Mapping for Quantitative Trait Loci and Major Genes Associated with Fresh-cut Browning in Apple

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Abstract. Flesh browning is an important negative trait for quality preservation of fresh-cut fruits. To obtain a better understanding of the inheritance and genetic control of flesh browning in apple, the phenotype of a hybrid population derived from ‘Jonathan’ × ‘Golden Delicious’ was studied for 2 successive years. The inheritance of the flesh browning trait was analyzed by the frequency distribution of the phenotypes. Flesh browning-associated major genes were then mapped by screening genome-wide simple sequence repeat (SSR) markers. Flesh browning is inherited quantitatively and showed a clear bimodal frequency distribution, indicating that the segregation of major genes is involved in the variation. The segregation ratio of light and heavy browning was 7:1 in 2010, 2011, and 2010 + 2011, suggesting that the inheritance of the trait in apple involves three segregated loci of major genes. The heritability of the major gene effect was 72.14% and 72.76% in 2010 and 2011, respectively. SSR markers were screened from 600 pairs of SSR primers located on 17 apple linkage groups (LGs). The three major genes were mapped on LG10, 15, and 17 on the apple genome, respectively, by linkage analysis of flesh browning phenotypes and the genotypes of SSR markers. Two quantitative trait loci (QTLs) for flesh browning were mapped on LG15 of ‘Jonathan’ and LG17 of ‘Golden Delicious’, respectively, which are the same linkage groups that two major genes mapped on.

In the last decade, the consumer’s requirement for convenience fruits and vegetables such as fresh-cut apples has increasingly expanded. However, the shelf life and quality

preservation of fresh-cut fruits are challenging. One limiting factor is post-harvest flesh browning in many fruits, including apple.

The browning of fresh-cut apple mainly results from enzymatic browning of phenolic compounds, catalyzed by polyphenol oxidase (PPO) in the flesh tissue (Gil et al., 1998). Therefore, flesh browning is affected by many factors related to polyphenol oxidation. As a substrate of enzymatic browning, the content of phenolic compounds directly affects flesh browning. The main phenolic compounds causing apple browning are chlorogenic acid and flavan-3-alcohols (Amiot et al., 1992). Once oxidized, phenolic compounds turn into quinone and polymerize to produce brown,

red, and black pigments (Martínez and Whitaker, 1995). The activity of relevant enzymes also affects the rate of flesh browning. PPO activity is directly related to the rate of flesh browning in ‘Classic Delicious’, ‘Rhode Island Greening’, ‘McIntosh’, and other apple cultivars (Coseteng and Lee, 1987). In addition to polyphenols and PPO, ascorbic acid, like many other antioxidants in the cells, inhibits enzymatic browning, and its content is an important factor influencing apple browning (Nicolas et al., 1994).

Several techniques are currently used to control flesh browning, e.g., thermal treatment, low O₂–high CO₂ environment, and immersion with color-protection agents. Among these techniques, heat treatment can inactivate the PPO, thus reducing flesh browning. However, heat treatment also changes the structure of flesh tissue and damages some nutrients (Lamikanra, 2002). Low O₂ and high CO₂ environments can reduce the browning of fresh-cut apple slices (Gunes et al., 2001); a high CO₂ level has a negative impact on the physiological function of flesh tissue (Gorny et al., 2002). As color-protection agents, ascorbic acid and its derivatives as well as N-acetylcysteine, 4-hexylresorcinol, and calcium propionate can inhibit enzymatic browning in apple (Buta et al., 1999; Dorantes-Alvarez et al., 1998). However, chemical treatment increases the cost and may have potential food safety problems by inappropriate use or excessive residual of these agents. Therefore, selection of cultivars resistant to flesh browning is the best solution to reduce flesh browning and to maintain maximum fruit quality during fresh-cut apple processing.

Post-harvest flesh browning in apples varies significantly among cultivars. During fresh-cut apple processing, ‘Golden Delicious’, ‘Delicious’, and ‘Jonagold’ had a low rate of flesh browning, and ‘McIntosh’ and ‘Braeburn’ had a high rate of flesh browning (Kim et al., 1993; Luo and Barbosa-Cánovas, 1997). The rate of browning varies because of different levels of phenolic compounds in different cultivars of apple. Total content of phenolic compounds in ‘Golden Delicious’ were lower than in ‘Fuji’ and ‘Starkrimson’ (Nie and Sun, 2005).

The content and composition of phenolic compounds differ in 10 apple processing cultivars; four cider apple cultivars, Marie Menard, Frequin Rouge, Kermerrien, and Douce Coetlignel, and six juice cultivars, Judeline, Judaine, Judestar, Juliana, Jurella, and Granny Smith which were grown in the same orchard at Xiaojinkou, Shandong, China; whereas total phenolic compounds content in cider cultivars is significantly higher than that in juice cultivars (Song et al., 2007). In addition, the PPO activity varies with substrate, leading to changes in the rate of flesh browning in different apple cultivars. The PPO activity in ‘Starkrimson’ is higher than other cultivars when using chlorogenic acid as a substrate but is higher in ‘Orin’ when catechin is used as a substrate (Nie et al., 2004).

The flesh browning trait is thought to be inherited quantitatively, and is controlled by

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polygenes. Flesh browning-associated QTLs have been mapped in a hybrid population derived from 'Telamon' × 'Braeburn' by using the data collected in 2005, and two relevant major QTLs were located on LG3 and 17 of the apple genome (Kenis et al., 2008). The two major QTLs accounted for 66.2% and 76.9% of the total variation in this segregating population, respectively. However, the authors identified no QTLs in 2004, indicating that the results of QTL mapping were completely different in these 2 years. On the other hand, some QTLs identified from different hybrid population may differ apparently (Kenis et al., 2008; Kumar et al., 2012; Liebhard et al., 2003). QTLs with robustness between hybrid populations and consistency between years are more satisfactory for potential use in marker-assisted breeding.

Previously, the rate of flesh browning was determined to be higher in 'Jonathan' and lower in 'Golden Delicious' (Kim et al., 1993). In the present study, to further understand the inheritance of the apple flesh browning trait, phenotypes of seedlings in a segregating population from 'Jonathan' × 'Golden Delicious' were studied in 2010 and 2011. The data were analyzed by the frequency distribution of phenotypes and assisted by SSR marker-based major gene mapping and QTL mapping for flesh browning.

Materials and Methods

Plant material. The segregating population used in this study consisted of 1733 hybrid seedlings of 'Jonathan' × 'Golden Delicious' from the Changli Institute of Pomology, Hebei Academy of Agriculture and Forestry Sciences. The hybridization was made in 2002 and the seedlings on their own roots were transplanted at a density of 0.5 m × 2 m in 2003. The seedlings were maintained under conventional management and pest control. Ripened apple fruit, determined by the seeds becoming dark brown in color, was harvested from different positions of the canopy on each seedling for flesh browning scoring in 2010 and 2011.

Phenotyping for flesh browning. The experimental variation during phenotyping was estimated using analysis of variance (ANOVA) with 30 apples (with three replicates totally and 10 apples for each replicate) from each seedling fruiting in both years. However, because of alternate bearing, 429 seedlings in 2010 and 108 seedlings in 2011 set enough fruit for phenotyping, and 31 seedlings set fruit in both 2010 and 2011. Therefore, 30 seedlings, excluding one producing less than 30 apples, were used for ANOVA.

Fully ripened fruit were randomly collected from each seedling, and the flesh browning score was measured using an HP-200 colorimeter (Hanpu, Shanghai) following the method described by Wang et al. (2004). The apple was first cut into two equal halves along the longitudinal axis of the pedicel and calyx. The flesh surface of each half was exposed to the air for 40 min at room temperature to let

them brown base on the preliminary experiments. Each half fruit was then cut longitudinally into two equal pieces along the same axis; the newly cut surface is perpendicular to the previous one. The CIE coordinates, L^* (lightness), a^* (red–green), and b^* (yellow–blue), of fresh-cut surface and browning surface were measured for each piece of fruit using the HP-200 colorimeter. The flesh browning score was calculated using the formula $\Delta E^*a^*b^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{0.5}$ (Choi et al., 2002).

Analysis of inheritance. The inheritance of flesh browning was analyzed using Microsoft Excel 2003 (Microsoft Inc., Redmond, WA) by the frequency distribution of phenotype values collected in 2010 and 2011 as well as 2010 + 2011. The value of intersection point (if there was) of the two Gaussian curves in the distribution diagram was determined as the cutoff point for light and heavy browning. The individuals in which flesh browning score was less than the cutoff point were categorized as light browning and otherwise the individuals with browning scores larger than the cutoff value were treated as heavy browning. The segregation ratio between light and heavy browning was examined by a χ^2 test. Heritability of flesh browning was estimated using variance analysis in the segregating population following the method of Sun et al. (2012). The parental phenotype values of flesh browning were scored with 30 fruit from each maternal parent 'Jonathan' and paternal cultivar Golden Delicious. The environmental effect variance (Se) was presented as the mean value of the parental variances. Then, the mean values (Sm) of the internal variances of the two independent distributions were calculated. Theoretically, the average values (Sm) included polygene effect variance (Si) and environmental effect variance (Se) only. Thus, the equation was $Si = Sm - Se$. The major gene effect variance (Sa) was calculated using average phenotype values of two distributions with low and high flesh browning score. The interaction between the environment and genes was ignored because it is impossible to be estimated by this experimental design. Total population variance was $S = Sa + Si + Se$. Finally, the heritability of relevant major gene effect (Sa/S) and polygene effect (Si/S) was calculated.

Construction of the segregating population. According to the frequency distribution diagram of phenotypic values of flesh browning in 2010, randomly selected 100 seedlings with light-browning phenotype and all of the 50 seedlings with heavy-browning phenotype were used to construct the segregating population for linkage analysis of relevant major genes. Young leaves were sampled from each seedling of the segregating population in 2010. Total genomic DNA was extracted from young leaves using the modified CTAB method (Maguire et al., 1994). DNA quality was checked using 1% agarose electrophoresis gels. The concentration and purity of the DNA were determined using a ultraviolet spectrophotometer. The final DNA concentration was adjusted to 100 ng·μL⁻¹.

SSR genotyping. Six hundred pairs of primers were selected from previously published SSRs and expressed sequence tag (EST)–SSRs based on the apple EST library (Gianfranceschi et al., 1998; Guilford et al., 1997; Hokanson et al., 1998; Liebhard et al., 2002; Silfverberg-Dilworth et al., 2006; Zhang et al., 2012). Total genomic DNA of the two parents and eight randomly selected offspring seedlings were used as templates for screening primer polymorphisms.

SSR genotyping was performed with selected polymorphic primers in the segregating population comprising 150 seedlings. The polymerase chain reaction (PCR) amplification reaction (20 μL) contained 20 ng template DNA, 2.0 μL 10 × buffer, 200 μM each dNTP, 0.4 μM forward and reverse primers, and 0.4 U Taq DNA polymerase. The amplification program was: denaturation at 94 °C for 4 min; 32 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 40 s, and extension at 72 °C for 1 min; final extension at 72 °C for 5 min; and holding at 4 °C. The PCR products were separated using 8% polyacrylamide gel electrophoresis and then visualized by silver staining. The DNA ladder was "pucl8/Msp I" (RealTimer BioTech Co., Beijing, China).

Mapping for major genes and genotypes of the parents. In the segregating population, seedlings with light-browning phenotype were selected randomly and will thus have one or more gene alleles for light browning. In contrast, the seedlings with heavy-browning genotype will lack all the three dominant loci. So the markers that are present in most seedlings of light browning but are completely or almost absent in the seedlings with heavy-browning phenotype were selected. The polymorphic markers present at low frequency in the seedlings with heavy-browning phenotype were treated as recombinant between the targeted gene and linked markers.

Linkage between related genotypes of SSR markers and flesh browning phenotypes of all 50 seedlings with heavy-browning phenotype was analyzed using Mapmaker/EXP3.0 software (Lincoln et al., 1992). Map distances in centi Morgans (cM) were calculated from recombination frequencies by using Kosambi's mapping function, and the maximum distance and the likelihood of odd (LOD) threshold were set 37.2 cM and 3.0, respectively. The linkage mapping of the major gene loci involved in flesh browning was generated by MapDraw2.0 (Liu and Meng, 2003).

The parental genotypes were estimated according to the segregation ratio of the markers in the 100 seedlings with light-browning phenotype and the appearance of them in samples of the parents.

QTL analysis for flesh browning. The QTL analysis was carried on using the two parental maps constructed with 242 seedlings randomly selected from the same hybrid population (Zhang et al., 2012). The analysis software was MapQTL 6.0 (Van Ooijen et al., 2009). Two different models, interval

mapping and Kruskal-Wallis analysis, were used for QTL detection. For interval mapping, the genome-wide significant threshold according to the 1000 permutation tests was an LOD score of 2.8, but the chromosomal threshold was also considered to declare QTL locus (Xu et al., 2012). In Kruskal-Wallis analysis, QTL-associated markers were obtained at the *P* value of 0.01.

Results

Sample size for reliable phenotyping. The ANOVA of phenotypic values showed a highly significant variation in flesh browning score among apple seedlings ($F = 4.94$, $F_{0.01} = 2.42$), but not between years ($F = 3.59$, $F_{0.05} = 4.18$) and replicates ($F = 1.03$, $F_{0.05} = 3.33$). This indicated that the flesh browning trait segregated significantly in this hybrid population but was invariable between sampling years. Because the variation between replicates with 10 apples was not significant, 10 apples would be sufficient sample size for reliable phenotyping.

Analysis of frequency distribution. Parental phenotypic value of flesh browning was 5.68 for ‘Jonathan’ and 4.41 for ‘Golden Delicious’, but flesh browning segregated as 0.84 to 11.05 and 0.95 to 11.73 in 2010 and 2011, respectively. As shown in Figure 1, the frequency distribution diagrams of flesh browning were typically bimodal in both 2010 and 2011 as well as in the combined data of 2010 + 2011, indicating not only robustness over years, but also the involvement of major genes in the inheritance of this trait.

As listed in Table 1, the cutoff criteria of light and heavy browning were 5.70, 6.88, and 5.77 in 2010, 2011, and 2010 + 2011, respectively. The segregation ratios between light- and heavy-browning seedlings were 379:50, 91:17, and 435:71 in 2010, 2011, and 2010 + 2011, respectively. Results of a χ^2 test indicated that each of these ratios was significantly different from 3:1 and 15:1 but not different from 7:1. The segregation of one major gene locus (e.g., $Aa \times aa$) may result in half of offspring individuals with a recessive phenotype and the ratio of dominant vs. recessive phenotypes will be 1:1. The phenotypic segregation ratio of 7:1 is caused by the segregation of two or three major gene loci, e.g., $AaBb \times Aabb$ or $AaBbcc \times aabbCc$. The results therefore indicated that the variation in flesh browning was caused by the segregation of two or three independently segregating major genes, three in the case of different single parental loci (e.g., $AaBbcc \times aabbCc$) and two in case one locus was segregating in both parents (e.g., $AaBb \times Aabb$) and that light browning is dominant against heavy browning.

Heritability estimates. The heritability estimates of flesh browning in the hybrid population of ‘Jonathan’ $\times$ ‘Golden Delicious’ are listed in Table 2. The heritability of major gene effect was 72.14%, 72.76%, and 67.72% in 2010, 2011, and 2010 + 2011, respectively, whereas heritability of polygene effect was 13.23%, 15.32%, and 18.77% in 2010, 2011, and 2010 + 2011, respectively.

Fig. 1. Frequency distribution diagram of flesh browning in a hybrid population of ‘Jonathan’ $\times$ ‘Golden Delicious’. The three frequency distributions were generated from data in 2010, 2011, and 2010 + 2011, respectively.

Table 1. The cutoff point, segregation ratio, and χ^2 test of light and high heavy browning in the hybrid population of ‘Jonathan’ $\times$ ‘Golden Delicious’.

Yr	Cutoff point	Observed segregation ratio	Expected ratio	χ^2	$\chi^2_{0.05}$	$\chi^2_{0.01}$
2010	5.70	379:50	3:1	39.87 b ^a	3.84	6.64
			7:1	0.2 1	3.84	6.64
			15:1	20.48 b	3.84	6.64
2011	6.88	91:17	3:1	4.46 a	3.84	6.64
			7:1	0.76	3.84	6.64
			15:1	15.02 b	3.84	6.64
2010 + 2011	5.77	435:71	3:1	31.39 b	3.84	6.64
			7:1	1.02	3.84	6.64
			15:1	49.45 b	3.84	6.64

^aaP < 0.05; bP < 0.01.

Screening for microsatellite markers and major gene mapping. Of the 600 pairs of SSR and EST-SSR primers, 266 pairs of polymorphic primers were identified in the hybrid population. These polymorphic markers were well distributed on all of the 17 linkage groups with 10 to 27 on each. Any marker closely

linked to light browning, i.e., the specific electrophoretic band amplified with the polymorphic primers, was expected to be absent in all seedlings with heavy-browning phenotype. The 100 seedlings with light-browning phenotype were randomly chosen; therefore, the recessive phenotype was excluded, and the

Table 2. Heritability estimates of flesh browning in a hybrid population of ‘Jonathan’ × ‘Golden Delicious’.

Yr	Population variance (S)	Major gene variance (Sa)	Polygene variance (Si)	Environmental variance (Se)	Heritability of major gene effect (%)	Heritability of polygene effect (%)
2010	4.221	3.045	0.559	0.617	72.14	13.23
2011	5.180	3.769	0.794	0.617	72.76	15.32
2010 + 2011	4.569	3.094	0.858	0.617	67.72	18.77

Table 3. Microsatellite markers linked to the trait of light browning in hybrid population of ‘Jonathan’ × ‘Golden Delicious’.

Marker	Size of the polymorphic fragments (bp)	Jonathan	Golden Delicious	Observed segregation ratio	Expected ratio	χ^2	Linkage group	Recombination rate (%)	Map distance (cM)
Hi22f04	147	+	–	56:44	4:3	0.02	10	21.7	23.3
CH02a10	216	+	–	63:37	4:3	1.17	10	19.6	20.7
CH02b07	188	+	–	53:47	4:3	0.54	10	23.9	26.0
CN911379	275	+	–	54:46	4:3	0.29	15	10.9	11.0
CH03b01	425	+	–	49:51	4:3	2.39	15	21.7	23.3
AJ001681-SSR	187	–	+	55:45	4:3	0.11	17	10.9	11.0
AT000174-SSR	186	–	+	48:52	4:3	3.05	17	10.9	11.0
CH04c06	186	–	+	58:42	4:3	0.01	17	23.9	26.0

$\chi^2_{0.05} = 3.84$.

ratio of presence/absence of the specific bands should be 4:3 or 6:1 theoretically. In cases in which the marker was not absent in a few seedlings with heavy-browning phenotype, the marker-present seedlings were regarded as genetic recombinants.

Eight SSR markers (Hi22f04, CH02a10, CH02b07, CN911379, CH03b01, AJ001681-SSR, AT000174-SSR, and CH04c06) of 266 pairs of primers were unambiguously identified as being linked to the light-browning trait (Table 3). The segregation ratio of all eight markers in the 100 seedlings with light-browning phenotype was 4:3. Markers Hi22f04, CH02a10, CH02b07, CN911379, and CH03b01 appeared in the maternal parent ‘Jonathan’, whereas AJ001681-SSR, AT000174-SSR, and CH04c06 appeared in the pollen parent ‘Golden Delicious’ (Table 3).

Markers Hi22f04, CH02a10, and CH02b07 were located on LG10 (Silfverberg-Dilworth et al., 2006). Their PCR amplification yielded three polymorphic fragments of 147 bp, 216 bp, and 188bp, respectively. Of the 46 seedlings (four seedlings were suspected as outbreeders and were excluded) with heavy-browning phenotype, 10, nine, and 11 seedlings appeared as recombinants and the recombination rate of the markers Hi22f04, CH02a10, and CH02b07 was 21.7%, 19.6%, and 23.9%, respectively. Thus, the map distances between the light-browning major gene locus and markers Hi22f04, CH02a10, and CH02b07 were estimated as 23.3, 20.7, and 26.0 cM, respectively. Based on the distance (44 cM) between Hi22f04 and CH02a10, also the distance (15.8 cM) between CH02a10 and CH02b07 on the linkage map of Discovery (Silfverberg-Dilworth et al., 2006), Hi22f04, and CH02a10 putatively flanked the target gene B_{10} (B: dominant locus for the light browning; subscript number: linkage group) and CH02b07 located at the same side with CH02a10 (Fig. 2).

Both the markers CN911379 and CH03b01 were located on LG15 according to Zhang et al. (2012). CN911379 and CH03b01 generated specific fragments of 275 bp and 425 bp, respectively. Five and 10 seedlings appeared as

Fig. 2. Mapping of light browning-related major genes in a hybrid population of ‘Jonathan’ × ‘Golden Delicious’. Numbers on the left of each linkage group (LG) indicate the map distance (cM). Bold letters on the right of each LG indicate major gene loci and associated markers of flesh browning. J = ‘Jonathan’; G = ‘Golden Delicious’ and following number means the linkage group on apple genome.

recombinants in the 46 seedlings of the heavy-browning population, and the recombination rate was 10.9% and 21.7% for CN911379 and CH03b01, respectively. The map distances for the two markers CN911379 and CH03b01 were 11.0 and 23.3 cM, respectively. The distance between these two markers was calculated as 18.8 cM using Mapmaker software. Thus, CN911379 and CH03b01 were postulated to be located at the same side of the target gene B_{15} (Fig. 2).

Markers AJ001681-SSR, AT000174-SSR, and CH04c06 on LG17 yielded three specific fragments of 187 bp, 186 bp, and 186 bp, respectively. Of the 46 seedlings of the heavy-browning population, five, five, and 11 recombinant seedlings contributed to 10.9%, 10.9%, and 23.9% of the recombination and the map distances between the light-browning major gene locus and markers AJ001681-SSR, AT000174-SSR, and CH04c06 were estimated as 11.0, 11.0, and 26.0 cM, respectively. The map distance between AJ001681-SSR and CH04c06 was calculated 16.6 cM using Mapmaker software, and the distance between AJ001681-SSR and AT000174-SSR was only 4.1 cM. Thus, AJ001681-SSR, AT000174-SSR, and CH04c06 were mapped on the same side of the target gene B_{17} (Fig. 2).

Summarizing the results of inheritance and major gene mapping for apple flesh

browning in this hybrid population, the genotype of maternal parent, ‘Jonathan’, was presumed to be $B_{10}b_{10}B_{15}b_{15}b_{17}b_{17}$ and that of paternal parent, ‘Golden Delicious’, was $b_{10}b_{10}b_{15}b_{15}B_{17}b_{17}$ (B: dominant locus for the light browning; b: recessive locus for the heavy browning; subscript number: linkage group).

Identification of QTLs for flesh browning.

By interval mapping model using the genome-wide significant threshold LOD 2.8, no QTL was detected for flesh browning. However, using the LG-specific LOD threshold, two minor QTLs, qtl1 and qtl2, were mapped on LG15 of ‘Jonathan’ and LG17 of ‘Golden Delicious’, respectively, using the fruit fresh-cut browning phenotyping data in 2010. The peak LOD value of qtl1 was 1.75, which was higher than the LG-specific LOD threshold value (1.7); qtl1 contributed with 11.6% of variance explanation. The peak of qtl1 was associated with marker CN911379 ($K = 6.482$ and $P < 0.01$) based on the Kruskal-Wallis analyses. Because there was a gap on the LG17 linkage map of ‘Golden Delicious’ (Zhang et al., 2012), qtl2 was identified on the part that only has two SSR markers NZmsEB137525 and CN938125 with an LG-specific LOD threshold value of 1.1. The QTL was located in the 11-cM interval between these two markers supported with a high peak

Table 4. Quantitative trait loci (QTLs) for fresh-cut browning detected by interval mapping and Kruskal-Wallis in 2010 in the hybrid population of 'Jonathan' × 'Golden Delicious'.

QTL	LG	Interval mapping			Kruskal-Wallis analysis			
		Peak Position (cM)	LOD/LG-specific	LOD threshold	Variance explained (%)	Marker	Position (cM)	Significance levels
qtl1	J15 ²	108.02	1.75/1.7		11.6	CN911379	108.02	6.482
qtl2	G17	3.00	1.85/1.1		14.0	NZmsEB137525	0.00	7.880
						CN938125	11.91	7.447
								0.005

²J15: linkage group 15 of 'Jonathan'; G17: linkage group 17 of 'Golden Delicious'.

LOD = likelihood of odd; LG = linkage group.

LOD value of 1.85 and explaining 14.0% phenotypic variation. According to the Kruskal-Wallis analysis, both NZmsEB137525 and CN938125 linked with qtl2 ($K = 7.880$, 7.447 and $P < 0.001$, 0.005 , respectively) (Table 4). As a result of alternate bearing between years, only 12 of the 242 mapping populations set fruit in 2011; no QTLs were identified successfully.

Discussion

Environmental effects have more influence on QTLs, or even major QTLs, but relatively less influence on the qualitative/quantitative traits involving major genes. Two major QTLs associating the rate of apple flesh browning were identified in a hybrid population of 'Telamon' × 'Braeburn' in 2005 (Kenis et al., 2008). However, the major QTLs were not stable from year to year, because neither of the two flesh browning-associated major QTLs were detected in 2004. In addition, only one of the 17 major QTLs for several fruit quality traits including fruit size and weight, flesh firmness, acidity and sugar content, and harvest date was year-stable (Kenis et al., 2008). Flesh browning is mostly caused by the oxidation of phenolic compounds. Both the content and antioxidant capacity of phenolic compounds are influenced by multiple factors such as maturity, temperature, and light (Gómez et al., 2010; Marta et al., 2011; Murata et al., 1995). This may explain the difference in QTLs for flesh browning previously reported by Kenis et al. (2008). Major gene segregation and polygenetic effects were detected in flesh browning in the present hybrid population, and the heritability of the major gene effect reached 72.14% and 72.76% in 2010 and 2011, respectively. These suggested that major genes contributed more to the variation in flesh browning in our hybrid population, and a good robustness of the segregation ratio of major genes was confirmed between years.

Besides quantitative loci, major genes can also be identified by QTL analysis (Soufflet-Freslon et al., 2008). In our case, two QTLs related to fruit fresh-cut browning were mapped using phenotyping data in 2010. Although we could not define them as major QTLs because the contribution to phenotypic variation was lower than 20% (Mellidou et al., 2012), the results of the QTL were perfectly coordinated with that of major gene mapping. qtl1 and major gene B15 were both associated with marker CN911379. Both qtl2 and major gene B17 were mapped on LG17 of 'Golden Delicious'.

PPO activity is controlled by polygenes in many plants such as wheat, tomatoes, and apples (Boss et al., 1995; Demeke and Morris, 2002; Newman et al., 1993). The major gene that controls wheat PPO activity has been identified on chromosome 2D (Tigst et al., 2001). In the hybrid population of 'Telamon' × 'Braeburn', a normal distribution in the ascorbic acid content implied a quantitative genetic control of ascorbate in apple. Several QTLs associated with ascorbic acid content have been identified on LG6, 10, and 11 in the apple genome (Davey et al., 2006). The QTL identified by Davey and colleagues on LG10 (LOD 8.4) could explain 28.3% of total phenotypic variation, and the marker for this QTL, CH03d11, is only 11 cM from our marker for the light browning-associated major gene, Hi22f04, according to the published map (Silfverberg-Dilworth et al., 2006). Oxidation of polyphenols and polyphenol concentrations were closely related to flesh browning (Amiot et al., 1992; Coseteng and Lee, 1987). In apple cultivar Braeburn, QTL for p-coumaroyl quinic acid in fruit cortex has been mapped on LG15, associated with single nucleotide polymorphism markers GDSNP01265 and GDSNP00932 (Chagne et al., 2012). The distance between the marker CN911379 for qtl1 in our results and the two markers, GDSNP01265 and GDSNP00932, was not longer than 5 Mb on the apple genome. A previous study indicated that one of the QTLs for the rate of flesh browning in the 'Telamon' × 'Braeburn' population is located on LG17 of 'Telamon'. This QTL accounts for 66.2% phenotypic variation and is a major QTL (Kenis et al., 2008). It may be related to the major gene locus B₁₇ in the present study.

Molecular marker-assisted breeding has been successfully used in apple, e.g., powdery mildew resistance and fireblight resistance (Evans and James, 2003; Khan et al., 2007). Marker-assisted inheritance analysis has been attempted for traits such as fruit shape index and ring rot susceptibility (Sun et al., 2012; Zhuang et al., 2011). In this study, we identified three major gene loci associated with flesh browning, which indicated that the trait of flesh browning is controlled by three major genes in this hybrid population. However, the map distances between the markers and the major gene loci in this report are all larger than 10 cM, and further study is required to reduce the map distance before their application in marker-assisted breeding practice.

In conclusion, the apple flesh browning is inherited quantitatively, and the inheritance involves three segregating loci of major genes. The three major genes were mapped on LG10,

15, and 17, but two minor QTLs were detected on the LG15 and LG17 on the apple genome, respectively, by using SSR markers.

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