Journal of Science and Technology 13(June 2015): 118-121

GENETIC CHARACTERIZATION OF RED PERICARP TRAIT IN RICE

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ABSTRACT

Red rice is the type of rice which accumulates high level of pigments on the pericarp and form the pericarp red. Genetic analysis from segregating population of the cross between red pericarp rice and wild type white pericarp rice revealed that the red pericarp color is dominant in nature and seed color of F_1 plant is determined by its female parent. Cross between the red pericarp rice and the white pericarp rice followed the segregation ratio of 3 red: 1 white. Consequently, the red pericarp rice and the white pericarp rice genotypes might be *RdRdRcRc* and *RdRdrcrc*, respectively. The red color deposited from milking stage to maturation stage of the seed development. The red pigment was accumulated only in the pericarp layer remaining completely white endosperm in the seeds from parent as well as progeny.

Key words: Proanthocyanidin, *RdRc*, rice pericarp, segregation

INTRODUCTION

The outer most layer of the dehulled rice grain, the pericarp, is usually white in most cultivated rice (*Oryza sativa* L.). Rice with white pericarp is derived from the domestication of red rice and the white rice is ubiquitous throughout the world. It is postulated that the dominant allele *Rc* form red, and the recessive allele *rc* and mutation in the dominant allele confer the white pericarp in rice (Furukawa *et al*. 2007). Whereas, the *Rc-g* is dominant allele which was derived from the mutation in rc allele that turned white pericarp to red pericarp (Brooks *et al*. 2008). This red rice is the wild ancestors of cultivated rice and pigmentation on pericarp is due to the presence of proanthocyanidins as well as tannin (Oki *et al*. 2002; Scalbert 1991). A Basic Helix-Loop-Helix protein encoding gene *Rc* has been identified that is responsible for Anthocyanidin synthesis and corresponding red color development on the pericarp (Sweeney *et al*. 2006, 2007). Lee *et al*. (2009) stated, including 14 bp deletion in the *Rc* gene one more G base was deleted that restores the reading frame for the *Rc* gene and confer red color pericarp in the mutant. Red color is determined either by single or double copy of *RdRc* and brown color is determined by either single or double copy of *Rc* (in absence of *Rd*). On the other hand single or double copy of *Rd* (in absence of *Rc*) and homozygous recessive rdrdrcrc determines the white pericarp color in rice (Furukawa *et al*. 2007).

Reciprocal crosses between red and achromatic rice revealed that the seed color of F_1 was determined by its female parent and pericarp color was controlled by dominant gene (Lei *et al*. 2006). However, steps toward understanding of the molecular mechanisms of linkage relationship between the *Rc*, *Rd*, *RdRc* and some other domestication traits the genetic analysis could give the clue to get into insight. For the domestication of any important trait from their progenitors red rice could be used.

The aim of this study was to determine the genetic basis of red color pericarp. For the better understanding for the molecular basis of development of pigment on pericarp, phenotype of red rice was widely investigated. Moreover, genetic analysis of the red pericarp color was performed. This information might be useful for identification of the functions of genetic factors in the Mendelian inheritance.

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MATERIALS AND METHODS

Plant material

Several red rice germplasms collected from China, and white pericarp rice from BRRI were grown in paddy field of Hajee Mohammad Danesh Science and Technology University, Dinajpur during the period from May to November 2009. From the marker line collection, one rice line was selected with red pericarp and seed coat and was used for genetic analysis of color formation.

Genetic analysis

In this present study, red pericarp seed plant was crossed fertilized with white pericarp pollen parent. A total of 20 F_1 seeds were produced from the cross and color of matured F_1 seeds was visually examined. The F_1 plants were grown and self fertilized to produce F_2 seeds. F_2 seed was collected from a single F_1 plant and 268 F_2s were germinated and transplanted in the field under natural conditions. Phenotypic data of genes segregation were documented. At mature stage, F_3 seeds from single panicle were harvested separately from each F_2 plant. To confirm the heterozygosity, 20 seeds from each dominant plant were further grown and phenotypic data from each plant were documented. The genotype (dominant, heterozygous, and recessive) of the F_2 progeny was also determined.

Phenotypic analysis

Phenotype of F_1 plants and pericarp color of F_2 seeds produced from the F_1 were documented. Similarly, phenotype of all F_2 plants and F_3 seeds produced from F_2 plant were documented. For segregation analysis, at least 50 seeds from every plant were dehulled and colors of pericarp were detected manually based on red and white color of the parents.

Agronomic data scoring of parents and progeny

Beside the pericarp color of the materials, several agronomic traits including days to heading (DH), tiller number (TN), culm length (CL), leaf length (LL), plant height (PH), panicle length (PL), panicle number (PN), spikelet number (SN), spikelet fertility (SF) and 100 grains weight were evaluated according to Rahman *et al*. (2013). Twenty five days old seedlings were transplanted in a single manner to the experimental field.

RESULTS AND DISCUSSION

Genetic analysis of red pericarp in rice

Genetic study was performed in order to analyze the inheritance pattern of the pericarp color of rice, as well as to confirm that pigments were accumulated in maternal tissues only. In the study, pericarp color of matured seeds from F_1, F_2, and F_3 populations for individuals with red and white seed coat pigmentation including control with white pericarp were usually examined. A total of 20 crossed fertilized seeds were obtained and the color of all F_1 seeds were red as female parent which revealed that the F_1 phenotype conferred by maternal plant genotype. The pericarp color of all F_2 seeds displayed red phenotype as same as female parent, suggesting dominant nature of the red pericarp phenotype with maternal effects. Matured F_2 seeds were collected from one F_1 plant and were grown in the field to produce F_2 progeny. Phenotypic data were collected throughout the developmental stages of the pericarp. Out of 268 F_2 plants studied, seed pericarp of 204 plants was red (female parent type), and seed pericarp of 64 plants was white (male parent type) (Table 1). Further the best fitted data using Chi-square (χ^2) test for testing goodness of fit for number of gene segregating in the population were analyzed. Here it was hypothesized that the red color was segregating according to 3: 1 (red pericarp: white pericarp) ratio. The χ^2 results of segregation

Table 1. Chi square ($\chi2$) analysis for the phenotypic and genotypic segregation of seed pericarp color of red and white pericarp rice cross at F_2 generation

Cross	F_1 phenotype	F_2 segregation				$\chi2$ (3:1)	p-value
		Number	red	white	Total		
Red rice/ White rice	Red pericarp	Observed	204	64	268	0.178	0.9 - 0.1
		Expected	201	67	268		
		Genotypic	segregation			$\chi2$(1:2:1)	p-value
	Number	Dominant	Heterozygous	Recessive	Total		
	Observed	60	144	67	268	1.6	0.9 - 0.1
	Expected	67	134	67	268		

data of red: white fit with 3:1 (χ^2 =0.178, p-value, 0.90-0.10, (Table 2). With 1 degree of freedom, 5% critical value is 3.841. Thus, computed value of χ^2 (0.178) indicated that the hypothesis of gene segregation is not rejected.

Further, F_3 seeds were harvested separately from each F_2 plant. To confirm heterozygosity, 20 seeds from each dominant (204 red pericarp) and recessive (64 white pericarp) plants were further grown and phenotypic data from each plant were documented. Among the 204 red pericarp plants, 60 plants produced progeny where all of them had red pericarp indicating the homozygous dominant plants, and the other 144 plants produced progeny with segregation of red and white pericarp, indicating heterozygous plants. Whereas, all of the 64 recessive plants produced progenies had white pericarp indicating homozygous recessive plants. From this data, genotype (dominant, heterozygous, and recessive) of F_2 progeny was determined. The results indicating, the genotypic segregation followed Mendelian 1:2:1 (60: 144: 64) ratio (χ^2 =1.6, p-value, 0.90-0.10, (Table 2) which ensure the inheritance pattern of red pericarp color in rice.

Red coloration in rice grains is determined by the complementary effect of two genes, *Rc* and *Rd* (Nagao and Takahashi 1947) and the F_2 generation segregating at a ratio of 9 red: 3 brown: 4 white. However, no brown pericarp was noted in F_2 and F_3 segregation generations (Table 1). The red pericarp segregation of 3 red: 1 white is possible if both parents have homozygous dominant *RdRd* genes. In this cross, only *Rc* gene segregates as a ratio of 3 red: 1 white. The genotype of red rice might be *RdRdRcRc* and white rice might be *RdRdrcrc* (Table 2).

Table 2. The probability method for predicting genotype *RdRdRcRc* for red rice and *RdRdrcrc* for white rice

Cross	Red rice (*RdRdRcRc*) X	White rice (*RdRdrcrc*)
Gametets	*RdRc* ↓	*Rdrc*
F_1	*RdRdRcrc* red	
F_2		
Gamets	*RdRc* (1/2)	*Rdrc* (1/2)
RdRc (1/2)	*RdRdRcRc* (1/4) red	*RdRdRcrc* (1/4) red
Rdrc (1/2)	*RdRdRcrc* (1/4) red	*RdRdrcrc* (1/4) White

Pigment accumulation in the maternal tissue

The pericarp is a maternally determined tissue of the seed, which is the protective tissue of the ovule and surrounds the endosperm (West and Harada 1993). In this study, several reciprocal crosses were made in order to confirm the pigment accumulation in the maternal tissue. Homozygous plant of red pericarp seed was crossed with wild type plant having white pericarp and vice versa (Table 3). The mature seeds were observed for pigment formation. The F_1 was red when mother plant was red and pollen donor was white whilst F_1 was white when mother was white and donor was red pericarp. The results showed all of the F_1 seeds exhibited the genotype of the mother plant. Furthermore, The F_2 seeds from self fertilized F_1 of all combinations had the red pericarp color, even if the F_1 was white indicating the maternal effect of color accumulation on the pericarp. This mode of colorization is the same as other experiment on rice and Arabidopsis seed coat (Lei *et al*. 2006).

Table 3. The seed coat color of parents, F_1 and F_2 generations in reciprocal crosses

Cross	Phenotype			
	♀	♂	F_1	F_2
White rice /red rice	white	red	white	red
red rice/White rice	red	white	red	red

Phenotypic analysis of red rice

The red rice germplasm was identified from a large mutant collection which had red pericarp and seed coat at mature stage. Faint red pigment started to form at dough stage on the pericarp and become dark red at mature stage which might be due to the complementary action of *Rc* and *Rd*. *Rd* does not have any phenotype, however, in presence of *Rc*, *Rd* enhances the pigmentation and pericarp color become deep red. The color of the endosperm was white as in that of wild type rice. Apart from the pericarp phenotypes of the germplasm, several important agronomic traits were observed. There were significant differences between red rice and wild type control regarding days to heading, plant stature, tillering ability, leaf structures, grain structure and quality, and panicle structure (Table 4). The red rice exhibited a significant shorter life span than that of wild type control. Plant stature, tillering ability, panicle formation ability were slightly lower, as well as lower trait value of spikelets per panicle, and spikelet fertility were found in red rice than that of wild type rice.

Table 4. Several important agronomic traits of the studied red rice germplasms.

Plant ID	DH	CL	PH	LL	LW	TN	PL	PN	SN	SF	GW
Red rice	73±4	58±2	111.2±3	48±2.3	1.9±.06	12±3	25.3±1	11±2	121±5	82.1±2.3	2.5±.81
White rice	80±2	62±1.2	125.3±4	58±0.8	1.7±.05	20±3	25.4±.6	18±1	128±4	95.5±0.5	2.5 ±.15
Hybrid	80±4	70±2.1	132.2±3	60±2.3	2.0±.06	25±3	28.3±1	23±2	160±5	32.2.±2.3	2.7±.81

Note: DH; days to heading (defined as duration from transplantation to emergence of the first panicle), 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, PL; panicle length in cm, PN; panicle number per hill, SN; spikelet number per panicle, SF; spikelet fertility percentage, GW; 100 grains weight in gram, ±; standard error of five observation for each trait.

High seed shattering tendency was found in red rice than control. There was significantly lower rate of seed germination of the red rice than the white rice which might be because of high accumulation of pigment on the pericarp.

CONCLUSION

In this genetic analysis, we demonstrated that the *Rd* and *Rc* genes were involved in the red pigmentation of the rice pericarp with recessive epistatic interactions. The studied red and white rice genotypes might be *RdRdRcRc* and *RdRdrcrc*, respectively. The genetic constitutions would be promising approach in the breeding of red rice.

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