

Transformed Apples: Traits of Significance to Growers and Consumers

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ABSTRACT

The first report of transformed apple plants in 1989 raised expectations for new apple cultivars that would be better tasting, healthier and easier to grow. Although, many different traits have been now been introduced successfully into apple, no transformed cultivars have yet made it to commercial production. Most early reports on transformed apple described 'proof of concept' experiments involving the development of regeneration protocols, and the choice of appropriate promoters and selectable markers. More recently the focus has moved onto functional testing of traits of potential commercial interest. These traits can be grouped into two categories: horticultural production traits and fruit-focussed traits. Production traits of interest include bacterial, fungal and pest resistance, dwarfing, propagation, stress resistance, precocity, storage life and self fertility. Examples of fruit-focussed traits include novel health properties, flavour, reduced browning, colour, and reduced allergenicity. This review will consider reports of characters introduced into apple that are useful to growers and consumers, and looks toward future trends, targets and challenges.

Keywords: allergenicity, dwarfing, flavour, herbicide resistance, pest and disease resistance, precocity, storage life, stress resistance

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INTRODUCTION

Apples (*Malus pumila* synonym. *Malus x domestica*; Maberley *et al.* 2001) comprised 13% (5.2 million Ha; 62 million metric tons) of total world fruit production in 2005 (World Apple Report 2006, Belrose Inc.) and are widely

grown in temperate areas throughout the world. The fruit are borne on generally self incompatible perennial trees with long generation times, which makes retaining multiple desirable characters through conventional breeding very difficult and time consuming (Brown 1992). Therefore there was great excitement and expectation with the first

report of genetic transformation by James *et al.* (1989). Suddenly it seemed possible that existing cultivars with completely novel, or with slightly altered characters could be produced within a much shorter timeframe than by conventional breeding.

In the eighteen years since apple transformation was first reported, many common scion and rootstock cultivars have been successfully transformed, yet no transformed cultivars have progressed through to commercial production. With hindsight it was probably optimistic to have expected the latter to occur quickly. Most early reports on transformed apple described 'proof of concept' experiments involving the development of regeneration and transformation protocols, and the choice of appropriate promoters and selectable markers (summarised in Bulley and James 2004; Malnoy and Aldwinckle 2007). More recently, attention has focussed on functional testing of traits of scientific and potential commercial interest. Today, genetic engineering is seen as another powerful tool in the breeder's 'toolbox'. It can be used to introduce variation from genetic resources within and outside of apple and complements the rapid advances (saturated genetic maps, genome sequencing, etc.) being made in marker-assisted breeding.

This review will consider the reports of transformed apples having direct application to producers, consumers, or

both. It will also briefly discuss consumer attitudes to genetically engineered (GE) apples and consider the implications of these attitudes to both the traits that might be introduced into apple and appropriate delivery technologies. Transformation protocols are not discussed as they have been covered extensively in other reviews; however a summary of transformation frequencies is presented in **Table 1** to give the reader an idea of the relative difficulty in transformation of various cultivars.

HORTICULTURAL PRODUCTION TRAITS INTRODUCED INTO APPLE

Bacterial disease resistance

Engineering to increase resistance to fire blight is the focus of research in bacterial disease resistance (also reviewed recently in Malnoy and Aldwinckle 2007). Fire blight, caused by the necrotic bacterium *Erwinia amylovora*, is arguably the most devastating disease of apple, with some outbreaks resulting in the destruction of whole orchards. The disease was first reported in the northeast of USA in 1784, and from there it has spread through infected material throughout most global apple growing regions, except China, India, South America and Australia. Fire blight bacteria are capa-

Table 1 Transformation efficiencies of various apple cultivars and rootstocks.

Type	Cultivars	Efficiency of transformation	Reference	
Scions	'Ariane'	2%	Faize <i>et al.</i> 2003, 2004	
	'Braeburn'	1.20%	de Bondt <i>et al.</i> 1994, 1996	
	Red Delicious'	1-2%	Sriskandarajah <i>et al.</i> 1994, 1998	
	'Elstar'	1.20%	de Bondt <i>et al.</i> 1994, 1996	
		0.4-0.8%	Schaart <i>et al.</i> 1995; Puite and Schaart 1996	
		0.17%	Szankowski <i>et al.</i> 2003	
	'Falstaff'	7.50%	Wilson and James 1998	
	'Florina'	7.90%	Radchuck and Korkhovoy 2005	
	'Fuji'	1.20%	de Bondt <i>et al.</i> 1994, 1996	
		1.10%	Murata <i>et al.</i> 2001	
		1.4-6.5%	Seong <i>et al.</i> 2005	
	'Gala'	1.20%	de Bondt <i>et al.</i> 1994, 1996	
	'Galaxy'	5-20 %	Malnoy <i>et al.</i> 2007a	
	'Royal Gala'	0.7-0.9%	Schaart <i>et al.</i> 1995	
		0.7-8.7%	Puite and Schaart 1996	
		0.4-4.6%	Yao <i>et al.</i> 1995	
		1-1.4 %	Faize <i>et al.</i> 2003, 2004	
		1.70%	Liu <i>et al.</i> 2001	
	'Golden Delicious'	0.2-6%	Schaart <i>et al.</i> 1995	
		1.60%	Puite and Schaart 1996	
	'Greensleeves'	0.1-0.5%	James <i>et al.</i> 1989	
		8-16%	S.M. Bulley <i>et al.</i> unpublished results	
	'Holsteiner Cox'	0.5-2.68%	Szankowski <i>et al.</i> 2003	
	'Liberty'	0.03-0.4%	Hanke <i>et al.</i> 2000	
	'McIntosh'	5-20 %	Norelli <i>et al.</i> 1996	
	'Wijcik'	3.50%	Wilson and James 1998	
	'Melba'	13.30%	Dolgov <i>et al.</i> 2000	
	'Orin'	0.10%	Murata <i>et al.</i> 2000	
		0.50%	Kanamaru <i>et al.</i> 2004	
		0.25%	Kotoda <i>et al.</i> 2006	
	'Pink Lady'	1-2%	Sriskandarajah and Goodwin 1998	
	'Pinova'	0.03-0.4%	Hanke <i>et al.</i> 2000	
'Queen Cox'	0.5-2.2%	Wilson and James 2003		
'Remo'	0.03-0.4%	Hanke <i>et al.</i> 2000		
'Reka'	0.03-0.4%	Hanke <i>et al.</i> 2000		
Rootstocks	'A2'	0.33%	Zhu <i>et al.</i> 2001a	
	'JET-H'	5.60%	Sule <i>et al.</i> 2002	
	'Jork 9'	6.50%	Sedira <i>et al.</i> 2001, 2005	
	'M.7'	5-20 %	Norelli <i>et al.</i> 1996	
	'M.26'	2.5-7.1%	Welander <i>et al.</i> 1998	
		0.9-3.9%	Holefors <i>et al.</i> 1998, 2000	
	'M9/29'	0.02%	Zhu <i>et al.</i> 2001b	
	'N545'	1.5-7.2%	Dolgov <i>et al.</i> 2000, 2004	
	<i>Malus prunifolia</i>	'Ringo Asami'	2%	Masuda <i>et al.</i> 2004
	<i>Malus robusta</i> Rehd.	'Balenghaitang'	1.20%	Qu <i>et al.</i> 2005

ble of infecting blossom, fruit, vegetative shoots and rootstock crowns through natural openings or wounds. Current disease management strategies rely on a mix of cultural practice and application of copper compounds and antibiotics. The most effective antibiotic is streptomycin. However, streptomycin is not registered for commercial use in many countries and streptomycin-resistant *E. amylovora* strains have arisen where streptomycin was used frequently (Jones and Schnabel 2000). Fire blight resistant rootstocks have been commercially released (e.g. the Geneva® series); but conventional breeding of scions is hampered by the very poor quality of resistance sources.

A number of genes have been expressed in apple to improve bacterial resistance, with varying degrees of success. Researchers have followed three different strategies: producing antimicrobial proteins, inhibiting bacterial pathogenicity factors, and silencing DspE-interacting proteins.

Producing antimicrobial proteins. Cecropins act as antimicrobial proteins because they contain amphipathic helices which associate to induce pore formation in bacterial membranes (Hultmark *et al.* 1982; Boman and Hultmark 1987; Flink *et al.* 1989; Noorden *et al.* 1992; Jaynes *et al.* 1993). The cecropin B analogs SB-37 and Shiva 1 (from the giant silk moth, *Hyalophora cecropia*) were expressed in 'Royal Gala' and 'M.7' apple rootstock under control of wound-inducible and constitutive promoters with mixed success (Aldwinckle *et al.* 1996; Norelli *et al.* 1996). One transgenic line (T245) showed significantly increased resistance compared with the control 'Royal Gala' (12% vs. 67% shoot length infected), and several other lines developed less disease than the controls. Enhanced resistance to fire blight in tissue culture tests was achieved by expressing a modified cecropin SB37 gene, fused to a secretory coding sequence from barley α -amylase under the control of a wound-inducible osmotin promoter from tobacco, but no data from whole plants were reported (Liu *et al.* 2001).

Attacin E (*AttE* gene) is an antimicrobial protein from *H. cecropia* that has been expressed in apple (Hanke *et al.* 2000; Ko *et al.* 2000). In the study by Ko *et al.* (2000), four binary plasmids were transformed separately into 'Galaxy' (a sport of 'Gala'). The authors found a correlation between attacin content and resistance in lines with *AttE* under control of the *Pin2p* promoter, and showed that the AMV untranslated leader sequence increased attacin content three times that of the *AttE* construct without AMV. Most interesting though, was the result of directing secretion into the intercellular space. These lines had the lowest attacin content but highest fire blight resistance. Degradation assays indicated that 60% of the attacin was degraded after 1 hr. This study appears to agree with the proposition that antibacterial proteins localised in the intercellular space should be more effective for bacterial resistance than expression within the plant cell (Alfano and Collmer 1996; Düring 1996; Bogs *et al.* 1998). Increasing the longevity of the secreted proteins in the intercellular space should presumably increase resistance.

Lysozyme proteins are bacteriolytic enzymes that are only effective against gram-positive bacteria (Boman and Hultmark 1987). Transgenic 'Galaxy' expressing either T4 lysozyme or *AttE* had significant fire blight disease reduction compared with non-transgenic 'Galaxy', but none of the lines expressing both were significantly more resistant than the 'Galaxy' control (Ko *et al.* 2002). Hanke *et al.* (2000) also reported that increased resistance to fire blight was achieved by expression of the phage T4 lysozyme in the fire blight susceptible apple 'Pinova'.

Inhibiting bacterial pathogenicity factors. The capsular exopolysaccharide (EPS) layer is very important to bacterial physiology. The EPS has roles in bacterial pathogenicity (Belleman and Geider 1992; Bernhard *et al.* 1993) as well as binding and absorbing nutrients, and avoiding host detection (Romeiro *et al.* 1981). The polysaccharide Φ Ea1-depolymerase gene (*Dpo*) from an *E. amylovora* phage (driven by *CaMV35S*) was transferred into nine apple cultivars, resulting in increased (but not complete) resistance to

fire blight (Hanke *et al.* 2000; Flachowsky and Hanke 2006). Sule *et al.* (2002) transferred *Dpo* into the apple rootstock 'JTE-H' and reported resistance of transgenic lines to fire blight infection compared with controls. Greater resistance was observed when *Dpo* with a signal peptide for secretion into the intercellular space was transformed into 'M.26' rootstock (Borejsza-Wysocka *et al.* 2006).

Silencing DspE-interacting proteins. Borejsza-Wysocka *et al.* (2004) silenced endogenous apple genes whose products interact with the *dspE* gene of *E. amylovora*. The *dspE* gene encodes a pathogenicity effector essential for development of the fire blight. DspE-interacting proteins (DIPM) are conserved in all hosts of *E. amylovora* tested, but not in any non-host plants tested so far (Borejsza-Wysocka *et al.* 2006). The authors reported that resistance to *E. amylovora* strain Ea273 was increased in certain lines with silenced DIPM genes.

Besides resistance to fire blight, resistance to only one other bacterial disease, crown gall (*Agrobacterium tumefaciens*), has been engineered in apple. Viss *et al.* (2003) produced crown gall resistant transformed 'Jonagold' apple trees by expressing double-stranded RNA from *IaaM* and *Ipt* oncogene sequences from *A. tumefaciens*. These genes are involved in phytohormone production and subsequent gall formation. Increased resistance to *A. tumefaciens* would be most useful for rootstocks, where gall formation is most problematic.

Fungal disease resistance

Work on resistance to fungal disease has focused on apple scab (*Venturia inaequalis*), the most serious fungal disease of apple in growing areas with wet springs. It attacks both the foliage and fruit, resulting in reduced yield, and un-saleable blemished fruit. Scab is mainly controlled by the use of fungicides, although this method of control is threatened by increasing fungicide resistance. Conventional breeding has relied on one major resistance gene (*Vf*). Race 6 of the *V. inaequalis* that can overcome this resistance occurs in Europe, but apparently not in North America. Researchers have also introduced antifungal proteins from fungal and plant sources with interesting results.

Wong *et al.* (1998) reported increased scab resistance in 'Royal Gala' over-expressing an endochitinase and chitinobiosidase from the bio control fungus *Trichoderma atrovirida*. Resistance was engineered into the very scab susceptible 'Marshall McIntosh' apple by introducing the same two genes (Bolar *et al.* 2000, 2001). The levels of chitinase expression were positively correlated with resistance to scab and the endochitinase was more effective than exochitinase, although lines with high endo-chitinase gene expression had reduced vigour. Double transformant lines with high exo-chitinase and low endo-chitinase expression were resistant to scab and had negligible effects on growth (Bolar *et al.* 2001). Similar effects were reported by Faize *et al.* (2003) in 'Galaxy' and 'Ariane' (carrying *Vf*) transformed with the same endo- and exo-chitinase genes. Additionally, introduction of the chitinases gave a pyramiding of resistance because transformed 'Ariane' plants gained resistance to scab race 6 (*Vf* in 'Ariane' confers resistance to race 1). Race specific resistance was seen in an experiment where and antifungal cysteine-rich wheat seed protein called puroindoline-b (*pinB*) was transformed into 'Galaxy' and 'Ariane' resulting in resistance to race 6, but not to race 1 in the case of 'Galaxy' (Faize *et al.* 2004). The differing modes and differential nature of scab resistance emphasises the need to pyramid multiple sources of resistance to ensure that durable resistance can be achieved.

Extracts from transgenic 'Jonagold' shoots over-expressing an antifungal peptide from radish and an antimicrobial peptide from onion showed 8 to 32 and 4 fold increases, respectively, in fungal growth inhibition activity over controls *in vitro* (de Bondt *et al.* 1998), but nothing has been published regarding their performance in the field.

The introduction of the apple scab resistance gene *Vf*

from a wild into a cultivated apple began as early as the 1950s (Hough *et al.* 1953; Schmidt and van de Weg 2005). Despite more than 50 years of traditional breeding, the new apple cultivars carrying this gene have yet to acquire the same fruit quality in terms of taste and texture as elite susceptible cultivars, because of linkage drag. The genes at the *Vf* locus have recently been cloned (Vinatzer *et al.* 2001; Xu and Korban 2002). One of these *Vf* paralogs, *HcrVf2*, was transformed into the susceptible 'Gala' cultivar and shown to confer scab resistance when driven by the strong constitutive CaMV35S promoter (Belfanti *et al.* 2004). Recently, Malnoy *et al.* (unpublished results) undertook a study of the function of each of the three *Vfa* full-length paralogs at the *Vf* locus in the resistance response of apple to *V. inaequalis* by complementation tests. Intact genomic candidate genes of each of the *Vfa* paralogs under the control of their own promoter were integrated into the genome of the scab-susceptible apple cultivars 'Galaxy' and 'McIntosh'. They found that transformed lines expressing *Vfa4* were susceptible to apple scab; whereas, those expressing either *Vfa1* or *Vfa2* were partially resistant to apple scab.

Enhancing natural disease resistance mechanisms to pathogenic bacteria and fungi

During the infection process pathogens secrete compounds that elicit a range of plant host defence responses that occur at different speeds. Researchers have tried to induce plant defence responses by introducing elicitors or by speeding up the defence response with various promoters. The harpin N_{EA} gene from *E. amylovora*, is an elicitor (and pathogenicity factor), that induces a hypersensitive response (HR) as well as systemic acquired resistance (SAR) (Wei *et al.* 1992; Dong *et al.* 1999). This gene was introduced into the fire blight susceptible 'M.26' rootstock under the control of either the weak constitutive *nos* or the pathogen-inducible *gst1* promoters (Abdul-Kader *et al.* 1999; Aldwinckle *et al.* 2003). In the *gst1* promoter driven plants, an increase in resistance equivalent to that of the fire blight resistant rootstock 'M.7' was observed over a number of years (Malnoy and Aldwinckle 2007).

Another approach has been to over-express master regulators of the plant disease response. The *NPR1* gene is a key mediator of SAR (Cao *et al.* 1994, 1997, 1998). An additional copy of the apple orthologue, *MpNPR1*, was introduced into 'Galaxy' and the rootstock 'M.26' (Malnoy *et al.* 2006). In test chamber challenges with *E. amylovora*, the transformed 'Galaxy' clones had 17.5 to 35.5% shoot length infected compared with 80% in controls. In addition, there was increased resistance to two other pathogens [*V. inaequalis* and *Gymnosporangium juniperi-virginianae* (cedar apple rust)] (Malnoy *et al.* 2004, 2006). The increased, broad spectrum resistance produced by the introduction of an additional copy of a gene sourced within apple, makes the use of *MpNPR1* (and similar strategies) very attractive, as all the other genes for resistance employed previously have been of viral, bacterial, fungal or animal origin.

Insect pest resistance

Apple plants have been transformed with lepidopteran-specific insecticidal crystal proteins (ICPs) from *Bacillus thuringiensis* (Dandekar *et al.* 1992, 2002). Initially, this approach was unsuccessful because of a lack of expression caused by codon usage, cryptic splice sites, and motifs causing mRNA instability (Escobar and Dandekar 2000; Dandekar *et al.* 2002), but this was rectified by using ICPs with a re-synthesised gene sequence (Perlak *et al.* 1991). Under licence from Monsanto, Dry Creek Laboratories developed transgenic apple trees expressing synthetic ICP genes. High mortality rates of codling moths were observed on these trees (Dandekar *et al.* 1992; Clark *et al.* 2004). Interestingly, these trees were used as trap plants in walnut orchards with great effect and in a 4-year trial "worm damage to the walnuts was almost completely controlled without pesticide ap-

plications, equivalent to that in the plots sprayed three times per season with pesticides" (Clark *et al.* 2004).

The use of the biotin-binding proteins avidin and streptavidin gave resistance to the economically significant pest lightbrown apple moth (LBAM), *Epiphyas postvittana* (Markwick *et al.* 2003). Expression of the transgene protein was directed to the vacuole using potato proteinase inhibitor targeting sequences (Murray *et al.* 2002). Targeting to the vacuole was crucial because production of biotin is essential for plant growth (Hood *et al.* 1997). The use of biotin-binding proteins is likely to confer resistance to a wide variety of insects (Morgan *et al.* 1993; Markwick *et al.* 2001; Burgess *et al.* 2002), including some not targeted by ICPs from *B. thuringiensis*. Therefore, the deployment of both types of genes would not only confer broad insect resistance but would also give a better chance of durable resistance.

Dwarfing

The ability to control plant vigour made modern high intensity apple production systems possible. In general, sufficient vigour control of dessert apple cultivars is imparted by the use of rootstocks. Bittersweet apple cultivars, on the other hand, tend to be very vigorous and require the application of chemical growth retardants such as paclobutrazol (Cultar™, Syngenta) or Apogee®, in addition to grafting onto rootstocks. Both these growth retardants are known to be inhibitors of gibberellin (GA) biosynthesis. With this in mind, dwarf (reduced internode length) 'Greensleeves' scions were produced by silencing an endogenous GA 20-oxidase (Fig. 1, Bulley *et al.* 2005). The size of the transformed trees ranged between 50 and 80% of non-transformed controls and more pertinently, the dwarfing effect was retained in the T₀ plants after grafting onto normally invigorating rootstocks (M.25 and MM.106). Transformed lines typically produced normal sized fruit over several seasons (S.M. Bulley *et al.*, unpublished results).

Earlier work also reported dwarf apple trees. Holfors *et al.* (2000) transformed 'M.26' rootstock with the light dependent photoreceptor *phytochrome B* gene (*PhyB*) from *Arabidopsis* (Sharrock and Quail 1989). *PhyB* regulates developmental light responses and over-expression of *PhyB* in *Arabidopsis* and tobacco resulted in dwarf plants (Wagner *et al.* 1991; Halliday *et al.* 1997). Transformed apple plants over-expressing *PhyB* had reduced stem lengths down to 2/3 that of control (for the most dwarfed), due to slightly reduced internode length and presumably internode number (internode number data not given) (Holfors *et al.* 2000). This result is more like the dwarfing effect imparted by rootstocks, where internode number is reduced but internode length is *not*, and there is an increased probability of the bud becoming floral (Seleznova *et al.* 2003). No further data have been published regarding the ability of the *PhyB* dwarfed rootstock to impart a dwarfing effect to the scion.

The *rol* genes from *A. rhizogenes* have been used to dwarf two cultivars of rootstock. The *rolA* gene was transformed into the vigorous 'A2' rootstock (Zhu *et al.* 2001a) and the semi-vigorous 'M.26' rootstock, resulting in slower relative growth rates and shortened internode lengths (Holfors *et al.* 1998; Zhu and Welander 2000). The *rolC* gene was transferred into the 'Marubakaidou' rootstock and the resulting dwarf trees fell into two groups. The first group had reduced height with normal internode lengths, while the second group displayed both reduced height and shortened internodes (Igarashi *et al.* 2002). Again, no further data have been published regarding any ability of the *rolC* dwarfed rootstock to impart the same dwarfing effect to the scion.

Introduction of the *rolB* gene can also lead to dwarfing in some cases. However, this gene has been used mainly to increase rooting ability, as discussed below.

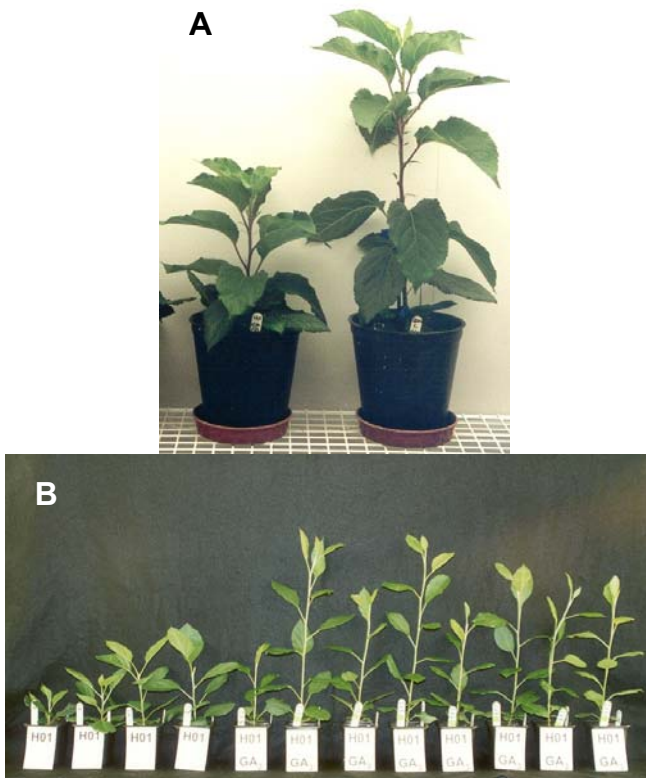


Fig. 1 (A) Dwarfed transgenic line (left) compared with a non-transgenic control of same age (right). Plants were dwarfed using a sense-suppression strategy with a partial sequence (316 bp) of an apple gibberellin 20-oxidase gene (Bulley *et al.* 2005). The dwarfing effect is a result of reduced internode length, as the number of internodes remained unchanged. (B) GA₃ application can rescue the phenotype. All the plants are the same dwarf line as in 'A' and of the same age. The eight plants on the right had GA₃ applied at weekly intervals (approx. 200 mg.L⁻¹), while the four on the left were sprayed with water only (Bulley *et al.* 2005).

Propagation

Some apple cultivars are difficult to propagate from cuttings. The *rolB* gene from *A. rhizogenes* has been transferred into cv. 'Florina' (Radchuck and Korkhovoy 2005), and the rootstocks 'M.26' (Welander *et al.* 1998; Zhu and Welander 2000), 'M.9'/'M29' (Zhu *et al.* 2001b) and 'Jork 9' (Pawlicki-Jullian *et al.* 2002). Expression of *rolB* conferred increased adventitious root formation on hormone-free medium (80-100% explants rooting compared with 0-50% for controls). In some cases, reduced apical dominance with slow initial growth and shorter and fewer internodes were seen (Zhu and Welander 2000; Zhu *et al.* 2001b; Pawlicki-Jullian *et al.* 2002), although the degree of dwarfing was not as strong compared with *rolA* transformants (Zhu and Welander 2000). No difference in shoot length was seen in *rolB* transformed 'Florina' (Radchuck and Korkhovoy 2005).

Stress resistance

Apple trees with greater resistance to abiotic stresses would allow more sustainable methods of production as well as productive use of more marginal lands. To date, only two traits have been modified using genetic engineering. Increased tolerance to heat and cold stress and UV-B was achieved by over-expressing a cytosolic ascorbate peroxidase from pea in 'Royal Gala'. The greatest improvement in tolerance was in response to long-term exposure to sub-lethal heat stress, with lethal temperature 50 values (LT₅₀) increased to between 52 to 58°C in transformed plants compared with 51°C in controls (Wisniewski *et al.* 2007).

Transformed *Malus robusta* Rehd rootstock over-expressing a single copy of the tomato iron transporter gene (*LeIRT2*) showed increased tolerance to iron-deficient

growing conditions in hydroponic culture, with the transformed plants having approximately 21% more fresh weight than controls (Qu *et al.* 2005).

Precocity

An attractive feature of many clonal rootstocks is that they reduce the length of juvenility and promote heavier flowering of grafted scions when compared with scion cultivars grown on their own roots or on seedling rootstocks. Kotoda and colleagues (Kotoda and Wada 2005; Kotoda *et al.* 2006) reported that transformed 'Orin' apple plants expressing the *MdTFL1* gene in antisense orientation (MdTFL is an endogenous transcription factor that delays flowering), flowered 8-22 months after transfer to the greenhouse, whereas non-transgenic plants flowered 69 months after transfer to the greenhouse. However, the magnitude of the early flowering effect is unclear, since first flowering was obtained ~22 months after transformation in *AttE*-transgenic 'Gala' plants grafted on 'M.9' rootstock and grown for six months in a screenhouse, given a 3-month cold treatment and then planted in the field (H.S. Aldwinckle and J.L. Norelli, unpublished results). The *AttE* gene is not known to affect the onset of first flowering. An interesting effect of this modification was that the architecture of the trees changed because buds that were normally vegetative were instead now floral, leading to a less vigorous tree with shorter branches compared with controls. Might this explain, at least in part, how dwarfing rootstocks exert their effect?

Flachowsky *et al.* (2007a, 2007b) reported that expression of the *BpMADS4* gene from silver birch (*Betula pendula* Roth.) in 'Pinova' apple caused flower buds to develop on *in vitro*-cultured transgenic plants 13 weeks after transformation, and morphologically normal flowers were produced. Flowers were also produced on own-rooted plants of three *BpMADS4*-transgenic lines after 3-4 months growth in a greenhouse. These flowers produced viable pollen, and when pollinated set fruit with 8-10 seeds per fruit. This research is promising for producing early flowering model apple lines for the study of genomics of flowering regulation and fruit quality characters.

Storage life

Good storage potential is an important quality of any modern dessert apple cultivar. Whilst controlled atmosphere storage technology and chemicals such as 1-methylcyclopropane (MCP) can mitigate poor storage in some cultivars, these technologies have drawbacks in terms of energy use (refrigeration and atmosphere control), expense, and chemical application is subject to regulatory control. Transformed 'Gala', 'McIntosh' and 'Greensleeves' down-regulated for either ACS (ACC synthase; 1-aminocyclopropane-1-carboxylic acid synthase) or ACO (ACC oxidase) have been produced (Hrazdina *et al.* 2000; Dandekar *et al.* 2004; Schaffer *et al.* 2007). These genes encode key enzymes in biosynthesis of ethylene (Yang and Hoffman 1984; Gray *et al.* 1992), which is a key hormone initiating and mediating ripening in climacteric fruit.

Both Dandekar *et al.* (2004) and Defilippi *et al.* (2004) analysed fruit from the same or similar transformed anti-sense ACO and ACS apple lines, which ranged in ethylene production rate (nL C₂H₄ kg⁻¹.h⁻¹) from 4 to 40% that of the 'Greensleeves' control ('Greensleeves' has 'James Grieve' x 'Golden Delicious' parentage). They both observed greatly increased shelf life and increased firmness: 75-83 N compared with 50 N for controls after 12 days at 20°C. The fruit also retained more of its green colour under the same conditions (101-107 hue angle vs 97 for control; *p*<0.001). Dandekar *et al.* (2004) reported no significant difference in soluble solids (°Brix) and acidity at harvest and after storage, whereas sugar and acid composition was different from controls in fruit stored without ethylene treatment, but was no different from controls for ethylene-treated fruit (Defilippi *et al.* 2004). Production of volatile esters such as



Fig. 2 Ripening put on hold by disrupting ethylene biosynthesis: increased storage life without controlled atmosphere storage could allow large energy savings. Fruit from untransformed 'Royal Gala' (left) and ACC oxidase antisense line AO3 (right), after storage at 22 °C for 85 days (Schaffer *et al.* 2007). Image ©2007 American Society of Plant Biologists. A similar effect was reported in 'Greensleeves' apples silenced for either ACC synthase or ACC oxidase (Dandekar *et al.* 2004).

butyl 2-methylbutanoate, hexyl acetate, hexyl propanoate, and hexyl butanoate, as well as α -farnesene was suppressed (total ester production was down to 29% of control and α -farnesene was down to 40% of control). Alpha-farnesene is a major component of the 'green apple' smell and its production is regulated by ethylene (Dandekar *et al.* 2004; Pechous and Whitaker 2004). The volatile esters are important components of the fruit flavour complex, but a similar level of suppression of volatile ester production was observed when control fruit was treated with 1 $\mu\text{L}\cdot\text{L}^{-1}$ 1-MCP for 20 h at 20°C (Defilippi *et al.* 2005). Ethylene treatment allowed ester and alcohol synthesis to recover to 70% of the control fruit values, whereas ester and alcohol synthesis only slightly recovered in MCP-treated fruit (Defilippi *et al.* 2005).

In a separate study, Schaffer *et al.* (2007) found that in ACC oxidase down-regulated 'Royal Gala', ester and alcohol synthesis completely recovered after ethylene treatment. Their microarray data revealed that ethylene controlled only the last biosynthetic steps of aroma biosynthesis. Genotypic factors such as ethylene sensitivity might explain the difference in recovery of ester and alcohol synthesis. Being able to apply ethylene to control ripening offers a situation similar to that already being used for banana, and increased storage life without refrigeration could allow large energy savings (Fig. 2). These studies have provided valuable insights into the complexities of fruit ripening and flavour development. However, as ethylene is an important phytohormone that is also involved in mediating plant response to stress (flooding, drought, chilling, wounding, and pathogen attack), flower opening and abscission, more work is needed for commercial applications to be realised.

In another experiment to affect storage life, the apple polygalacturonase gene, *MdPG*, was expressed in sense orientation to co-suppress expression of the endogenous *MdPG* protein (Atkinson *et al.* 2002). A range of phenotypes were observed in the over-expressing 'Royal Gala' lines related to pectin changes in the leaf cell walls. However, although mature enough, none of the *MdPG* transformants developed flowers and, as a consequence, did not set any fruit. Therefore the effect of *MdPG* on fruit storage was not determined.

Herbicide resistance

A BASTA® (phosphinothricin) herbicide-resistant N545 apple rootstock has been produced by introducing the *bar* gene (phosphinothricin acetyl transferase). Plants transformed with the *bar* gene were resistant to a 1% solution of BASTA® (Dolgov and Skryabin 2004). Herbicide-resistant scions and rootstocks would facilitate the use of herbicides in nurseries and young orchards.

Self fertility

The majority of apples cultivars and many other tree crops are self-incompatible due to a system which specifically prevents self-pollen from fertilising self egg cells. In apple, a key gene in the self incompatibility system resides at the *S*-locus which encodes *S*-RNases (Broothaerts *et al.* 1995). One allele isolated from 'Elstar' (S3; Broothaerts *et al.* 1995) was over-expressed under the control of the CaMV35S promoter in 'Elstar' to silence the endogenous allele. Self-compatible transformed lines were obtained that were silenced for the S3 allele but also the S5 allele – presumably due to sequence similarity or methylation of the S5 allele (Broothaerts *et al.* 2004). Over a three year trial the S3/S5 silenced lines were equally fertile with self (32% set) and non-self pollen (31% set), and to controls pollinated with non-self pollen (30%). Fertility of self-pollinated controls was very low (4%) (Broothaerts *et al.* 2004). Self-fertile apples would be attractive to growers, particularly if pollination by bees was not required. The recent outbreak of varroa mite (*Varroa destructor*) in New Zealand highlights this issue. With the decimation of wild bee populations, orchardists must now bear additional costs for managed bee hives to be brought into orchards for pollination. Self-fertile apple cultivars could also be useful to apple breeders and researchers for producing homozygous breeding lines.

FRUIT-FOCUSED TRAITS INTRODUCED INTO APPLE

Novel health properties

'Elstar' and 'Holsteiner Cox' were transformed with a grape stilbene synthase gene under the control of its own promoter with the aim of increasing scab resistance (Szankowski *et al.* 2003; Rühmann *et al.* 2006). The resulting transgenic lines contained piceid (a glycoside of resveratrol). Resveratrol and its glycosides (storage form) occur naturally in grapes and wine and have a wide range of purported beneficial properties. These include being anti-inflammatory, estrogenic, antiplatelet, and anticarcinogenic (Bertelli *et al.* 1995; Gehm *et al.* 1997; Jang *et al.* 1997; Subbaramaiah *et al.* 1998; Manna *et al.* 2000), and in laboratory studies these compounds increased the life spans of yeast, *Caenorhabditis elegans*, *Drosophila melanogaster*, fish, and mice fed a calorie-rich diet (Howitz *et al.* 2003; Wood *et al.* 2004; Baur *et al.* 2006; Valenzano *et al.* 2006). However, it is unknown whether similar benefits will also be seen in humans, and the action of resveratrol as a phytoestrogen may also have deleterious effects in certain individuals (Gehm *et al.* 1997). No further information has been published regarding the degree of scab resistance conferred by expressing a stilbene synthase, but it is promising that apples containing increased levels of antioxidant compounds beneficial to human health might also promote resistance to fungal pathogens such as *V. inaequalis* (scab).

Flavour

The gene for the super-sweet tasting thaumatin II protein (from *Thaumatococcus danielli*) was transformed into the 'Melba' scion cultivar to increase sweetness and phytopathogen resistance (Dolgov *et al.* 2004). In an organoleptic test, the leaves of transgenic lines were reported to be modified in their taste. There was no mention of testing for changes to phytopathogen resistance or for changes to the taste of fruit. Introducing pathogenesis-related (PR) genes such as thaumatin to engineer flavour or phytopathogen resistance needs to be treated with care as many PR proteins are potentially allergenic (Hoffmann-Sommergruber 2000).

Alteration of sorbitol biosynthesis can impact on sugar partitioning in fruit. Sorbitol is the principal sugar translocated from source to sink organs in apple (Webb and Burley 1962). Transformation of 'Orin' with a sense sorbitol-6-phosphate dehydrogenase gene (*S6PDH*) under the control

of the CaMV35S promoter resulted in lines either silenced or up-regulated in S6PDH activity (Kanamaru *et al.* 2004). Plants that were silenced for S6PDH had reduced levels of sorbitol (to almost zero in some lines) and increased steady-state concentrations of sucrose compared with controls. In lines with increased S6PDH activity, both sorbitol *and* sucrose were increased compared with controls (Kanamaru *et al.* 2004). Conversion of triose-3-phosphate of the translocated photosynthate, in this case sorbitol, to 3-phosphoglyceric acid, results in NADPH production, which in turn reduces mannose-6-phosphate via mannose-6-phosphate reductase, was proposed to drive an apparent increase in photosynthetic rate (Stoop and Pharr 1996; Kanamaru *et al.* 2004). It will be interesting to see what effects there are on fruit constituents in the lines with increased S6PDH activity.

In a similar experiment, transformed 'Greensleeves' down-regulated for S6PDH showed marked changes in overall sugar metabolism (Cheng *et al.* 2005; Teo *et al.* 2006). The concentration of sorbitol was reduced in mature leaves while the concentration of sucrose and starch was increased. Although partitioning of newly fixed carbon to sucrose was unchanged, partitioning was increased for starch in S6PDH down-regulated lines. The increase in sucrose concentration was suggested to be due to breakdown of starch to glucose and maltose in chloroplasts for synthesis of sucrose during the night (Cheng *et al.* 2005). Fruit of S6PDH down-regulated lines had reduced sorbitol and increased sucrose concentrations. The fruit also had altered patterns of glucose, fructose, starch, and malic acid accumulation. Net carbon assimilation was also decreased in S6PDH down-regulated lines. Zhou *et al.* (2006) reported that these plants had reduced sorbitol dehydrogenase and increased sucrose synthase activities.

These studies have shown that altering expression of a key enzyme in sugar metabolism can have wide-ranging effects on fruit flavour such as the sugar acid balance. It would be of interest to determine how these changes affect sensory perception.

Reduced browning potential

Freshly cut apple fruit are particularly prone to browning of the flesh. Browning is caused by enzymatic oxidation of *o*-diphenols to their corresponding quinones, which then polymerise to form brown pigments (Mayer and Harel 1979). The chief catalysts are polyphenol oxidases (PPO). Transformed 'Fuji' and 'Orin' shoots and callus down-regulated for *PPO7* (Haruta *et al.* 1998) showed significantly reduced browning potential (Murata *et al.* 2000, 2001).

Various commercial apple cultivars have been transformed with a chimeric *PPO* with the aim of producing a non-browning phenotype. The chimeric gene was composed of sequence elements from four divergent apple *PPO* genes under the control of a constitutive promoter. This coordinated down regulation of the entire apple *PPO* gene family resulted in reduced levels of total PPO enzyme activity in the leaf and fruit of transformed lines (>90%) and non-browning phenotypes were achieved in multiple lines of several apple cultivars (Okanagan Specialty Fruits [OSF], unpublished results). Multiple years of field testing of this material confirm the stability of the non-browning phenotype and have identified no negative impacts on horticultural traits, or on resistance to diseases and insects when grown under field conditions. The non-browning technology developed at OSF has been incorporated into a new enabling platform that: (i) eliminates the selectable marker, (ii) uses only plant-derived gene sequences and control elements, and (iii) improves the efficiency of gene silencing. Plants arising from this series of transformations are now entering field trials and may be the first transformed apples to reach market. Such fruit would be attractive to both the consumer and the food processing industry.

Colour for novelty and health

Apple skin and flesh colour patterns attract the attention of consumers and serve to differentiate cultivars. The pigments involved in fruit colouration have also been reported to provide protection from cardiac disease (decrease lipid peroxidation and cholesterol level) and inhibit cancer cell proliferation (Boyer and Liu 2004; Schijlen *et al.* 2004). The distinctive skin and flesh colour of each cultivar is made up of combinations of coloured flavonoids and carotenoids, although there is much less diversity in flesh colour compared with skin colour. Recent work by Espley *et al.* (2007) has revealed a Myb transcription factor named *MdMYB10* that is likely to be the controlling factor for anthocyanin production in apple. When *MdMYB10* was over-expressed in 'Royal Gala' under the control of the CaMV35S promoter, the resulting callus and transformed plants were a deep red colour (Espley *et al.* 2007). Changes to fruit skin and flesh were not reported, but pigmentation would be expected to be similar to the cultivar 'Red Field' (Fig. 3) from which *MdMYB10* was isolated. Messenger RNA transcripts for *MdMYB10* and the associated anthocyanin biosynthetic genes that it regulates are much more abundant in skin and cortex tissue in 'Red Field' than in the white-fleshed and partially red-skinned 'Pacific RoseTM' (Espley *et al.* 2007). This work demonstrates the potential for manipulation of skin colour and patterning in apple. Transcription factors controlling the formation of other colour pigments such as carotenoids have been investigated in other species (Davuluri *et al.* 2005) and have potential in apple to increase health properties and visual attractiveness.



Fig. 3 An apple of the future? Visually attractive, distinctive branding and improved health qualities all rolled into one. While this 'Red Field' apple is *not* GE, but Espley *et al.* (2006) have identified the cause of the red flesh colouration as being due to increased expression of a Myb transcription factor named *MdMyb10*. 'Royal Gala' plants engineered to over-express *MdMyb10* have dark red leaves and have just flowered for the first time (A.C. Allan *et al.*, unpublished results).

Reducing allergenicity

The prevalence of allergies to foods is lower than that of airborne pollen. Roughly 8% of children, particularly in the first 3 years of life, exhibit an allergic reaction to certain foods, but this drops to around 2% in adults. The prevalence of allergy to apple is uncertain, but it varies by geographic region. In Northern Europe, most apple allergic patients (>90%) present oral allergy symptoms resulting from cross reaction of IgE to the birch pollen allergen Betv1 (Fernandez-Rivas *et al.* 2006). The protein in apple that they react to is Mald1 which is an orthologue of Betv1 (Ebner *et al.* 1995; Vanek-Krebitz *et al.* 1995). The allergic symptoms are typically far less severe than peanut allergy because the Mald1 proteins are labile and therefore broken down by the oral mucosa (Astwood *et al.* 1996). In Spain, apple allergic patients are predominantly sensitised to Mald3 (non-speci-

fic lipid transfer protein), which is less easily degraded than Mald1, and so patients can develop much more severe allergic reactions (Fernandez-Rivas *et al.* 2006).

The Mald1b gene has been silenced in 'Elstar' and the silenced lines showed a greater than 10-fold reduction of Mal d 1 protein in leaf. Skin prick tests on allergic patients showed significantly reduced allergenicity, compared with the non-transformed control (Gilissen *et al.* 2005). Immunoblotting of protein extracts from Mald1-silenced lines with a mixture of allergic patient IgE showed little or no reaction to Mald1 in the silenced lines but did show reactivity to other bands (in the regions of ~20-26 KDa, ~39-43 KDa and ~50-58 KDa) (Gilissen *et al.* 2005). Mald1 is encoded by a large multi-gene family of at least 18 members in apple (Atkinson *et al.* 1996; Beuning *et al.* 2004; Gao *et al.* 2005), and it is unknown at this stage which members are allergenic and if all were down-regulated in the silenced lines.

Assessment of the commercial feasibility of producing 'hypoallergenic' apples needs to include the potential market of between 2-10% of the population (although many consumers might choose hypoallergenic products even if they were not required). Allergic people typically avoid the foods they are allergic to and will require persuasion and assurance that a hypoallergenic fruit is safe. Allergic patients can also react to multiple proteins and so multiple genes would need to be silenced. The function of many of these genes is unknown and not all of the allergens present in apple have been characterised or identified. Nevertheless, there is the possibility that low allergenic cultivars may be given preference by food producers and processors because of food health and safety regulations and thus set the benchmark for future cultivars.

CONSUMER PERCEPTIONS OF TRANSFORMED APPLES

Gaining consumer acceptance is vital for the success of GE apples and realisation of the benefits that they potentially offer. Thus development of GE apples will be driven by the market rather than by science (Gamble 1999). Securing a market for any GE food product (including apples) will require consumer perceptions to be addressed, which will be difficult in some markets, given the negative publicity surrounding the technology that has built up over the last ten or so years.

It is also well known that people's intentions can be quite different from their actions. In one topic-blind study from the UK, 93% of participants willingly tasted an apple they believed to be GE and 48% said they would buy GE food when it became available (Townsend and Campbell 2004). The majority (85%) of the 49% of consumers who said they would not purchase GE food in the future willingly tasted and ate an apple they believed to be GE. Therefore, the proportion of consumers who reject genetic engineering outright might be smaller than previously reported and the negative perceptions of the GE food (fruit in this case) may not be as strong as was thought. GE apples that address the fears and wants of consumers may have a better chance of acceptance. In an experimental auction setting with New Zealand consumers, a majority (68-70%, depending on type of GE apple) were willing to pay for the two types of GE apples offered, with only 15% stating specific concern about the use of genetic modification as a reason for not bidding (Kassardjian *et al.* 2005).

What may lessen the perception of risk of genetic engineering and thus make consumers more comfortable and accepting of a specific product?

1. Provide some specific benefit to the consumer (Blaine *et al.* 2002; Brown and Ping 2003; Mucci *et al.* 2004; Miles *et al.* 2006). Benefits combining societal relevance and personal tangible benefits (e.g. can be consumed by diabetics combined with low calories for example) do best in compensating for risks and negative associations (Grunert *et al.* 2001). In addition, the benefit should be unique and not provided by other food products (Brown and

Ping 2003).

2. Increase convenience. The level of convenience had a strong effect on consumer disposition to biotechnology (Lockie *et al.* 2005).

3. Only transfer genes within kingdom. Transfers of a gene(s) within a kingdom (e.g. one plant species to another plant species) are more acceptable than transferring between kingdoms (an animal derived gene introduced into plants) (Burton *et al.* 2001; Lusk and Sullivan 2002; Nielsen 2003). Presumably, only altering the expression of apple genes would be even more acceptable.

4. Distance dimension has importance (Grunert *et al.* 2001), some examples are given below:

- GE component not present in the final product: product is derived from transformed fruit but does not contain DNA or introduced protein (e.g. oil or chemical extracts).

- GE component present in the final product: introduced DNA is present but introduced protein is only found in vegetative tissues but not in fruit. How this could be achieved is discussed in the following sections.

- GE component present and 'living' in the final product: definition was originally used to describe an example of live GE bacteria present in a yoghurt but in this case it can be thought of as both the introduced DNA and protein is present in the fruit.

How can GE apple lines be produced to make them more acceptable to consumers?

Limiting the transformed product to a particular tissue

It is important for any transformed product to have the correct spatial and temporal expression pattern for proper function and avoid any deleterious effects on plant properties/growth/health. This can be achieved by including signal tags to direct secretion to particular cellular compartments/spaces and using appropriate promoters to drive expression. Altering codon usage, expressing micro RNAs, and changing mRNA secondary structures (including the use of intron sequences) can increase transcript longevity or alter the rate of translation.

By limiting the transformed product to a particular tissue (e.g. a disease resistance gene to leaves), negative perception of GE apple fruit may be lessened when presented to a consumer, especially if the promoter sequence driving transgene expression is also derived from apple (or other another plant). This fits with the distance dimension concept discussed previously. It should be recognized that many promoters are leaky, and absence of the 'transprotein' in the fruit would have to be thoroughly assayed in each cultivar under various growing conditions.

Unfortunately, only a few plant-derived promoters have been characterised for their expression patterns in apple (Gittins *et al.* 2000, 2001; Malnoy *et al.* 2006). However, the ability to isolate apple promoters and genes will soon become much easier with the public release of apple genome sequence (R. Velasco *et al.*, unpublished results). It is already possible to select an apple orthologue of a gene of interest by mining publicly available apple expressed sequence tags (EST) (Newcomb *et al.* 2006).

Limiting the amount of integrated non-plant genetic material

The amount of integrated non-plant genetic material can be limited by producing transgenic plants without selectable marker genes (Malnoy *et al.* 2007a) and by using plant border sequences (P-DNA) that replace bacterial T-DNA sequences (Rommens *et al.* 2004). For research purposes, genes conferring antibiotic or herbicide resistance are typically included in the T-DNA (transfer DNA) transferred via *Agrobacterium* transformation to allow selection of transformed plants. However, for release of a GE cultivar, antibiotic resistance marker-free plants may be required. Interestingly, transformation without a selectable marker gave

12% frequency of transformation of ‘Galaxy’ and up to 25% for ‘M.26’ (Malnoy *et al.* 2007a). Thus some of the more complicated post-transformation selectable marker excision strategies (e.g. using inducible recombinases) may now be superfluous for producing transformed lines. However, identifying chimeras and vector backbone-free transformants will still be required.

In more difficult to transform cultivars where selection is still required, protocols utilising non-antibiotic based selection may be preferred. Two types of positive/non-antibiotic selection have been reported in literature on apple. The first used the *phosphomannose isomerase* gene with selection on media supplemented with mannose (Flachowsky *et al.* 2004; Zhu *et al.* 2004; Degenhardt *et al.* 2006). The second used the *Vr-ERE* selectable marker gene to detoxify benzaldehyde, which inhibits adventitious bud formation (Chevreau *et al.* 2007), although the authors reported that this selection system requires more development. Herbicide selection (discussed earlier) is another option.

Limiting transformed gene expression to rootstocks

One of the most promising strategies for apple may be limiting transformed expression to the rootstock, meaning that the scion and hence fruit are not GE. Orchard practice does not allow the rootstock to grow leaves, shoots and flowers, thus the potential for transgene spread is significantly reduced. Mechanisms of long distance RNA/protein/metabolite transport could allow ‘remote control’ of gene expression in the non-transformed scion (reviewed by Lough and Lucas 2006). Utilising this long distance transport system would be beneficial, because a suite of generic rootstocks could be produced that serve particular applications and environments. It would also reduce negative consumer perceptions about directly eating a GE product, and concerns about transgene spread. As noted above, for use of tissue specific promoters, the absence of the transprotein in the fruit of trees on transformed rootstocks would have to be thoroughly assayed in each cultivar/rootstock combination under various growing conditions.

Affecting single base pair mutations

It is possible to affect single base pair changes using chimerical RNA/DNA oligonucleotide-directed gene targeting (chimeric oligonucleotides). Success has been reported for maize, tobacco and rice (Beetham *et al.* 1999; Zhu *et al.* 1999, 2000; Kochevenko and Willmitzer 2003; Okuzaki and Toriyama 2004; Iida and Terada 2005); however, the use of this technique has not been reported in apple. This technique has potential for breeding by introducing a premature stop codon, or modulating gene expression by introducing a rare codon or changing mRNA secondary structure. Furthermore, it is reasonable to say that the resultant plants are non-transgenic, because such polymorphisms can occur without human intervention.

CONCLUSIONS

A great deal of progress has been made by researchers trying to confront important horticultural problems using genetic transformation. Unfortunately data on fruit quality and sensory attributes has been published for only a few of the GE apples discussed here. This is understandable because it takes time for the apple trees to reach maturity and so more data should eventuate as the lines start producing fruit. These studies will be vital in determining whether or not to release a novel cultivar.

Nevertheless, it is now feasible to engineer resistance to some of the most important bacterial and fungal diseases of apple and furthermore, important insect pests can also be controlled without the need to use environmentally unfriendly pesticides. Promising developments have been made in controlling excess vigour, self fertility, storage life

and increasing precocity. These advances translate into a reduction in chemical use, greater productivity and a smaller environmental footprint.

On the consumer side, there has been less progress, although advances in colour, reduced browning and novel health properties (including reduced allergenicity) offer interesting and desirable traits to the consumer. A non-browning apple may be the first GE apple to market. Analysis of consumer perception tells us that combining a tangible personal benefit with a trait conferring societal benefit (such as reducing pesticide use) has a better chance of acceptance (Grunert *et al.* 2001). It is time to put more emphasis on developing a GE product that will be acceptable and provide direct benefits to the consumer. As GE products with such consumer benefits become more widely available and accepted, there may be more room to release products which address production issues.

Aside from consumer perceptions, there are other challenges to the development and use of transgenic apples. The global apple market is extremely competitive and generally oversupplied. As a consequence, margins for producers are typically low. When the costs of research, development and regulatory approval are included, the cost of bringing a GE apple cultivar to market is high. This limits the number of applications (Alston 2004). Licensing all the intellectual property (IP) required to obtain freedom-to-operate is also a major barrier, as no institution has all the IP required for commercialisation of a transformed cultivar (Delmer 2004). To those that do have freedom to operate, the economic benefits of producing a GE apple are reduced by the crops small market size, diversity of cultivars and the small acreages grown (Graff *et al.* 2004).

Any GE apple crop will almost certainly require deployment of multiple genes. For cost effectiveness, the development of a GE cultivar may require the free exchange of materials and IP between researchers and companies. Examples of such sharing already exist, e.g. the ‘Open Source’ movement that exists in the computer software industry and PIPRA (www.pipra.org/) which develops and distributes subsistence crops for humanitarian purposes in the developing world and specialty crops in the developed world. Such initiatives bridge freedom-to-operate barriers to and may also help improve consumer perceptions of biotechnology (i.e. often seen as being the domain of big business).

In summary, the potential benefits of GE apples include apples grown with fewer inputs and that are more environmentally friendly, apples with additional health benefits and nutritive quality, and apples with novel flavours and characters for the consumer to enjoy. However, before these benefits can be achieved, public perceptions must change. We have discussed some of the changes that we as researchers can address, but it may also require development of new technologies and attitudes to exchange of information before these benefits can be realised.

ACKNOWLEDGEMENTS

The work of Sean Bulley and Ross Atkinson was funded by the Foundation for Research, Science and Technology, New Zealand. The work of Mickael Malnoy and Herb Aldwinckle was supported by New York apple growers through a grant from the New York Apple Research and Development Program, and by a special grant from USDA-CSREES. The authors would also like to thank Martin Heffer, Andrew Allan and Robert Schaffer for supplying images.

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