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- Review -

Allelic Gene Interaction and Anthocyanin Biosynthesis of *Purple Pericarp* Trait for Yield Improvement in Black Rice

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Rice (*Oryza sativa* L.) is one of the major cereal crops for consumption by the world's population. Recently, various colored rice, such as white, red, brown, green, and black rice, have caught the attention of world consumers. The commercial name 'black rice' contains a high amount of anthocyanins in pericarp, which increases nutritional value. Moreover, anthocyanin in black rice possesses biomedical properties, including anti-oxidant, anti-cancer, and anti-inflammatory effects in humans. In genetics, black rice has a dominant *PURPLE PERICARP* (*Prp*) trait governed by two genes, *Pb* and *Pp*, which are involved in the synthesis of cyanidin-3-O-glucoside (C3G). Since the publication of a report by Nagai at 1921, the genetics and physiological studies of black rice driven by *Prp* traits are still unable to understand the relevant genes and their roles. However, with the increased demand for anthocyanin-rich black rice as a functional food for human health, it has become urgent to develop high-yielding anthocyanin-rich varieties of rice. We explored many years in the genetics of purple pericarp trait, anthocyanin biosynthesis in pericarp during seed development, and, consequently, their products in relation to different physiological and agronomic traits. In this review, we summarized the anthocyanin biosynthesis in pericarp, emphasizing the inheritance pattern of the trait and functions of their products on different physiological and agronomic traits, including the yield of black rice.

Key words : Anthocyanin, black rice, complementation gene, cyanidin-3-O-glucoside, purple pericarp

Introduction

Pericarp is the outermost layer of rice seed and located just beneath the hull of rice. All over the world, the rice varieties mostly possess white pericarp and few are red, brown, green, black or purple pericarp [9, 12, 17, 25]. Among them, black rice is characterized by dark purple pigments of anthocyanin accumulation in the pericarp [18, 22, 38].

Genetically, the pigmentation in pericarp of black rice requires two dominant complementary genes, *PURPLE PERICARP A* (*Pp*, *Prpa* and *Prp1*) and *PURPLE PERICARP B* (*Pb*, *Prpb* and *Prp2*) located on chromosomes 1 and 4, respectively [14, 28, 47]. The existence of two dominant genes *Pb* and *Pp* result purple color of rice pericarp. However, *Pb* in the absence of *Pp* produces brown pericarp and *Pp* alone produces ordinary white pericarp rice [13, 34, 45]. Additionally, the intensity of pericarp color in black rice also affected by the number of dominant *Pp* alleles in presence of dominant *Pb* alleles [34].

In black rice, high level of anthocyanins accumulation occurs during seed development stages result in a dark purple color pericarp [37, 41]. Anthocyanins are a group of natural pigments that belong to the family of flavonoids. Two major anthocyanin pigments namely cyanidin-3-O-glucoside (C3G) and peonidin-3-glucoside (P3G) are found in rice seed pericarp [22, 34]. Among them, C3G anthocyanin possesses biomedical properties including anti-oxidant, anti-cancer, and anti-inflammatory effects in human [15, 26, 29].

Although black rice has increased demand, the yield potentiality of colored rice is much lower as compared with white or hybrid rice [23, 31, 48]. The probable reasons for the low yield potentiality of black rice in comparison with white rice might be due to small sink size [16]. We conducted a pilot project to observe the pigments deposition pattern during grain filling stage and its influence on yield. Therefore, it is urgent to explore the facts regarding the genetic makeup and deposition of anthocyanins in pericarp,

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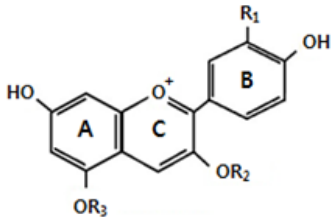
and their relations with development of rice grain. In this review, we summarized the anthocyanin biosynthesis in pericarp, emphasizing inheritance pattern of the trait and the functions of their products on different physiological and agronomic traits including yield of black rice.

Anthocyanin pigments in pericarp of black rice

Black rice has a high level of purple pigments in pericarp layers [47], which results in dark purple color seed. This purple pigmentation in the black rice is the color of anthocyanin [1]. Anthocyanins are a group of natural pigments that belong to the family of flavonoids which synthesized by a secondary metabolic pathway from the amino acid phenylalanine [36]. Early studies reported that, cyanidin-3-O-glucoside (C3G) as a major anthocyanin in black rice, but the minor ones either malvidin-3-glucoside [46] or peonidin-3-glucoside (P3G) [38]. Recently, four anthocyanins were identified in black rice namely cyanidin-3-O-glucoside, peonidin-3-glucoside, cyanidin-3-5-diglucoside, cyanidin-3-rutinoside (Fig. 1). A strong and identical peak of cyanidin-3-O-glucoside (kuromanin) was found in pericarp extract of purple pericarp rice, a trace amount of peonidin-3-glucoside was also found as well [34]. In contrast, the accumulation of anthocyanin was not detected in pericarp extract of white and brown pericarp rice (Fig. 2). Cyanidin-3-O-glucoside as major pigment and peonidin-3-glucoside as minor pigment are also the core anthocyanins in seed pericarp of the grains such as purple wheat and purple rye [6].

Genes involved in anthocyanin biosynthesis in black rice

Anthocyanins are derived from one molecule of *p*-coumaroyl-CoA and three molecule of malonyl-CoA which is originated by using phenylpropanoid and acetate pathway, respectively (Fig. 3). Several genes are involved in anthocyanin biosynthesis of rice and divided into two types; the structural genes (Table 1) are directly participating in the biosynthesis of anthocyanins, and the regulatory genes (Table 2) that control the expression of structural genes [11].



Anthocyanins	Substitution pattern		
	R_1	R_2	R_3
Cyanidin-3-glocoside	OH	glucose	H
Peonidin-3-glocoside	OCH_3	glucose	H
Cyanidin-3-rutnoside	OH	rutinose	H
Cyanidin-3,5-glocoside	OH	glucose	glucose

Fig. 1. Chemical structures of most common anthocyanin in the pericarp of black rice [19, 42].

Structural genes for black rice

Anthocyanins with significant biological activities and a number of structural genes isolated from rice have been described in Table 1. The anthocyanin biosynthesis is catalyzed by chalcone synthase (CHS), chalcone isomerase (CHI), flavanone-3-hydroxylase (F3H), flavonoid-3′-hydroxylase (F3′H), flavonoid-3′,5′-hydroxylase (F3′5′H), dihydroflavonol-4-reductase (DFR), anthocyanidin synthase (ANS), and flavonoid-3-O-glucosyltransferase (UFGT) [19, 21, 32, 42]. Generally, the first step in anthocyanin biosynthesis is condensation of three malonyl-CoA molecules and one p-coumaroyl-CoA

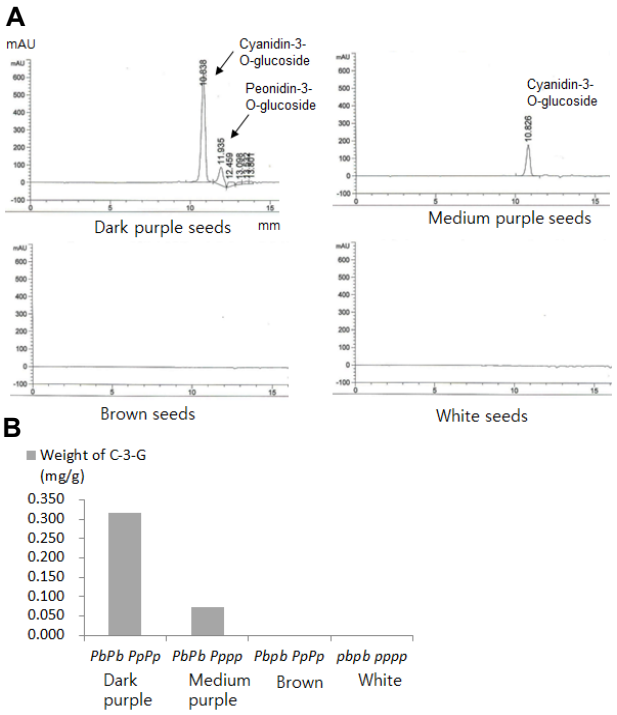


Fig. 2. Anthocyanin profile in progenies of black rice and white rice extract analyzed via HPLC. (A) HPLC profiles of the extracts from the dark-purple to white pericarp of rice seeds in F_2 progenies. (B) Measurement of cyanidin-3-O-glucoside (C3G)in each pericarp of rice seeds. The high amount of C3G was extracted in dark purple seeds. However, relatively smaller amounts of C3G were extracted in the medium purple seeds of the F_2 progenies. The brown and white seeds of F_2 progenies contained no anthocyanin [34].

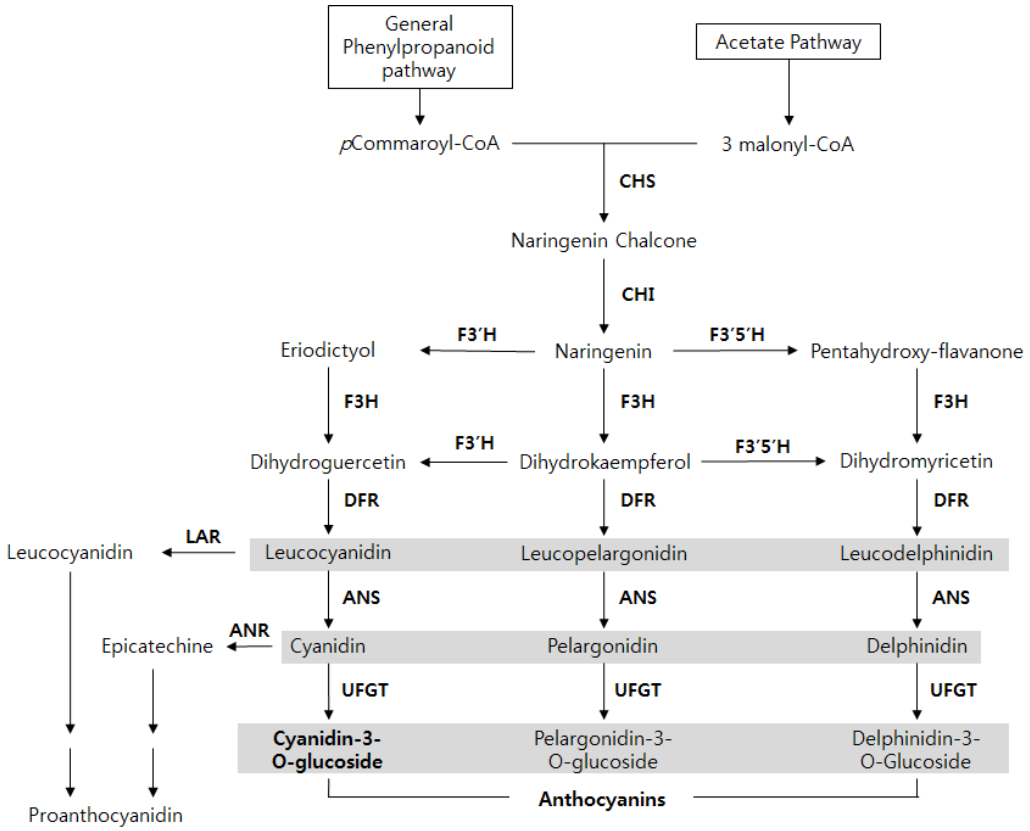


Fig. 3. The anthocyanin biosynthesis pathway in rice. CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavanone3β-hydroxylase; F3'H, flavonoid 3'-hydroxylase; F3'5'H, flavonoid 3', 5'-hydroxylase; DFR, dihydroflavonol 4-reductase; ANS, Anthocyanidin synthase; ANR, anthocyanidin reductase; LAR, leucoanthocyanidin reductase and UFGT, Flavonoid 3-0-glucosyltransferase [32, 37, 42].

Table 1. Structural genes involved in anthocyanin biosynthesis pathway in rice

Enzyme	Gene name	Locus ID	Accession numbers	References
Chalcone Synthase	*OsCHS1* *OsCHS2*	Os11g0530600 Os07g0214900	X89859	Reddy et al. (1996) Shih et al. (2008)
Chalcone isomerase	*OsCHI*	Os03g0819600	AF474922	Druka et al. (2003)
Flavanone 3-hydroxylase	*OsF3H1* *OsF3H2* *OsF3H3*	Os04g0662600 Os10g0536400 Os04g0667200	NM_001060692 AAL58118 CAE02796	Kim et al. (2008)
Flavanone 3'-hydroxylase	*OsF3'H*	Os10g0320100	AK064736	Shih et al. (2008)
Dihydroflavonol reductase	*Rd*	Os01g0633500	AB003496	Furukawa et al. (2006)
Leucoanthocyanidin dioxygenase	*OsANS1* *OsANS2,*	Os01g0372500 Os06g0626700	Y07955	Reddy et al. (2007) Shih et al. (2008)
UDP-glycosyl transferase	*OsUFGT*	Os06g0192100	NM_001063569	Tanaka et al. (2008)

molecule by the action of enzyme chalcone synthase (CHS) to produce a yellow naringenin chalcone. In rice (*O. sativa*), there are at least two genes *CHS1* (LOC_Os11g32650) and *CHS2* (LOC_Os07g11440) encoding CHS which are located on different chromosomes [42]. After the action of chalcone isomerase (CHI), naringenin chalcone isomerized into a colorless naringenin flavanone stereospecifically and quickly. The naringenin flavanone is then hydroxylated by flavanone 3-β-hydroxylase (F3H) to produce an unpigmented dihydroflavonol. In rice, three genes *F3H1* (NM_001060692), *F3H2* (AAL58118) and *F3H3* (CAE02796) encoding *F3H* were identified and characterized [20]. The dihydroflavonol is reduced

Table 2. Regulatory genes involved in anthocyanin biosynthesis in rice seed pericarp

Genetics study	Chromosome	Gene locus	Cloned or possible gene
Kato and Ishikawa 1921 Hsieh and Chang 1964 Hu et al. 1996 Yoshimura et al. 1997 Wang et al. 2009 Rahman et al. 2013	1	*A* (Nagao and Takahashi 1963)	
		Rd (Furukawa et al. 2006)	*DFR* (Furukawa et al. 2006)
		Pp (Wang and Shu 2007)	
		Kala1 (Maeda et al. 2014)	
	3	*P* (Nagao and Takahashi 1963)	Unknown
		Kala3 (Maeda et al. 2014)	
	4	*C* (Nagao and Takahashi 1963)	
		Pb (Wang and Shu 2007; Rahman et al. 2013)	*OSB1* (Wang and Shu 2007; Rahman et al. 2013)
		pl^{w} (Sakamoto et al. 2001)	*OSB1 and OSB2* (Sakamoto et al. 2001)
		Kala4 (Maeda et al. 2014)	*OSB2* (Oikawa et al. 2015)

to colorless leucoanthocyanidin by dihydroflavonol- 4-reductase (DFR). This leucoanthocyanidin is then converted into a colored anthocyanidins, catalyzed by anthocyanidin synthase (ANS). Two highly conserved ANS genes *ANS1* (Os01g0372500) and *ANS2* (Os06g0626700) located on different chromosomes were identified and characterized in rice [42]. In the final step, anthocyanidins are glycosylated to form anthocyanins, catalyzed by the enzyme UDP glucose flavonoid-3-oxy‐glucosyl transferase (UF3GT) (Fig. 3).

Over the last few years, many structural genes (Table 1) encoding the key enzymes involved in anthocyanin biosynthesis in rice have been cloned. Among them, two genes *CHS* [36] and *CHI* [8] were identified in rice based on sequence homology that involve in anthocyanin biosynthesis. A functional gene, *DFR* is responsible for red color in pericarp was established by over-expressing transgenic lines in rice [9]. Similarly, transient assays showed that *ANS* gene of rice can complement the function of mutated *a2* gene in maize kernels [35]. Furthermore, biochemical roles of six genes of rice (*CHS, CHI, F3H, F3'H, DFR* and *ANS*) were well established by complementation assays in the Arabidopsis *tt* mutants [42].

Regulatory genes for anthocyanin of black rice

Regulatory genes (Table 2) influence the intensity and tissue specific pattern of anthocyanin accumulation and generally regulate expression of different structural genes of anthocyanin biosynthesis pathway at the transcriptional level [10]. Two major classes of regulatory gene family, namely *R/B* family gene encode basic helix-loop-helix (bHLH) myc type protein [3] and *C1/PI* family gene encode the myb type protein [5] are known to regulate anthocyanin biosynthesis pathway in many plants. At least one gene from each family is required for anthocyanins pigmentation in tissue specific manner. Physical interactions between the R/B and C1/PI proteins are necessary to activate the expression of the key structural genes in anthocyanin pathway. However, the genes encoding a WD40 protein are also required for the expression of anthocyanin biosynthetic genes in maize seed [2]. These are connected to form a complex in a hierarchical network to regulate the expression of the structural genes involved in the pathway [10].

Two basic genes namely, chromogen gene *C* and activator gene *A* are essential in the formation of anthocyanin pigments [43]. The organ-specific regulatory gene *P* is necessary to confer tissue-specific accumulation of anthocyanin in plants [44]. They are termed as localizer or distributor genes or regulatory genes of the pathway conditioning tissue specificity.

Rice seed pericarp becomes brown in color in presence of *Pb* alone (in absence of *Pp*), purple color in presence of both genes, and white color in absence of *Pb* (even in presence or absence of *Pp*) [34]. Recently, three loci namely *Kala1*, *Kala3* and *Kala4* were identified to involve in pericarp pigmentation of black rice [24]. It is also proposed that *Kala1*, *Kala3* and *Kala4* might correspond to *C*, *A* and *P*, respectively. *Kala4* contains *Pb* gene that encodes a bHLH protein which is essential for color development in black rice pericarp [30]. The Pl^{w} locus composed two highly homologous genes *OSB1* and *OSB2* encoding bHLH protein and confers pigmented leaf and pericarp of black rice [40]. The *Pb* gene is probable to be the same gene *OSB1* which is located in the Pl^{w} locus [44]. Therefore, investigation of the Pl^{w} locus is still endured. *Pp* may be an allele of *Kala1* as the genetic interaction of *Kala4* and *Kala1* is similar with that of *Pb* and *Pp* [24]. However, *Kala4* and *Kala1* are urgent for anthocyanin biosynthesis in rice pericarp. Furthermore, *Kala1* corresponds *A* gene which encodes the dihydrofavonol-4-reductase (DFR) enzyme and mapped on rice chromosome 1. *DFR* is a widely conserved gene which encodes an enzyme involved in both anthocyanin and pro-anthocyanin synthesis pathway [42]. *Kala3* might be a myb family transcription factor, act as a tissue-specific regulator and may be similar to *P* genes. Thus, *Kala1* and *Kala4* are necessary for anthocyanin biosynthesis and *Kala3* might possess distinct seed pericarp tissue-specific transcription pattern [24]. Molecular cloning has been conducted for the regulatory genes involved in anthocyanin biosynthesis in rice seed pericarp. The rice genes *Ra1* and *Rb* [14], *C1* [39] and *Pb* [30, 34, 40, 44] have been cloned. Further, *Pp* gene cloning is required to complete understanding the genetic regulation of *Prp* trait.

Genetic analysis of purple pericarp traits in black rice

The inheritance of purple pericarp trait of rice is found to be dominant over white. The purple coloration in rice pericarp is determined by the complementary effect of two genes namely, *Pp* and *Pb* whose are located on chromosome 4 and 1, respectively [14, 47]. The *Pp* gene acts in a recessive epistasis fashion with the *Pb* gene for the production of purple pericarp in rice [13, 44]. However, the *Prp* trait has been defined as complementary interaction between *Pb* and *Pp* genes recently (Lee et al. 2016 under publication). The *Pb* gene was mapped on rice chromosome number 4 and suggested that, *Ra* gene may be similar to *Pb* gene, and purple pericarp characteristic of rice is caused by two bases deletion (GT) within exon 7 of gene *Ra* [34, 43]. The *Pb* gene alone is responsible for the pigments accumulation in the pericarp of brown colored seed was described in figure 5. Furthermore, purple pericarp rice requires the *Pp* gene to increase the content of the pigment from brown to purple (Fig. 5). We proposed that *Pp* gene regulate the anthocyanidin syn-

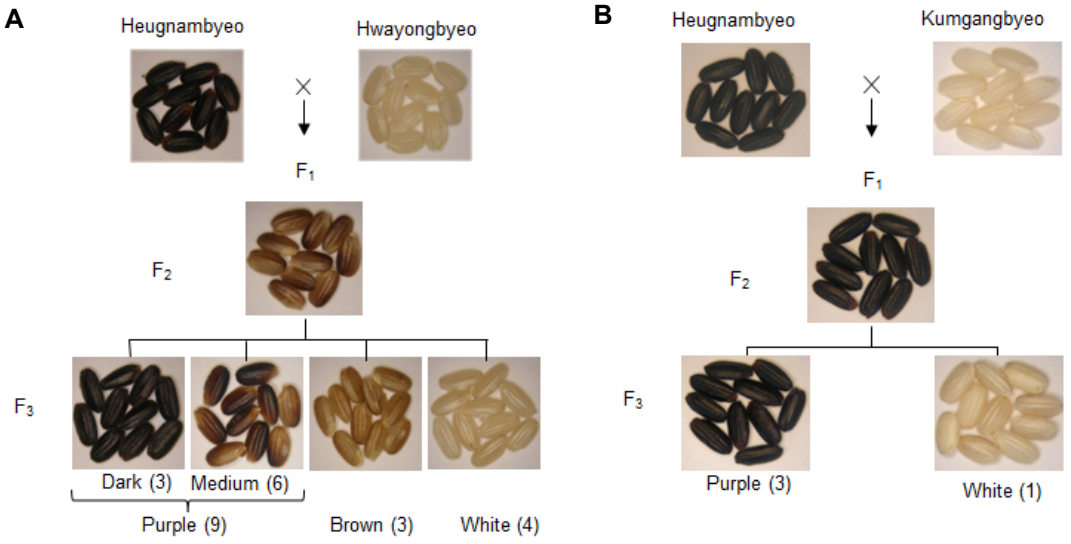


Fig. 4. Segregation analysis of pericarp color of black rice. (A) Purple pericarp rice *Oryza sativa* L. *japonica* var. 'Heugnambyeo' was crossed with a white pericarp rice. F_1 plants become purple color pericarp. Further selfing of F_2 plants produced F_3 seeds with purple pericarp, brown color pericarp, and white pericarp. (B) Phenotypes of the pericarp of cross materials. Purple pericarp rice was crossed with white-colored pericarp wild-type 'Kumgangbyeo' rice [34].

A

Cross	Dark Purple black (*PbPbPpPp*)	X	White (*pbpbpppp*)	
Gamets	*PbPp*		*pbpp*	
F_1	*PbpbPppp* (Medium Purple)			
F_2				
Gamets	*PbPp*	*pbPp*	*Pbpp*	*pbpp*
PbPp	*PbPbPpPp* Dark Purple	*PbpbPpPp* Dark Purple	*PbPbPppp* Medium Purple	*PbpbPppp* Medium Purple
pbPp	*PbpbPpPp* Dark Purple	*pbpbPpPp* White	*PbpbPppp* Medium Purple	*pbpbPppp* White
Pbpp	*PbPbPppp* Medium Purple	*PbpbPppp* Medium Purple	*PbPbpppp* Brown	*Pbpbpppp* Brown
pbpp	*PbpbPppp* Medium Purple	*pbpbPppp* White	*Pbpbpppp* Brown	*pbpbpppp* White

B

Cross	Dark Purple black (*PbPbPpPp*)	X	White (*pbpbPpPp*)
Gamets	*PbPp*		*pbPp*
F_1	*PbpbPpPp* (Dark Purple)		
F_2			
Gamets	*PbPp*		*pbPp*
PbPp	*PbPbPpPp* Dark Purple		*PbpbPpPp* Dark Purple
pbPp	*PbpbPpPp* Dark Purple		*pbpbPpPp* White

Fig. 5. Genetic analysis of crosses among black rice. (A) Genetic analysis of a cross between black rice *PbPb PpPp* and white rice *pbpb pppp* in their genotypes. (B) Genetic analysis of a cross between black rice *PbPb PpPp* and white rice *pbpb PpPp* in their genotypes [34].

thase which mediated the conversion of leucoanthocyanidin to anthocyanidin during anthocyanin biosynthetic pathway in rice. Therefore, *Pb* and *Pp* genes are involved in the purple pigmentation in rice pericarp, *Pb- Pp-* for purple pericarp, *Pb- pppp* for brown pericarp, and *pbpb Pp*-or *pbpb pppp* for white pericarp [34]. Here, homozygous recessive allele of *pb* gene may be masked the expression of either allele of the *Pp* gene. Consequently, *Pp* itself does not produce any pigment in presence of *pbpb*. Therefore, the *Pb* and *Pp* genes involved in the purple pigmentation of the rice pericarp with epistatic gene interaction having segregation ratio of 9 purple: 3 brown: 4 white (Fig. 4). This gene interaction was also observed in inheritance of purple pericarp traits in wheat. It is recognized that two dominant complementary genes, *Pp1* and *Pp3*, control purple pigments deposition in wheat pericarps [7].

Interestingly, we found that the intensity of pericarp color in black rice varies from dark purple to medium purple [34]. Medium purple seeds of F_1 and F_2 plants showed purple color deposition in brown background. The purple pericarp progeny followed a segregation ratio of 1 dark purple (*PpPp*): 2 medium purple (*Pppp*): 1 brown (*pppp*), suggesting that the dominant *Pp* allele was incomplete over the recessive *pp* allele of the *Pp* gene (Fig, 5). Based on the genotype analysis shown in figure 5, the pericarp color phenotypes of the F_3 progeny match with the genotypes exactly (Fig. 6). A strong peak of cyanidin-3-O-glucoside (kuromanin) was observed in the dark purple pericarp rice having the *Pb_PpPp* genotypes. But, cyanidin-3-O-glucoside peak was detected significantly lower in the medium purple pericarp rice (*Pb_Pppp*), and no cyanidin-3-O-glucoside peak was identified in the pericarp extraction of the brown pericarp rice (*Pb_pppp*) (Fig. 2). Hence, it is clear that the level of cyanidin-3-O-glucoside was determined by the number of cop-

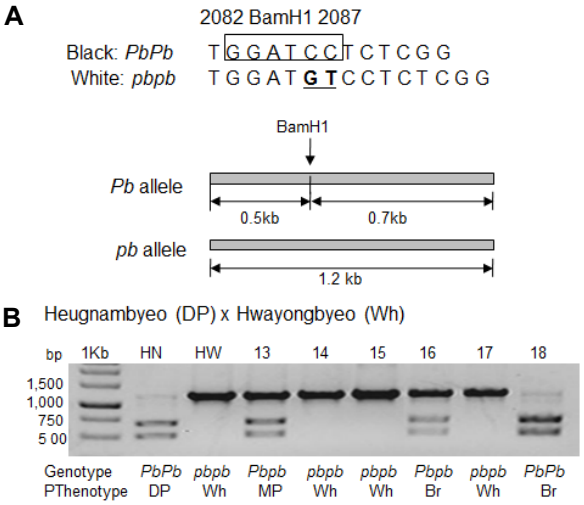


Fig. 6. Analysis of the allelic polymorphism among progeny of the crosses between a black pericarp rice and a white pericarp rice. (A) DNA sequence fragments of the amplified Pb gene (OsB1 DNA sequence). The black pericarp rice 'Hugnambyeo' was composed of two homologous dominant *Pb* alleles and presented a *Bam*H1 restriction enzyme site in the amplified DNA sequences of each corresponding allele. The white pericarp rice 'Hwayoungbyeo' was composed of two homologous recessive *pb* alleles without any BamH1 restriction enzyme site in the amplified DNA sequences. The *pb* allele of the white pericarp rice has a 2bp (GT) insertion in the BamH1 site of the same DNA sequences in the *Pb* allele. Schematic drawings are presented after BamH1 digestion in the DNA of the *Pb* allele resulting in division into two fragments of DNA. (B) Genetic analysis of the genotypic and phenotypic constitutions of the F_3 progeny of the crosses between a black pericarp rice and a white pericarp rice. Phenotypes are indicated by the pericarp colors, with DP indicating dark purple, MP indicating medium and mixed purple, Wh indicating white, and Br indicating brown. Genetic analysis of *Prp* trait was approved that phenotypes of progenies has been corresponded completely to their genotypes [34].

ies of *Pp* alleles of the genotype in each progeny [34]. Furthermore, the dominant *Pp* allele was found incompletely dominant over the recessive *pp* allele in rice and the concentration of cyanidin-3-O-glucoside in the pericarp of black rice also affected by the number of dominant *Pp* alleles [34].

Accumulation of anthocyanins in rice pericarp during grain development

The green pigments accumulated in seed from fertilization to dough stage followed by continuous degradation of the deposited green pigments which finally disappeared in mature seed of white rice. Fading or disappearing of green pigment in pericarp of white rice by 25-30 days after pollination (DAP) in field conditions were also reported [4]. When green pigment in seed started to degrade, color pigments started to accumulate simultaneously at the beginning of the desiccation stage and continued to intensify up to physiological maturity of seed development [33]. Brown pigment deposition was started at 15 DAP adjacent to the embryo and the entire seed turned into brown color followed by the complete disappearing of green pigment at 25 DAP in the brown pericarp rice. Furthermore, brown pigment accumulation continued to intensify the color of deep brown pericarp rice seed at harvest. In purple pericarp rice, the pigments accumulation was occurred in the pericarp at the beginning of the dough stage and persistently increased the accumulation of purple pigments until physiological maturity and begun to decrease slightly until at harvest.

An increasing trend of seed chlorophyll content was observed from 5th day after pollination (DAP) which reached to maximum level at 10th DAP and thereafter decreased sharply. The highest amount of chlorophyll were recorded in white rice which were found statistically identical with brown rice, whereas the lowest chlorophyll content was observed in black rice both at 10 DAP and 15 DAP (Fig. 7A). Grain anthocyanin content of purple pericarp rice was revealed much higher than the brown and white pericarp rice throughout the growth season of rice (Fig. 7B). A gradual increase in anthocyanin content was observed from 5th DAP and the whole rice seed become purple in color after 15 DAP. Furthermore, the anthocyanin content of purple pericarp rice was reached maximum at 25 DAP followed by a gradual decrease until harvest. Finally, the endosperms of black, brown and white rice were appeared fade purple, fade brown and white color, respectively.

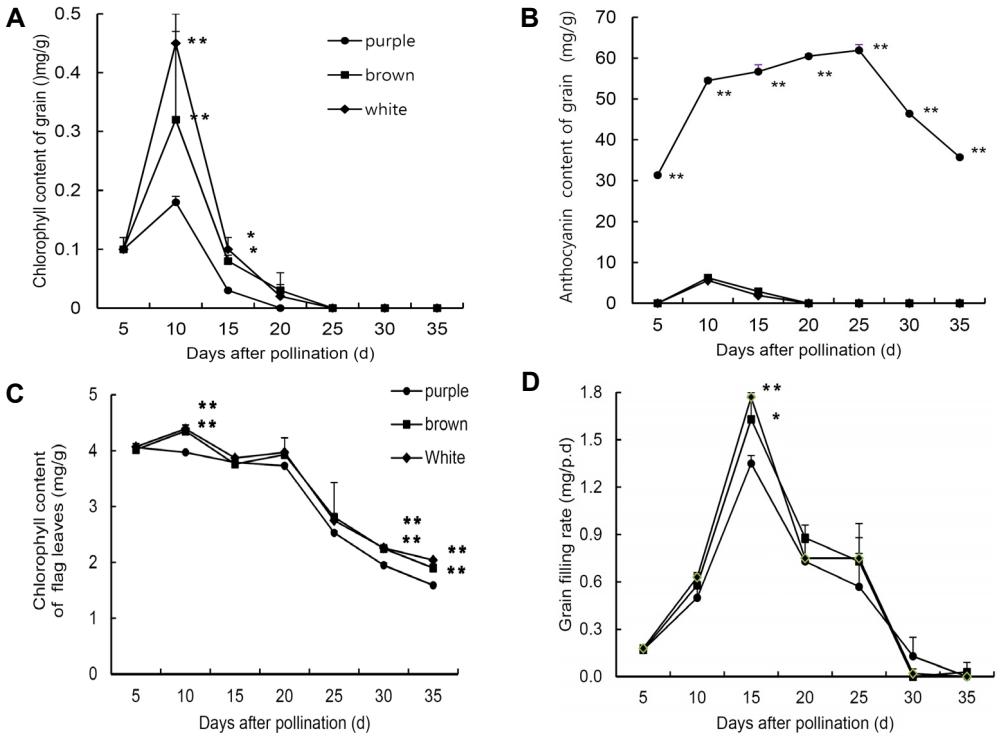


Fig. 7. Changes in chlorophyll and anthocyanin contents of seed extracts from the purple, brown and white pericarp rice. (A) Changes in grain chlorophyll content. (B) Changes in grain anthocyanin (C) Changes in chlorophyll content of flag leaves. (D) Changes in grain filling rate (mg/p.d.) [33].

Anthocyanins pigment accumulation in pericarp decrease rice yield

The yield of colored rice is much lower compared to hybrid and white rice [31]. Significantly lower seed weight of rice having purple pericarp in compare with rice having white pericarp was also reported [46]. We also hypothesized that the lower seed weight of purple pericarp rice might be due to the accumulation of anthocyanin in pericarp [33]. As we observed that pigments deposition in colored rice may be the reason for decrease of chlorophyll content in spikelet (Fig. 7A), which could be responsible for reduced yield [33]. In our previous study, inverse relationship between chlorophyll and anthocyanin content in the pericarp were found [33]. The probable reasons may be the deposition of carbohydrates stimulates anthocyanin metabolism and chlorophyll degradation [27]. There is a significant relationship between seed length and seed weight. The reduced sink size may be resulted in the decreased 100 seed weight, which suggested that purple pericarp rice possessed reduced sink size (Fig. 8). It was also reported that purple pericarp rice found to give low yield as compared with white pericarp rice that might be due to reduced sink size [16]. The reduced grain thickness and lower yield in black rice near isogenic lines (NILs) found in Japan [24]. So, the small seed size of black rice resulting in its reduced yield potential may not be correlated with yield related genetic traits rather than it may related to physiological factors associated with anthocyanin synthesis in pericarps. These factors would reduce the photosynthetic rate and grain filling rate in seed which ultimately reduced the yield of black rice [33].

Conclusion and future prospects

Anthocyanins are a major group of flavonoid compounds which are fundamentally responsible for the purple color of rice tissues and organs. There is an increasing trend to develop functional food rich in anthocyanin. To fulfill the increasing demand, the identification of regulatory proteins should be conducted together with the investigation of the parameters controlling their expression, and a better understanding of the regulatory mechanism involve in tissue specific anthocyanin synthesis in rice are the key prerequisite. As well, the endogenous factors which trigger the expression of the regulatory genes should also be determined. Mark genes of allelic variabilities are useful for black rice quality improvement. Understanding about the accumulation of anthocyanin during chlorophyll degradation, distribution of assimilates, source and sink relationship as well as grain development and yield of black rice is essential.

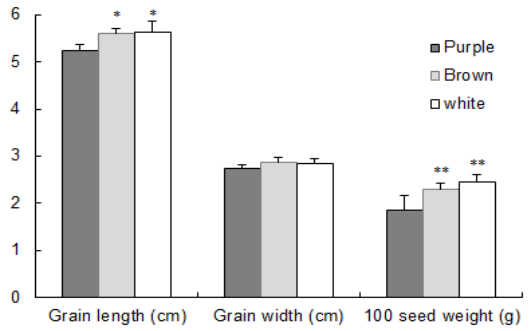


Fig. 8. Comparison of seed length (mm), seed width (mm) and 100 seed weight (g) of the purple, brown and white pericarp rice [33].

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References

1. Abdel-Aal, E. S. M., Young, J. C. and Rabalski, I. 2006. Anthocyanin composition in black, blue, pink, purple, and red cereal grains. *J. Agric. Food Chem.* **54,** 4696-4704.
2. Carey, C. C., Strahle, J. T., Selinger, D. A. and Chandler, V. 2004. Mutations in the *pale aleurone color1* regulatory gene of the *Zea mays* anthocyanin pathway has distinct phenotype relative to the functionally similar *Transparent Testa Glabra1* gene in *Arabidopsis thaliana*. *Plant Cell* **16,** 450-464.
3. Chandler, V. L., Radicella, J. P., Robbins, T. P., Chen, J. and Turks, D. 1989. Two regulatory genes of the maize anthocyanin pathway are homologous: isolation of b utilizing r genomic sequences. *Plant Cell* **1,** 1175-1183.
4. Chu, S. H., Lee, H. H., Ryu, S. N. and Koh, H. J. 2004. Grain characteristics and inheritance of green kerneled rice (*Oryza sativa* L.). *Kor. J. Breed.* **36,** 222-228.
5. Cone, K. C., Cocciolone, S. M., Burr, F. A. and Burr, B. 1993. Maize anthocyanin regulatory gene *pl* is a duplicate of *c1* that functions in the plant. *Plant Cell* **5,** 1795-1805.
6. Dedio, W., Hill, R. D. and Evans, L. E. 1972. Anthocyanins in the pericarp and coleoptiles of purple wheat. *Can. J. Plant Sci.* **52,** 977-980.
7. Dobrovolskaya, O. B., Arbuzova, V. S., Lohwasser, U., Roder, M. S. and Borner, A. 2006. Microsatellite mapping of complementary genes for purple grain colour in bread wheat (*Triticum aestivum* L.). *Euphytica* **150,** 355-364.

8. Druka, A., Kudrna, D., Rpstoks, N., Brueggeman, R., von Wettstein, D. and Kleinhofs, A. 2003. *Chalcone synthase* gene from rice (*Oryza sativa*) and barley (*Hordeum vulgare*): physical, genetic and mutation mapping. *Gene* **302,** 171-178.
9. Furukawa, T., Maekawa, M., Oki, T., Suda, I., Iida, S., Shimada, H., Takamure, I. and Kadowaki, K. 2006. The *Rc* and *Rd* genes are involved in proanthocyanidin synthesis in rice pericarp. *Plant J.* **49,** 91-102.
10. Hichri, I., Barrieu, F., Bogs, J., Kappel, C., Delrot, S. and Lauvergeat, V. 2011. Recent advances in the transcriptional regulation of the flavonoid biosynthetic pathway. *J. Exp. Bot.* **62,** 2465-2483.
11. Holton, T. A. and Cornish, E. C. 1995. Genetics and biochemistry of anthocyanin biosynthesis. *Plant Cell* **7,** 1071-1083.
12. Nagai, I. 1921. A genetico-physiological study on the formation of anthocyanin and brown pigments in plants. *Tokyo Univ. College Agric. J.* **8,** 1-92.
13. Hsieh, S. C. and Chang, T. M. 1964. Genic analysis in rice. IV. Genes for purple pericarp and other characters. *Japan J. Breed.* **14,** 141-149.
14. Hu, J., Anderson, B. and Wessler, S. R. 1996. Isolation and characterization of rice *R* genes: evidence for distinct evolutionary paths in rice and maize. *Genetics* **142,** 1021-1031.
15. Hyun, J. W. and Chung, H. S. 2004. Cyanidin and malvidin from *Oryza sativa* cv. Heugjinjubyeo mediate cytotoxicity against human monocytic leukemia cells by arrest of G2/M phase and induction of apoptosis. *J. Agric. Food Chem.* **52,** 2213-2217.
16. Ji, Z. U., Wang, X. G., Zeng, Y. X., Ma, L. Y., Li, X. M., Liu, B. X. and Yang, C. D. 2012. Comparison of physiological and yield traits between purple and white pericarp rice using SLs. *Breed. Sci.* **62,** 71-77.
17. Kang, S. G., Pandeya, D., Kim, S. S. and Suh, H. S. 2006. Morphological characters of panicle and seed mutants of rice. *Kor. J. Crop Sci.* **51,** 348-355.
18. Kato, H. and Ishikawa, J. 1921. On the inheritance of pigment of rice. *Japan J. Genet.* (Supp. **1**) 1-7.
19. Kim, B. G., Kim, J. H., Min, S. Y., Shin, K. H., Kim, J. H., Kim, H. Y., Ryu, S. N. and Ahn, J. H. 2007. Anthocyanin content in rice is related to expression level of anthocyanin biosynthetic genes. *J. Plant Biol.* **50,** 156-160.
20. Kim, J. H., Lee, Y. J., Kim, B. G., Lim, Y. and Ahn, J. H. 2008. Flavanone 3-beta-hydroxylases from rice: key enzymes for favonol and anthocyanin biosynthesis. *Mol. Cells* **25,** 312-316.
21. Koes, R., Verweij, W. and Quattrocchio, F. 2005. Flavonoids: a colorful model for the regulation and evolution of biochemical pathways. *Trends Plant Sci.* **10,** 236-242.
22. Lee, J. H. 2010. Identification and quantification of anthocyanins from the grains of black rice (*Oryza sativa* L.) varieties. *Food Sci. Biotechnol.* **19,** 391-397.
23. Liu, J. P., Ouyang, L. and Li, J. N. 1998. The preliminary report on the breeding of three-1ines for black rice. *Jiangxi Agril. Sci. Technol.* **2,** 7-9.
24. Maeda, H., Yamaguchi, T., Omoteno, M., Takarada, T., Fujita, K., Murata, K., Iyama, Y., Kojima, Y., Morikawa, M., Ozaki, H., Mukaino, N., Kidani, Y. and Ebitani, T. 2014. Genetic dissection of black grain rice by the development of a near isogenic line. *Breed. Sci.* **64,** 134-41.
25. Matin, M. N. and Kang, S. G. 2010. Morphological characteristics of the rice (*Oryza sativa* L.) with red pigmentation. *J. Life. Sci.* **20,** 22-26.
26. Min, B., McClung, A. M. and Chen, M. H. 2011. Phytochemicals and antioxidant capacities in rice brans of different color. *J. Food Sci.* **76,** 117-126.
27. Momose, T. and Ozeki, Y. 2013. Regulatory effect of stems on sucrose-induced chlorophyll degradation and anthocyanin synthesis in Egeria densa leaves. *J. Plant Res.* **126,** 859-867.
28. Nagao, S. and Takahashi, M. 1963. Trial construction of twelve linkage groups in Japanese rice. *J. Fac. Agri. Hokkaido Univ.* **53,** 76-131.
29. Nam, S. H., Choi, S. P., Kang, M. Y., Koh, H. J., Kozukue, N. and Friedman, M. 2006. Antioxidative activities of bran from twenty one pigmented rice cultivars. *Food Chem.* **94,** 613-620.
30. Oikawa, T., Maeda, H., Oguchi, T., Yamaguchi, T., Tanabe, N., Ebana, K., Yano, M., Ebitani, T. and Izawa, T. 2015. The birth of a black rice gene and its local spread by introgression. *Plant Cell* **27,** 2401-2414.
31. Peng, Z. M., Zhang, M. W. and Tu, J. M. 2004. Breeding and nutrient evaluation on three-lines and their combination of indica black glutinous rice hybrid. *Acta Agronomica Sinica* **30,** 342-347.
32. Petroni, K. and Tonelli, C. 2011. Recent advances on the regulation of anthocyanin synthesis in reproductive organs. *Plant Sci.* **181,** 219-229.
33. Rahman, M. M., Lee, K. E. and Kang, S. G. 2015. Studies on the effects of pericarp pigmentation on grain development and yield of black rice. *Indian J. Genet.* **75,** 426-433.
34. Rahman, M. M., Lee, K, E., Lee, E. S., Matin, M. N., Lee, D. S., Yun, J. S., Kim, J. B. and Kang, S. G. 2013. The genetic constitutions of complementary genes *Pp* and *Pb* determine the purple color variation in pericarps with cyanidin-3-0-glucoside depositions in black rice. *J. Plant Biol.* **56,** 24-31.
35. Reddy, A. M., Reddy, V. S., Schezer, B. E., Wienand, U. and Reddy, A. R. 2007. Novel transgenic rice overexpressing anthocyanidin synthase accumulates a mixture of flavonoids leading to an increased antioxidant potential. *Metab. Eng.* **9,** 95-111.
36. Reddy, A. R., ScheZer, B. E., Nadhuri, G., Srivastava, M. N., Kumar, A., Sathyanarayanan, P. V., Nair, S. and Mohan, M. 1996. Chalcone synthase in rice (*Oryza sativa* L.): detection of the CHS protein in seedlings and molecular mapping of the *chs* locus. *Plant Mol. Biol.* **32,** 735-743.
37. Reddy, V. S., Dash, S. and Reddy, A. R. 1995. Anthocyanin pathway in rice (*Oryza sativa* L.): identification of a mutant showing dominant inhibition of anthocyanins in leaf and accumulation of proanthocyanidins in pericarps. *Theor. Appl. Genet.* **91,** 301-312.

38. Ryu, S. N., Park, S. Z. and Ho, C. T. 1998. High performances liquid chromatographic determination of anthocyanin pigments in some varieties of black rice. *J. Food Drug Anal.* **6,** 1710-1715.
39. Saitoh, K., Onishi, K., Mikami, I., Thidar, K. and Sano, Y. 2004. Allelic diversification at the *C* (*OsC1*) locus of wild and cultivated rice: nucleotide changes associated with phenotypes. *Genetics* **168,** 997-1007.
40. Sakamoto, W., Ohmori, T., Kageyama, K., Miyazaki, C., Saito, A., Murata, M., Noda, K. and Maekawa, M. 2001. The *Purple leaf* (*Pl*) locus of rice: the Pl^{w} allele has a complex organization and includes two genes encoding basic helix-loop-helix proteins involved in anthocyanin biosynthesis. *Plant Cell Physiol.* **42,** 982-991.
41. Shao, Y., Jin, L., Zhang, G., Lu, Y., Shen, Y. and Bao, J. 2011. Association mapping of grain color, phenolic content, flavonoid content and antioxidant capacity in dehulled rice. *Theor. Appl. Genet.* **122,** 1005-1016.
42. Shih, C. H., Chu, H., Tang, L. K., Sakamoto, W. Maekawa, M., Chu, I. K., Wang, M. and Lo, C. 2008. Functional characterization of key structural genes in rice flavonoid biosynthesis. *Planta* **228,** 1043-1054.
43. Takahashi, M. E. 1957. Analysis on apiculus color genes essential to anthocyanin coloration in rice. *J. Fac. Agric. Hokkaido Univ.* **50,** 266-362.
44. Wang, C. and Shu, Q. 2007. Fine mapping and candidate gene analysis of purple pericarp gene *Pb* in rice (*Oryza sativa* L.). *Chinese Sci. Bull.* **52,** 3097-3104.
45. Wang, X., Ji, Z., Cai, J., Ma, L., Li, X. and Yang, C. 2009. Construction of near isogenic lines for pericarp color and evaluation on their near isogenicity in rice. *Rice Sci.* **16,** 261-266.
46. Yoon, H. H., Paik, Y. S., Kim, J. B. and Hahn, T. R. 1995. Identification of anthocyanidins from Korean pigmented rice. *Agric. Chem. Biotechnol.* **38,** 581-583.
47. Yoshimura, Y., Zaima, N., Moriyama, T. and Kawamura, Y. 2012. Different localization patterns of anthocyanin species in the pericarp of black rice revealed by imaging mass spectrometry. *PLoS One* **7,** e31285.
48. Zhang, M. W., Zhou, J. and Peng, Z. M. 1994. Effects of different sowing date on yield traits and pigment contents in purple pericarp rice. *J. Hubei Agril. Sci.* **1,** l-4.

초록 : 흑미의 자색종자과피 형질을 결정하는 대립유전자와 안토시아닌 생성의 상호관계

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쌀은 인류가 가장 많이 먹고 있는 주식이다. 최근 백색미, 적색미, 갈색미, 녹색미 그리고 흑색미 등 다양한 품종들이 소비되고 있다. 고농도의 안토시아닌이 함유되어 있는 흑미는 영양가뿐만 아니라 암 발생 예방 효과가 있어 세계적으로 그 소비가 증가되고 있다. 유전학적으로 흑미는 cyanidin-3-*O*-glucoside (C3G)의 합성경로를 조절하는 두 개의 유전자 *Pb*와 *Pp*가 관여하는 *Purple Pericarp* (*Prp*) 유전형질에 의하여 종자과피의 색이 결정된다. 1921년 Nagai가 흑미의 유전현상을 일으키는 *Prp* 형질을 보고한 이 후 *Prp* 형질에 관여하는 유전자들과 그 기능 분석에 관한 많은 연구가 보고 되었으나 아직도 많은 부분이 밝혀지지 않았다. 그러나 안토시아닌의 함량이 높은 기능성 흑미의 소비는 증가하고 있는 반면 흑미는 일반 재배 벼에 비하여 상대적인 수확량이 매우 적어 농경제적인 문제가 있다. 본 논문에서는 흑미 종자가 성숙하는 과정에서 *Prp* 유전자의 유전현상의 특성과 안토시아닌 합성경로 그리고 수확량에 관한 유전학적 관점에서 내용을 정리하였다.