

Improving Fruit Quality in *Rubus* and *Ribes* through Breeding

Rex Brennan* • Julie Graham

Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK

Corresponding author: * rex.brennan@scri.ac.uk

ABSTRACT

The breeding of new berry fruit cultivars in the UK has in recent years focused more on fruit quality traits than agronomic characters, for both fresh and processing markets. This trend is aligned with increasing consumer awareness of cultivar differentiation in key traits and the perceived health benefits of berry fruit consumption, mainly linked to antioxidant activity of the fruit. The development of markers linked to QTLs for fruit quality traits and the identification of the genes involved has been progressed in berry fruits such as *Rubus* and *Ribes* for a number of nutritionally-relevant components and also for sensory characters. Using the phenotypic diversity across the range of germplasm available to breeders, combined with the increasing knowledge of the underlying genetics and regulation of these traits, new breeding strategies are now under development to enable a more rapid production of improved cultivars.

Keywords: anthocyanins, antioxidants, ascorbic acid, blackcurrant, raspberry

Abbreviations: **AsA**, ascorbic acid; **BAC**, bacterial artificial chromosome; **bHLH**, basic-helix-loop-helix; **CHS**, chalcone synthase; **CHI**, chalcone isomerase; **FUL**, *fruitful*; **GA**, gibberellic acid; **GLA**, γ -linolenic acid; **IAA**, indole-3-acetic acid; **LG**, linkage group; **PKS**, polyketide synthase; **QTL**, quantitative trait locus; **REML**, residual maximum likelihood; **SHP**, *shatterproof*; **SNP**, single nucleotide polymorphism; **SSR**, simple sequence repeat

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INTRODUCTION

Berry fruit cultivation in the UK has relied for many years on new cultivars offering improvements in yield, cropping season and resistance to damaging pests and diseases. While these characters remain of key importance to the success of newly-released cultivars, there has been a growing demand from growers, processors and consumers for improvements in fruit quality attributes, to the point where these traits are now equally important for cultivars and indeed may even affect decisions regarding commercial release (Graham and Jennings 2009).

Fruit quality covers a range of traits, including physical characters such as berry size, berry colour, berry conformation (drupelet structure and cohesion), firmness and shelf-life in the case of fresh fruit, and characters linked to chemical composition, such as colour, sweetness, sourness and flavour intensity and the levels of nutritionally important compounds.

After strawberry, the main berry fruits that are cultivated in the UK are raspberry and blackberry (*Rubus* spp.)

and blackcurrant (*Ribes nigrum* L.). These have differing requirements in terms of quality attributes for commercial production, in part due to their different end-uses, since raspberry and blackberry are predominantly grown for the fresh market in the UK, while blackcurrant is mainly grown for processing. However, this market differentiation is changing, mainly as a result of increasing interest in berry fruits from a human health standpoint (Hancock *et al.* 2007a).

Consumer demand for high quality fruit now extends across a wide season, which can partly be addressed by the use of environmentally adaptable cultivars that can be manipulated with regard to cropping season. However, the changing climate is already a major consideration for soft fruit growing in northern Europe, with a succession of mild winters leading to poor bud break in certain cultivars of some species, notably blackcurrant, leading to a reduction in harvested quality (Jones and Brennan 2009). Additionally, environmental factors, notably light intensity, spectral quality and abiotic stress, are known to affect the regulation of flavonoid biosynthesis (Jenkins 2008).

The potential health benefits of berry fruit consumption is now the subject of considerable public investment by various bodies including governments, following the success of the intervention programme in North Karelia, Finland (Puska *et al.* 1990). This initiative encouraged increased dietary intake of berries which then led to a reduction in the incidence of cardiovascular disease of around 60%. Berries, especially those with darker colours, are now sought by consumers globally due to the high antioxidant content of berries (Clifford 2000; Wu *et al.* 2004) and the reported benefits on human health (Matsumoto *et al.* 2006; Stewart *et al.* 2007). *Rubus* and *Ribes* fruits are known to contain very high levels of antioxidant compounds (Mazza and Miniati 1993), increasing their desirability to consumers. Despite ongoing issues regarding the *ex vivo* nature of many of the trials and resultant bioavailability of antioxidant compounds such as anthocyanins (Aura *et al.* 2005; McDougall *et al.* 2005a), large yearly increases in sales have been reported for both established fruits such as blackcurrant and also berries that have only in recent years been grown in the UK in significant quantities, e.g. highbush blueberry, whose sales doubled in the two years to 2007 (Doward 2007). However, for plant breeders, it is essential that enhanced levels of anthocyanins and other desirable components are coupled with further quality attributes (e.g. sensory factors) and agronomic traits (yield, cropping season and pathogen resistance) to produce new cultivars of berry fruit with a wider commercial and consumer acceptability.

RASPBERRY (*Rubus*)

The primary objectives in modern raspberry breeding include high quality fruit, good yield, shelf life and suitability for shipping for the fresh market and for mechanical harvesting for processing. Although many other characteristics such as agronomic performance remain important in the successful acceptance of new cultivars, fruit quality must be considered the premier factor.

Flavour, appearance and shelf life are the main attributes of fresh market quality and are essential for repeat purchase of fruit by consumers. Flavour can be broken down into multiple measurable descriptors for taste, texture and other sensory characteristics. Good acceptable flavour in raspberry tends to be fruity, sweet and floral with some acidity but without bitterness (Harrison *et al.* 1999).

Physical traits are highly relevant to fruit quality breeding, and colour, brightness, size and shape are crucial to attract the initial purchase of fruit by consumers. The colour of fruit, as well as affecting appearance, desirability and associated freshness, is also important for flavour perception. Many attempts have been made to evaluate the role of colour cues (for review see Clydesdale 1993; Delwiche 2004) which have been shown to dramatically affect perceptions in a variety of different foods and drinks (DuBose *et al.* 1980; Roth *et al.* 1988; Garber *et al.* 2000; Zellner and Durlach 2003; Zampini *et al.* 2007). The addition of red colouring to strawberry-flavoured sucrose solutions, for example, was found to increase perceived sweetness (Johnson *et al.* 1983), and colours that are typically associated with fruit ripening may be particularly effective at modulating sweetness perceptions (Maga 1974). Colour in raspberry is a complex trait with anthocyanin content thought to be a major contributing factor, but flavonols, enzymatic reactions, pH, vitamin C and organic acid content (Strack and Wray 1994; Melo *et al.* 2000) may also be important. Apart from colour, large fruit size is attractive to both consumers and also to producers, for whom picking becomes more cost-effective.

The antioxidant activity of raspberry fruits is attributable mainly to anthocyanins (particularly cyanidin and pelargonidin derivatives), proanthocyanidins and ellagitannins. It was reported by Beekwilder *et al.* (2005) that the relative levels of these compounds vary according to degree of ripeness, and also that significant genetic differences exist between raspberry cultivars in the content of individual

antioxidant compounds.

Quality across a wide cropping season

In recent years, consumer demand for high quality fresh raspberries beyond the main summer production season has increased, with high premiums paid for raspberries outwith the traditional cropping peak. Protected cultivation techniques have been developed to improve fruit quality but also to extend the season of availability, both earlier and later.

The ability to understand and manipulate the ripening period of raspberry crops would allow targeted scheduling of production across a wide season. With marker-assisted selection and allele mining across a range of germplasm, a reduction in the timescale to develop suitable high quality varieties becomes feasible. However, understanding the key genetic control points across the ripening period is a major challenge in raspberry (Graham *et al.* 2009).

Work on the genetic basis of fruit formation and development has been carried out in *Arabidopsis* (Pinyopich *et al.* 2003), whereas investigation of ripening and shelf life have been carried out on tomato (Giovannoni 2004), with ripening mutants playing a key role in understanding the ripening control (Giovannoni 2007). The MADS box family of transcription factors play a key role in flower and fruit development (Seymour *et al.* 2008). Currently the link between transcription factors modulating ripening and hormonal regulation remains unclear. Suppression of *IAA9* in tomato results in parthenocarpic fruit (Wang *et al.* 2005; Molesini *et al.* 2009). Transgenic raspberry expressing the *DefH9-iaaM* gene shows an increase in plant fecundity and fruit production (Mezzetti *et al.* 2004). Tomato fruit development can also be uncoupled from fertilisation by silencing repressors of GA signalling, the DELLA proteins (Marti *et al.* 2007). Epigenetic regulation of gene expression has also been recognised as a fundamental mechanism for regulation of development (Manning *et al.* 2006; Henderson and Jacobsen 2007). Gene regulation through microRNAs has also been recognised (Jones-Rhoades *et al.* 2006; Pilcher *et al.* 2007). Other classes of small RNAs play a role in targeted gene regulation and epigenetic silencing (Zilberman and Henikoff 2005). Recent studies in strawberry have shown that a gene encoding a polygalacturonase, transcribed in both soft and firm strawberry cultivars, is alternatively spliced across the different types (Villarreal *et al.* 2008).

In raspberry, dormancy induction and release are genetically and environmentally controlled (Lang 1987; Darnell *et al.* 2003; Olsen 2003; Serce and Hancock 2005; Mazzitelli *et al.* 2007). Floral initiation is largely dependent on the developmental stage of the plant with photoreceptors and transcription factors acting on the various environmental cues (Simpson and Dean 2002) and flower development can be divided into four steps that occur in a temporal sequence (for reviews see Jack 2004; Tan and Swain 2006). Fruit development results in an increase in size through both cell division and expansion accompanied by changes in texture, colour and flavour. Squamosa promoter binding protein genes and their putative MADS-box promoter appear to regulate fruit tissue development (Unte *et al.* 2003; Manning *et al.* 2006). Phenolic compounds signal when a fruit is ripe and ready for consumption. They are central components in phenylpropanoid metabolism that contribute to fruit pigmentation but also the formation of the building blocks for lignin polymers (Tani *et al.* 2007) and certain volatile components such as 4-(4-hydroxyphenyl)-butan-2-one or raspberry ketone. Expression studies of *FUL* and *SHP* MADS box transcription factors suggest temporal regulation may be involved in modulating the properties of lignified endocarp (Tani *et al.* 2007). Major raspberry volatiles have been studied to determine the effect of season and production environment, and the relationship with flavour is being explored (Kassim *et al.* 2008).

Phytochemicals

Raspberries were first used in Europe for medicinal purposes (Jennings 1988), and there is now heightened interest in these fruits as a major source of antioxidants, such as anthocyanins, catechins, ellagitannins, flavonols, flavones and ascorbic acid (AsA) - compounds that have been reported to protect against a wide variety of human diseases, particularly cardiovascular disease and some cancers (Deighton *et al.* 2000; Moyer *et al.* 2002). There has been an explosion of data on antioxidant components of berry fruit and their potential impact on human health (e.g. Marinova and Ribarova 2007; Rupasinghe and Clegg 2007).

In particular, regular consumption of raspberry anthocyanins is also reported to improve cognitive brain functions, age-related degeneration of eye-sight and influence cardiovascular disease (Stone *et al.* 2007), and as a result the consumption of these berries is expected to increase substantially in the near future as their perceived value in the daily diet is supported by further biomedical studies and recognised by the public.

Raspberry ellagitannins were found by Ross *et al.* (2007) to have very high antioxidant activity which had an antiproliferative effect on human cervical carcinoma (HeLa) cells *in vitro*. Ellagic acid, from which ellagitannins are synthesised, has been reported to also have protective effects against cancers of the colon (Rao *et al.* 1991), lung and oesophagus (Stoner and Morse 1997). A proposed mechanism for the action of ellagic acid released from dietary ellagitannins is that it induces apoptosis through the intrinsic mitochondrial pathway (Larrosa *et al.* 2005).

Phenolic compounds

Ellagitannins are the predominant phenolic components in raspberries, comprising between 30-50% of the total phenolic content (Kähkönen *et al.* 2001, Beekwilder *et al.* 2005), with compounds such as sanguin H6 (Mullen *et al.* 2003)

and lambertianin C particularly important. The genetic control of the biosynthetic pathway leading to ellagitannins is complex and not well-characterised, at least in raspberry. Most of the ellagic acid in plants is present in the form of ellagitannins (Atkinson *et al.* 2006), and biosynthesis occurs via gallic acid. Conjugation with carbohydrates then forms gallotannins, and the coupling of galloyl units then produces ellagitannins (Niemetz and Gross 2003). Environmental and cultivation effects on ellagic acid concentration have been reported in strawberry by Atkinson *et al.* (2006), although similar effects were not detected by Kähkönen *et al.* (2001) in raspberry grown in Finland.

Anthocyanins are synthesized from precursors from two biosynthetic pathways: the shikimate pathway, producing phenylalanine, and a second pathway generating malonyl-CoA. These two precursors are linked by chalcone synthase (CHS) via a polyketide folding mechanism, to form an intermediate chalcone, a substrate for chalcone isomerase (CHI) generating naringenin, subsequently oxidized by a series of enzymes such as flavanone 3-hydroxylase, flavonoid 3'-hydroxylase and flavonoid 3',5'-hydroxylase, then reduced by dihydroflavonol-4-reductase to leucoanthocyanidins, and finally converted to anthocyanidins by leucoanthocyanidin dioxygenase. Unstable anthocyanidins are coupled to a small range of sugars by activities such as UDP-glucose:flavonoid 3-O-glucosyltransferase and O-methyltransferase to yield relatively stable anthocyanins (Sullivan 1998). Cyanidins, with B ring 3', 4' dihydroxy substituents and conjugation between A and B rings, have antioxidant potentials four times that of a vitamin E analogue (Evans and Miller 1995). An outline of the anthocyanin biosynthetic pathway is shown in Fig. 1.

Red raspberries typically contain 23-59 mg total anthocyanin 100 g fw⁻¹ fruit (Torre and Barritt 1977), with cyanidins the most abundant followed by pelargonidins (Jennings and Carmichael 1980). Whilst raspberry anthocyanin levels are low in comparison fruits such as blackcurrant or blackberry, if the genes underlying production can be deter-

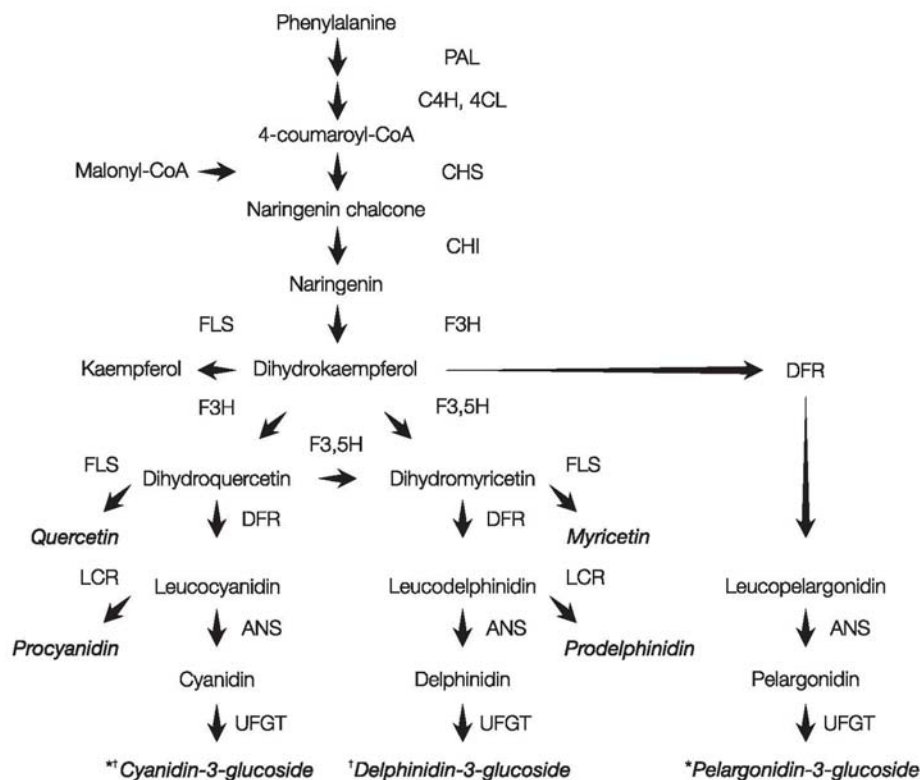


Fig. 1 The anthocyanin biosynthetic pathway, adapted from Jaakola *et al.* (2002) Zufall and Rausher (2004), leading to the formation of cyanidin⁺, delphinidin⁺ and pelargonidin^{*} glucosides. (* - found in raspberry; + found in blackcurrant). Enzyme abbreviations: ANS, anthocyanidin synthase; C4H, cinnamate 4-hydroxylase; CHI, chalcone isomerase; CHS, chalcone synthase; 4CL, 4-coumaroyl:CoA ligase; DNS, dihydroflavonol 4-reductase; F3H, flavonoid 3'-hydroxylase; F3,5H, flavonoid 3',5'-hydroxylase; LCR, leucoanthocyanidin reductase; MT, methyltransferase; PAL, phenylalanine ammonia-lyase; UFGT, UDP-glucose:flavonoid 3-O-glucosyltransferase.

mined, then marker-assisted breeding strategies could be deployed to enhance anthocyanin and by extension antioxidant content. McGhie *et al.* (2002) stated that considerable potential exists of breeding of new *Rubus* cultivars with enhanced content of specific anthocyanins, through a study of the composition of 300 *Rubus* selections including red, black and purple raspberries.

Anthocyanin biosynthesis is influenced by factors including light exposure (Lister and Lancaster 1996), temperature (Mori *et al.* 2005; Pasqua *et al.* 2005), phytohormones and sugar (Pasqua *et al.* 2005) and concentration of certain ions (Naumann and Horst 2003). Although anthocyanin production in raspberry is genetically regulated (Stewart *et al.* 2007), significant seasonal effects were observed (Kassim *et al.* 2009). Variation was also observed in a given season between field and different tunnel environments (Kassim *et al.* 2009). Light dosage (UV-factor) may explain most differences in anthocyanin contents observed between different seasons and the slight variation found between field grown and those under protected cultivation. Two major cyanidins, cyanidin-3-glucoside and cyanidin-3-rutinoside, were significantly more abundant in the season with more daylight hours (Kassim *et al.* 2009). Cyanidins are effective in absorbing UV (Luthria *et al.* 2006) and there is a report of a rapid stimulation in anthocyanin biosynthesis in potato subjected to a higher dosage of light (Lu and Yang 2006).

Most enzymes involved in anthocyanin biosynthesis and their encoding genes have been characterized in plants including maize, *Arabidopsis*, petunia and snapdragon; also, many regulatory genes directly controlling transcription of structural genes have been identified (Koes *et al.* 2005). In apple, genes involved in anthocyanin synthesis are co-ordinately induced during fruit development (Lister and Lancaster 1996), suggesting these genes are regulated by one or a small number of regulatory proteins. In apple, efficient anthocyanin production depends upon co-expression of both MYB and two distinct basic-helix-loop-helix (bHLH) transcription factors (Espley *et al.* 2007). In raspberry, key genes in polyketide synthase (PKS) class 3 have been allocated enzymatic activities; PKS genes 1 and 5 as chalcone synthases; PKS 4 as benzyl acetone synthase, which is important for synthesis of raspberry ketone (Zheng *et al.* 2001, 2008). The PKS 1 gene sequence has been mapped to raspberry chromosome 7 but does not underlie the anthocyanin QTLs identified (Kassim *et al.* 2009), which suggests transcription factors are more important than the structural genes in regulating anthocyanin production.

QTL mapping

Progress in any breeding programme is based on the amount of genetic variability available and the effectiveness of the selection and evaluation of the trait in question. A more accurate way of selection is to screen at the genetic level for markers linked to the underlying genes or QTLs (for reviews see Graham *et al.* 2007, 2008). A prerequisite for genotypic selection is the establishment of associations between traits of interest and genetic markers and requires markers and linkage maps to allow this to proceed effectively. A reference mapping population has been developed in raspberry and replicated within and across different sites (Graham *et al.* 2004). This population is being utilised to map QTLs for various quality traits. Increasingly EST resources have been used to develop functional gene-based markers (Woodhead *et al.* 2008), through a more knowledge-based approach linking genes to markers.

QTLs that describe the overall fruit ripening process have been located using summary statistics derived from scoring the stages of ripening in both field and protected growing environments across different seasons (Graham *et al.* 2009). Interestingly Gene *H* on chromosome 2 (Graham *et al.* 2006) is known to determine cane pubescence but is also associated with a slowing down of ripening across all stages from open flowers to the green/red fruit. Hairs and

spines are both outgrowths of epidermal cells and their early development is inter-related (Peitersen 1921). It would therefore seem likely that Gene *H* acts early in development and has been postulated to delay cell maturity. Chromosome 3 had a strong effect on ripening with a broad range of significant markers identified suggesting that more than one QTL is involved. A number of ESTs and candidate genes fall within the region of the mapped QTLs on chromosome 3; ESTs of significance including ERubLR_SQ07_4_D05 (which is similar to a cDNA of unknown function from apple leaf), ERubLR_SQ07_1_E10 (similar to a petal EST from *Rosa*) and ERubLR_SQ01_P18 (similar to *PpAz89* a pathogenesis-related EST from *Prunus* which is up-regulated during flowering, fruit ripening and senescence in flowers, fruit and leaves (Ruperti *et al.* 2002).

Also associated with the QTL for fruit set and ripening are the *RiMYB* gene and a BAC clone, Bac9022 SSR01 (Graham *et al.* 2009). The latter contains a MYB gene which is similar to MdMYB11 from *Malus x domestica* (Accession Number AAZ20431). R2R3 MYB genes are known to control phenylpropanoid metabolism including anthocyanin accumulation in fruit (Espley *et al.* 2007; Allan *et al.* 2008) but also have important roles in many other aspects of plant development, including trichome development, signal transduction, disease resistance and cell division (reviewed by Jin and Martin 1999). Recent work in apple has demonstrated that efficient induction of anthocyanin production during ripening depends upon the co-expression of both MdMYB10 and two bHLH transcription factors (MdbHLH3 and MdbHLH33; Espley *et al.* 2007). A bHLH gene similar to the bHLH33 from apple has recently been mapped to chromosome 1 in raspberry and underlies a QTL for 8 anthocyanin pigments (cyanidin-3-sophoroside, cyanidin-3-glucoside, cyanidin-3-glucosylrutinoside, cyanidin-3-rutinoside, pelargonidin-3-sophoroside, pelargonidin-3-glucoside, pelargonidin-3-glucosylrutinoside and pelargonidin-3-rutinoside) (Kassim *et al.* 2008). Seven of the eight antioxidants (not cyanidin-3-glucosylrutinoside) also mapped to a region on LG 4 across different seasons and growing sites (Kassim *et al.* 2008). Other candidate genes including NAM/CUC2 like protein and bZIP transcription factor underlying the mapped anthocyanins were identified (Kassim *et al.* 2009). Colour is being mapped and the relationship with anthocyanins and other components determined (McCallum *et al.* 2008). To date a number of candidate genes and transcription factors have been identified.

A MADS box gene, *RiMADS_01* on chromosome 5, significantly impacts on ripening, especially the early stages of fruit development (Graham *et al.* 2009). The raspberry *RiMADS_01* gene had the greatest homology with *dam6* from *Prunus persica*, one of six MADS box genes located in a cluster in a 132 kb region and thought to be responsible for regulating growth cessation and terminal bud formation in peach in response to dormancy-inducing conditions (Bielenberg *et al.* 2008). The gene was also similar to short vegetative phase MADS box genes which act to negatively control the expression of the *FLOWERING LOCUS T (FT)* modulating the timing of the developmental transition to flowering phase in response to temperature (Lee *et al.* 2007). This has implications for modulating flowering time in response to changing environments.

Sensory and compositional analysis is underway on progeny from the raspberry reference mapping population (Zait *et al.* 2008). Preliminary QTLs have been identified for sensory and compositional data and work is in progress to confirm the map locations and the identity of candidate genes.

BLACKCURRANT (*Ribes*)

Blackcurrant cultivation in the majority of European countries including the UK is largely geared towards juice production, and as a consequence cultivar requirements and breeding objectives are closely aligned with the needs of the juice processing industry. In the UK processors have a

strong preference for cultivars with fruit high in AsA but low overall acidity (Brennan and Gordon 2002). There is also increasing and specific demand for cultivars with high levels of polyphenols, particularly anthocyanins, due to their high antioxidant activity.

Vitamin C

Ascorbic acid (vitamin C) (AsA) is one of the main nutritional components found in *Ribes*, and blackcurrants are particularly rich in it, with levels in commonly grown cultivars ranging from 130–200 mg/100 ml juice, to over 350 mg/100 ml in some breeding lines (Brennan 2008). Levels of AsA in some wild accessions, particularly of *R. nigrum* var. *sibiricum* (Volunez and Zazulina 1980), can be even higher, although conversely it has been found that the fruit of some Scandinavian cultivars contain reduced quantities of AsA compared to western European types (Kuusi 1965; Heiberg *et al.* 1992).

Some reports suggest that the AsA contained in blackcurrant fruit is more stable than most other sources, possibly due to the protective effects of anthocyanins and other flavonoids within the berries (Hooper and Ayres 1950; Morton 1968). The main period of AsA accumulation is at the berry expansion phase, occurring shortly after flowering, and the AsA content of the berries is established at this early stage and remains constant thereafter (Viola *et al.* 2000; Hancock *et al.* 2007b). Continuing from the biosynthetic pathway for plant vitamin C proposed by Wheeler *et al.* (1998), it was shown by Hancock *et al.* (2007b) that the biosynthesis of AsA occurs within the berries from photosynthetically-derived sugars via the galactose pathway. It was also demonstrated that the AsA content of specific cultivars is genetically determined, so that whilst the actual amounts of AsA can vary from year to year due to the influence of environmental factors, cultivar rankings remain fairly constant (Walker *et al.* 2010).

Little is currently known about the heritability of fruit quality traits in blackcurrant; studies in New Zealand showed that additive genetic effects were significant for nine antioxidant traits from processed blackcurrant fruit (Currie *et al.* 2006), and initial analysis of a full diallel experiment at SCRI revealed that some traits including AsA content show a large component of genetic variance (Vagiri *et al.* unpublished). AsA content has a high narrow-sense heritability, so it is clear, therefore, that breeding for increased AsA content is an achievable objective within the context of a blackcurrant breeding programme, and that parental combinations should be chosen to reflect this and also other antioxidant traits (Currie *et al.* 2006).

Other phenolic compounds

Ribes species, especially blackcurrants, contain high levels of polyphenolic compounds within the fruit (Bordonaba and Terry 2008), including flavonoids such as anthocyanins and flavonols. The levels of these compounds present in the fruit are influenced by cultivar, environment and agronomic practices. Cultivars with elevated levels of anthocyanin are now in demand from processors and consumers due to increasing interest in the antioxidant activity and potential health benefits of berry fruits (Lister *et al.* 2002).

The main anthocyanins in blackcurrant are the 3-*O*-glucosides and 3-*O*-rutinosides of cyanidin and delphinidin (Macheix *et al.* 1990), and the relative proportions vary between genotypes. For example, a survey of *Ribes* germplasm found that western European cultivars contain more cyanidin derivatives, whilst Scandinavian cultivars contain a higher proportion of delphinidin derivatives (Taylor 1989). Other compounds that have been found, albeit in lower concentrations, include the 3-*O*-glucosides and 3-*O*-rutinosides of pelargonidin, peonidin and malvidin (Slimestad and Solheim 2002; Nielsen *et al.* 2003; Wu *et al.* 2004), and McDougall *et al.* (2005b) found a new class of flavanol-anthocyanin condensation products in extracted juice that

may influence the colour and palatability of processed products.

In terms of breeding strategy, the utility of specific blackcurrant cultivars to end-use juice production is enhanced by increasing colour stability, and as a result some breeders preferentially select for a higher proportion of delphinidin derivatives within the available germplasm (Brennan 1996). This strategy may produce unexpected benefits regarding the downstream effects on human health; recent research by Yun *et al.* (2009) has demonstrated significant effects of delphinidins on the growth and development of colon cancer cells *in vitro*, although there remains a need for efficacy of anthocyanins to be demonstrated in clinical trials *in vivo* (Aura *et al.* 2005). However, from a breeding viewpoint, selection of high total polyphenolic content may prove to be an easier target depending on the range of germplasm available.

Other *Ribes* species such as *R. valdivianum* (Moyer *et al.* 2002) and *R. sanguineum* have levels of total polyphenolics and antioxidant activity considerably in excess of the highest cultivated blackcurrants, raising the possibility of breeding for improved antioxidant activity, although other less desirable traits found in these species, e.g. astringent flavour, must also be considered.

Sensory characters

The selection of new blackcurrant cultivars with improved sensory profiles, leading to enhanced consumer acceptability, has become one of the main objectives in most contemporary breeding programmes, although the generation of high-throughput phenotyping for large populations remains problematic.

There is a high level of variation in sensory profile within blackcurrant (Brennan *et al.* 1997), and further research showed that the sensory attributes associated with particular genotypes persist in processed juice products even at juice concentrations of 25% (Brennan *et al.* 2003). The use of other *Ribes* species in breeding for agronomic traits such as disease or pest resistance can adversely influence the sensory characters of the fruit, at least in the early generations – for example, off-notes have been noted in the flavour profiles of progenies developed from *R. ussuriense* and *R. petiolare* (Anderson 1977; Melekhina *et al.* 1980).

Essential fatty acids

Blackcurrant seed oil is marketed as a health supplement due to its high content of γ -linolenic acid (GLA) and other nutritionally important fatty acids. The content of GLA varies between genotypes, with a maximum of over 20% of total fatty acids in some lines (Ruiz del Castillo *et al.* 2002). The content of two other nutritionally important fatty acids, stearidonic acid and α -linolenic acid, varied from 2 to 4% and 10-19%, respectively across the same range of germplasm.

Mapping of traits and gene identification

The use of marker-assisted breeding techniques is developing in many fruit breeding programmes, since effective deployment of markers can reduce the lengthy timescales involved in the breeding of perennial crops. In this context, the development and use of markers for quality traits in berry fruits is particularly attractive, since selection for key fruit quality attributes at the seedling stage prior to the first fruit appearing would dramatically increase breeding efficiency and reduce the time to cultivar.

QTLs for fruit quality traits including ascorbic acid content and fruit acidity have been mapped onto the blackcurrant linkage map by Brennan *et al.* (2007), and work is in progress using key markers most closely linked to these QTLs to provide tools for breeding and selection in blackcurrant. The markers most closely associated with acidity are separate from those associated with the QTLs for ascor-

bic acid content, since malic and citric acids are by far the most abundant in blackcurrant. The potential linkage of these markers to genes at control points within the biosynthetic pathway is under investigation, with SNPs in the coding region of one of the pathway enzymes, GDP-mannose epimerase showing a putative link with high AsA content in blackcurrant genotypes (Walker *et al.* 2010).

In the case of anthocyanins, there has been some progress towards the development of markers linked to high levels of anthocyanins, either total or specific. Residual maximum likelihood (REML) analysis of the mapping and phenotypic data originally used by Brennan *et al.* (2007) has revealed markers linked to QTLs for anthocyanin content. Also, differential screening of a cDNA library from developing berries of cv. 'Ben Alder' enabled Woodhead *et al.* (1998) to isolate clones of five genes showing enhanced expression in fully ripe fruit compared with green fruit.

Other fruit quality traits have also been mapped by Brennan *et al.* (2007), including physical quality traits such as berry size, where associated SSR markers have been identified. Further work to confirm the utility of these markers as a selection tool in blackcurrant breeding is required, but overall there is an emerging framework for the development of marker-assisted breeding strategies for blackcurrant for complex quality traits as well as agronomic characters.

FUTURE DIRECTIONS

The move towards higher quality fruit, with elevated content of specific components, is likely to remain for all berry fruit crops into the future. Already, cultivars are being selected solely on quality rather than agronomic traits by some end-users (Brennan *et al.* 2008), and as clinical evidence for the beneficial effects of berry fruit components on human health accumulates, the need by breeders to select appropriately enhanced phenotypes will increase accordingly. Fruit quality characteristics are key drivers for breeding programs and a greater understanding of the processes and genes involved in quality will enable more targeted breeding in the future. Biotechnology has resulted in a fundamental shift in detecting and monitoring genetic variation in plant breeding and genetic studies. A variety of molecular marker techniques have been employed in genetic studies of *Rubus* and *Ribes*. Additionally, many genomics tools are becoming available, including BAC libraries in *Rubus*. Additionally there has been several gene expression libraries created from various organs of raspberry (fruit and roots) and blackcurrant (buds and fruit).

Prior to the advent of molecular markers, inheritance and genetic mapping studies were limited to simple morphological traits (Ourecky 1975; Jennings 1988). These advances have resulted in a fundamental shift in the development of genetic linkage maps and their use in variety development. Classical breeding, which selects parents and their desirable offspring based on an observable phenotype, is being integrated with techniques that can identify and manage genetic variability at the molecular level. The ability to detect genome wide variability has led to the characterization of genetic variation within, not only coding regions (i.e. genes and their morphological manifestations), but also in non-coding regions as well, which make up large portions of plant genomes.

The identification of QTLs and associated markers/genes linked to key traits is enabling breeders to select desirable phenotypes faster and with greater precision, and recent initiatives to establish massively-parallel sequencing platforms for both *Rubus* and *Ribes* will accelerate the development of both markers and gene discovery.

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