Developmental Transitions to Fruiting in Red Raspberry

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Abstract

Climate change is impacting soft fruit crops. In raspberry, uneven bud break, greater variability in time to fruit ripening and crumbly fruit are already in evidence. Understanding the developmental process and how the environment impacts will be crucial in sustaining the industry in this changing climate against a background of biotic stresses. This chapter reviews regulation of processes leading to flowering time and fertilisation, developing fruit, ripening, colour, flavour and size. Recent developments of genomic and transcriptome tools which will have a significant role in breeding of the next generation of raspberry fruit are considered.

14.1 Introduction

Red raspberry has seen a significant increase in consumer demand while facing considerable grower challenges. Many of these challenges are associated with the fruit developmental process. For example, growers, breeders and propagators are seeing an increased occurrence of crumbly fruit, a lack of evenness of bud break and shifts in ripening period across seasons. Understanding the developmental process and how the environment impacts on it will be crucial in sustaining the industry in a changing climate and a background of increasingly aggressive biotic stresses. This chapter will review what is known about regulation of processes leading to flowering time and fertilisation, developing fruit, ripening, colour, flavour and size. It will also consider explanations for the increasing occurrence of crumbly fruit, a serious threat to consistent fruit harvest. The chapter will further review the recent development of genomic and transcriptomic tools which will have a significant role in breeding of the next generation of raspberry fruit.

14.2 Life Cycle

Raspberry bears short-lived woody shoots on a long-lived perennial root system bearing juvenile and mature shoots (canes) simultaneously on an individual plant. In biennial-fruiting cultivars, the canes have a two-year life cycle contrasting with primocane cultivars (annual fruiting) which complete the cycle of vegetative growth, flowering and fruiting in a single season (Jennings 1988). Low temperatures and/or short days are required for flowering in biennial cultivars, whereas the annual-fruiting raspberries initiate

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flower buds under the high temperature and long-day conditions of summer. With the absence of dormancy in a certain proportion of the initiated buds, primocane varieties flower in the first year unlike the biennial-fruiting cultivars where floral initiation is accompanied by growth cessation and bud dormancy. Dormancy release requires exposure to low temperatures or winter conditions, thus conferring a two-year life cycle upon the shoots. Shoots originating from root adventitious buds in biennial cultivars are juvenile and must develop 15–20 leaves before they can be induced to flower. In contrast, the juvenile phase is absent in annual-fruiting cultivars, which respond to a vernalisation treatment at the five-leaf stage (Sonsteby and Heide 2011).

14.3 Bud Break

Timing of bud break is of significant economic importance influencing realised fruit yields. Bud break in raspberry crops is often uneven with many of the sub-apical buds remaining in a dormant state (White et al. 1998). The control of bud break is a key ecological factor in woody perennial plant survival and is dependent upon exposure to a particular duration of cool temperatures (chilling) to release dormancy, followed by an appropriate temperature to permit growth in the spring (Rallo and Martin 1991). Such a mechanism prevents premature bud burst during warmer winter days that could result in subsequent frost damage. Three temporally overlapping stages of dormancy have been distinguished (Lang et al. 1987). In paradormancy, growth suspension is maintained by plant tissues outside of the dormant meristem, often by the apical bud. Endodormancy is controlled from within the bud itself and is characterised by a requirement for sustained exposure to low, near freezing temperatures before active growth can resume. Ecodormancy is where growth is prevented by environmental factors, e.g. low temperatures. Current theories of dormancy suggest that endodormancy is followed by a period of ecodormancy, where buds are held in a dormant state until temperatures rise in the spring allowing growth resumption (Lang et al. 1987; Faust et al. 1997; Arora et al. 2003). Raspberry exhibits a high degree of paradormancy caused by apical dominance, which is manifested in the typical unbranched form of the canes (Måge 1975). White et al. (1998) carried out experiments on intact canes, single nodes and cane trisections after different lengths of chill units to determine whether the buds were in an endodormant or paradormant state. Buds on the lower parts of the intact canes remained in a dormant state long after buds from higher up the intact cane and the single nodes from all parts of the cane had emerged from the deepest phase of endodormancy. No significant differences were apparent in the trisected cane portions in bud break levels throughout the experiment when compared with the single nodes. A period of secondary dormancy was also observed in the intact canes not seen in the single nodes or the trisected canes which indicates that treatments which reduce paradormancy may also minimise the risk of secondary dormancy. Methods which overcome paradormancy in protected crops therefore might include tipping (removal of the cane apex), horizontal training methods, more efficient chilling methods and chemical treatments. Phenological models of endodormancy release and subsequent bud break, based on the relationship between climate and periodic plant growth, have been formulated (Fuchigami and Wisniewski 1997). This requirement is being met sporadically with ongoing climate warming leading to erratic bud break and less consistent fruit yields being reported (Jennings pers comm). An understanding of the molecular and cellular basis of signals that control the processes of dormancy induction or dormancy release in woody perennial plants remains elusive (Dennis 1994; Chao 2002). Hormonal and environmental signals exert their effect on the induction, maintenance and release of dormancy, via activation and repression of diverse gene activities (reviewed in Olsen 2003). In order to understand the regulation of dormancy transition at the level of gene expression, Mazzitelli et al. (2007) measured bud burst in a growth-permissive environment following exposure to chilling

(4 °C cold storage). A microarray approach was then used to follow changes in gene expression that occurred. Sequence analysis of over 5000 clones, in many cases, enabled their functional categorisation and the development of hypotheses concerning the mechanisms of bud dormancy release.

14.4 Flowering Control

The timing of flowering is a key component of plant adaptation, affecting geographical distribution and geographical range. There is now evidence that red raspberry flowering time has shifted in response to changes in climate (Fitter and Fitter 2002; Amano et al. 2010). To understand this important change in flowering time requires a detailed understanding of the regulatory processes. Many plant species, including raspberry, are unable to respond to the florally inductive cues until they have reached a certain developmental stage. They have a juvenile phase which only responds to a prolonged period of cold (vernalisation) after a certain stage of development. Sonsteby and Heide (2014) determined the conditions necessary for full dormancy release and concluded that 20 or more weeks of chilling at temperatures near freezing were required for full dormancy release and promotion of flowering along the entire length of the cane. Much is known about the genetic pathways regulating flowering, and recent advances have now shed light on the mechanisms underlying vegetative phase change (Wu et al. 2009). The transition to flowering is regulated by a range of environmental and physiological cues (Fornara et al. 2010; Pin and Nilsson 2012; Song et al. 2012) that need to be fully understood in perennial crops. Flowering time variation is highly relevant to yield, quality and environmental considerations as flowering at the appropriate time ensures best use of the available growing season, promoting sustainability and reducing the need for inputs. Activity of CONSTANS (CO) a key component in leaves of the photoperiodic pathway accumulates under long-day conditions and activates transcription of FLOWERING LOCUS T (FT) (Simon et al. 2015) which interacts with bZIP transcription factors (Abe et al. 2005; Cao et al. 2016) activating a cascade of downstream genes leading to flowering. This basic flowering process is impacted by a number of autonomous and stress-related signals. For example, the MADS box FLOWERING LOCUS C (FLC) and short vegetative phase proteins (SVP) form a complex to repress flowering until the plant is exposed to the appropriate level of cold. The small non-coding RNA, miRNA156, acts by repressing members of the SQUAMOSA PROMOTER BINDING LIKE (SPL) family of transcription factors. Flowering repression is lost as miRNA156 is reduced as the plant ages (Wang 2014; Wu et al. 2009). The plant thus requires both down-regulation of miRNA156 and FLC for flowering to occur. In raspberry, RiMADS_01 was identified as a potential candidate affecting vernalisation (Graham et al. 2009a). The gene is similar to SVP modulating the timing of the developmental transition to flowering phase in response to temperature (Lee et al. 2007), and in colder seasons, RiMADS_01 was associated with earlier flowering. An SPL homolog has also been identified in raspberry within a QTL associated with floral transition. In terms of raspberry life cycle, the difference between annual and biennial cultivars is in whether floral initiation is linked to the induction of bud dormancy or whether floral initiation is followed by direct flower development. Although this is genetically determined, it is a plastic trait under environmental control. Thus, at low temperatures and short photoperiods, the majority of initiated buds also enter dormancy in annual-fruiting cultivars, with tip-flowering as a result (Heide and Sonsteby 2011).

14.5 Flower Morphology

Raspberry flowers have five sepals and petals, the sepals persisting until the fruit is ripe. Flower parts develop during active growth processes, and then, growth is arrested until pollination and fertilisation occurs, initiating fruit development.

Major genes have been identified with a role in modifying the number and size of the sepals. The recessive gene sx_3 (Keep 1964) induces either an extra whorl of sepals with the normal number of petals but fewer anthers, to two extra whorls of sepals where petals and anthers are completely suppressed or just a few anthers present. Another gene L_1 gives very large sepals with lobes contracted to a narrow point (Jennings 1988). In raspberry, the sepals reflex away from the fruit, but in other *Rubus* spp., they close after pollination until the fruit is ripe. Raspberry stamens arise in whorls of 60–90 with the numbers of both stamens and styles affected by ploidy, genotype and major genes (Jennings 1988). Most raspberries are hermaphrodite (genotype *FM*), but some male (*fM*) and some female (*Fm*) genotypes and sterile genotypes (*fm*) where recessive genes *f* and *m* suppress development of the female and male parts (Jennings 1988) occur. Styles arise spirally on the terminal part of the receptacle and determine the size and shape of the fruit. Mezetti et al. (2004) reported results from transgenic raspberry plants expressing the DefH9-iaaM auxin-synthesising gene. DefH9-iaaM plants had an increased number of flowers per inflorescence and an increased number of inflorescences per plant resulting in an increased number of fruits. The weight and size of transgenic fruits were also increased. The DefH9-iaaM gene is expressed in the flower buds. The total IAA (auxin) content of young flower buds expressing the DefH9-iaaM gene was increased in comparison with non-transgenic flower buds.

14.6 Fertilisation

The current model of fruit set implies that ovary growth is blocked before pollination and that auxin is a key regulator of ovary growth de-repression at fruit set (Goetz et al. 2007; Pandolfini et al. 2007). Following pollination in raspberry, there is a period of rapid growth due to cell division. This is followed by a period of slow growth during which the embryo develops and the endocarp becomes hardened, until finally cell enlargement results in a period of rapid growth. Other phytohormones (gibberellin, cytokinin, brassinosteroids, ethylene, and abscisic acid) play a role in fruit initiation and development (Schwabe and Mills 1981; Vriezen et al. 2008). The carpel has an ovary with two ovules one of which usually aborts. Fertilisation usually occurs the day after pollination, and the endosperm nucleus begins to divide a day later. The egg cell however does not begin to divide until the fourth day after pollination. Initially, the whole system behaves as a unit with the embryo, endosperm, testa and endocarp growing alongside each other (Topham 1970). Common to many fleshy fruits, once raspberry fruit growth has started, it is largely independent of the seed as the seed becomes dormant (McAtee et al. 2013). Raspberry fruits are aggregates of all the drupelets formed after fertilisation from each ovary from the same flower adhering to a common receptacle. In effect, each drupelet is a complete fruit and the control of drupelet formation must be co-ordinated in the aggregate fruit. The cohesion of the drupes depends on the entanglement of epidermal hairs. In some *Rubus* spp., wax on the outside of the epidermal cells also plays a role. A good set of drupelets and optimum early development of the seed appear to depend on the interactions between the gametes of the two parents. The maternal genotype has significant influence especially on timing of embryo sac differentiation where late differentiation is associated with low drupelet set (Jennings 1988). Self-incompatibility (gene *S*) is common among the diploid *Rubus* spp but domesticated forms are self-compatible due to mutation where the pollen of cultivated forms has changed to a self-compatible state.

14.7 Fruit Ripening

Raspberry fruits ripen from 30 to 60 days after pollination with variations in duration to ripe fruit dependant on genotype and environmental conditions. Fruits are generally divided into climacteric, which show a rise of respiration and ethylene formation at the beginning of ripening,

while non-climacteric fruit lacks this increase and other phytohormones may have a greater role to play. Reports vary on the classification of raspberry ripening as climacteric (Iannetta et al. 1999) or non-climacteric (Perkins-Veazie and Nonnecke 1992; Zheng and Hrazdina 2010). Evidence suggests that fruit ripening and abscission are controlled by ethylene from the recepticle and respiration continues even after the fruit is picked (Iannetta et al. 2000; Fuentes et al. 2015). Many raspberry cultivars differ in the amount of ethylene they produce with the ease of fruit abscission related to those cultivars producing higher amounts (Jennings 1988). But, there is no strong evidence of a large climacteric peak of respiration and ethylene at the start of raspberry fruit ripening. Auxin treatment delays fruit ripening in strawberry and grape, supporting a role for auxin in non-climacteric fruit and over-expression of an *F*-box auxin receptor in tomato enhanced fruit softening through upregulation of cell wall-degrading enzymes (Davies et al. 1997; Aharoni et al. 2002; El-Sharkawy et al. 2016). A microarray transcriptomic analysis of ripening strawberry recepticles challenged with phytohormones found auxin-regulated genes led to recepticle fruit growth and development and abscisic acid (ABA)-regulated genes involved in ripening (Medina-Puche et al. 2016). ABA and pyrabactin applied directly to raspberry fruit after fruit set did not alter fruit development and ripening, but doubled vitamin C content in fruit (Miret and Munné-Bosch 2016). Ethylene formation therefore may have a minor role in raspberries that may be co-ordinated with auxin and ABA formation as part of the mechanism that regulates timing of ripening in different fruit species (Trainotti et al. 2007; McAtee et al. 2013; Tadiello et al. 2016).

As part of the ripening process, fruits progressively lose firmness associated with fruit expansion when the thin-walled mesocarp cells become distended resulting in a loss of skin strength, the separation of the drupelets from the receptacle and a breakdown of cell walls in the mesocarp (Sexton et al. 1997; Vicente et al. 2007; Zheng and Hrazdina 2010). Abscission layers form in tissues as the fruit ripens at the point of attachment of each drupelet to the receptacle. A number of key enzymes have been shown to significantly impact on the degree and speed of the fruit-softening process; β-galactosidase and expansin genes act early in the ripening process and restrict or control the activities of other ripening-related hydrolases including polygalacturonases (PG), pectin-methylesterases (PME), endo-1,4-β-glucanases, xyloglucan endotransglycosylases and pectate lyases (Sexton et al. 1997; Iannetta et al. 2000, Jimenez-Bermudez et al. 2002; Costa et al. 2008; Santiago-Domenech et al. 2008; Uluisik et al. 2016). Studies on fruit softening have shown an absence of change in hemicellulosic polymers and an increased solubility of cell wall polyuronides accompanied by depolymerisation. Pectic compounds, therefore, seem to be the cell wall polymers undergoing extensive modifications during raspberry ripening (Stewart et al. 2001; Vicente et al. 2007).

Ripening stages across different years and environments from a raspberry mapping population were examined to identify QTLs for the overall ripening process, as well as for the time to reach each stage. QTLs were identified across four chromosomes for ripening and the time to reach each stage. A MADS box gene RiMADS, Gene H and several raspberry ESTs were associated with the QTLs (Graham et al. 2009a). It was interesting that Gene *H*, known to be associated with cane morphology, was also associated with a slowing down of ripening across all stages (Graham et al. 2006, 2009a). Sequencing of the Gene *H* region (McKenzie et al. 2015) identified a DIVIA like Myb transcription factor (Werewolf) which has been shown to be a post-transcriptional regulator of FT (Seo et al. 2011). Ripening-related QTL and underlying genes have also been identified for anthocyanin production (Kassim et al. 2009) colour development (McCallum et al. 2010) and volatile production (Patterson et al. 2013). Recently, fruit softening was examined using both qualitative scoring and quantitative scoring of fruit firmness, length, mass and resistance to applied force to identify QTL in a raspberry mapping population (Latham × Glen Moy). QTLs were located

primarily on linkage group (LG) 3 with other significant loci on LG 1 and LG 5 which showed mostly additive effects between the two parents (Simpson et al. 2016). The expression of key genes that underlie these QTLs, with roles in cell wall solubility, water uptake, polyamine synthesis, transcription and cell respiration, showed variable expression patterns across fruit development. Highly significant positive and negative correlations between genes supported precise regulation of different cell processes throughout raspberry fruit development. Variable timing in expression was also found in some genes between soft (Latham) and firm (Glen Moy) cultivars (Simpson et al. 2016).

14.8 Fruit Colour

Considerable fruit colour variation exists in different raspberry varieties, and this influences consumer perception of ripeness. Although the genetics of the anthocyanin pathway is well documented, the regulatory controls of both quantitative and qualitative variations of fruit anthocyanin content are less advanced (Castellarin and Di Gaspero 2007). This is an issue for fruit breeding, which is a lengthy and costly process in woody perennials (Graham and Jennings 2009). Anthocyanin consists of an aglycone (cyanidin and pelargonin) with a varying number of sugar residues attached. The concentration of anthocyanins in raspberry is known to be determined by the interplay of a number of genes. The recessive allele of gene *t* results in a low anthocyanin concentration. Homozygous *tt* interacts with a recessive inhibitory gene *i* to produce apricot coloured fruits, while gene *P* prevents *tt* from completely blocking anthocyanin synthesis (Jennings 1988). In the field, anthocyanin concentrations vary as a result of genotype, varying seasonal sunlight variation and as a result of growth under polytunnels (Kassim et al. 2009; Mazur et al. 2014). The final expression of fruit colour may be attributed to varying proportions of individual co-pigments and to pH, which has a significant influence on the colour of anthocyanin solutions (Jennings 1988). Co-pigmentation allows the formation of complex interactions between pigments and colourless compounds, which enhance colour intensity. Several compounds may act as co-pigments including flavonoids, alkaloids, amino acids, polysaccharides, metals, organic acids, nucleotides and other anthocyanins (Castaneda-Ovando et al. 2009). The enzymes involved in flavonoid biosynthesis, which includes anthocyanins and flavonols, are well characterised (Jaakola 2007). Flavonols are derived from dihydroflavonols by flavonol synthase (FLS) (Nielsen et al. 2002). Following flavonoid synthesis in the cytoplasm, anthocyanins and proanthocyanins are transported to the vacuole where they can be permanently stored. This transfer is facilitated by a glutathione S-transferase (GST), the gene for which has been identified in many plant species including *Petunia hybrida* (AN9 gene) (Mueller et al. 2002) and maize (Bz2 gene) (Alfenito et al. 1998). GST is involved in the last genetically defined step in anthocyanin biosynthesis by adding a glutathione onto anthocyanins such as cyanidin-3-glucoside. Major structural genes (F3'H, FLS, DFR, IFR, OMT, GST) and transcription factors (bZIP, bHLH, MYB) influencing flavonoid biosynthesis have been identified, mapped and shown to underlie QTL for quantitative and qualitative anthocyanin composition. Measures of individual anthocyanins mapped to the bHLH gene on LG 1 and a bZIP gene on LG 4 (Kassim et al. 2009; Bushakra et al. 2013), whereas colour and total anthocyanins mapped to different overlapping QTL on LG 2, LG 3, LG 4 and LG 6 (McCallum et al. 2010). Chalcone synthase (PKS1 and PKS5) genes mapped to LG 7 and did not underlie the anthocyanin QTLs identified (Kassim et al. 2009). Transcription factors related to the C1 and R genes, which belong to the MYB and bHLH family, respectively, have also been shown to regulate the flavonoid accumulation pathway in other plant species including apple (Espley et al. 2007) and grape (Lijavetzky et al. 2006). Other MYB genes regulate other flavonoid genes and a MYB12 gene from Arabidopsis has been shown to regulate flavonol and caffeoylquinic acid synthesis when expressed in

tomato fruit (Luo et al. 2008). In raspberry, two MYB genes underlie a QTL on LG 3 with a major impact on fruit ripening, importantly at the transition from the green to the green/red stage (Graham et al. 2009a, and these are implicated in the expression of fruit colour. Gene families like aquaporins may also have a role in colour as these are water channel proteins capable of transporting water and small molecules across cellular membranes. Tonoplast intrinsic proteins have been shown to act as water channels expressed predominantly within storage tissues. As glucose is accompanied by the transport of water, these genes are strong candidates for quantitative differences relating to the storage and transport of sugar molecules (Martinoia et al. 2000). McCallum et al. (2010) identified aquaporins on LG 2 in a QTL for colour scores and total anthocyanin measures (McCallum et al. 2010).

14.9 Fruit Composition

The composition of raspberry fruit is a key factor in taste. Raspberry fruits are mainly water with about 9% soluble solids. Pectins make up 0.1–1.0% of the soluble fraction, but this amount decreases with ripening due to hydrolysis. The main sugars are glucose, fructose and a smaller amount of sucrose. A typical ripe raspberry fruit will contain 5–6% sugar. Citric acid is the second largest component of the soluble fraction; raspberries contain very little malic acid, but at least ten other acids in trace amounts. The amount of acid in the fruit increases early in development and then decreases as the fruit begins to ripen. Flavour is central to quality in soft fruit and is determined by the content and ratios of sugars and acids as well as the volatiles. In raspberries, the two main flavour attributes sweetness and sourness (Harrison et al. 1999; Brennan and Graham 2009) vary with season and environment where flavour excellence relates to weather conditions (Jennings pers comm). Little work has been carried out in raspberry to correlate sensory evaluations with fruit composition. A study by Zait (2012) aimed to understand the association between sugars and acids and sweetness and sourness perceptions. Data from Patterson et al. (2013) on volatiles content and from Kassim et al (2009) on anthocyanin content was correlated with sensory data to develop a preliminary flavour model. Sweetness, sourness and flavour intensity traits were not adequately explained by singular contributions of either sugars or acids content, but through synergistic relationships between all flavour metabolites. Raspberry fruit accumulates the phenylpropanoid p-hydroxyphenylbutan-2-one (raspberry ketone) with content correlated with those of anthocyanins and soluble solids. Although only a small proportion of total volatiles, it has been reported to be a key determinant of raspberry flavour (Larsen et al. 1991). Fruit flavour volatile contents are generally continuous traits which are found to display a normal pattern of distribution which may be controlled by several genes of small effect or one or two genes conferring a large effect, or a combination of both. In the study of Kassim et al. 2013, raspberry volatile production appears to be significantly influenced by environmental as well as complex genetic factors and this work has provided a basis from which to proceed towards identifying the important variables contributing to desirable flavour/aroma characters at the genetic level. The demonstration that the concentrations of volatiles change across seasons and environments coupled with the shift or loss of significant QTL significantly associated with volatiles content across seasons and environments highlights the complex regulatory nature of volatile regulation.

In raspberry, the high levels of polyphenols and their distinct phytochemical profile have been associated with health benefits in humans (Rao and Snyder 2010; McDougall and Stewart 2012; Burton-Freeman et al. 2016). Levels of polyphenols are under genetic control subject to modulation by environmental factors such as temperature, sunlight and rainfall and can vary from season to season (Mazur et al. 2014). A number of tools have been developed for the rapid spectrophotometric quantification of specific compound classes such as total polyphenols, total anthocyanins and total

antioxidant capacity in fruit (Nwankno et al. 2012). Chromatographic methods have been developed for the analysis of key quality components such as individual sugars and organic acids (Nwankno et al. 2012), ascorbic acid (Walker et al. 2006), individual polyphenols and anthocyanins (Stewart et al. 2007) and volatiles (Patterson et al. 2013). A GC/MS method has been adapted from potato profiling (Hancock et al., 2014) to profile berry fruit metabolites, allowing the identification and quantification of a range of sugars, organic acids, amino acids, fatty acids, fatty alcohols and phytosterols. A study of ten different cultivars grown over three seasons in Western Norway found the main phenolic compounds in raspberry fruit were ellagitannins and anthocyanins with Cyanidin-3-sophoroside the most abundant anthocyanin (Mazur et al. 2014). An evaluation of the antioxidants in 14 different raspberry cultivars during fruit ripening identified the dominant antioxidants as ellagitannins, anthocyanins and proanthocyanidin-like tannins (Beekwilder et al. 2005). During the ripening process, some anthocyanins were newly produced, while others, like cyanidin-3-glucoside, were already present early in fruit development. The level of tannins, both ellagitannins and proanthocyanidin-like tannins, was reduced strongly during fruit ripening. Among the 14 cultivars, major differences were observed in the levels of pelargonidin-type anthocyanins and some proanthocyanidin-type tannins and the content of ellagitannins varied approximately threefold (Beekwilder et al. 2005). These findings suggest that the content of individual health-promoting compounds varies significantly in raspberry, due to both developmental and genetic factors. Large variation among 64 genotypes analysed for soluble sugars, titratable acids, pH and phenolic compounds was observed, highlighting the breeding and health potential within the germplasm (Weber et al. 2008). Using a mapping population, total phenol content (TPC) and total anthocyanin content (TAC) in ripe fruit was examined over five seasons under two environments to examine variability (Dobson et al. 2012). Corresponding measurements of antioxidant capacity using FRAP and TEAC were highly correlated with TPC over the entire dataset. The subset of anthocyanin content was genotype-dependent and also correlated with TPC, but the proportion of different anthocyanin compounds contributing to total phenolic pool varied from progeny to progeny. QTL was identified on linkage groups 2, 3, 5 and 6. The QTL that specifically influences TPC is of particular interest to boost the antioxidant capacity of raspberry fruits, which is often related to their bioactivities.

14.10 Fruit Enlargement

Fruit size is a key quality attribute due to its effect on yield and picking costs. Raspberry genotypes show a wide variation in fruit size, cell number and/or volume, which may be due to a number of genetic, environmental and growth management factors. Cells increase in volume by uptake of water and loosening and extension of the cell wall through the action of expansins and cell wall hydrolysing enzymes (Cosgrove 2016). Gibberellins are regulators of cell expansion and applied to grapes in the early stage of seedless berry development to increase berry size (Fortes et al. 2015). In raspberry, mutation of a major gene designated L_1 was identified in a large fruited 'Malling Jewel' mutant which resulted in an increase in both drupelet number and size. The gene itself however proved to be unstable mutating back to its normal-sized form (Jennings 1988). In tomato, a single major QTL for fruit weight was identified on chromosome 2, in close proximity to a cloned fruit weight gene *fw* 2.2 (Frary et al. 2000; Zygier et al. 2005). Some recent work has been carried out in raspberry to understand the basis of the genetic control of fruit size. QTL was identified on three linkage groups in the reference 'Glen Moy' by 'Latham' mapping population (Graham et al. 2004), across four seasons and three environments. Candidate genes for auxin, ethylene and specific size regulatory genes (fruit weight) and transcription factors were identified (McCallum et al. 2010). Initial analysis has found one marker explained 23% trait variation. In a *Rubus*

parvifolius × Tulameen cross one QTL was identified for fruit size (Molina-Bravo et al. 2014).

14.11 Disorders

In relation to fruit development, the disorder known as 'crumbly' fruit has become a serious problem in the raspberry industry. Drupelets are generally reduced in number and greatly enlarged or, in the case of small reductions, cohere imperfectly such that fruit readily crumbles when picked (Daubeny et al. 1967; Jennings 1988). There have been a number of causes suggested for the crumbly condition including infection with certain viruses (Jennings 1988; Murant et al. 1974; Daubeny et al. 1978). A genetic cause was demonstrated when the crumbly phenotype appeared in virus-clear mother plants (Jennings 1988). The cultivar 'Latham' can show a crumbly phenotype and this is thought to be due to a mutation of the dominant allele at a heterozygous gene locus, causing plants to become homozygous for a deleterious recessive gene (Jennings 1967b). From a 'Latham' self, Jennings (1967b) demonstrated that seedlings obtained could be classified into three groups: normal, crumbly and sterile. Studies have also shown that extensive tissue culturing of plants may increase the emergence of the condition (N. Jennings pers comm.). In addition, environmental factors appear to play a role as variation in the extent of crumbliness is apparent from year to year (A. Dolan pers comm). A study on the 'Latham' × 'Glen Moy' population examined the occurrence of crumbly fruit over a six-year period, in both open field and under polytunnel. This highlighted that seasonal, environmental and genetic factors all influence the condition. Two QTLs that are important for the genetic control of the condition were located on linkage groups one and three. Contrary to the suggestion by Jennings (1967a) that the crumbly fruit syndrome was related to the gene H region, no genetic association with this region on LG 2 could be identified. However, the longer the fruit takes to set fruit and reach green fruit stage, the more likely it is to be crumbly. This may explain the association hypothesised by Jennings, as the *Hh* genotype of gene H is associated with a slowing down of ripening across all stages from open flowers to the green/red stage compared to the *hh* genotype (Graham et al. 2009b).

Poor fruit set that affects raspberry fruit crumbliness has been associated with over frequent bee visits that impair fruit or seed production and/or quality by damaging flowers during visitation. Pollination and drupelet set in 16 raspberry fields was assessed along a gradient of bee abundance (Saez et al. 2014). Using pollen supplementation, they found pollen loads on stigmas increased with visit frequency in a subset of six fields. Drupelet set was not therefore pollen limited, but decreased with the proportion of damaged styles. In fields with the highest bee frequency equivalent to 300 visits per flower per day, 80% of styles were damaged and these developed into fruits with up to 30% fewer drupelets compared to flowers in fields with the lowest bee visitation rates of four visits per flower per day. Extreme bee visitation, particularly by *Bombus terrestris*, damaged the styles of raspberry flowers, precluding ovule fertilisation by deposited pollen and limiting crop production by reducing drupelet set (Saez et al. 2014).

14.12 Systems Biology Approaches to Studying Fruit Development

The development, improvement and integration of large-scale sequencing technologies and gas/liquid mass spectrometry techniques into horticultural breeding are changing our understanding of fruit developmental physiology, metabolic pathways and leading us to new important genes to important traits. Genomes of important *Rosaceae* species have been sequenced https://www.rosaceae.org/ and are providing the physical genetic maps that allow comparative differences to be discovered within these economically important species. Most recently, a 243 Mb whole genome assembly was established for black raspberry (*Rubus occidentalis)*, a

relative of red raspberry (VanBuren et al. 2016). The assembly consists of 2226 scaffolds spanning over an estimated 83% of the genome, which is largely collinear with the strawberry genome. In comparison with genetic maps, 87% of the selected red raspberry genetic markers match the position of the black raspberry markers on the physical map (VanBuren et al. 2016). The assembly of a red raspberry genome sequence will allow comparison between these closely related species and cultivar re-sequencing will further establish cultivar-specific genes significant to different traits.

A red raspberry 'fruit transcriptome' comprising of a comprehensive database of 56,000 unigenes has recently been established by combining sequences from Roche 454 transcripts, Illumina GAII assembled transcripts, Sanger expressed sequence tags and BAC coding sequences (unpublished). Probes were designed for the generation of a custom Agilent microarray and used to screen Glen Moy and Latham across five stages of fruit development. Developmental and genotype-specific gene expression patterns were found with expected roles in cell wall hydrolysis, water movement, fruit ripening and cell wall flexibility (unpublished). QTL mapping analysis for a selection of 20 candidate genes showed a distribution throughout all seven *Rubus* linkage groups, with the majority located on LG 3, 5 and 7, and several, for example, Pectinmethylesterase (PME) and β-1,4 xylan hydrolase (XL), were significantly associated with softening QTLs. Expression analysis of selected genes across the same fruit stages found that aquaporin and pectinmethylesterase show a steady rate of decline during ripening while polygalacturonase and β-1,4 xylan hydrolase significantly showed highest transcriptional levels in ripe fruit (Simpson et al. 2016). The evidence from microarray analysis indicates a co-ordinated expression between many genes during fruit development and ripening.

The analysis of a whole range of fruit phytochemicals associated with quality or health beneficial traits has now become relatively facile using chromatographic methods linked to mass spectrophotometry to aid the identification and facilitate quantification of compounds. It is well established that during the transition from an organ that functions to protect developing seeds to one that functions to act as an attractant to seed-dispersing organisms, fruit undergoes huge changes in their phytochemical profile. For example, in tomato fruit a range of primary metabolites including sugars, organic acids and amino acids see a tenfold or greater changes in concentration between 25 and 55 days after pollination (Osorio et al. 2011). Secondary metabolites see even greater changes; for example, levels of delphinidin the most abundant anthocyanin in blueberry change more than 80-fold over the course of development (Zifkin et al. 2012). These changes in phytochemical profile are accompanied by vast changes in transcript abundance with over 4000 transcripts over-expressed at least threefold or higher at specific stages of grape development, while over 1300 transcripts were expressed at least tenfold or higher and almost 350 50-fold or higher (Sweetman et al. 2012). Such vast changes in both phytochemical and transcript profiles can be effectively utilised to associate specific genes with the accumulation of specific compounds, and work illustrating such an approach is presented elsewhere in the current volume.

Integration of data from genomics, transcriptomics, proteomics and metabolomics (and other 'omic' technologies) from the same developmental fruit series, creates comparable reference datasets. For example, transcriptional results from the *Rubus* microarray allow the correlation of gene expression patterns with the accumulation of specific phytochemicals in developing fruit samples. With knowledge of biochemical pathways, an association of specific genes with important raspberry fruit components can be constructed and integrated network pathways developed. Using genomic information, the identification of gene transcripts has the potential to identify and validate gene markers by associating allelic variation within specific chromosomal regions with genes shown to correlate with phytochemical accumulation. Alternatively, such methods can be used to identify specific genes that are likely to influence the level of a specific

phytochemical or class of phytochemicals within fruit, thereby providing specific targets for the identification of allelic variation and molecular markers. These system biology approaches expand the reliability and predictability of raspberry breeding strategies to improve fruit yield and quality. These approaches will further offer the basis for the development of genetic manipulation and novel breeding strategies that improve and produce new varieties of this economically important and health beneficial crop.

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