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Research Paper

A genomic region harboring the *Pl1* allele from the Peruvian cultivar JC072A confers purple cob on Japanese flint corn (*Zea mays* L.)

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Purple corn is a maize variety (*Zea mays* L.) with high anthocyanin content. When purple corn is used as forage, its anthocyanins may mitigate oxidative stresses causing lower milk production in dairy cows. In this study, we analyzed quantitative trait loci (QTLs) for anthocyanin pigmentation of maize organs in an F_2 population derived from a cross between the Peruvian cultivar 'JC072A' (purple) and the inbred line 'Ki68' (yellowish) belonged to Japanese flint. We detected 17 significant QTLs on chromosomes 1–3, 6, and 10. Because the cob accounts for most of the fresh weight of the plant ear, we focused on a significant QTL for purple cob on chromosome 6. This QTL also conferred pigmentation of anther, spikelet, leaf sheath, culm, and bract leaf, and was confirmed by using two F_3 populations. The gene *Pl1* (*purple plant 1*) is the most likely candidate gene in this QTL region because the amino acid sequence encoded by *Pl1-JC072A* is similar to that of an Andean allele, *Pl-bol3*, which is responsible for anthocyanin production. The markers designed for the *Pl1* alleles will be useful for the breeding of F_1 lines with anthocyanin pigmentation in cobs.

Key Words: maize, Peruvian cultivar, Japanese flint cultivar, anthocyanin pigmentation, QTL analysis, *purple plant 1* (*Pl1*).

Introduction

Maize (*Zea mays* L.) is grown worldwide as a cereal and fodder crop, and grain color variants (white, yellow, and purple) are also used to extract colorants in food and beverages. Purple corn is originated from the species "Kculli", still cultivated in Peru (Grobman 1961). Its color is determined by anthocyanins, which are used to color jellies and syrups and as a health supplement in Japan (Aoki *et al.* 2002). Anthocyanins from purple corn have high antioxidant activity (Cevallos-Casals and Cisneros-Zevallos 2003), inhibit colorectal carcinogenesis in male rats (Hagiwara *et al.* 2001), reduce blood pressure in male spontaneously hypertensive rats (Shindo *et al.* 2007) and reduce AST (aspartate aminotransferase) activity and an increase SOD (superoxide dismutase) activity in lactating dairy cows (Hosoda *et al.* 2012). Then, anthocyanins from purple corn may mitigate oxidative stresses in dairy cows.

In purple cobs, the anthocyanin content ranges from 30% to 47% of total phenolic components (Jing *et al.* 2007), and the accumulation of anthocyanins are increased by drought, low temperature, visible light, and UV radiation (Chalker-Scott 1999). The regulatory genes *R*, *B*, *C1*, and *Pl* are responsible for anthocyanin synthesis; by sequence similarity, they are grouped into two families, *R/B* and *C1/Pl* (Chandler *et al.* 1989, Cone *et al.* 1986). The interaction of each gene of these families changes the temporal and spatial pattern of anthocyanin pigmentation.

The *R* and *B* genes encode proteins homologous to the basic-helix-loop-helix (*bHLH*) DNA-binding–protein dimerization domain of Myc oncoproteins (Chandler *et al.* 1989, Consonni *et al.* 1993, Ludwig *et al.* 1989). The *C1* and *Pl* genes encode R2R3 MYB transcription factors (Cone *et al.* 1986, 1993a, 1993b, Paz-Ares *et al.* 1986). Anthocyanin pigmentation of maize organs is controlled by at least 20 loci, comprising genes encoding DNA-binding-protein and transcriptional factors (Mol *et al.* 1998). It is necessary to understand the genetic architecture responsible for anthocyanin synthesis in breeding materials.

A program to breed Japanese elite flint cultivars with

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anthocyanin pigmentation has been designed to mitigate the oxidative stress causing the lower milk production in dairy cows. We used an unpigmented inbred line, 'Ki68' belongs to Japanese flint (Tamaki *et al.* 2014), and an anthocyanin-containing cultivar, 'JC072A', in the breeding program. 'Ki68' has green bract leaf, culm, and leaf sheath and white cobs, whereas 'JC072A' shows them with dark purple. Using F_2 progeny of a cross between 'Ki68' and 'JC072A', we identified QTLs for anthocyanin synthesis and elucidated the genetic mechanisms that contribute to anthocyanin pigmentation.

Materials and Methods

Plant materials and pigmentation grading

The F_2 and F_3 populations were established from a cross between the inbred line 'Ki68' belonged to Japanese flint and the Peruvian cultivar 'JC072A'. Anthocyanin pigmentation was assessed at the time of self-pollination (anther, spikelet, and silk), at the kernel dent stage (leaf sheath, culm, midrib of leaf blade, prop root, and bract leaf), and at the physiological matured stage (top and dorsal sides of grain, and glumes of cob). Anthocyanin pigmentation was graded from 1 (absent or very weak) to 9 (very strong).

Genotyping of F_2 and F_3 populations

Genomic DNA of 116 F_2 individuals was extracted by CTAB method and PCR was carried out for indel amplification at 148 loci with (1) SSR markers available at MaizeGDB (http://www.maizegdb.org/), (2) markers designed on the basis of BAC sequences with linkage mapping information (**Supplemental Table 1**), and (3) a *Pl1* marker designed as described below. PCR was performed in a 384-well thermal cycler (Applied Biosystems, Foster City, CA, USA) with KAPA2G Fast Ready Mix (Kapabiosystems, Boston, MA, USA) as follows: 95°C for 1 min; 35 cycles at 95°C for 10 s, 55°C for 10 s, and 72°C for 1 s; and a final 72°C for 30 s. PCR products were electrophoresed in 3% agarose gels in TBE (Tris–borate–EDTA) buffer. Gels were stained with ethidium bromide and the banding patterns were photographed under ultraviolet light for genotyping. Linkage mapping and QTL analysis were performed as described by Takai *et al.* (2012).

To validate the QTL for cob anthocyanin pigmentation on chromosome 6, we selected two F_3 populations: F3-46 (67 plants) and F3-82 (70 plants). These populations were derived from F_2 individuals with heterozygous alleles at the significant QTL region on chromosome 6 and homozygous 'JC072A' alleles at the QTL region with a high LOD peak on chromosome 10 (**Supplemental Fig. 1**). Genotypes and anthocyanin pigmentation of F3-46 and F3-82 were compared.

Isolation of Pl1 *and marker development*

Genomic DNA was extracted from 'Ki68' and 'JC072A' seedlings. *Pl1* was isolated using the primers pl1myb_s_86 and pl1myb_as_87 (**Supplemental Table 2**). The *Pl1* fragments amplified from 'Ki68' and 'JC072A' were purified with ExoSAP-IT (Affymetrix, Santa Clara, CA, USA) according to the manufacturer's instructions. The purified PCR products were directly sequenced on an ABI PRIME 3130xl genetic analyzer (Applied Biosystems) with primers newly designed on the basis of the determined nucleotide sequences: pl1_389-s, pl1_34-s, pl1myb_696-s, pl1_355-s, pl1myb_361-as, pl1_720-as, pl1_407-as, and pl1_495-as (**Supplemental Table 2**). The nucleotide sequences of the *Pl1* alleles of 'JC072A' and 'Ki68' were assembled from the sequenced fragments and were registered in DDBJ (LC384358 and LC384359, respectively). To distinguish between the *Pl1* alleles from 'Ki68' and 'JC072A', we performed fragment analysis with pl1_389-s and pl1_720-as primers as described by Yonemaru *et al.* (2009).

Phylogenetic analysis of Pl1

Known *Pl1* (*Pl*) sequences (Cocciolone and Cone 1993, Cone *et al.* 1993b, Pilu *et al.* 2003) were obtained from GenBank/EMBL/DDBJ/NCBI: L19496 (*Pl-Tx303*), L19494 (*Pl-Rh*), Q41842 (*Pl-Bh*), AY135018 (*Pl-bol3*), AY135019 (*Pl-W22*), and L19495 (*Pl-McC*). Amino acid sequences were aligned with CLUSTALW 2.1 software (Larkin *et al.* 2007). Phylogenetic analysis was conducted by the Neighbor Joining method in MEGA 7.0 software (Kumar *et al.* 2016).

Results

Detection of QTLs for anthocyanin pigmentation in maize organs

Significant QTLs were detected in 5 regions of chromosomes 1–3, 6, and 10; they affected anthocyanin pigmentation in 10 of the organs assayed (**Table 1**). No significant QTLs were found for pigmentation of the dorsal side of the grain. The effect of the QTL on chromosome 1 (*qACprr1*) was detected only in prop roots [LOD score (LOD) 4.2; additive effect (AE) 0.6]. The effects of significant QTLs on chromosome 2 (48.9–61.6 cM) were detected in spikelets (*qACspk2*), leaf sheaths (*qAClsh2*), culms (*qACcul2*), and bract leaves (*qACbrl2*). The values of LOD (10.5–13.6) and AE (2.0–2.3) were similar in these 4 organs. We only found the QTL on chromosome 3 for spikelets (*qACspk3*); it had the lowest values of phenotypic variance explained among all detected QTLs (**Table 1**).

The QTL region on chromosome 6 (92.7–107.8 cM) significantly affected most organs: anther (*qACant6*), spikelet (*qACspk6*), leaf sheath (*qAClsh6*), culm (*qACcul6*), prop root (*qACprr6*), bract leaf (*qACbrl6*), glumes of cob (*qACglc6*), and the top side of grain (*qACtsg6*). Among all QTLs, *qACtsg6* had the highest LOD (30.8) and *qACglc6* had the highest AE (2.7).

The QTL region on chromosome 10 (73.4–81.5 cM) significantly affected pigmentation of anthers (*qACant10*), silks (*qACsil10*), and midrib of leaf blade (*qACmlb10*). The

Table 1. QTLs for anthocyanin pigmentation of maize organs

Organ	QTL Name	Chr.	Marker interval		LOD score	Additive effect[a]	Dominant effect[a]	% PVE[b]	Bin	No. of listed genes per Bin	Candidate genes
Anther	*qACant6*	6	92.7	107.8	4.9	0.8	0.5	3.4	6.04	40	*pl1*, *sm1*
Anther	*qACant10*	10	73.7	76.1	19.8	–1.9	0.1	52.0	10.06	51	*lc1*, *lcm1*, *Nc1*, *Nc2*, *Nc3*, *p*, *r1*, *S*, *Sc*, *sn1*
Spikelet	*qACspk2*	2	49.9	59.7	13.6	2.1	1.0	16.8	2.02–2.04	42, 35, 53	*b1*
Spikelet	*qACspk3*	3	19.5	30.7	4.9	–0.5	–1.0	0.0	3.01–3.04	25, 21, 16, 62	no candidates
Spikelet	*qACspk6*	6	93.7	101.8	12.4	1.5	0.7	14.6	6.04	40	*pl1*, *sm1*
Silk	*qACsil10*	10	73.4	80.0	18.4	–0.6	0.0	40.6	10.06	51	*lc1*, *lcm1*, *Nc1*, *Nc2*, *Nc3*, *p*, *r1*, *S*, *Sc*, *sn1*
Leaf sheath	*qAClsh2*	2	49.4	60.2	10.5	2.3	0.3	23.8	2.02–2.04	42, 35, 53	*b1*
Leaf sheath	*qAClsh6*	6	96.0	102.5	7.3	1.4	0.4	10.1	6.04	40	*pl1*, *sm1*
Culm	*qACcul2*	2	48.9	59.5	11.0	2.0	0.8	9.6	2.02–2.04	42, 35, 53	*b1*
Culm	*qACcul6*	6	93.2	103.8	8.1	1.6	0.3	12.5	6.04	40	*pl1*, *sm1*
Midrib of leaf blade	*qACmlb10*	10	73.5	81.5	5.3	0.4	0.0	15.0	10.06	51	*lc1*, *lcm1*, *Nc1*, *Nc2*, *Nc3*, *p*, *r1*, *S*, *Sc*, *sn1*
Prop root	*qACprr1*	1	181.3	193.2	4.2	0.6	–0.2	12.7	1.07–1.09	79, 69, 69	*bz2*
Prop root	*qACprr6*	6	92.9	101.4	7.8	0.9	0.4	9.9	6.04	40	*pl1*, *sm1*
Bract leaf	*qACbrl2*	2	49.9	61.6	12.1	2.2	1.4	12.3	2.02–2.04	42, 35, 53	*b1*
Bract leaf	*qACbrl6*	6	94.1	102.7	10.8	1.6	1.1	7.9	6.04	40	*pl1*, *sm1*
Glumes of cob	*qACglc6*	6	94.3	100.2	22.2	2.7	2.8	7.1	6.04	40	*pl1*, *sm1*
Top side of grain	*qACtsg6*	6	95.7	100.2	30.8	2.6	2.4	11.7	6.04	40	*pl1*, *sm1*

[a] Positive values show that the JC072A allele increases values.
[b] Percentage of phenotypic variance explained.

AE values of *qACant10* (–1.9) and *qACsil10* (–0.6) and that of *qACmlb10* had opposite signs (0.4). In this region, no significant LOD peaks were observed for glumes of cob or grains (**Supplemental Fig. 1**).

Development of the Pl1 *marker and association between* Pl1 *alleles and cob anthocyanin pigmentation*

Because the cob accounts for most of the fresh weight of the plant ear, we focused on cob pigmentation. To avoid the interaction of the QTL on chromosome 10 with *qACglc6*, we selected two F_3 populations (F3-46 and F3-82) for the validation of *qACglc6* with the same alleles in the QTL region on chromosome 10 as in 'JC072A'. In maize, *Pl1* on chromosome 6 is required for anthocyanin synthesis in dark purple cobs (Hollick *et al.* 1995), implying that the functional *Pl1* allele is responsible for anthocyanin pigmentation in 'JC072A'. To detect the association between *qACglc6* and *Pl1*, we developed a PCR marker (primers pl1_389-s and pl1_720-as). The segregation of the *Pl1* genotype coincided with that of cob anthocyanin pigmentation at a 3:1 ratio in both F_3 populations (consistency of 0.94 and 0.91, respectively; **Fig. 1**). These results confirm that the *Pl1* gene on chromosome 6 is responsible for anthocyanin synthesis in cobs.

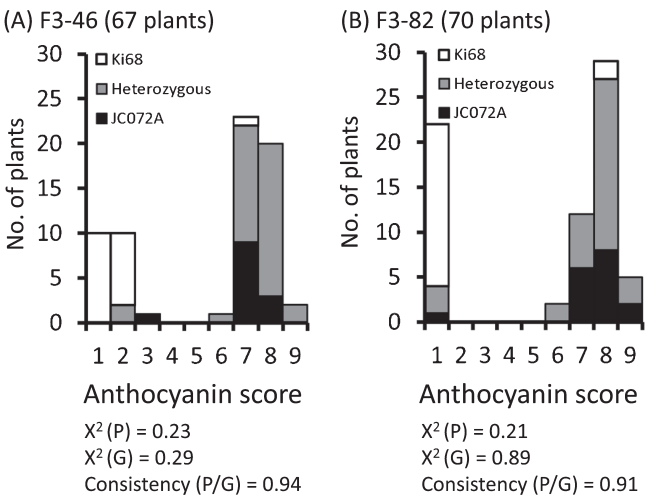

Fig. 1. Relationship between cob anthocyanin score and *Pl1* genotype in two F_3 populations. The degree of anthocyanin pigmentation of cobs in F_3-46 (67 plants) and F_3-82 (70 plants) ranged from 1 (absent or weak) to 9 (very strong). Chi-squared tests assuming a 3:1 ratio were conducted on segregation of the phenotype ($x \leq 3$, non-colored; $X \geq 6$, colored) and *Pl1* genotype (Ki68-type allele, non-colored; JC072A-type and heterozygous, colored). χ^2 (P), chi-squared *P* value of the phenotype; χ^2 (G), chi-squared *P* value of the genotype; consistency (P/G), consistency ratio between phenotype and genotype.

Comparison of Pl1 *sequences between 'JC072A' and 'Ki68'*

We used the reported nucleotide sequences of *Pl1* (Cone *et al.* 1993b) to obtain the sequences of *Pl1* of 'Ki68' and 'JC072A' and designated the alleles *Pl1-Ki68* and *Pl1-JC072A*, respectively. The comparison between the amplified fragments of *Pl1-Ki68* (1014 bp) and *Pl1-JC072A* (1030 bp) showed that *Pl1-Ki68* contained 2 SNPs in exon 1, 6 SNPs, and a 18-bp deletion in exon 3, 3-bp and 2-bp insertions in intron 1, and a 3-bp deletion in intron 2 (**Fig. 2**, **Supplemental Fig. 2**).

We aligned the deduced Pl1 amino sequences from 'Ki68' and 'JC072A' with those encoded by *Pl-Rh*, *Pl-McC*, *Pl-Tx303*, *Pl-Bh*, *Pl-bol3*, and *Pl-W22* (**Fig. 3**). *Pl1-Ki68* and *Pl-Tx303* differed by substitutions of 2 amino acid residues and deletion of 1 residue in *Pl-Tx303* (**Supplemental**

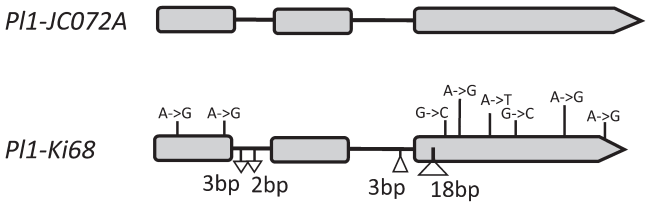

Fig. 2. Comparison of *Pl1* gene structures between 'JC072A' and 'Ki68'. Boxes, exons; horizontal bars, introns. The positions of insertions (▽), deletions (△), and single nucleotide polymorphisms in 'Ki68' are shown. The sequence alignment is shown in **Supplemental Fig. 2**.

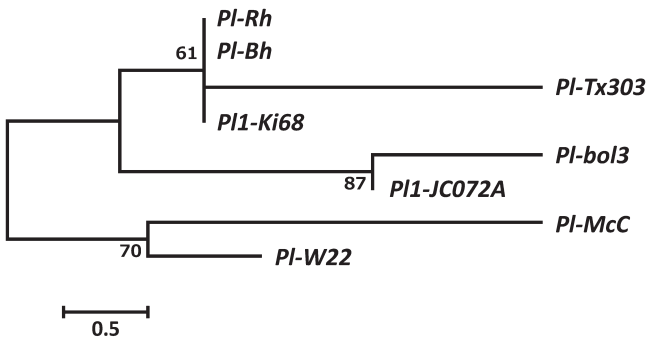

Fig. 3. Phylogenetic analysis of *Pl1* (*Pl*) based on amino acid sequences. Numbers indicate the bootstrap confidence values from 1000 replicates.

Fig. 3). *Pl1-JC072A* and *Pl-bol3* were of the same length with an amino acid substitution (**Supplemental Fig. 3**). In *Pl-McC* and *Pl-W22*, 3 residues around amino acid 190 were deleted and a residue was inserted relative to those of *Pl-TX303* and *Pl1-Ki68*; the cobs of these cultivars are white. To check whether the identified *Pl1* alleles mapped to chromosome 6, we designed a pair of primers for the *Pl1* marker based on the identified nucleotide sequences of 'Ki68' and 'JC072A'. Mapping and QTL analysis showed that *Pl1* is located on chromosome 6, as previously reported, and detected a large-effect QTL at the *Pl1* locus (**Supplemental Fig. 4**).

Discussion

In the F_2 population, we detected 17 QTLs for anthocyanin pigmentation on chromosomes 1–3, 6, and 10 (**Table 1**). For 6 out of 10 organs, at least 2 QTLs were found, one on chromosome 6 (92.7–107.8 cM) and the other(s) on other chromosomes. These 6 combinations confirmed that anthocyanin pigmentation is controlled by the interaction of two different members, *R/B* and *C1/Pl* (**Table 1**).

Four QTLs (*qACspk2*, *qAClsh2*, *qACcul2*, and *qACbl2*) detected on chromosome 2 (48.9–61.6 cM) were located in bins 2.02–2.04. Out of 130 genes listed in these bins, only *b1* is associated with anthocyanin synthesis (**Table 1**); dominant *B1* alleles determine anthocyanin synthesis in most aboveground plant parts (Chandler *et al.* 1989). Two major dominant *B1* alleles, *B-I* and *B-Peru*, have been reported; *B-I* determines pigmentation of spikelet, leaf sheath, culm, and bract leaf (Chandler *et al.* 1989, Radicella *et al.* 1992), whereas *B-Peru* determines pale color in these organs but strong purple aleurone as well as a transcriptional regulator of *R1* gene encoding bHLH protein. Although not significant, a QTL for anthocyanin pigmentation in both cob and grain was detected at a region that included *R1* on chromosome 10 (**Supplemental Fig. 1**). The significant QTLs (*qACant10*, *qACsil10*, *qACmlb10*) on chromosome 10 (73.4–81.5 cM) were located in bin 10.06. Among 51 genes listed in this bin, 10 are associated with anthocyanin synthesis (**Table 1**) and are related to the *R1* gene members of which occur as at least two tightly linked genes (Eggleston *et al.* 1995, Ludwig and Wessler 1990). Four *R1* gene family haplotypes—*R-g*, *R-st*, *R-sc* and *lc*—result in colored seed, but our QTLs coincided with colorless anthers (Petroni *et al.* 2000, Tonelli *et al.* 1994). The *R1* allele in the 'JC072A' haplotype may be any one of these four alleles.

Purple corn has a high content of anthocyanins, which are important natural colorants. Whole cobs contain about 55% of the anthocyanins and whole kernels contain about 45% (Cevallos-Casals and Cisneros-Zevallos 2003). Purple cobs have the highest contents of monomeric and acylated anthocyanins, which are considered natural food colorants. Only the QTL region on chromosome 6 (92.7–102.7 cM) located in bin 6.04 could result in cob and grain pigmentation. Among 40 genes listed in bin 6.04, *pl1* and *sm1* are functional genes for anthocyanin pigmentation (**Table 1**), but *sm1* does not confer cob and grain pigmentation (McMullen *et al.* 2004). Several alleles of *pl* (*pl1*) genes have been reported. The light-independent dominant allele *Pl-Rhoades* (*Pl-Rh*) contains a transposable CACTA element in the promoter and results in anthocyanin-rich seedlings with dark purple husks, culms, sheaths, tassels, and cobs (Cone *et al.* 1993b). A cultivar with unpigmented seedlings derived from McClintock's stock (McC) and inbred line Tx303 respectively carry the light-dependent recessive alleles *Pl-McC* and *Pl-Tx303*. The expression of the *Pl-bol3* allele is partially light independent, and *Pl-bol3* is active mainly at the juvenile phase (Pilu *et al.* 2003). The results of *Pl* marker mapping and analysis using two F_3 populations confirmed that the *Pl* allele of 'JC072A' (*Pl1-JC072A*) confers the anthocyanin pigmentation of cobs (**Fig. 1**, **Supplemental Fig. 4**). Sequence analysis of *Pl* genes showed that *Pl1-JC072A* is similar to the functional allele *Pl-bol3* (**Fig. 3**, **Supplemental Fig. 3**). Therefore, *Pl1* is the most likely candidate gene responsible for anthocyanin production in cobs.

Although cobs with high anthocyanin content are useful for anthocyanin production, they tend to be injured by mold, which hampers harvesting F_1 hybrid seeds. Each of the parents of an F_1 hybrid with a different gene of two genes could sustain seed production of F_1 hybrids. Our markers designed for the *Pl1* genes will be useful for the breeding of F_1 parents with anthocyanin coloration in cobs.

Acknowledgments

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Literature Cited

Aoki, H., N. Kuze and Y. Kato (2002) Anthocyanins isolated from purple corn (*Zea mays* L.). Foods Food Ingredients J. Japan 199: 41–45.

Cevallos-Casals, B.A. and L. Cisneros-Zevallos (2003) Stoichiometric and kinetic studies of phenolic antioxidants from Andean purple corn and red-fleshed sweetpotato. J. Agric. Food Chem. 51: 3313–3319.

Chalker-Scott, L. (1999) Environmental significance of anthocyanins in plant stress responses. Photochem. Photobiol. 70: 1–9.

Chandler, V.L., J.P. Radicella, T.P. Robbins, J. Chen and D. Turks (1989) Two regulatory genes of the maize anthocyanin pathway are homologous: isolation of *B* utilizing *R* genomic sequences. Plant Cell 1: 1175–1183.

Cocciolone, S.M. and K.C. Cone (1993) *Pl-Bh*, an anthocyanin regulatory gene of maize that leads to variegated pigmentation. Genetics 135: 575–588.

Cone, K.C., F.A. Burr and B. Burr (1986) Molecular analysis of the maize anthocyanin regulatory locus *C1*. Proc. Natl. Acad. Sci. USA 83: 9631–9635.

Cone, K.C., S.M. Cocciolone, F.A. Burr and B. Burr (1993a) Maize anthocyanin regulatory gene *pl* is a duplicate of *c1* that functions in the plant. Plant Cell 5: 1795–1805.

Cone, K.C., S.M. Cocciolone, C.A. Moehlenkamp, T. Weber, B.J. Drummond, L.A. Tagliani, B.A. Bowen and G.H. Perrot (1993b) Role of the regulatory gene *pl* in the photocontrol of maize anthocyanin pigmentation. Plant Cell 5: 1807–1816.

Consonni, G., F. Geuna, G. Gavazzi and C. Tonelli (1993) Molecular homology among members of the *R* gene family in maize. Plant J. 3: 335–346.

Eggleston, W.B., M. Alleman and J.L. Kermicle (1995) Molecular organization and germinal instability of *R-stippled* maize. Genetics 141: 347–360.

Grobman, T.A. (1961) Races of maize in Peru: Their origins, evolution and classification. National Academy of Sciences-National Research Council, Washington.

Hagiwara, A., K. Miyashita, T. Nakanishi, M. Sano, S. Tamano, T. Kadota, T. Koda, M. Nakamura, K. Imaida, N. Ito *et al.* (2001) Pronounced inhibition by a natural anthocyanin, purple corn color, of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP)-associated colorectal carcinogenesis in male F344 rats pretreated with 1,2-dimethylhydrazine. Cancer Lett. 171: 17–25.

Hollick, J.B., G.I. Patterson, E.H. Coe, Jr., K.C. Cone and V.L. Chandler (1995) Allelic interactions heritably alter the activity of a metastable maize *pl* allele. Genetics 141: 709–719.

Hosoda, K., B. Eruden, M. Matsuyama and S. Shioya (2012) Effect of anthocyanin-rich corn silage on digestibility, milk production and plasma enzyme activities in lactating dairy cows. Anim. Sci. J. 83: 453–459.

Jing, P., V. Noriega, S.J. Schwartz and M.M. Giusti (2007) Effects of growing conditions on purple corncob (*Zea mays* L.) anthocyanins. J. Agric. Food Chem. 55: 8625–8629.

Kumar, S., G. Stecher and K. Tamura (2016) MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33: 1870–1874.

Larkin, M.A., G. Blackshields, N.P. Brown, R. Chenna, P.A. McGettigan, H. McWilliam, F. Valentin, I.M. Wallace, A. Wilm, R. Lopez *et al.* (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23: 2947–2948.

Ludwig, S.R., L.F. Habera, S.L. Dellaporta and S.R. Wessler (1989) *Lc*, a member of the maize *R* gene family responsible for tissue-specific anthocyanin production, encodes a protein similar to transcriptional activators and contains the *myc*-homology region. Proc. Natl. Acad. Sci. USA 86: 7092–7096.

Ludwig, S.R. and S.R. Wessler (1990) Maize *R* gene family: tissue-specific helix-loop-helix proteins. Cell 62: 849–851.

McMullen, M.D., H. Kross, M.E. Snook, M. Cortés-Cruz, K. Houchins, T.A. Musket and E.H. Coe (2004) *Salmon silk* genes contribute to the elucidation of the flavone pathway in maize (*Zea mays* L.). J. Hered. 95 3: 225–233.

Mol, J., E. Grotewold and R. Koes (1998) How genes paint flowers and seeds. Trends Plant Sci. 3: 212–217.

Paz-Ares, J., U. Wienand, P.A. Peterson and H. Saedler (1986) Molecular cloning of the *c* locus of *Zea mays*: a locus regulating the anthocyanin pathway. EMBO J. 5: 829–833.

Petroni, K., E. Cominelli, G. Consonni, G. Gusmaroli, G. Gavazzi and C. Tonelli (2000) The developmental expression of the maize regulatory gene *Hopi* determines germination-dependent anthocyanin accumulation. Genetics 155: 323–336.

Pilu, R., P. Piazza, K. Petroni, A. Ronchi, C. Martin and C. Tonelli (2003) *pl-bol3*, a complex allele of the anthocyanin regulatory *pl1* locus that arose in a naturally occurring maize population. Plant J. 36: 510–521.

Radicella, J.P., D. Brown, L.A. Tolar and V.L. Chandler (1992) Allelic diversity of the maize *B* regulatory gene: different leader and promoter sequences of two *B* alleles determine distinct tissue specificities of anthocyanin production. Genes Dev. 6: 2152–2164.

Shindo, M., T. Kasai, A. Abe and Y. Kondo (2007) Effects of dietary administration of plant-derived anthocyanin-rich colors to spontaneously hypertensive rats. J. Nutr. Sci. Vitaminol. 53: 90–93.

Takai, T., J.-i. Yonemaru, H. Kaidai and S. Kasuga (2012) Quantitative trait locus analysis for days-to-heading and morphological traits in an RIL population derived from an extremely late flowering F_1 hybrid of sorghum. Euphytica 187: 411–420.

Tamaki, H., T. Matsumoto, S. Mitsuhashi, N. Okumura, T. Kikawada and H. Sato (2014) A study on 'Genomewide Selection' for maize (*Zea mays* L.) Breeding in Japanese public sectors: Single nucleotide polymorphisms observed among parental inbred lines. Bull. NARO Inst. Livest. Grassl. Sci. 14: 1–11.

Tonelli, C., S. Dolfini, A. Ronchi, G. Consonni and G. Gavazzi (1994) Light inducibility and tissue specificity of the *R* gene family in maize. Genetica 94: 225–234.

Yonemaru, J.-i., T. Ando, T. Mizubayashi, S. Kasuga, T. Matsumoto and M. Yano (2009) Development of genome-wide simple sequence repeat markers using whole-genome shotgun sequences of sorghum (*Sorghum bicolor* (L.) Moench). DNA Res. 16: 187–193.