



## The genetics of anthocyanin reddening in apple fruit skin

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### Abstract

Among apple fruit characters, the red coloration of the fruit skin derived mainly from a class of flavonoids called anthocyanins, is of great importance. This fruit quality characteristic largely determines the consumer appeal and impacts significantly on the market value of the produce. Anthocyanin reddening in apple fruit skin has been a subject of genetic studies since the 1930's. Several studies have indicated a monogenic mode of inheritance and a single dominant gene,  $R_f$ , proposed to control anthocyanin reddening on apple fruit skin. Recent molecular genetic studies strongly corroborate the hypothesis of a major gene inheritance as has been reported in the earlier literature. However, there has been much controversy among researchers and many of the studies have been inconclusive. Additional genetic studies based on common standards for color categorization in apple, relatively larger population sizes, and biochemical genetic studies especially those based on the main anthocyanin pigment in apple skin, cyanidin-3-galactoside, would be needed to confirm the inheritance of color in apple skin. In this article, an updated summary of published reports on the inheritance of anthocyanin reddening in apple fruit skin is presented.

**Key words:** Anthocyanin, apple fruit, red color, genetics, molecular markers.

### Introduction

The domesticated apple (*Malus x domestica* Borkh) is one of the most widely cultivated temperate fruit crops<sup>9</sup>. Since the 1970's, apple production has more than doubled world-wide, from ~21 to ~55 million tons in 1997<sup>5</sup>. The complex genetic system, the prolonged juvenile phase, the large field space requirement and associated cost continues to make the development of improved cultivars a slow and laborious task<sup>12</sup>.

Improvement of the quality characteristics of fresh fruits and processed products is a major objective in apple breeding. The red coloration of apple skin is derived mainly from a class of flavonoids called anthocyanins. Although the pigments that compose fruit color vary by fruit, the amount and composition of anthocyanins present in the skin, is the major determinant of apple fruit skin reddening.

The anthocyanins in apples are predominantly glycosylated cyanidins, although some peonidin [a methoxylated form of cyanidin] and pelargonidin glycosides have been found<sup>11</sup>. The main cyanidin pigment which accounts for apple skin reddening is cyanidin-3-galactoside (~80%), while cyanidin-3-arabinoside, cyanidin-3-rutinoside, cyanidin-3-xyloside, and cyanidin-3-glucose exist in minor amounts in some apple cultivars<sup>6,11</sup>.

Anthocyanins in apple skin appear at the beginning of fruit development, then disappear and then reappear at the fruit maturation stage in red cultivars<sup>7,11</sup>. In contrast, anthocyanins are generally not detected at the mature stage in non-red cultivars, although they appear at the juvenile stage. The red pigment in the fruitlet is cyanidin-3-galactoside, as in mature fruit of red cultivars<sup>11</sup>. The mechanism of coloring at the fruitlet stage, as well as their disappearance during the fruit enlargement

stage in both red and non-red cultivars, is yet unclear. It is the intensity and extent of the anthocyanin reddening at the mature fruit stage that is most important as it impacts greatly on the marketable value of the produce and has a direct bearing in the economics of apple production.

The accumulation of anthocyanins is influenced by external stimuli<sup>11,16</sup> and by genetic factors. Anthocyanin reddening in apple fruit skin has been a subject of genetic studies since the 1930's<sup>1, 2, 8, 13, 17, 22, 23</sup>. However, most of the studies on the inheritance of this important fruit quality trait have been rather contradictory.

This review is a summary of published reports on the inheritance of anthocyanin reddening in apple fruit skin. The objective is to update our knowledge on the genetics of this important fruit quality trait in the light of available molecular genetic information.

### Classical Genetic Studies on Anthocyanin Coloration in Apple Fruit Skin

Albeit the fact that apples are complex polyploids in nature, the majority of the domesticated apples are perceived to be functionally diploids ( $2n=34$ ), and many traits including anthocyanin reddening in the fruit skin have been shown to be under single gene control with diploid segregation<sup>4,10</sup>. Anthocyanin production in all plant tissues has also been shown to be under the control of a single dominant gene<sup>15</sup>. Brown<sup>1</sup> reviewed the earlier literature on the genetics of anthocyanin coloration in apple skin. A single dominant gene,  $R_f$ , was

proposed to control anthocyanin on the apple skin<sup>1</sup>. Although many researchers have studied the inheritance of apple fruit skin color, the results are contradictory.

Based on the analysis of segregation data in the cross 'Richared' x 'Reinette di Landsberg', Lespinasse et al.<sup>13</sup> proposed that two dominant complementary genes, A and B (i.e. genotype A\_B\_) control the yellow phenotype, while the presence of either dominant gene without the other (i.e. A\_bb or aaB\_) produces the red phenotype. A third dominant gene, C, was suggested to control russet, however, only when the genes A and B are recessive. However, White and Lespinasse<sup>23</sup> changed this hypothesis and postulated that red fruit color was also determined by two complementary genes, A and B (i.e. genotype A\_B\_) with yellow fruit produced by A or B alone (i.e. genotypes A\_bb or aaB\_), and yellow or green phenotypes resulting from the expression of a homozygous recessive genotype. A third gene L was suggested to produce a blush when dominant and stripes when recessive. They indicated that blush and red color did not appear to assort independently, and proposed that a single dominant gene, L (the third gene), was linked to A or B. An A\_B\_L\_ genotype has red blush fruit; an A\_B\_ll genotype has red stripe fruit; and an aabll genotype has green or yellow fruit.

From an examination of previous reports on the occurrence of a high frequency of red fruited seedlings from yellow x yellow crosses, and a mutant of 'Golden Delicious' with red fruits, Lespinasse et al.<sup>14</sup> suggested that red is recessive and yellow dominant as proposed by Schmidt and Kruger<sup>18</sup>. If genes in yellow and green cultivars are dominant, the percentage of recessive combination (red fruited trees) in their F<sub>1</sub> should be extremely low, yet high frequency (30%) of red-colored F<sub>1</sub> progeny was obtained from the combination 'Guoshuai' (yellow) x 'White Winter Pearmain' (green), which is not in support of this hypothesis<sup>8</sup>.

On the basis of anthocyanin analysis in 'Red Delicious' apple peel by Sun and Francis<sup>19</sup>, Lespinasse et al.<sup>14</sup> proposed that three dominant genes A, B or C control anthocyanin synthesis. The presence of any gene A, B or C would involve one pigment: I (cyanidin-3-galactoside) or II (cyanidin-3-arabinoside) or III (cyanidin-7-arabinoside) and produce yellow fruit. The combination of any of these genes (A\_B\_cc, A\_bbC\_, aaB\_C\_) would involve two or three pigments and lead to the production of red fruit. Yellow-green or green fruit would be produced by the homozygous recessive genotype aabbcc, and they would not contain any of these three pigments. However, this hypothesis did not fit the segregation data observed by Ju et al.<sup>8</sup> and other studies from the earlier literature<sup>1</sup>. Ju et al.<sup>8</sup> found 34% of the F<sub>1</sub> trees from the cross 'Guoshuai' (yellow) x 'White Winter Pearmain' (green) bearing red fruit. This result was unexpected if the yellow cultivar contains only one dominant gene and there is no dominant gene in the green cultivar. There should also be no anthocyanin synthesis under any conditions if green fruit were the result of pure recessive genes. However, the two green cultivars ('Indo' and 'White Winter Pearmain') examined by Ju et al.<sup>8</sup> accumulated a certain amount of anthocyanin when harvest was delayed and the yellow cultivars also accumulated anthocyanin under such conditions. Although these studies provided important developments on genetic studies in anthocyanin reddening in apple fruit skin, the results were rather contradictory.

### Insights From Molecular Genetic Studies

Molecular markers can provide unambiguous evidence for the inheritance of traits. The study of Cheng et al.<sup>2</sup> based on co-dominant RAPD markers provided the strongest support for the hypothesis in the earlier literature that anthocyanin reddening in apple fruit skin was controlled by a single dominant gene<sup>1</sup>. Cheng et al.<sup>2</sup> found no indication of a modifier gene(s) that could reverse the effect of the locus,  $R_f$ , linked to the markers. A segregation ratio of 3 : 1, red and yellow, respectively, was obtained from the crosses 'Rome Beauty' x 'White Angel', 'Wijcik McIntosh' x NY 75441-23 and 'Esopus Spitzenburg' x NY 75441-67, indicating that both parents were heterozygous. The cross NY 489 x NY 61343-1 gave a 1:1 segregation ratio, indicating that one of the parents (NY 489) was homozygous yellow and the other (NY 61343-1) heterozygous.

Since the cultivars used as parents in the crosses for this study were of diverse genetic background, Cheng et al.<sup>2</sup> suggested that red skin color in domestic apple is primarily controlled by one or more genes at a single locus,  $R_f$ . The  $R_f$  locus was located within 2 cM of both the isozyme marker, *Idh-2* reported by Weeden et al.<sup>21</sup> and the DNA fragments generated by the universal primers described by Cheng et al.<sup>2</sup>. Chevreau et al.<sup>3</sup> identified isozyme markers linked to anthocyanin production in all tissues [flowers, fruit, foliage and bark] which segregated as a single dominant gene,  $R_f$ , in four apple crosses ('Fiesta' x '3762', 'Fiesta' x 'SA 572/2', 'Double Red Northern Spy' x 'SA 572/2' and 'Crimson Spy' x '3762') examined. Interestingly, the *Idh-2* locus which is linked to  $R_f$ <sup>21</sup> is also found in linkage group I where the  $R_i$  gene is located<sup>3</sup>, suggesting that the  $R_f$  and  $R_i$  genes are located in the same linkage group. Furthermore, an analysis of 56 cultivars and advanced breeding selections<sup>2</sup> further lends support to the single gene hypothesis as the DNA marker genotypes from the universal primers could be used to predict fruit skin color in most cases.

More recently, Wakasa et al.<sup>20</sup> used the set of universal PCR primers described by Cheng et al.<sup>2</sup> which amplified three DNA markers (A1, a1 and a2) linked to skin color in 'Jonathan', 'Narihokoh' and 'Golden Delicious', respectively. Wakasa et al.<sup>20</sup> found these marker genotypes to be correlated with the skin color as earlier reported<sup>2</sup>, with marker A1 co-segregating with red skin color and a1 and a2 associated with yellow skin color. The sequence information indicated that the a1 and a2 fragments are virtually identical to A1 (except for their respective insertions), and are alleles of the red skin gene,  $R_f$ <sup>2</sup>. This study further corroborates the report of Cheng et al.<sup>2</sup> for a single gene hypothesis for apple skin reddening.

### Limitations of The Hypothesis of a Monogenic Mode of Inheritance

Although molecular genetic studies<sup>2,20</sup> have provided the strongest support for a single dominant gene control for anthocyanin reddening in apple fruit skin, Cheng et al.<sup>2</sup> were neither able to predict the incidence of green-skinned phenotypes nor correlate the presence or absence of stripes on red-skinned fruit or with the intensity of anthocyanin pigmentation based on the DNA marker genotypes. They suggested that an entirely different mechanism, such as delayed

ripening response, could be involved in the production of green-skinned fruit. The appearance of a small fraction of trees with red fruit in a population produced from a yellow x yellow cross as has been earlier reported by Schmidt<sup>17</sup>, was attributed to a likely outcome of a low level of pollen contamination which is often observed in controlled crosses in apples<sup>2</sup>.

The hypothesis of a monogenic mode of inheritance<sup>2</sup> occurred in the study of Ju et al.<sup>8</sup> with a close fit segregation ratio of 1:1, red/yellow, from the cross 'Golden Delicious' (proposed genotype aa) x 'Giant Jeniton' (proposed genotype A). However, the great variation in coloration observed among red-fruited cultivars or among the red-fruited progeny (which might all be called red-fruited progeny) could not be resolved by this hypothesis<sup>8</sup>. For instance, there was a wide variation in the intensity of red coloration within the 50% of red-fruited trees obtained with about half of the trees having fruits with < 20% red color, one third of the trees having fruits with > 50% red colour, and one tree with fruit as red as the red cultivar 'Delicious'<sup>8</sup>. Moreover, red-fruited trees were also obtained in the F<sub>1</sub> progeny from the yellow x yellow cross. Although a low level of pollen contamination could explain the occurrence of colored trees in non-colored crosses<sup>17</sup>, Ju et al.<sup>8</sup> indicates that it is also possible that yellow cultivars like 'Golden Delicious' contains 'red' gene since its fruit also synthesized anthocyanin under certain conditions.

It is, however, worth noting the fact that the evaluation system used to categorize color differences in apples<sup>2</sup>, and insufficient definitions of anthocyanin expression or varying systems used by different authors<sup>17</sup>, could make it difficult to unambiguously assign a particular color class or compare published data from different authors. Ju et al.<sup>8</sup> also used small population sizes that further hindered their investigation.

Cheng et al.<sup>2</sup> and Ju et al.<sup>8</sup> concluded that anthocyanin synthesis in apples, like that in other plant systems, is most probably controlled by a family of regulatory genes which link tightly and their hereditary behavior follows the same pattern as one gene with red as dominant. Additional genetic studies and especially those based on the main pigment in apple skin, cyanidin-3-galactoside would be needed to provide insight into the inheritance of color in apple skin.

### Conclusions

Although apples fruit color has been the subject of genetic studies for over 8 decades, there has been much controversy among researchers. Most of the studies on the genetics of color formation in the apple fruit have also been inconclusive. A number of hypotheses have been proposed by many researchers, with a monogenic mode of inheritance being the most plausible. The strongest support for a monogenic mode of inheritance has also been provided by evidence from molecular genetic studies. However, the hypothesis of a monogenic mode of inheritance has yet to be confirmed in subsequent studies. The evaluation system used to categorize color differences in apples, insufficient definitions of anthocyanin expression or varying systems used by different authors, and small population sizes could confound interpretation of genetic data. Biochemical genetic studies especially based on the main pigment in apple

skin, cyanidin-3-galactoside, could provide further insights into the genetic basis of color in apples.

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