 2003). Under these circumstances, the commercialization of transgenic apple carrying a DNA from different species or genera, in the near future is certain.

A recent multidisciplinary EU project entitled 'Sustainable production of transgenic strawberry plants: Ethical consequences and potential effect on producers, environment and consumers' was published (Iversen, 2003).

This project surveyed and gathered the opinion regarding the attitude of the consumers on the use or consumption of genetically transformed plants. The attitude of the consumers in Norway, Denmark and the UK towards genetic modification was rather negative, but for the type of genetically modified strawberry plants and the traits involved in the transformation process. For instance, consumer acceptance increased when the modified trait was perceived beneficial to them and when the own strawberry DNA was utilized to transform the plant. A recent consumer survey in the USA showed that a majority of the respondents would eat vegetables containing a gene from the same species (81%), or from another vegetable species (61%), in comparison to those from other sources like viral genes (14%) (Lusk and Sullivan, 2002).

Sociological studies (Iversen, 2003) showed that public perception of GM crop is driven by emotions, rather than an open discussion on the advantages or possible limitations in each case.

In order to fully utilize the possibilities of genetic modifications for crop improvement, it would be interesting to discuss what is the minimal genetic distance required for the genome manipulation to ensure sufficient public acceptance. Until now, there is no public concern when breeders make crosses within o between genotypes that belong to the same species, including wild and domesticated germplasm. In several cases, conventional breeders utilize germplasm coming from gene pool I, genotypes closely related where crosses do not present any biological limitations), or from gene pool II; where breeders use some techniques such embryo culture to obtain fertile offspring when cross genotypes more distantly related or protoplast fusion when they need to obtain a progeny from different species.

Cisgenic plants. In order to increase acceptance of the genetically modified plants by consumers, a group of researchers at Plant Research International (Wageningen University) and at Research Centre in The Netherlands developed a new series of biotechnological strategies to reduce the limitations of the conventional genetic engineering tools available (Schaart, 2004; Schaart *et al*., 2004; Krens *et al*., 2004).

This innovation consisted in the use of genes from the same species or closely related species, along with their own native promoters and the markerless DNA transformation technology, where no selectable marker, like antibiotics and herbicide resistance genes are used to select the transformed lines (Schouten *et al*., 2006a; 2006b). All these innovations are expected to facilitate the acceptability and commercialization of genetically engineered plants by consumers, growers, and regulatory agencies. This new innovation was named as "Cisgenesis", and it is considered as a friendly technology and an excellent strategy to improve plant resistance and to complement conventional breeding programs (Schouten *et al*., 2006b; Jacobsen and Schouten, 2007; Haverkort *et al*., 2008; Schouthen and Jacobsen, 2008).

To date, the majority of established regulations on genetically modified organisms (GMOs) worldwide have not discriminated cisgenic from transgenic plants. This may be because until now, few cisgenic plants have been developed and submitted for approval. Canada is one of the countries that has a product-based regulation rather than a process-based regulations, therefore, it is possible that cisgenic plant could be treated less stringently than transgenic plants (Schouten *et al*., 2006b).

Cisgenic plants are fundamentally different from transgenic plants. In the case of transgenesis, a foreign gene, from different species or genera, is introduced into a plant. Therefore, it is postulated that a transgenic plant has a phenotypic trait that did not occur in the species (wild and domesticated) and could affect its fitness through the traits itself or by the gene flow from the domesticated transformed plant to its wild relatives (Schouten and Jacobsen, 2008).

In contrast, in cisgenesis the introduced gene of interest with its native promoter has already been present in the domesticated or wild species for centuries. Therefore, cisgenesis does not add an extra trait to the species. It does not invoke a fitness change that could not also occur through traditional breeding or in nature. The same holds true for other environmental risks, such as effects on non target organisms or soil ecosystems, and for usage in food or feed. As a result, deliberate release of cisgenic plants into the environment could be as safe as the deliberate release of traditionally bred plants (Jacobsen and Schouten, 2008).

Nowadays, modern fruit production and a higher consumer demand require cultivars with a better productivity, uniformity, long-term storage, resistance to diseases and pests and good quality to ensure a commercial success. In Chile, apple is one of the most important fruit exported to different markets and it plays a major economic and social role in the agricultural sector and in Chilean economy. Unfortunately, pests and diseases are one of the major factors that are limiting the potential growing of this industry.

Based on those facts, use of such cisgenic apples plants could aid to develop a new way of sustainable crop production practices. Recently, the Instituto de Investigaciones Agropecuarias INIA Quilamapu started to develop a platform to produce cisgenic Royal Gala and Granny Smith apple cultivars to improve their resistant to *V. inaequalis*.

CONCLUSIONS

Nowadays, a high market demand apple cultivars with high productivity and fruit quality, and a reduced pesticide application and recent advances in plant biotechnology could help to satisfy this consumer demand.

Transformation technology in apple has a long history but it practical application has been limited by the consumer acceptance. The development of a new "clean vector technology", the sequencing of gene controlling agronomic traits, under the control of their own promoters will allow to produce transformed plants with better consumer acceptance.

Applications of this technology could support and complement the apple breeding program in the development of a more sustainable crop apple production practices and to improve its productivity and competitiveness in the world market.

RESUMEN

Avances de la tecnología de transformación del manzano para conferir resistencia a enfermedades fungosas en su cultivo: Una perspectiva chilena. La manzana (*Malus domestica* Borkh.) es una de las frutas más consumidas en el mundo. La transformación genética es un proceso clave para sustentar esta demanda, permitiendo el mejoramiento potencial de los cultivares existentes, así como el desarrollo de nuevas variedades resistentes a plagas, enfermedades y problemas de almacenamiento que se originan en las zonas de producción más importantes. Esta revisión resume los avances de la ingeniería genética aplicada al desarrollo de variedades de manzana resistentes a enfermedades fungosas, con especial atención en la generación de manzanas resistentes a *Venturia inaequalis* (Cooke) G.Winter, el principal problema fitosanitario que afecta a los cultivos de manzana en Chile.

Palabras claves: manzana, enfermedades fungosas, transgénico, cisgénesis.

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