3rd New Zealand and German CELL WALL SYMPOSIUM

SCION Next generation biomaterials

New Zealand Journal of Forestry Science

39 (2009) 207-216

http://nzjfs.scionresearch.com

Actinidia eriantha: A Parental Species for Breeding Kiwifruit with Novel Peelability and Health Attributes†

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(Submitted for publication 11 November 2008; accepted in revised form 26 March 2009)

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Abstract

Consumers will pay a premium for fruit that have excellent flavour, high nutritional value, store well and are convenient to eat. In kiwifruit, the opportunity to breed fruit with these characteristics exists within the ~50 species that make up the *Actinidia* genus. *Actinidia eriantha* has been identified as having desirable convenience and health attributes that could be introgressed into commercial kiwifruit species by conventional breeding. *Actinidia eriantha* has an 'easy-to-peel' phenotype that increases the level of convenience associated with kiwifruit by making fruit easier to eat. In this review we describe analysis of biochemical and chemical differences in cell walls of easy-to-peel versus poor-peeling genotypes, and how these relate to their mechanical, structural and chemical features. We also discuss the health attributes of *A. eriantha*, including its content of vitamin C, oxalate, triterpenoids and allergens.

Keywords: *Actinidia eriantha;* cell wall; kiwifruit; peelable; vitamin C

† Based on a paper presented at the 3rd Joint New Zealand – German Symposium on Plant Cell Walls, 13–15 February 2008, Auckland, New Zealand

Introduction

Compared with other mainstream fruits, consumers have little choice when purchasing kiwifruit (*Actinidia* spp. Lindl.). Currently the international market is dominated by fruit obtained from two species *A. deliciosa* (A. Chev.) C.F. Liang et A.R. Ferguson (green-fleshed kiwifruit e.g. 'Hayward') and *A. chinensis* Planch. (gold-fleshed kiwifruit e.g. 'Hort16A' and 'Jintao'). Small quantities of *A. arguta* Sieb. et Zucc. (baby kiwifruit, e.g. 'HortGem Tahi') are available in some markets. Extensive genetic variation exists within the *Actinidia* genus for plant breeders to use as parental material to develop new kiwifruit cultivars for commercialisation. Key attributes of interest include those for flavour (high dry matter, low acid, and novel volatiles), colour (red or purple flesh or skin), health (high vitamin C, quinic acid, folic acid) and convenience (long storage, improved shelf life, edible or peelable skins). To a large extent, the convenience attributes are related to changes in the fruit cell wall.

Actinidia eriantha Benth. is a diploid (2n = 58) species that can be crossed with other members of the *Stellatae* section of the *Actinidia* genus including *A. deliciosa* (2n = 176) and *A. chinensis* (2n = 58 or 116). Like all other *Actinidia* species, *A. eriantha* vines are functionally dioecious. The vines are sometimes

ISSN 0048 - 0134

sold as ornamentals in China because they flower prolifically and produce eye-catching pink or red flowers with an attractive aroma (Wang et al., 1993). The vines have a short juvenile period and a low requirement for winter chilling (Wang et al., 2006). *Actinidia eriantha* vines produce the third largest fruit of the *Actinidia* genus. The fruit typically weigh around 25 g and are covered with fine, white hairs. The flavour is normally described as being bland and not highly palatable. Figure 1A shows fruit of a selection of *A. eriantha* genotypes, illustrating slight differences in colouring, shape and size between fruit.

New kiwifruit cultivars may be developed directly from within *A. eriantha* germplasm or by introgression of valuable characteristics through interspecific hybridisation with existing commercial species, followed by recurrent selection (Seal, 2003). Already one cultivar of *A. eriantha* has been released in China, and another cultivar 'Bidan' has been released in Korea (Jo et al., 2007). In this review we will discuss the potential for using *A. eriantha* to breed new kiwifruit cultivars that are peelable and have desirable health attributes.

Easy-to-Peel Characteristics of *A. eriantha*

Kiwifruit are frequently classed as inconvenient by consumers (Harker et al., 2007), and this barrier to consumption has excluded kiwifruit from substantial segments of the fruit-eating public. Fruit that fail to adapt to convenience drivers in the marketplace have an uncertain future (Jaeger, 2003). Consumers see kiwifruit as being inconvenient because the fruit needs to be 'prepared' e.g. peeled with a knife, or cut in half and the flesh scooped out with a teaspoon. In contrast, fruit with peelable skins such as banana or mandarin, or with edible skin such as apple or grapes, are perceived as more convenient and less messy to eat. Some consumers also prefer peelable fruit because removing the skin increases perceptions of fruit hygiene.

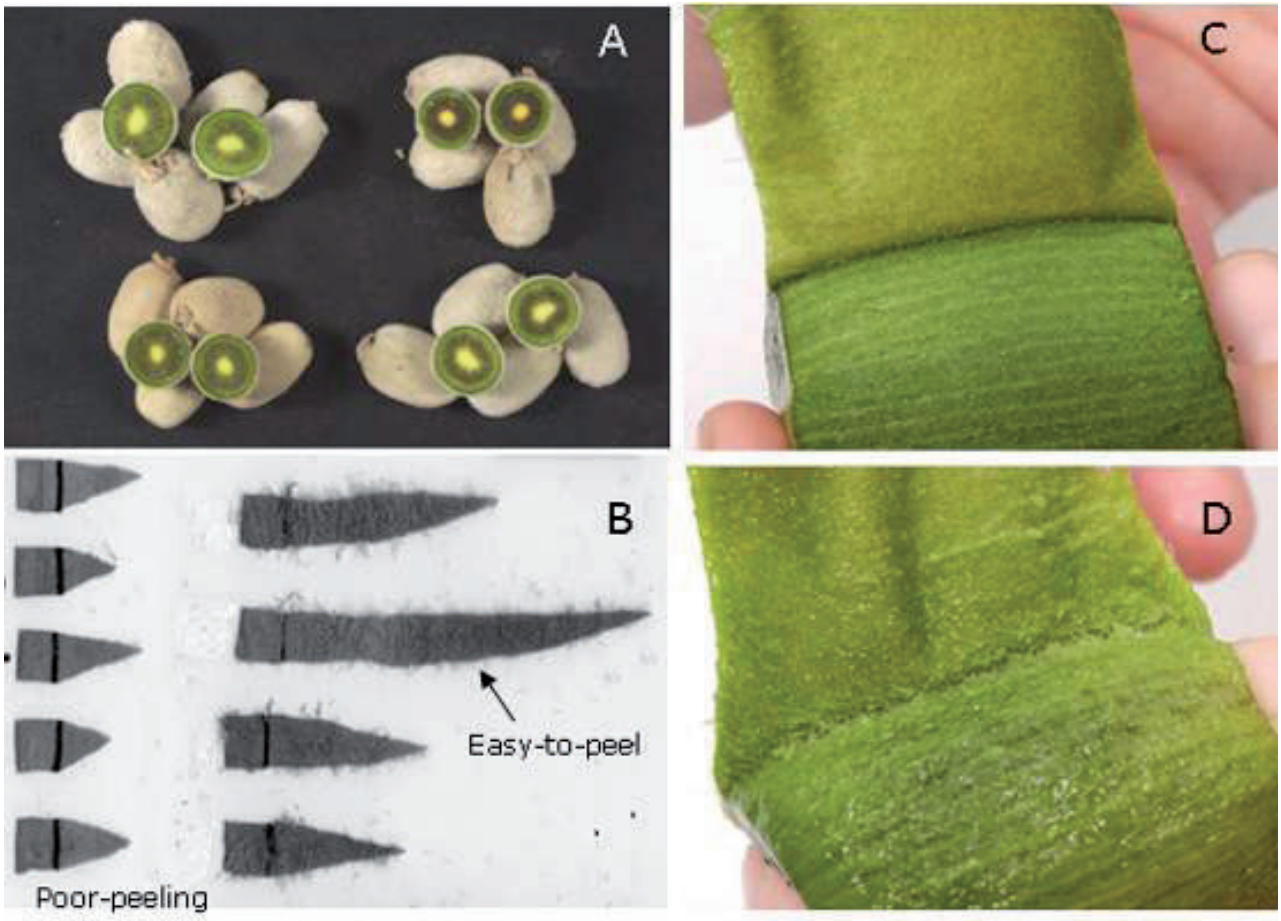

FIGURE 1: Characteristics of *Actinidia eriantha* fruit. (A) whole and cross-sectioned *A. eriantha* fruit from four different genotypes; (B) skin-tear lengths in *A. eriantha* genotypes. Short skin strips from poor-peeling genotypes are shown on the left and a long peel strip from an easy-to-peel genotype on the right. Image courtesy of A. White, Plant and Food; (C) detachment in an easy-to-peel genotype is characterised by a clean dry surface on both the skin and outer pericarp; and (D) detachment in a poor-peeling genotype is characterised by a moist surface and clumps attached to both skin and outer pericarp.

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Genetics and phenotyping

Within the *Actinidia* genus, only *A. eriantha* produces fruit that can be considered easy-to-peel. All accessions of *A. eriantha* in New Zealand produce fruit that show some degree of peelability, with different genotypes within the species showing variation in ease and cleanliness of the peeling action. Peelability only develops once the fruit reaches eating softness. Unripe *A. eriantha* fruit are not peelable. Fruit from *A. chinensis* and *A. deliciosa* are also considered non-peeling because the skin tends to come off with a significant amount of flesh and only small fragments of skin can be taken off at one time. These two commercial species do not produce easy-to-peel fruit. Interspecific hybrids of *A. deliciosa* x *A. eriantha* can show inheritance of the peelable phenotype found in the *A. eriantha* parent, although (not unexpectedly) fruit from these seedling progeny show considerable segregation for peeling characteristics. If seedlings produce peelable fruit, however, the peeling characteristics are usually reproducible between years. Many other fruit characters such as flavour, size and appearance tend to reflect the *A. deliciosa* parent – perhaps because of the greater genetic contribution of the *A. deliciosa* parent to the progeny (C. Bulley & A. Seal, Plant and Food, unpublished results).

In kiwifruit, peelability is described as the function of two processes – peel number and detachability. Peel number is the number of peeling actions required to remove the peel completely starting at the stylar end, whereas detachability is a measure of how cleanly the peel can be detached from the underlying flesh (A. Seal, Plant and Food, personal communication). In 'easy-to-peel' fruit, the peel number is low and the peel can be removed in long strips (Figure 1B). Detachment is clean, with the surfaces of both the peel and the fruit remaining dry (Figure 1C). In fruit with a 'poor-peeling' phenotype, peel number is high, initiation is difficult and depending on the genotype, sometimes only short strips of peel can be removed (Figure 1B). Detachment is messy, with clumps of outer pericarp flesh being torn away with the peel, leaving a moist surface on the fruit (Figure 1D). Currently, peel number and detachability are assessed by hand. However, simple, repeatable, and operator-independent tests will be required to phenotype large breeding populations for this trait. One such method under development involves assessing the length of an equatorial strip that can be removed before it tears off (A. White, Plant and Food, personal communication).

Structural characteristics of peelability

Peelability requires a zone of weakness to develop below the skin, to allow clean separation of the peel from the fruit flesh. In citrus and banana, abscission-like zones develop between these tissues to allow separation to occur. In mandarin, peel adhesion to the juice sac membrane loosens during development and this appears to be related to changes in the cellular structure of the peel tissue and pectic substances in the cell walls of this tissue during peel development and maturation (Kita et al., 2000). In *A. eriantha*, the development of peelability is not connected with major changes in the structure of the skin, hypodermis, or flesh immediately below the skin (outer pericarp),

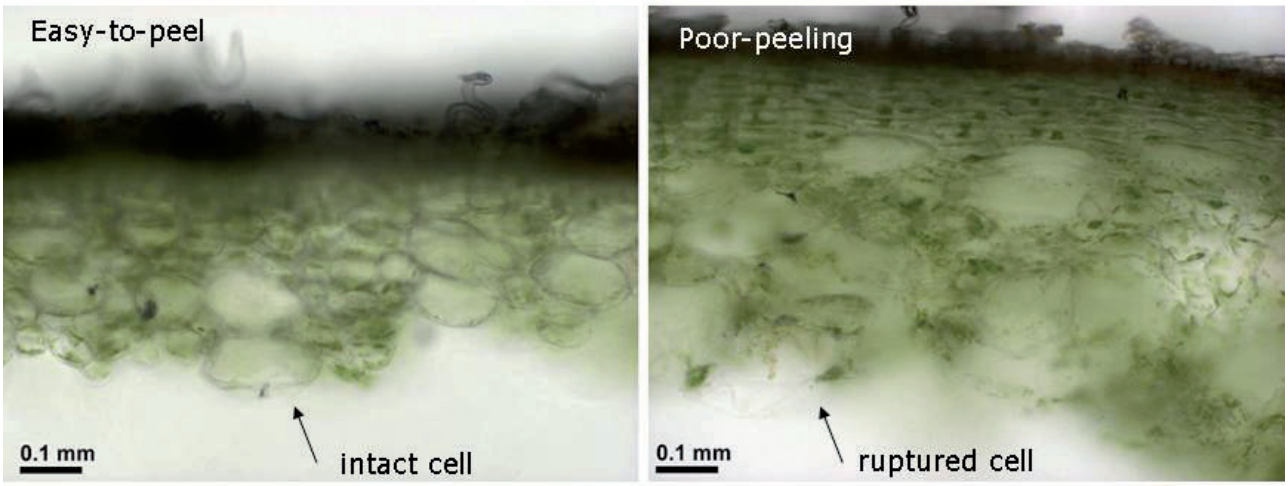

FIGURE 2: Cross-section of fresh peel from: (A) an easy-to-peel *Actinidia eriantha* genotype; and (B) a poor-peeling genotype at the peelable stage. Cells are still intact in the peel of the easy-to-peel genotype, whereas cells have ruptured in the poor-peeling genotype.

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suggesting that the easy-to-peel phenotype is not associated with development of a classic abscission zone or obvious tissue junction (Hallett & Sutherland, 2007). Instead, detachability appears to be related to differential changes in cell wall chemistry in the region where the fruit is peeling. This leads to cleanly separating cells in easy-to-peel fruit, whereas cells rupture in poor-peeling fruit. This is probably because the cell walls remain firmly adhered to each other, leading to damage of the flesh in the remaining fruit tissue (Figure 2).

Structural differences in the skin/outer pericarp region between easy-to-peel and poor-peeling genotypes are relatively small (Figure 3A). Often, easy-to-peel genotypes have a few layers of smaller cells just below the hypodermis, indicating a gradual transition between hypodermal cells to the outer pericarp, whereas in poor-peeling genotypes, the outer pericarp contains large cells directly below the hypodermis.

The different peeling behaviours observed between genotypes may result from changes in the distribution and types of pectin that are present in middle lamellae of the cell wall. Middle lamellae pectin is low-esterified, and, therefore, can easily create strong calcium gels, gluing the cells together. Using immuno-localisation with JIM5, an antibody that recognises specific patterns of esterification in low-esterified pectin, Hallett and Sutherland (2007) demonstrated that there were differences in pectin localisation between the non-peelable *A. deliciosa* 'Hayward' cultivar and a peelable *A. deliciosa* x *A. eriantha* hybrid. In non-peelable *A. deliciosa* 'Hayward', immuno-labelling with JIM5 showed an abundance of low-esterified pectin in the peel and the outer pericarp region, whereas in the peelable hybrid, immuno-staining was barely detectable, except very close to the skin (Figure 3B). This distribution of low-esterified pectin in the good-peeling phenotype would ensure that peel tissue holds together and separates cleanly from the outer pericarp during the peeling action. Together, these results suggest that one of the factors determining peelability in kiwifruit may be the strength with which the cell walls adhere together.

Hallett and Sutherland (2007) also reported a difference in immuno-labelling with LM5 antibodies that recognise epitopes associated with the large galactan side chains of pectin. As with JIM5 distribution, the non-peelable *A. deliciosa* 'Hayward' cultivar showed an abundance of LM5 immuno-staining throughout the peel and outer pericarp region, whereas in the peelable hybrid, immuno-staining could only be detected closer to the skin (Figure 3C). Current work suggests similar differences in immuno-staining patterns are found in easy-to-peel and poor-peeling *A. eriantha* genotypes (P. Sutherland & I. Hallett, unpublished results). Pectic galactan has previously been reported to be involved in determining fruit firmness (Brummell, 2006), and inhibition of galactose loss in transgenic tomato fruit lacking β-galactosidase activity resulted in firmer fruit than wild-type controls (Smith et al., 2002). These results suggest that firmness of the peel may be another factor determining peelability in kiwifruit, by preventing it from tearing during peeling.

Based on these structural data, the easy-to-peel phenotype might be explained if there was differential protein and gene expression between the peel and the outer pericarp region. These differential changes would be manifest in the chemical composition of the cell walls and lead to a zone of low cell adhesion between the outer pericarp and peel. Cell adhesion and cell wall strength would remain high in the peel, keeping it flexible and strong.

Differential changes in chemical composition of the cell wall

Peel and outer pericarp tissues were collected from an easy-to-peel genotype and a poor-peeling genotype of *A. eriantha* from three stages during the development of peelablity: (1) unripe fruit (firmness ~15 N) immediately before they became peelable; (2) fruit at eating firmness (~10 N) when they had just started to become peelable; and (3) soft fruit (~5 N) that were fully peelable. Fruit firmness was measured using an Instron, probe diameter 2 mm, after skin removal. Cell wall material was prepared after Jermyn and Isherwood (1956) and sugar composition determined (neutral sugars after Albersheim et al., 1967; uronic acids after Blumenkrantz & Asboe-Hansen, 1973).

The compositional analysis showed that differences between samples were small, not only between the two genotypes but surprisingly also between the peel and outer pericarp (Figure 4). Almost no change in sugar composition was observed during the development of peelability. This may be because, between the non-peelable stage and when the fruit are fully peelable, fruit firmness changes by only ~10 N. In this narrow softening window, even cell wall changes related to normal fruit softening are not marked.

Immuno-labelling with either JIM5 or LM5 suggested that there were differences in pectin or galactose content between a poor-peeling and an easy-to-peel genotype. However, this observation could not be verified by sugar composition analysis of their cell walls. The sensitivity of each method may explain the different results. With immuno-labelling, the distribution of polysaccharides in the walls of single cells can be assessed, whereas bulk tissue material is used for compositional analyses. Thus subtle differences between the walls of neighbouring cells or cell layers may not be picked up. Furthermore, in crude cell wall preparations, slight differences between wall components could be masked and would only be revealed by sequential extraction and compositional analyses in a more purified state.

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However, immuno-labelling is directed against epitopes, and may not pick up changes reflecting the loss of a polysaccharide but changes in the structure. Thus a degree of adhesion could be lost without losing the polysaccharide itself (see Sutherland et al., 2009).

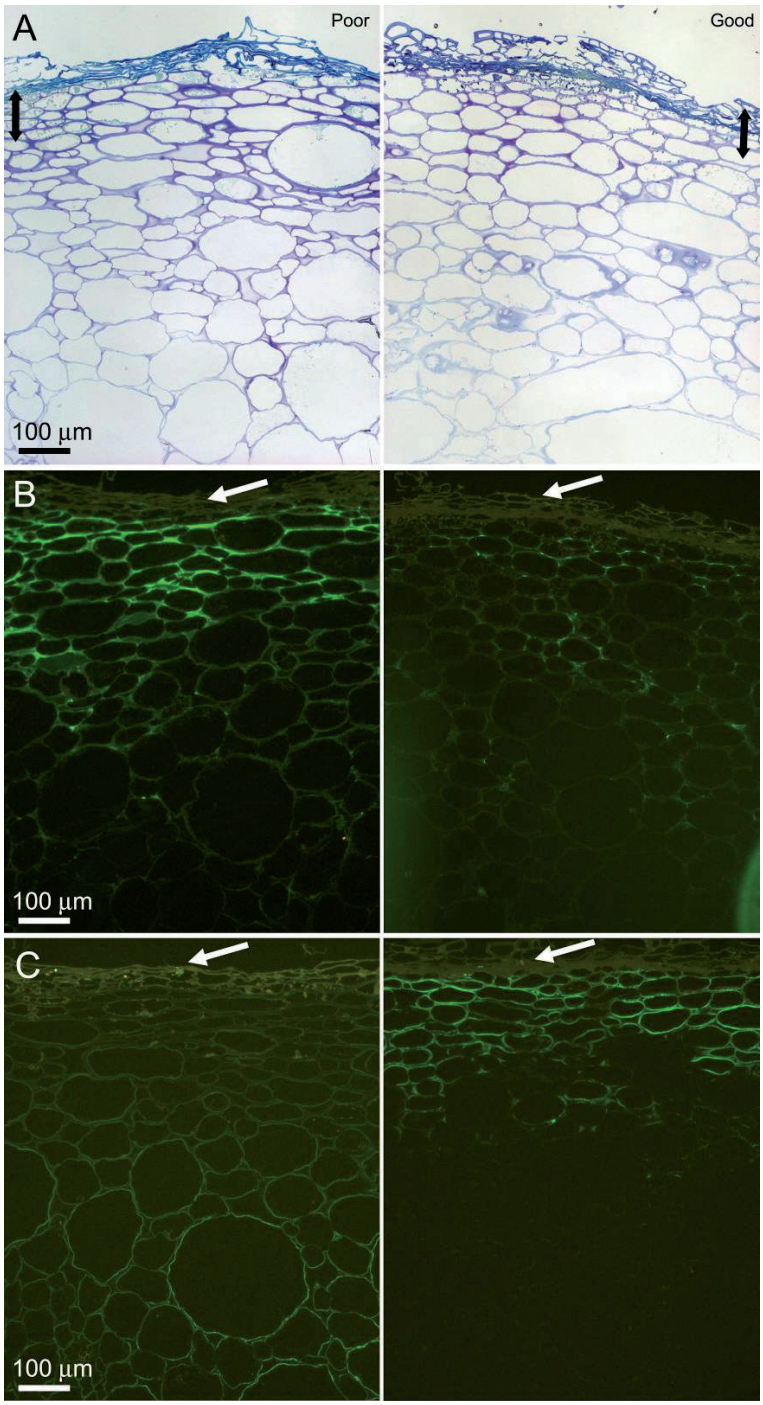

FIGURE 3: Comparison of cross-sections from the peel and outer pericarp of a poor-peeling *Actinidia eriantha* genotype (left) and an easy-to-peel genotype (right) at the peelable stage (firmness ~10 N): (A) stained with toluidine blue; (B) labelled with JIM5, an antibody recognising low-esterified homogalacturonan; and (C) labelled with LM5, an antibody recognising the 1,4-β-galactan side chains of pectin (Modified after Hallett & Sutherland, 2007). Single-headed arrows indicate skin surface. Double-headed arrows indicate extent of skin plus hypodermis.

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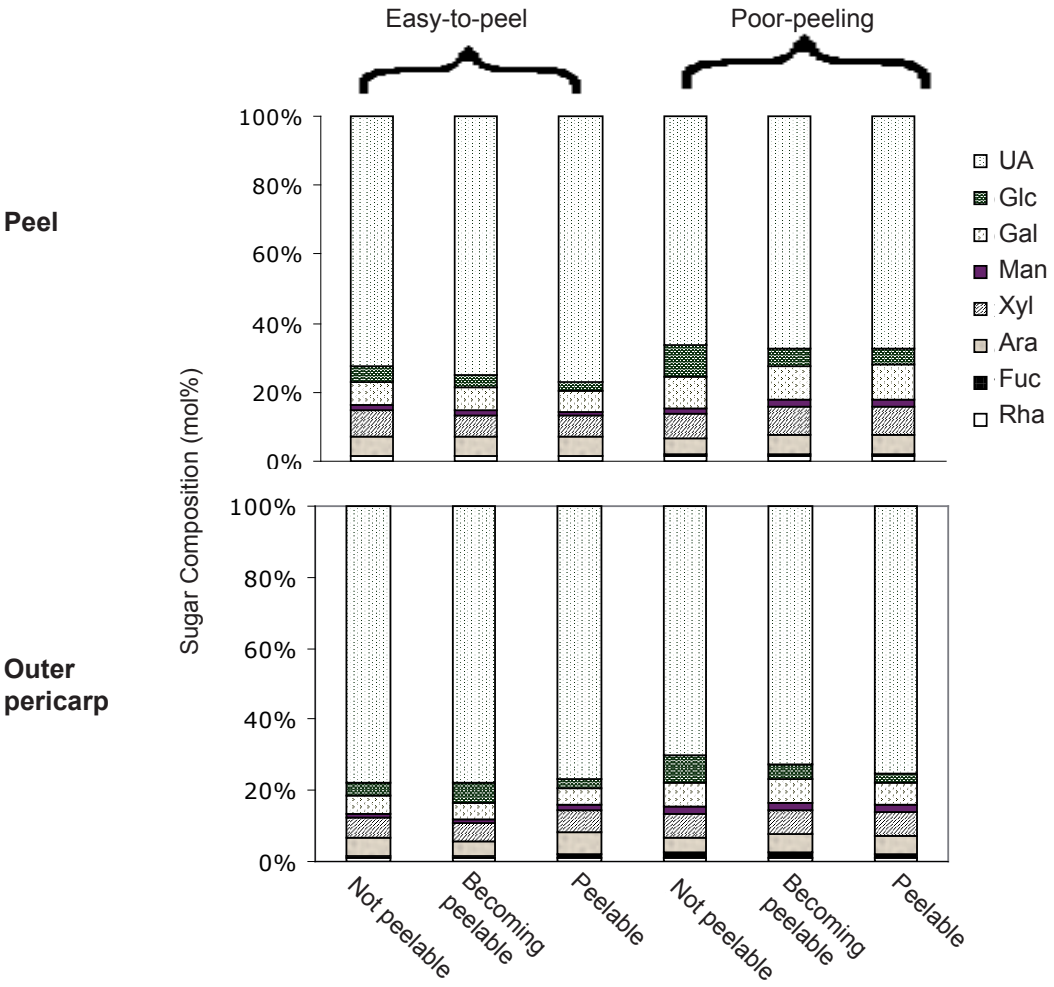

Figure 4: Sugar composition of cell wall material prepared from an easy-to-peel and a poor-peeling *Actinidia eriantha* genotype, using peel and outer pericarp tissue at three different ripening stages. Values are averages of two replicates (neutral sugars) and three replicates (uronic acids). Standard deviations were below 1 mol% for neutral sugars, and below 5 mol% for uronic acids.

Differential protein expression

To evaluate differences in protein expression during the development of peelability in *A. eriantha*, protein extracts were isolated, fluorescently-labelled and characterised by DIGE (difference gel electrophoresis). Protein samples were extracted from the peel of an easy-to-peel and a poor-peeling genotype at the same developmental stages used for sugar compositional analysis. Two-dimensional gel electrophoreses was used to separate proteins isolated from peel extracts by size (10-250 kDa) and by charge (pH 3-11) as described in Barraclough et al., (2004). The six individual protein extracts were compared sequentially by DIGE to a reference pool of fluorescently-labelled protein obtained by combining equal amounts of protein extracted from all six samples. Individual protein extracts and the reference pool were labelled using CyDyes following manufacturer's instructions (GE Healthcare).

The DIGE analysis revealed very few differences in protein expression as the fruit ripened and became peelable in either the easy-to-peel or poor-peeling genotype (Figure 5, A vs B comparisons). There were subtle differences in protein expression between the two genotypes (which are not isogenic) when the fruit were compared at similar ripening stages. However, the changes in protein amounts were minimal compared with the reference pool, and, in most cases, did not exceed a factor of two, either in increase or decrease. If spots differed significantly (by more than a factor of two), often protein amounts were insufficient to analyse for amino acid sequence.

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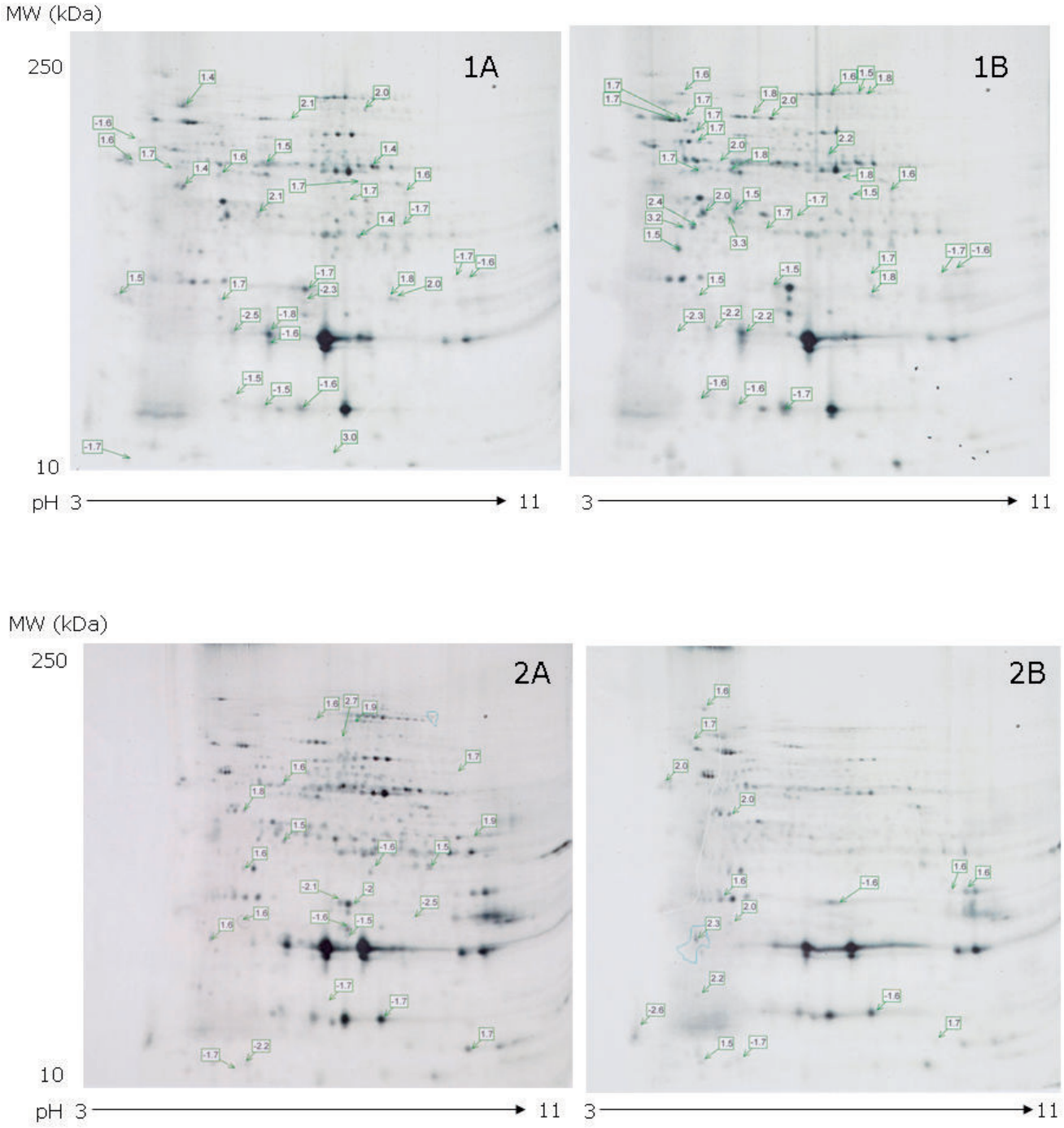

FIGURE 5: Differential protein expression in *Actinidia eriantha* peel samples. The comparison shown is for proteins extracted from the peel of the poor-peeling genotype (1A) when the fruit just became peelable and (1B) when the fruit were fully peelable, and from the easy-to-peel genotype (2A) when the fruit just became peelable and (2B) when the fruit were fully peelable. Boxed values are fold increases or decreases (-) in protein content of a spot compared with peel extracts from a reference pool (poor-peeling and easy-to-peel genotypes at the non-peelable stage, when fruit are becoming peelable and at the peelable stage).

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Differential gene expression

To help to identify the genes involved in development of the easy-to-peel phenotype a database of over 130,000 expressed sequence tag sequences (ESTs) obtained from a range of *Actinidia* species was utilised (Crowhurst et al., 2008). Of these sequences, 12,647 ESTs representing ~900 unique contigs were derived from *A. eriantha*. The sequences were obtained from libraries constructed from fruit tissues (11,259 ESTs) and flowers (1,388 ESTs). The fruit ESTs include sequences obtained from an easy-to-peel *A. eriantha* genotype from a mixture of softening stages before and after the appearance of the peeling phenotype. Initially, 1,000 sequences were obtained from the peel library, but these included a very high frequency (~40%) of just two sequences – an EST encoding the fruit allergen kiwellin and an EST encoding a protein of unknown function from *Ricinus communis* (castor bean). A second round of sequencing was performed after subtraction of these two sequences. The ten most abundant ESTs with high BLAST (Basic Local Alignment Search Tool) match scores ($E < 1.0 \times e^{-40}$) in the peel library and their putative functions are shown in Table 1.

The library was analysed for genes and enzymes involved in cell wall breakdown, cell wall synthesis and cell wall loosening as well as genes and enzymes that modify cell shape and structure, and genes involved in epidermal cell lignification and suberisation. Transcription factors that could potentially control multiple genes or pathways were also of particular interest. Overall, genes with these putative cell wall-related functions were typically not represented at high frequency in the *A. eriantha* peel library, although ESTs for xyloglucan endotransglucosylase/hydrolase (Atkinson et al., 2009), pectin methylesterase, β-galactosidase and α-expansin were observed. A selection of these genes are currently being characterised by quantitative PCR using RNA extracted from tissues corresponding to those used for protein and sugar compositional analysis.

A gene of particular interest encodes the enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase that is involved in the formation of the ripening hormone ethylene. The enzyme ACC oxidase was targeted because the easy-to-peel phenotype develops as the fruit ripen and is co-incident with ethylene production. Protocols for regeneration and *Agrobacterium*-mediated transformation of *A. eriantha* have recently been developed (Wang et al., 2006) that allow direct testing of genes for involvement in the easy-to-peel phenotype. Three transgenic lines strongly down-regulated for ACC oxidase gene and ethylene production have been obtained (R. Atkinson, unpublished results), but fruit production is required to test for changes to the easy-to-peel phenotype in these lines.

Health Attributes of *A. eriantha*

High vitamin C levels

Kiwifruit are recognised by consumers as being rich sources of vitamin C (ascorbate). Maintaining or increasing levels of vitamin C is an important criterion for selecting any new kiwifruit cultivar. Vitamin C levels vary widely within and between *Actinidia* species from 20 mg/100 g fresh weight to more than 1000 mg/100 g fresh weight (Ferguson & MacRae, 1992). Levels in *A. deliciosa* vary from 40-260 mg/100 g, in *A. chinensis*

TABLE 1: The ten most abundant expressed sequence tag sequences in the *Actinidia eriantha* skin library with high BLAST (Basic Local Alignment Search Tool) match scores ($E < 1.0 \times e^{-40}$) and their putative functions.

Rank	Nearest homologue	Description / Putative function	Frequency in library
1	P25096	Protein P21/thaumatin family, allergen	24
2	P50700	Osmotin-like protein OSM34/thaumatin family	17
3	P27054	Endochitinase PR4 precursor	12
4	Q9FHW7	SKP1-like protein 1B/ UFO-binding protein	9
5	Q64LH2	Profilin-2/ pollen allergen	5
6	P83326	Pectin methylesterase inhibitor	5
7	P63279	Ubiquitin-conjugating enzyme E2 I	5
8	Q9SQI3	Myristoyl-acyl carrier protein thioesterase	3
9	P00785	Actinidin precursor/cysteine protease, allergen	3
10	P93004	Aquaporin PIP2.7	3

Note: abundant ESTs with lower homology scores are not included in the table

 ISSN 0048 - 0134

from 51-211 mg/100 g, whilst some genotypes of *A. eriantha* are reported to contain exceptionally high levels of vitamin C with over 800 mg/100 g.

This wide range of vitamin C levels in kiwifruit has made it a good system to investigate the genetic basis for vitamin C production in plants. Laing et al. (2007) used kiwifruit to identify the last remaining enzyme step in the L-galactose pathway of ascorbate biosynthesis. They showed transient over-expression of the *A. chinensis* GDP-L-galactose transferase gene in tobacco resulted in a 3-fold increase in ascorbate, thus demonstrating that the gene was likely to be rate-limiting for ascorbate production. In *A. eriantha*, it has recently been shown that the very high levels of vitamin C in the fruit can be correlated with high expression levels of GDP-mannose-3',5'-epimerase and GDP-L-galactose transferase (Bulley et al., 2009).

Other health attributes

Triterpenoids are a group of structurally diverse natural compounds with a wide range of medicinal properties. Triterpenes have been reported from a number of kiwifruit species, most notably from *A. polygama*, where a compound with analgesic activity was isolated (Sashida et al., 1994). In *A. eriantha* roots, six different triterpenes have been isolated (Huang et al., 1988), including ursolic acid and two compounds unique to *A. eriantha* (eriantic acid A, eriantic acid B). Ursolic acid is a pentacyclic triterpenoid of particular interest for its anti-inflammatory, anti-tumour and anti-microbial properties (Liu, 2005).

Actinidia eriantha contains compounds that may have anti-nutritional effects e.g. allergens and oxalate. Allergy to kiwifruit has been recognised for over 25 years. In adults, the symptoms of kiwifruit allergy are typically mild, causing localised oral allergy syndrome. However in children, the condition can be occasionally more serious. An apparent increase in kiwifruit allergy has led to considerable activity in recent years to identify the allergens responsible (Lucas & Atkinson, 2008). Although allergy to *A. eriantha* has not specifically been reported, three common allergens (and ESTs encoding them) are present in the fruit: the cysteine protease actinidin (Liang et al., 1999; Table 1), thaumatin and kiwellin (R. Maddumage, Plant and Food, personal communication; Table 1).

Oxalate consumption can reduce trace element adsorption and can lead to the formation of kidney stones. Insoluble calcium oxalate raphide crystals are believed to be responsible for the unpleasant 'catch' sensation associated with eating kiwifruit (Walker & Prescott, 2003). *Actinidia eriantha* has extremely high levels of oxalate in its leaves, although in the fruit oxalate levels are similar to those found in *A. deliciosa* and *A. chinensis*. Although oxalate is thought to be a degradation product of ascorbate, there appears to be no correlation between oxalate and ascorbate levels in fruit (Rassam et al., 2007). This suggests that it should be possible to breed fruit with low oxalate and high vitamin C using *A. eriantha* as a parent.

Conclusions

Our understanding of what controls the easy-to-peel phenotype and high vitamin C trait in *A. eriantha* has been significantly increased by investigation of this species at the chemical, biochemical and molecular levels. This knowledge increases the potential for *A. eriantha* to be used as parental material to develop new kiwifruit cultivars with these beneficial traits.

Acknowledgements

We are very grateful to Alan Seal, Roger Harker and Anne White for allowing us to discuss some of their preliminary data on the peelability phenotype in kiwifruit. We would like to thank Roger Harker, William Laing and Alan Seal for critically reviewing the manuscript. This work was funded by New Zealand Foundation for Research, Science and Technology (C06X0202 and C06X0403).

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 ISSN 0048 - 0134