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Morphological Characteristics of the Rice (*Oryza sativa* L.) with Red Pigmentation

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Seven rice germplasms with red pigmentation within the pericarp were isolated from a large mutant collection. These red pericarp phenotypes resulted from the functions of the *Rc*, *Rd* and *RdRc* genes. Among them, two brown pericarp of the *Rc* type, four red pericarp of the *RdRc* type, and one white pericarp of the *Rd* type were identified. Morphological and agronomic characteristics of those rice germplasms were studied. The *Rc* type germplasms have the faint red or brown color pericarp and the *Rd* types produce the white pericarp, whereas the *RdRc* type germplasms have the dark red pericarp. Most of the important agronomic characteristics including plant stature, tillering ability, spikelet fertility, and total grain yield were lower in the colored rice than those of the wild-type control. All of the studied colored rice germplasms had a tendency of easy seed-shattering in comparison to the control. These characteristics of newly identified germplasms will be useful for identifying the genes responsible for pericarp color phenotype determination.

Key words : Red rice, rice mutants, *RdRc*, red pericarp, rice genetics

Introduction

The cultivated type white pericarp rice (*Oryza sativa* L.) is the principle source of cereal food in the world [4]. Along with a normal plant phenotype, rice may contain mutant phenotypes in the pericarp and seed coat including brown, red, and/or purple pericarp. Red-pericarped rice is ubiquitous among the wild ancestors of cultivated rice. The red pigmentation in the pericarp as well as other tissues is mostly due to the presence of two major groups of phenolic metabolites, the proanthocyanidins and the hydrolysable tannins [2,6,9,13,16,17]. Several genes related to pigmentation also have been described in plants. The *Rc* gene has been described in rice that encodes a basic Helix-Loop-Helix (bHLH) transcription factor [19]. The bHLH protein of the *Rc* gene is an up-regulator of proanthocyanidin expression in the rice seed coat and is responsible for the pigmentation of the brown pericarp [19]. The dominant allele *Rc* confers dark brown speckles on reddish brown background and accumulates pigments in the pigment layers. Thus, dominant *Rc* locus forms red pericarp in rice [19], whereas, domestication of the recessive *rc* allele and the mutations in the dominant *Rc* allele of the ancestral red rice confer the white pericarp [5]. Mutation in the *rc* alleles results in a loss of proanthocyanidin synthesis and lack of pigmentation on the pericarp and forms white pericarp which has been identified in many rice cultivars [3,5,8,18]. The other color related gene, the *Rd*, is not associated with any typical phenotype itself. However, when *Rc* co-exist with *Rd*, the entire surface of the rice pericarp becomes dark red [12]. Based on the dominant action of the *Rc* over *rc*, it is suggested that the cultivated white allele might actually be the mutant of the ancestral cultivars [5,18,19].

Beside white pericarp rice, there are many red pericarp rice varieties throughout the world, which may have lower qualities in taste and texture. Therefore, exploration of the agronomic traits from the red pericarp rice is needed to use the trait of red pericarp in adapted germplasms for rice breeding program. For the better understanding of the pigmentation on the rice pericarp, we have studied the phenotypes of pericarp in various rice varieties. These red rice germplasms showed distinct phenotypes from the wild type rice, and were studied for several important agronomic characteristics.

Materials and Methods

Plant materials

Several mutant rice germplasms and wild-type rice Ilpoombyeo (*japonica* cultivar), collected from Yeungnam University Genetic Resources Stock Center, Gyeongsan, Korea, were grown under natural field conditions between 30 to 35°C in the paddy field of Yeungnam University.

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Among them, two lines carrying *Rc* type, four lines carrying *RdRc* type, and one line carrying *Rd* type were isolated.

Phenotypic analysis and agronomical data scoring

The time of pericarp color development at different developmental stages were documented. Mature grains were collected at 90 days old and photographed using digital camera attached with simple Olympus SZ61 microscope (Olympus, Tokyo, Japan). Several agronomic characteristics including days to heading (DH), tiller number (TN) per hill, culm length (CL), panicle exertion ability (PE), panicle length (PL), panicle number (PN) per hill, panicle thresh ability (PT), spikelet number (SN) per panicle, spikelet fertility (SF) percentage and 100 grains (GW) weight were evaluated on the plant materials.

For each germplasm line, at least 15 seedlings of 25 days old were transplanted to the experimental field. Most of the agronomic data were evaluated at 60 days after transplantation during the end of vegetative growth. Fifteen plants for each accession were evaluated for agronomic traits. The number of tillers per hill was scored as the number of reproductive tillers per hill for each plant. The average tiller number and standard error (SE) were calculated from the data obtained from the 15 plants. The average number of spikelets was measured from the five plants using five panicles per plant. Spikelet fertility percentage was scored as the number of grains per panicle divided by the number of spikelets per panicle. The heading date for each plant of each accession was recorded when the first developing panicle emerged about 1 cm beyond the leaf sheath of the flag leaf. Heading time was monitored everyday. The mean days to heading of each plant were calculated by converting heading date and days to heading (DH) from the day of transplantation to mean heading date. For grain weight, 100 ripped spikelets were dehulled and gram weight were measured using an electronic balance.

Results and Discussion

Phenotypes of the red pericarp rice germplasms

We visually examined rice populations individually with red and white seed coat pigmentation. We have grouped the studied germplasms into *Rc*, *Rd* and *RdRc* type for pericarp color. Brown pericarp and seed coat, the *Rc* type, was observed in two rice accessions YUM033 and YUM034. Four accessions YUM016, YUM017, YUM041 and YUM068 had the red pericarp and seed coat, the *RdRc* type, and YUM063 had the *Rd* type pericarp which did not make any typical color in the pericarp (Fig. 1). At mature stage, the pericarp of the rice, the outermost layer of dehulled grain, was turned to reddish brown in the *Rc* and dark red in the *RdRc* type lines. The *RdRc* type had the deeper red color than the color of the *Rc* type (Fig. 1). On the other hand, the *Rd* type germplasm did not make any distinguished color on the pericarp. The accumulation of the dark red pigments in the *RdRc* type might be due to the complementary action of *Rc* and *Rd*. The co-existence of *Rc* with *Rd* turned the entire surface of pericarp into dark red [12]. However, the endosperm was white glutinous in all of the germplasms. The red color in the pericarp of the dehulled grain of the germplasms could be removed by polishing and thus the white endosperm was

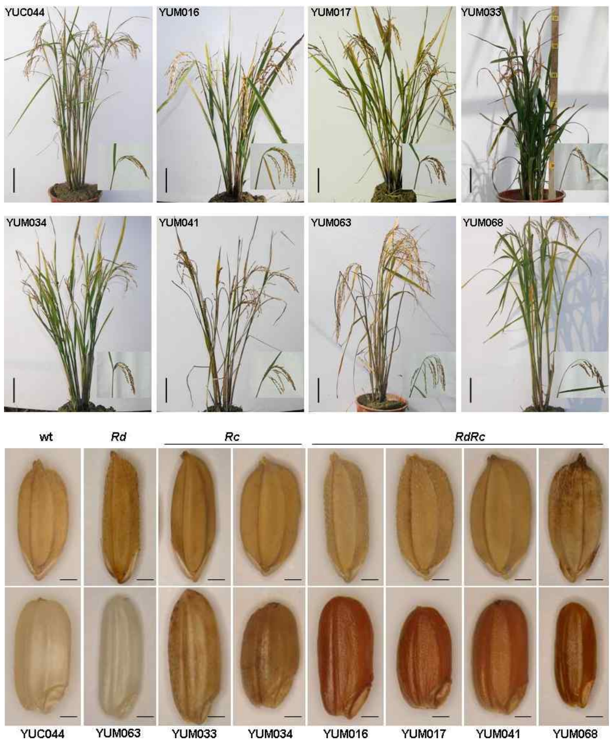

Fig. 1. Phenotypic and morphological features of the mutant germplasms. Photograph of the plant features and close-up images of paddy rice (up) and de-hulled seeds (bottom) of the wild type and red rice germplasms are shown. Field grown mature plants were photographed with digital camera. Close-up images of paddy rice and dehulled seeds were taken by digital camera attached with simple microscope at .67X magnification. Wild type cultivar (YUC044) and mutant germplasms are distinguished by corresponding YUM number. Panicles of the plants are illustrated in inset. Bars, 10 cm in the plant pictures and 1 mm in the seed pictures.

evident in the red rice, indicating that pigment accumulated on the maternally determined tissue pericarp only. Similar pattern of the color formation was identified in Arabidopsis where color was formed only on the maternally determined tissue testa of the seed [1].

The genetic mechanisms for color development in the pericarp indicated that red, brown, or white pericarp color is determined by the *RdRc*, *Rc* and *Rd* allele, respectively. Red color is determined either by single or double copy of both *Rd and Rc* genes and brown color is determined by either single or double copy of *Rc* [7,11,19]. However, the *Rd*, the other gene related to pigment synthesis which encodes a key enzyme for dihydroflavonol-4-reductase (DFR), does not form any detectable color itself [5]. Therefore, it is elucidated that, *Rc* itself makes brown to faint reddish color on rice pericarp but *Rd* alone does not make any color. Whereas, the presence of *Rc* together with *Rd* makes complementary effects that enhance the pigment accumulation and produce dark red color on the pericarp. Molecular genetic analysis indicated that a 14 bp deletion within the *Rc* gene caused a frame shift mutation and a premature stop codon [5,19]. This mutation results in a loss of proanthocyanidin synthesis forming white pericarp in the rice. On the contrary, a natural mutation in the *rc* allele (white pericarp) within the *rc* gene restored the reading frame of the gene and turned the pericarp color into red [3]. A high level of red pigment accumulation within the seed coat was identified in Arabidopsis *banyuls* (*ban*) mutant which indicated *BANYULS* protein is a negative regulator of flavonoid biosynthesis that prevents the accumulation of pigments in the seed coat [1]. In maize, deep red pericarp pigmentation was observed due to mutation in *pericarp color1* gene [15]. The chromosomal location of the *Rc* gene was identified on the chromosome 7 and the functional protein for this gene was Bhlh transcription factor containing protein [7,11,19]. The coloration in the studied germplasms was developmentally regulated where color formation started at a certain stage of development and increased until maturation. Similar color development was found in Arabidopsis [1]. We performed our visual observation on leaf sheath, leaf blade, mid rib, leaf margin, leaf tip, ligule, collar, auricle, node, internode, empty glumes, husk, apiculus, stigma, anther, and pericarp for pigment formation in our selected lines. Except pericarp, pigment formation was not exhibited in any other organs in the selected lines. Only one line, YUM068 had purple color on the hull up to dough stage which disappeared after matu-

Table 1. Phenotypic description of the rice germplasms with red pericarp

Plant ID	YU Bank ID	Germplasm	Genotype	Phenotypic description of the germplasm
YUC044	YC0110	Ilpoombyeo	wt	Wild type plant having color less seed coat and pericarp as well as white endosperm
YUM016	YC1420	Line 1020	*RdRc*	Dark red color in seed coat and pericarp. White endosperm. Hull is straw type to yellowish at maturation. Short stature and spreading type plant feature, broad leaves.
YUM017	YC1421	Line 1021	*RdRc, gh*	Dark red color in seed coat and pericarp with golden hull. Hull is golden due to presence of *gh* gene.
YUM033	YC1437	Line 1039	*Rc, gh gl, wx, drp*	Outer layer of grain is brown due to pigmentation on pericarp. *Rc* is responsible for the production of pigment in brown pericarp rice. Hull of spikelets also golden in color
YUM034	YC1438	Line 1040	*Rc, C, gh, gl, drp*	Outer layer of grain is brown due to pigmentation on pericarp. *Rc* is responsible for the production of pigment in brown pericarp rice. Hull of spikelets also golden in color.
YUM041	YC1446	CB817-2-1-1-2-1	*RdRc, gh, wx, Ac*	Dark red color in seed coat and pericarp. Hull golden, Endosperm white and waxy
YUM063	YC1469	Line 1165	*Rd, gh, C*	Pericarp color is whitish brown but not reddish due to lack of complementary effects of *Rc*. Hull become golden at maturation. Stature medium with normal wild type phenotype
YUM068	YC1474	Line 1174	*RdRc Pr, Lh, Ac*	Dark red color in the pericarp due to the complementary effects of *Rd* and *Rc*, hull become purple during dough stage and have hairy trichomes on the leaves and hull.

Note. Most germplasms have other mutant phenotype with red pericarp. Here, only the pericarp color was considered for analysis.

Table 2. Several important agronomic traits of the studied red rice germplasms

Plant ID	type	DH	CT	CL	PH	LL	LW	TN	PE	PL	PN	PT	SN	SF	GW
YUC044	Wt	79±2	3	66.4±1.2	128.3±4	56.9±0.8	1.76±0.05	16±3	1	24.40±0.6	16±1	1	123±4	94.50±0.5	2.46±0.15
YUM016	*RdRc*	73±4	3	58.0±2.0	111.2±3	48.0±2.3	1.90±0.06	12±3	1	25.30±1.0	11±2	3	121±5	82.10±2.3	2.50±0.81
YUM017	*RdRc*	74±4	3	62.8±0.7	116.2±3	50.4±1.3	2.10±0.06	13±3	3	22.50±0.8	10±2	3	110±6	85.48±2.0	2.20±0.08
YUM033	*Rc*	83±3	5	49.9±0.6	91.5±3	37.0±1.8	2.20±0.10	19±3	3	19.56±1.0	17±2	5	89±6	82.70±2.5	3.06±0.09
YUM034	*Rc*	78±4	3	59.0±1.2	113.4±4	49.0±0.8	1.72±0.02	14±4	3	19.40±0.5	12±4	5	112±6	78.20±3.3	2.10±0.89
YUM041	*RdRc*	79±3	3	63.9±0.6	102.0±2	38.0±1.8	1.04±0.10	11±3	5	18.56±1.0	10±2	5	87±6	87.70±2.5	3.02±0.09
YUM063	*Rd*	75±3	3	64.9±0.6	126.7±4	55.0±1.8	1.80±0.10	11±2	1	26.60±1.0	9±2	3	97±6	82.70±2.5	3.20±0.08
YUM068	*RdRc*	89±5	3	67.9±0.6	129.4±3	56.0±1.8	2.00±0.10	9±2	1	25.51±1.0	9±2	3	103±6	72.70±2.5	3.06±0.09

Note: Wt; wild type cultivar (Ilpoombyeo), DH; days to heading (defined as duration from transplantation to emergence of the first panicle), CT; culm thickness (Scale: 1; thin, 3; medium, 5; thick), CL; culm length in centimeter (cm), PH; plant height in cm, LL; leaf length in cm, LW; leaf width in cm, TN; number of reproductive tiller per hill, PE; panicle exsertion ability (Scale: 1; well exserted, 3; moderately well exserted, 5; just exserted, 7; partly exserted, 9; enclosed), PL; panicle length in cm, PN; panicle number per hill, PT; panicle thresh ability [Firmly grasp and pull the hand over the panicle and estimate the percentage of shattered grains. Scale: 1; difficult (less than 1%), 3; moderately difficult (1-5%), 5; intermediate (6-15%), 7; loose (26-50%), 9; easy (51-100%)]. SN; spikelet number per panicle, SF; spikelet fertility percentage, GW; 100 grains weight in gram, SE; standard error of five observation for each trait.

ration leaving faint purple color in the apiculus and glumes. Our observations indicated that those liens were unique for pigment accumulation.

Agronomic characteristics of the mutant germplasms

Beside the pericarp color of the mutant germplasms, several important agronomic traits were also observed and significant differences between the mutants and wild-type plants as well as among the mutants were found. Few mutants exhibited a life span that was significantly shorter than those of the normal white pericarp cultivars grown in the same environmental conditions. The flowering times of YUM033 and YUM068 were 83 and 89 days after transplantation, respectively. The flowering times of the rice including YUM016, YUM017 and YUM063 were 73, 74 and 75 days after transplantation, respectively. However, the flowering times of most wild-types were 79 days after transplantation (Table 2). In most germplasms, the plant stature was short; 111.2, 116.2, 91.5, 113.4, and 102.0 cm for YUM016, YUM017, YUM033, YUM034, and YUM041, respectively. All mutants showed various tiller numbers (Fig. 1, Table 2). Interestingly, all germplasms had very easy seed shattering tendency. Lower trait values of spikelets per panicle and spikelet fertility were found in most cases in comparison to those of wild-type rice which made lower yield in the mutants (Table 2). It is concluded that plant stature, tillering ability, and panicle formation ability were slightly lower in most of the mutants than in wild-type. Moreover, those mutants exhibited significantly reduced rate of seed germination compared to wild-type which might be the consequence of a high level of pigment in the mutants. It also might be due to a high degree of seed dormancy, since it was demonstrated that genotypes having a high intensity of black and red pigmentations on the pericarp had a relatively high degree of seed dormancy [6]. Due to their importance in rice breeding, extensive studies have been carried out to understand about many agronomic characteristics including high tillering [21], culm structure [10], and spikelets per panicle [20]. It also makes the study on colored rice very interesting that anthocyanin is involved in the plant defense mechanisms against pathogens [16] and the antioxidant functions against abiotic stress [14].

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References

1. Albert, S., M. Delseny, and M. Devic. 1997. *BANYULS*, a novel negative regulator of flavonoid biosynthesis in the Arabidopsis seed coat. *Plant J.* **11**, 289-299.
2. Bate-Smith, E. C. 1973. Tannins of herbaceous leguminosae. *Phytochemistry.* **12**, 1809-1812.
3. Brooks, S. A., W. Yan, A. K. Jackson, and C. W. Deren. 2008. A natural mutation in *rc* reverts white-rice-pericarp to red and results in a new, dominant, wild-type allele: *Rc-g*. *Theor. Appl. Genet.* **117**, 575-580.

4. Clay, J. W. 2004. *World Agriculture and the Environment:* A Commodity by-Commodity Guide to Impacts and Practices. Island Press, Washington.
5. Furukawa, T., M. Maekawa, T. Oki, I. Suda, S. Iida, and H. Shimada. 2007. The *Rc* and *Rd* genes are involved in proanthocyanidin synthesis in rice pericarp. *Plant J.* **49,** 91-102.
6. Gu, X. Y., S. F. Kianian, G. A. Hareland, B. L. Hoffer, and M. E. Foley. 2005. Genetic analysis of adaptive syndromes interrelated with seed dormancy in weedy rice (*Oryza sativa*). *Theor. Appl. Genet.* **110,** 1108-1118.
7. Kinoshita, T. 1993. Report of the committee on gene symbolization, nomenclature and linkage groups. *Rice Genet. Newsl.* **10,** 7-39.
8. Lee, D., E. Lupotto, and W. Powell. 2009. G-string slippage turns white rice red. *Genome* **52,** 490-493.
9. Lei, H., Z. H. Yu, X. P. Zhou, L. Yun, W. X. Dong, and W. X. Jun. 2006. Genetic analysis and histological study of red seed in rice. *Acta Genetica Sinka.* **33,** 559-564.
10. Li, X., Q. Qian, Z. Fu, Y. Wang, G. Xiong, D. Zeng, X. Wang, X. Liu, S. Teng, F. Hiroshi, M. Yuan, D. Luo, B. Han, and J. Li. 2003. Control of tillering in rice. *Nature* **422,** 618-621.
11. Nagato, Y. and A. Yoshimura. 1998. Report of committee on gene symbolization, nomenculture and linkagemap. *Rice Genet. Newsl.* **15,** 13-74.
12. Nagao, S., M. Takahashi, and T. Kinoshita, 1962. Genetics studies on rice plant, XXVI. *J. Fac. Agric. Hokkaido Univ.* **52,** 20-50.
13. Oki, T., M. Masuda, M. Kobayashi, Y. Nishiba, S. Furuta, I. Suda, and T. Sato. 2002. Polymeric procyanidins as radical-scavenging components in red-hulled rice. *J. Agric. Food Chem.* **50,** 7524-7529.
14. Reddy, V. S., K. V. Goud, R. P. Sharma, and A. R. Reddy. 1994. Ultraviolet-B-responsive anthocyanin production in a rice cultivar is associated with a specific phase of phenylalanine ammonia lyase biosynthesis. *Plant Physiol.* **105,** 1059-1066.
15. Robbins, M. L., R. S. Sekhon, R. Meeley, and S. Chopra. 2008. A *mutator* transposon insertion is associated with ectopic expression of a tandemly repeated multicopy *Myb* gene *pericarp color1* of maize. *Genetics* **178,** 1859-1874.
16. Scalbert, A. 1991. Antimicrobial properties of tannins. *Phytochemistry* **30,** 3875-3883.
17. Sylvie, A. M. Delseny, and M. Devic. 1997. BANYULS, a novel negative regulator of flavonoid biosynthesis in the *Arabidopsis* seed coat. *Plant J.* **11,** 289-299.
18. Sweeney, M. T., M. J. Thomson, Y. G. Cho, Y. J. Park, S. H. Williamson, C. D. Bustamante, and S. R. McCouch. 2007. Global dissemination of a single mutation conferring white pericarp in rice. *PLoS Genet.* **3,** e133.
19. Sweeney, M. T., M. J. Thomson, B. E. Pfeil, and S. McCouch. 2006. Caught red-handed: *Rc* encodes a basic helix-loop-helix protein conditioning red pericarp in rice. *Plant Cell* **18,** 283-294.
20. Xing, Y. Z., W. J. Tang, W. Y. Xue, C. G. Xu, and Q. Zhang. 2008. Fine mapping of a major quantitative trait loci, *qSSP7,* controlling the number of spikelets per panicle as a single Mendelian factor in rice. *Theor. Appl. Genet.* **116,** 789-796.
21. Zou, J., Z. Chen, S. Zhang, W. Zhang, G. Jiang, X. Zhao, W. Zhai, X. Pan, and L. Zhu. 2005. Characterizations and fine mapping of a mutant gene for high tillering and dwarf in rice (*Oryza sativa* L.). *Planta* **222,** 604-612.

초록 : 적색종피 돌연변이 벼의 형질특성조사

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벼의 적색 종피 표현형질은 *Rc* 혹은 *Rd* 그리고 *RdRc* 두 개의 복수유전자에 의하여 결정된다. 본 연구에서는 종피에 색소가 있는 돌연변이 벼 7계통을 벼 표현형 돌연변이 군들로부터 분리하였다. 이들 종피색의 돌연변이 형질을 조사한 결과 두 개의 변이체가 갈색의 종피형질을 가졌으며 네 개의 변이체가 적색의 종피형질을 가졌다. 그리고 한 개의 변이체는 종피에 색깔이 없었다. 수확 후 종자의 종피색 표현형질에 관한 정밀조사를 실시한 결과 두개의 갈색종피 돌변이체는 *Rc* 유전자형 이었으며 네개의 적색종피 돌변이체는 *RdRc* 유전자형이었다. 또한 종피에 색깔이 없는 한 개의 돌변이체가 *Rd* 유전자형이었다. 이들 중 동일한 종피색깔을 나타내는 돌연변이 개체들이 단일 돌연변이 개체의 후손인지 여부를 판명하기 위하여 돌변이체들의 초형과 농업형질을 조사한 결과 동일 종피색 돌변이체들의 형질이 서로 상이하므로 각각 서로 다른 돌연변이 계통들로 확인되었다. 그리고 이들 종피색깔 돌연변이들은 초형, 분얼 특징, 임성과 수량성 등을 포함한 농업형질들이 정상 벼의 농업형질들보다 대체로 빈약하고 또한 높은 탈립성을 보였다. 이러한 종피색과 관련된 돌연변이 벼 유전자원은 안토시아닌과 폴리페놀이 풍부하므로 병충해 저항성 품종과 환경스트레스 내성 품종의 육종에 필요할 뿐만 아니라 종피의 색깔을 결정하는 유전자들의 기능을 밝히는 데 중요한 자료로 사용될 것이다.