Differences in the CaMYBA Genome Between Anthocyanin-Pigmented Cultivars and Non-pigmented Cultivars in Pepper (Capsicum annuum)

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Anthocyanin in pepper is beneficial as a food antioxidant compound and as a pigment for ornamentals, while unexpected anthocyanin accumulation in fruit, known as black spots, reduces the commercial quality of some cultivars. Previous studies demonstrated that the Anthocyanin (A) locus determines the anthocyanin accumulation in pepper fruits, and an MYB transcription factor, CaMYBA, was found to be located near the A locus. However, the causal gene sequence of the A locus has not yet been identified. With progress regarding genome information in pepper, two other homologous MYB genes were found to be located near CaMYBA, and they are also considered to be candidate genes for the A locus. In this study, we attempted to identify the causal gene sequence of the A locus by performing linkage analysis, genomic sequence analysis, and gene expression analysis of the three candidate MYB genes. A crossing experiment between pigmented ‘Peruvian Purple’ and non-pigmented cultivars confirmed that anthocyanin accumulation in the pigmented cultivar was controlled by a single locus. Gene expression analysis demonstrated that a basic helix-loop-helix transcription factor, CaMYC, and CaMYBA were expressed abundantly in pigmented cultivars, but the other two MYB genes were not. Genotyping of the F2 population derived from the cross demonstrated that the anthocyanin accumulation phenotype was highly linked to CaMYBA, but not to CaMYC. The DNA sequence of CaMYBA in pigmented cultivars had an insertion of a 4.3 kb retrotransposable element LINE-1 in the first intron, but that of non-pigmented cultivars did not. No pigmented cultivar-specific sequence was found in the promoter region of CaMYBA. Therefore, it was suggested that CaMYBA, but not the other two homologous MYB genes, is the A locus gene, and insertion of LINE-1 in CaMYBA appeared to be important for the regulation of anthocyanin accumulation, although the mechanism by which the LINE-1 insertion induces CaMYBA expression is unknown.

Key Words: delphinidin, LINE-1 retrotransposable element, MYB transcription factor.

Introduction

Peppers (Capsicum) belong to the Solanaceae family and originated in South and Central America. Generally, immature fruits of C. annuum are consumed as vegetables and mature fruits are consumed as spices all over the world. In addition, some Capsicum plants have purple flowers, foliage, and colourful fruits, and are used as ornamental plants.

The purple pigment of pepper is anthocyanin, a flavonoid compound, and its chemical structure was reported as delphinidin-3-(4-p-coumaroyl)-rutinoside-5-glucoside (Lightbourn et al., 2008). Anthocyanin accumulation in pepper is beneficial in terms of antioxidant capacity when consumed or as a pigment for ornamentals. However, unexpected anthocyanin accumulation in fruit causes black spots and could be a problem by reducing the commercial quality of some cultivars.

Anthocyanin is one of the most studied secondary metabolites in plants. In the flavonoid biosynthetic pathway, anthocyanin is synthesized from two substrate compounds, malonyl-CoA, and p-coumaroyl-CoA, by chalcone synthase (CHS), chalcone isomerase (CHI), flavanone 3-hydroxylase (F3H), dihydroflavonol 4-reductase (DFR), anthocyanidin synthase (ANS), and flavonoid UDP-glucosyltransferase (Tanaka et al., 2008). Although the regulatory mechanisms depend on the species, the expression of some enzyme genes are
regulated by transcription factors such as MYB, a basic helix-loop-helix (bHLH), and WD repeats (WDR) (Koes et al., 2005). Anthocyanin biosynthesis in pepper is controlled by mainly one locus known as the Anthocyanin (A) locus, which is an incomplete dominant gene for anthocyanin color in the foliage, flowers and immature fruit (Wang and Bosland, 2006). This A locus was mapped to chromosome 10 (Chaim et al., 2003), and afterwards mapped near to an MYB transcription factor (CaMYBA) that is orthologous to PhAN2 in petunia (Petunia hybrida) (Borovsky et al., 2004).

There are many reports analysing anthocyanin biosynthetic genes in pepper (Aza-González et al., 2013; Li et al., 2011; Lightbourn et al., 2007), but the causal gene sequence for anthocyanin pigmentation has not yet been identified. Recent reports in petunia and potato (Solanum tuberosum) indicate that several paralogous MYB genes (PhAN2, PhAN4, PhPHZ, and PhDPL in petunia, Stan1, StMYBA1, and StMYB113 in potato) can regulate anthocyanin biosynthesis (Albert et al., 2011; Liu et al., 2016). Genomic DNA sequences of several pepper cultivars, ‘Criollos de Morelos 334’ (CM334) (Kim et al., 2014), ‘Zunla-1’, Chiltepin (C. annuum var. glabriusculum) (Qin et al., 2014), and UCD10X (Hulse-Kemp et al., 2018), suggested that three MYB genes, including CaMYBA, are located near each other on the same chromosome. For example, in CM334 (version 1.55), CaMYBA, CaMYBB, and CaPHZ were allocated as CA10g11650 (Chr10 182955325..182956589), CA10g11690 (Chr10 184012261..184013487), and CA10g11710 (Chr10 184898196..184899719), respectively. Therefore, not only CaMYBA, but also the two homologous MYB genes, are considered as candidate genes for the A locus. In this study, we performed linkage analysis, genomic DNA sequence analysis and gene expression analysis of the three candidate MYB genes to identify the A locus gene sequence in anthocyanin-pigmented cultivars and non-pigmented cultivars of pepper.

Materials and Methods

Plant materials

C. annuum anthocyanin-pigmented cultivars ‘Peruvian Purple’, ‘Murasaki’, and ‘Purple Flash’, and non-pigmented cultivars ‘Takanotsume’, ‘Nikko’, and ‘Sapporo-Onaga’ were used for the experiments (Fig. 1A–F). For genomic PCR, anthocyanin-pigmented cultivars ‘Conga’ and ‘Nishiki-sango’, and non-pigmented cultivars ‘Shishihomare’ and ‘Manganji’ were also used. All plants were grown in the field or greenhouses (heated to keep the minimum temperature > 5°C from November to April) of the experimental farm at Kyoto University (Kyoto, Japan). For pot cultivation, plants were grown in 24-cm pots filled with mixed soil (akadama soil:leaf mold = 7:3) and IB Kasei (JCAM AGRI. CO., LTD., Tokyo, Japan) (N:P:K = 10:10:10) was used as a fertilizer.

HPLC analysis

The pigment compositions of flowers and fruits were measured by high-performance liquid chromatography (HPLC). The HPLC analysis process was according to Ohno et al. (2011). Delphinidin chloride (Nagara Science, Gifu, Japan) was used as a standard anthocyanin.

Crossing experiment

‘Peruvian Purple’ was crossed with ‘Takanotsume’ or ‘Nikko’, and then the F1 and F2 generations were produced. Segregation of pigmentation traits in flowers and fruits was recorded in both generations and they were used for genotyping.

Gene expression analysis

Total RNA was extracted from flowers and fruit peels using Sepasol RNA I Super G (Nacalai Tesque, Kyoto, Japan), and purified by precipitation with a High-salt solution (Takara Bio Inc., Shiga, Japan). It was reverse transcribed with ReverTra Ace (TOYOBO Co., Ltd., Osaka, Japan), and 1 μL of the resultant cDNA was diluted 10-fold and used as a template for semi-quantitative RT-PCR. Semi-quantitative RT-PCR
was performed with Blend Taq polymerase (TOYOBO) using the primers shown in Supplemental Table S1. The PCR program was set at 94°C for 2 min, followed by 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 1.5–2.5 min.

**Genotyping analysis of CaMYBA and CaMYC in the ‘Peruvian Purple’ × ‘Takanotsume’ populations**

To genotype the CaMYBA allele, PCR-RFLP was performed. The CaMYBA of ‘Takanotsume’ has a HaeIII restriction enzyme digestion site in the fourth exon (GGCC), but ‘Peruvian Purple’ does not (GACC). PCR was performed using Blend Taq polymerase (TOYOBO) using the primers Forward: 5'-GAAGCTATAACTGCTCCTCATCGA-3' and Reverse: 5'-CTTTTGATGGCTGTGTCCTTTGC-3'. Then, the PCR product was digested with HaeIII (Takara Bio) for 1 hr at 37°C and electrophoresed. As a result, the band of the CaMYBA allele from ‘Peruvian Purple’ was 615 bp, while the bands of the CaMYBA allele from ‘Takanotsume’ were 426 bp and 189 bp. To genotype the CaMYC allele, a PCR length polymorphism in the promoter region was used. ‘Peruvian Purple’ has two repeats of 119 bp sequence, while ‘Takanotsume’ has only one 116 bp sequence. PCR was performed using Blend Taq polymerase (TOYOBO) using the primers (Forward: 5'-CGTAGTAAGGAGTGTAAACTTTGAT-3' and Reverse: 5'-AATAGCTCATACACATGAACCTTTTTCATG-3') and electrophoresed. The PCR program for genotyping was set at 94°C for 2 min, followed by 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 1.5–2 min.

**Genomic analysis of CaMYBA**

Genomic DNA was extracted from leaves using MagExtractor Plant Genome (TOYOBO). To clone the full length CaMYBA genome, the PCR product was amplified with Primer-Forward: 5'-GAAGCTATAACTGCTCCTCATCGA-3' and Primer-Reverse: 5'-CTTTTGATGGCTGTGTCCTTTGC-3' using Blend Taq polymerase (TOYOBO) and cloned into a pTAC-1 vector (BioDynamics Laboratory Inc., Tokyo, Japan). The PCR program was set at 94°C for 2 min, followed by 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 3 min. To clone the insertion sequence in the CaMYBA genome, the PCR product amplified with Forward: 5'-AAAAGGACGTGCTGCTTATTAGCCTAAATCT-3' and Reverse: 5'-AGAAAAGCTGTGTTAATAGCATTTCATGAT-3' using a KOD FX Neo polymerase (TOYOBO) was cloned into a pDONR221 vector using Gateway™ BP Clonase™ II Enzyme mix (Invitrogen, Carlsbad, CA, USA). The PCR program was set at 94°C for 2 min, followed by 35 cycles of 98°C for 10 s, 55°C for 30 s, and 68°C for 8 min. To clone the promoter region, two primer sets (Forward: 5'-CACATGAGATGATAGCTCCATG-3' and Reverse: 5'-GGGACGTGCTGCTTATTAGCCTAATCT-3', Forward: 5'-AATAACCAACCGTTCATCATATCT-3' and Reverse: 5'-CTTATAAGAGCTGTGTCCTTTGC-3') were designed to amplify overlapping regions based on the genome sequence of ‘CM334’. Primers used for sequencing are listed in Supplemental Table S2. For genomic PCR of CaMYBA among different cultivars, PCR was performed with the above conditions using a KOD FX Neo polymerase (TOYOBO) with primers (Forward: 5'-CGGAAGGAGGTAGTCAATCAAAT-3' and Reverse: 5'-AATAGCAGTATTCAATGAT-3'). For genomic PCR of CaMYBA among ‘Peruvian Purple’ × ‘Nikko’ F2 populations, PCR was performed with the above conditions except the extension time was changed to 3 min using KOD FX Neo polymerase (TOYOBO) with primers (Forward: 5'-ACCTTACAAATATAAACAATT-3' and Reverse: 5'-AATAGCAGTATTCAATGAT-3'). The accession number of the ‘Peruvian Purple’ CaMYBA genome, including the promoter region, is deposited as LC473089.

**Results**

**Pigmentation locus of ‘Peruvian Purple’**

From HPLC analysis, pigmented cultivars accumulated delphinidin-based anthocyanin in their flowers and fruits, whereas non-pigmented cultivars did not accumulate any anthocyanins (Table 1).

In order to confirm that the pigmentation locus was identical to the A locus, ‘Peruvian Purple’, which has purple foliage, purple flowers and purple fruits was crossed with ‘Takanotsume’ or ‘Nikko’. As for flower color, all F1 plants of ‘Peruvian Purple’ × ‘Takanotsume’ had bicolor flowers (Fig. 1G), suggesting incomplete dominance of the pigmentation gene. Pigmentation in the F2 generation of ‘Peruvian Purple’ × ‘Takanotsume’ was found to be Purple:Bicolor:White = 35:87:47 (χ2 = 0.97, P > 0.05) (Fig. 1H–J; Table 2). The segregation ratio was not significantly different from 1:2:1, suggesting one locus is associated with pigmentation of these parent lines. Similar results were obtained from a crossing between ‘Peruvian Purple’ and ‘Nikko’. All F1 plants had bicolor flowers (Fig. 1K), and pigmentation in the F2 generation was Purple:Bicolor:White = 35:51:20 (χ2 = 4.40, P > 0.05) (Fig. 1L–N; Table 2). As for fruit color, all F1 plants had purple pigmentation and F2 plants had Purple pig-

| Cultivar    | Flower | Fruit |
|-------------|--------|-------|
| Peruvian Purple | +      | +     |
| Murasaki     | +      | +     |
| Purple Flash | +      | +     |
| Takanotsume  | -      | -     |
| Nikko        | -      | -     |
| Sapporo-Onaga| -      | -     |

+: Abundant, -: Not detected

| Cultivar     | Flower | Fruit |
|--------------|--------|-------|
| Peruvian Purple | +      | +     |
| Murasaki      | +      | +     |
| Purple Flash  | +      | +     |
| Takanotsume   | -      | -     |
| Nikko         | -      | -     |
| Sapporo-Onaga | -      | -     |

+: Abundant, -: Not detected
Flavonoid biosynthetic gene expression analysis

Semi-quantitative RT-PCR was performed for gene expression analysis. In flowers, as for enzymatic genes compared to pigmented cultivars, CaF3'5'H, CaDFR, and CaANS were down-regulated in non-pigmented cultivars (Fig. 2A). Downregulation of multiple structural enzyme genes suggested the involvement of transcription factors. We analyzed three MYB genes (CaMYBA, CaPHZ, and CaMYBB), two bHLH transcription factors (CaMYC and CaJAF13) and one WDR transcription factor (CaWDR), and only CaMYBA and CaMYC were down-regulated in non-pigmented cultivars (Fig. 2A). Similar data was obtained from fruits (Fig. 2B). These data suggested that CaPHZ and CaMYBB were not important for pigmentation of the cultivars used in this experiment, and that CaMYBA or CaMYC could be the anthocyanin determinant locus.

Genomic segregation analysis of CaMYBA and CaMYC

The genomic location of CaMYBA and CaMYC was analyzed using the pepper genome database. This indicated that CaMYBA is located on chromosome 10, while CaMYC is located on chromosome 9. Using ‘Peruvian Purple’ and ‘Takanotsume’ F2 populations exhibiting different pigmentation, PCR segregation analysis was performed. For CaMYC, PCR was performed using a sequence length polymorphism found in the promoter region between ‘Peruvian Purple’ and ‘Takanotsume’. When PCR was performed, however, no correlation was found between the genotype of CaMYC and anthocyanin pigmentation (Fig. 3), suggesting that CaMYC is not the determinant factor for pigmentation. For CaMYBA, PCR-RFLP was performed using a single sequence polymorphism found in the fourth exon between ‘Peruvian Purple’ and ‘Takanotsume’. When PCR-RFLP was performed, the genotype of CaMYBA and pigmentation phenotype were totally co-segregated (Fig. 3). Considering the results of semi-quantitative RT-PCR and this segregation analysis, CaMYBA is most likely to be the A locus, as reported previously (Borovsky et al., 2004).

Table 2. Phenotypic segregation of anthocyanin pigmentation in flowers.

| Parental cultivar and cross combination | Population size | Number of plants | Expected ratio (Purple:Bicolor:White) | Chi-square (P-value) |
|----------------------------------------|----------------|-----------------|-------------------------------------|---------------------|
| P1: Peruvian Purple                    | 10             | 10              | 0                                   |                     |
| P2: Takanotsume                        | 10             | 0               | 0                                   |                     |
| P3: Nikko                              | 10             | 0               | 0                                   |                     |
| F1: (P1×P2)                            | 8              | 0               | 8                                   | 0:1:0               |
| F1: (P1×P3)                            | 8              | 0               | 8                                   | 0:1:0               |
| F2: (P1×P2)                            | 172            | 38              | 87                                 | 1:2:1               | χ² = 4.40 (P = 0.15) |
| F2: (P1×P3)                            | 106            | 35              | 51                                 | 1:2:1               |                     |

Table 3. Phenotypic segregation of anthocyanin pigmentation in fruits.

| Parental cultivar and cross combination | Population size | Number of plants | Expected ratio (Purple:Green) | Chi-square (P-value) |
|----------------------------------------|----------------|-----------------|-------------------------------|---------------------|
| P1: Peruvian Purple                    | 10             | 10              | 0                             |                     |
| P2: Takanotsume                        | 10             | 0               | 0                             |                     |
| P3: Nikko                              | 10             | 0               | 0                             |                     |
| F1: (P1×P2)                            | 8              | 8               | 0                             | 1:0                 |
| F1: (P1×P3)                            | 8              | 8               | 0                             | 1:0                 |
| F2: (P1×P2)                            | 172            | 118             | 54                            | 3:1                 | χ² = 3.75 (P = 0.053) |
| F2: (P1×P3)                            | 106            | 81              | 25                            | 3:1                 | χ² = 0.11 (P = 0.74)  |
first intron, long-PCR was performed. We could amplify an approximately 4.3 kb longer sequence in pigmented cultivars than non-pigmentation cultivars (Fig. 4B), and this insertion sequence of ‘Peruvian Purple’ was cloned and sequenced. The full length of this insertion sequence was 4321 bp, and it contained an open reading frame for sense orientation and encoding a 907 amino acid polypeptide with a non-LTR (long terminal repeat) retrotransposon LINE-1 endonuclease domain (cd09076) and a non-LTR retrotransposon reverse transcriptase domain (cd01650) (Fig. 4A), which are typical of LINE L1 retrotransposons (Wicker et al., 2007). This polypeptide sequence shared high identity (53%) with *Zea mays* Retrovirus-related Pol polyprotein LINE-1 (ONM52836). We could detect an 18 bp (TGTTGTTTTAATTTTGGA) target site duplication sequence, but no terminal inverted repeat-like sequence was found. To confirm the relationship between the
LINE-1 insertion in CaMYBA and anthocyanin pigmentation, genomic PCR in the ‘Peruvian Purple’ × ‘Nikko’ F₂ population was performed. We could detect a longer band from ‘Peruvian Purple’ and F₂ populations that had purple flowers, a short band from ‘Nikko’ and F₂ populations that had white flowers, and both bands from F₁ and F₂ populations that had bicolor flowers (Fig. 4C).

To investigate whether there is a polymorphism in the CaMYBA promoter region specific to pigmented cultivars, we isolated and compared a region of approximately 1300 bp upstream from the putative transcription start site of the CaMYBA gene among six cultivars; however, there were no sequence polymorphisms specific to pigmented cultivars (Supplemental Fig. S1). Therefore, it was suggested that this LINE-1 insertion is important for the pigmentation allele of CaMYBA.

**Discussion**

Anthocyanin accumulation in pepper was reported to be determined by the A locus, and this locus was tightly mapped to CaMYBA (Borovsky et al., 2004). However, it remained unclear whether the causal gene of anthocyanin pigmentation in pepper was CaMYBA because the draft genome of pepper ‘CM334’ indicates that there are at least two homologous MYB genes (CaMYBB and CaPHZ) that are located near CaMYBA on chromosome 10. It was reported that four homologous MYB genes (PhAN2, PhAN4, PhDPL, and PhPHZ) in petunia, and three homologous MYB genes (StAN1, StMYBA1, and StMYB113) in potato regulate anthocyanin biosynthesis (Albert et al., 2011; Liu et al., 2016). A sequence comparison indicated that CaMYBA, CaMYBB, and CaPHZ are orthologous to StMYB113, StAN1, and StMYBA1, respectively (Supplemental Fig. S2). Therefore, it was considered that not only CaMYBA, but also CaMYBB and CaPHZ, were candidate genes for the A locus. From semi-quantitative RT-PCR, expressions of CaMYBB and CaPHZ were not detected from pigmented cultivars, while abundant expression of CaMYBA was detected from pigmented cultivars (Fig. 2). Therefore, it was suggested that CaMYBB and CaPHZ were not the determinant factors of anthocyanin biosynthesis, and that CaMYBA is a strong candidate for the A locus.

Previously, virus-induced gene silencing (VIGS) of the CaMYBA gene induced down-regulation of several enzyme genes (Aguilar-Barragán and Ochoa-Alejo, 2014; Zhang et al., 2015) and CaMYC (Zhang et al., 2015). However, VIGS of CaMYC reduced the expression levels of several enzyme genes, but not CaMYBA (Lu et al., 2019). This suggested that CaMYBA regulated expression of not only flavonoid biosynthetic enzyme genes, but also CaMYC, similar to the overexpression of the potato MYB gene StAN1 in tobacco, which induces endogenous bHLH NtAN1a and NtAN1b (Liu et al., 2016). In pigmented cultivars, expression levels of both CaMYBA and CaMYC were up-regulated in flowers (Fig. 2A) and fruit peels (Fig. 2B); however, only the CaMYBA genotype was linked to the pigmentation (Fig. 3), suggesting that CaMYBA functions upstream of CaMYC.

Several reports have mentioned the role of retrotransposable elements in anthocyanin biosynthesis. In grape (Vitis vinifera), a Ty3-gypsy-type retrotransposon Gret1 inserted in the 5’-flanking region of VvmybA blocks VvmybA expression (Kobayashi et al., 2004). In Sicilian blood orange ‘Tarocco’ (Citrus sinensis), a Copia-like retrotransposon activator of anthocyanin production, controls fruit-specific and cold-inducible Ruby expression (Butelli et al., 2012). In this case, the start of Ruby transcription lies within the LTR of Tcs1, indicating that the LTR sequence functions as a promoter and transcription start site. In apple (Malus domestica), a gypsy-type retrotransposon insertion upstream of MdMYB1, a core transcriptional activator of anthocyanin biosynthesis, is associated with a red-skinned phenotype (Zhang et al., 2019). Here, all pigmented cultivars and purple flower plants of the ‘Peruvian Purple’ × ‘Nikko’ F₂ population had a LINE-1 insertion in the first intron of CaMYBA, while all non-pigmented cultivars and white flower plants of the ‘Peruvian Purple’ × ‘Nikko’ F₂ population did not (Fig. 4B, C). The sequence of the CaMYBA promoter region was almost the same among cultivars tested in this study (Supplemental Fig. S1), indicating the difference in expression level of CaMYBA is due to a LINE-1 insertion in the first intron. Thus, two possibilities can be considered for the A locus: one is that CaMYBA is identical to the A locus, and the insertion of LINE-1 into the first intron induces CaMYBA expression. The other is that LINE-1 insertion is not related to the induction of CaMYBA expression and the A locus encodes another transcription factor which regulates CaMYBA expression. In the latter case, the gene must be located close to CaMYBA because the CaMYBA genotype perfectly coincided with the pigmentation phenotype (Figs. 3 and 4; Borovsky et al., 2004). Although, the latter possibility cannot be excluded, the former hypothesis is the more likely. As for the former possibility, the question arises as to how the LINE-1 inserted allele can express more CaMYBA than the non-inserted allele, despite the fact that both alleles contain the full coding region. The possibility that a LINE-1 insertion created a novel promoter is excluded because expression of the first exon of CaMYBA was detected (Supplemental Fig. S3). It is known that intron-mediated enhancement of gene expression exists (Gallegos and Rose, 2015). As a result, it is possible that the insertion promoted CaMYBA expression. The mechanism by which LINE-1 insertion in the first intron affects CaMYBA expression needs to be elucidated.
Literature Cited

Aguilar-Barragán, A. and N. Ochoa-Alejo. 2014. Virus-induced silencing of MYB and WD40 transcription factor genes affects the accumulation of anthocyanins in chili pepper. Biol. Plant. 58: 567–574.

Albert, N. W., D. H. Lewis, H. Zhang, K. E. Schwinn, P. E. Jameson and K. M. Davies. 2011. Members of an R2R3-MYB transcription factor family in Petunia are developmentally and environmentally regulated to control complex floral and vegetative pigmentation patterning. Plant J. 65: 771–784.

Aza-González, C., L. Herrera-Isidrón, H. G. Núñez-Palenius, O. M. De La Vega and N. Ochoa-Alejo. 2013. Anthocyanin accumulation and expression analysis of biosynthesis-related genes during chili pepper fruit development. Biol. Plant. 57: 49–55.

Borovsky, Y., M. Oren-Shamir, R. Ovadia, W. D. Jong and I. Paran. 2004. The A locus that controls anthocyanin accumulation in pepper encodes a MYB transcription factor homologous to Anthocyanin2 of Petunia. Theor. Appl. Genet. 109: 23–29.

Butelli, E., C. Licciardello, Y. Zhang, J. Liu, S. Mackay, P. Bailey, G. Reforgiato Recupero and C. Martin. 2012. Retrotransposons control fruit-specific, cold-dependent accumulation of anthocyanins in blood oranges. Plant Cell 24: 1242–1255.

Chaim, A. B., Y. Borovsky, W. De Jong and I. Paran. 2003. Linkage of the A locus for the presence of anthocyanin and fs10.1, a major fruit-shape QTL in pepper. Theor. Appl. Genet. 106: 889–894.

Gallegos, J. E. and A. B. Rose. 2015. The enduring mystery of intron-mediated enhancement. Plant Sci. 237: 8–15.

Hulse-Kemp, A. M., S. Maheshwari, K. Stoffel, T. A. Hill, D. Jaffe, S. R. Williams, N. Weisenfeld, S. Ramakrishnan, V. Kumar, P. Shah, M. C. Schatz, D. M. Church and A. Van Deynze. 2018. Reference quality assembly of the 3.5-Gb genome of Capsicum annuum from a single linked-read library. Hortic. Res. 5: 4.

Kim, S., M. Park, S. I. Yeom, Y. M. Kim, J. M. Lee, H. A. Lee, E. Seo, J. Choi, K. Cheong, K. T. Kim, K. Jung, G. W. Lee, S. K. Oh, C. Bae, S. B. Kim, H. Y. Lee, S. Y. Kim, M. S. Kim, B. C. Kang, Y. D. Jo, H. B. Yang, H. J. Jeong, W. H. Kang, J. K. Kwon, C. Shin, Z. Y. Lim, J. H. Park, J. S. Huh, J. S. Kim, B. D. Kim, O. Cohen, I. Paran, M. C. Suh, S. B. Lee, Y. K. Kim, Y. Shin, S. J. Noh, J. Park, Y. S. Seo, Y. Kwon, H. A. Kim, J. M. Park, H. J. Kim, S. B. Choi, P. W. Bosland, G. Reeves, S. H. Jo, B. W. Lee, H. T. Cho, H. S. Choi, M. S. Lee, Y. Yu, Y. D. Choi, B. S. Park, A. Deynze, H. Ashrati, T. Hill, W. T. Kim, H. S. Pai, H. K. Ahn, I. Yeam, J. J. Giovannoni, J. C. Rose, I. Sörensen, S. J. Lee, R. W. Kim, I. Y. Choi, B. S. Choi, J. S. Lim, Y. H. Lee and D. Choi. 2014. Genome sequence of the hot pepper provides insights into the evolution of pungency in Capsicum species. Nat. Genet. 46: 270–278.

Kobayashi, S., N. Goto-Yamamoto and H. Hirochika. 2004. Retrotransposon-induced mutations in grape skin color. Science 304: 982.

Koes, R., W. Verweij and F. Quattrochio. 2005. Flavonoids: a colorful model for the regulation and evolution of biochemical pathways. Trends Plant Sci. 10: 236–242.

Li, J. G., H. L. Li and S. Q. Peng. 2011. Three R2R3 MYB transcription factor genes from Capsicum annuum showing differential expression during fruit ripening. Afr. J. Biotechnol. 10: 8267–8274.

Lightbourn, G. J., R. J. Griesbach, J. A. Novotny, B. A. Clevendence, D. D. Rao and R. J. Stommel. 2008. Effects of anthocyanin and carotenoid combinations on foliage and immature fruit color of Capsicum annuum. L. J. Heredity 99: 105–111.

Lightbourn, G. J., J. R. Stommel and R. J. Griesbach. 2007. Epistatic interactions influencing anthocyanin gene expression in Capsicum annuum. Am. Soc. Hort. Sci. 132: 824–829.

Liu, Y., K. Lin-Wang, R. V. Espley, L. Wang, H. Yang, B. Yu, A. Dare, E. Varkonyi-Gasic, J. Wang, J. Zhang, D. Wang and C. A. Allan. 2016. Functional diversification of the potato R2R3 MYB anthocyanin activators AN1, MYBA1, and MYB113 and their interaction with basic helix-loop-helix cofactors. J. Exp. Bot. 67: 2159–2176.

Lu, B. Y., G. X. Cheng, Z. Zhang, J. T. Sun, M. Ali, Q. L. Jia, L. De-Xu, Z. H. Gong and D. W. Li. 2019. CaMYC, a novel transcription factor, regulates anthocyanin biosynthesis in color-leaved pepper (Capsicum annuum L.). J. Plant Growth Reg. 38: 574–585.

Ohno, S., M. Hosokawa, M. Kojima, Y. Kitamura, A. Hoshino, F. Tatsuazawa, M. Doi and S. Yazawa. 2011. Simultaneous post-transcriptional gene silencing of two different chalcone synthase genes resulting in pure white flowers in the octoploid dahlia. Planta 234: 945–958.

Qin, C., C. Yu, Y. Shen, X. Fang, L. Chen, J. Min, J. Cheng, S. Zhao, M. Xu, Y. Luo, Y. Yang, Z. Wu, L. Mao, H. Wu, C. L. Hu, H. Zhou, H. Lin, S. González-Morales, D. L. Trejo-Saavedra, H. Tian, X. Tang, M. Zhao, H. Zhang, A. Zhou, X. Yao, J. Cui, W. Li, Z. Chen, Y. Feng, Y. Niu, S. Bi, X. Yang, W. Li, H. Cai, X. Luo, S. Montes-Hernández, M. A. Leyva-González, Z. Xiong, X. He, L. Bai, S. Tan, X. Tang, D. Liu, J. Liu, S. Zhang, M. Chen, L. Zhang, L. Zhang, Y. Zhang, W. Liao, Y. Zhang, M. Wang, X. Lv, B. Wen, H. Liu, H. Luan, Y. Zhang, S. Yang, X. Wang, J. Xu, X. Li, S. Li, J. Wang, A. Palloix, P. W. Bosland, Y. Li, A. Krogh, R. F. Rivera-Bustamante, L. Herrera-Estrella, Y. Yin, J. Yu, K. Hu and Z. Zhang. 2014. Whole-genome sequencing of cultivated and wild peppers provides insights into Capsicum domestication and specialization. Proc. Nat. Amer. Soc. USA 111: 5135–5140.

Tanaka, Y., N. Sasaki and A. Ohmiya. 2008. Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids. Plant J. 54: 733–749.

Wang, D. and P. W. Bosland. 2006. The genes of Capsicum. HortScience 41: 1169–1187.

Wicker, T., F. Sabot, A. Hua-Van, J. L. Bennetzen, P. Capy, B. Chalhoub, A. Flavell, P. Leroy, M. Morgante, O. Panaud, E. Saavedra, H. Tian, X. Tang, D. Wang, Y. Tian, G. Liu, H. Gul, D. Wang, Y. Tian, C. Yang, M. Meng, G. Yuan, G. Kang, Y. Wu, K. Wang, H. Zhang, D. Wang and P. Cong. 2019. A unified classification system for eukaryotic transposable elements. Nat. Rev. Genet. 8: 973–982.

Zhang, L., J. Hu, X. Han, J. Li, Y. Gao, C. M. Richards, C. Zhang, Y. Tian, G. Liu, H. Gul, D. Wang, Y. Tian, C. Yang, M. Meng, G. Yuan, G. Kang, Y. Wu, K. Wang, H. Zhang, D. Wang and P. Cong. 2019. A high-quality apple genome assembly reveals the association of a retrotransposon and red fruit colour. Nat. Commun. 10: 1494.

Zhang, Z., D. W. Li, J. H. Jin, Y. X. Yin, H. X. Zhang, W. G. Chai and Z. H. Gong. 2015. VIGS approach reveals the modulation of anthocyanin biosynthetic genes by CaMYB in chili pepper leaves. Front. Plant Sci. 6: 500.