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**PJBS**

ISSN 1028-8880

**Pakistan  
Journal of Biological Sciences**

**ANSI***net*

Asian Network for Scientific Information  
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

## Pericarp Color and Haplotype Diversity in Weedy Rice (*O. sativa* f. *spontanea*) from Thailand

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**Abstract:** Weedy rice (*Oryza sativa* f. *spontanea*) is an important resource for breeding and evolutionary studies. The present study was carried out to identify the genotype of weedy rice strains responsible for variation of pericarp color by sequencing exon 6 of the Rc gene. Haplotype analysis revealed that rice strains with light red- and red-pericarp lacked the 14 bp deletion in the Rc gene, while rice strains with white-, brown and light green-pericarp contained the 14 bp deletion. A single nucleotide polymorphism (C→A SNP) in exon 6 of the Rc gene was not found in all the weedy rice strains examined. This indicates that white pericarp allele in weedy rice gene pools of today might have originated by gene flow from locally grown modern rice varieties with white pericarp into established gene pools of wild rice.

**Key words:** Weedy rice, seed color, Rc gene

### INTRODUCTION

Weedy rice (*Oryza sativa* L. f. *spontanea* Roshev) is commonly found in the rice fields of Thailand (Maneechote, 2004; Prathepha, 2009). Weedy rice has direct consequences on farmers income because it reduces the yield and lowers the commodity value of rice (Olofsdotter *et al.*, 2000). Genomic studies can be applied to improve understanding of weed science. Such information coupled with advances in molecular biology increases our understanding of weeds and the development of novel approaches to weed management (Weller *et al.*, 2001).

The Rc locus, located on exon 6, encodes a bHLH protein, which effects a change from red pigmentation to white in the rice bran layer (Sweeney *et al.*, 2006; Furukawa *et al.*, 2007). Studies showed that rice varieties with white pericarp contains the 14 bp deletion in the Rc gene; this deletion was found in none of those with red pericarp. Recently, Brooks *et al.* (2008) reported that a novel dominant allele (Rc-g) was found in cultivated rice variety (cv. Wells) that arose from natural mutation.

Morphotypes from weedy rice samples collected in Northeastern Thailand were identified based on the seed morphological traits (awn color, apiculus color, lemma and palea color and pericarp color). The samples showed combinations of morphological variation and diversity of the pericarp color. The weedy rice accessions showed red, light-red, white and a light-green pericarp (Prathepha, 2009).

Proper identification of mutation (s) that cause the variation of pigment in the weedy rice grains would required comparative studies of the mutations in cultivated and wild rice. Currently, little attention is paid to the development of such information. In this study, the frequency and distribution of the 14 bp deletion and/or a SNP (C→A) in the Rc gene that has shown to cause white pericarp in rice were determined in weedy rice samples. Thus, a set of diverse weedy rice accessions based on pericarp color were sequenced the exon 6 of the Rc gene.

### MATERIALS AND METHODS

**Plant material:** The pericarp is derived from the maternal tissue, hence its color is dependent on the maternal genotype. DNA samples from flag leaves of each weedy rice plant (maternal plant) that produced progeny seeds of a particular pericarp color were extracted and sequenced to determine the association between pericarp color and the InDel (the 14 bp deletion) and SNP (C→A mutation). These mutations occurred in exon 6 of the Rc gene (Sweeney *et al.*, 2007). Two hundred and fifteen weedy rice plants were collected from the North-eastern region of Thailand in 2007 and studied by Prathepha (2009). Among these, nine groups representative of different pericarp color (Fig. 1) were selected for investigation of the DNA sequence of exon 6 of the Rc gene.

**DNA extraction, amplification and sequencing:** Total DNA was extracted from fresh leaves, following the CTAB

method as described by Doyle and Doyle (1987). The Polymerase Chain Reaction (PCR) amplification of the target DNA fragment containing exon 6 was performed using primer pairs: Rc3s21: 5'-ccatttcctctgtcacag-3'; Rcex7R1: 5'-aaaggtaacaaagatcgcag-3' by Furukawa *et al.* (2007). The reaction mixture contained 10 pmol each of the primer pairs, 0.1 mM of each dNTP, 1.5 mM MgCl<sub>2</sub> and 0.5 units of Taq polymerase (Promega, Madison, WI, USA) in a final volume of 20 µL. The amplification conditions were 3 min at 94°C followed by 35 cycles of 94°C for 1 min, 60°C for 1 min and 72°C for 1.5 min. A final extension step at 72°C for 5 min was performed after the 35 cycles. The PCR products were resolved using 2.0% agarose gel electrophoresis. The amplified DNA fragments corresponding to the expected size (~428bp) were cut from the gel and purified with a purification kit according to the manufacturer's manual (Pharmacia, Biotech, Freiburg, Germany). The purified PCR products of amplified fragments were sequenced directly using BigDye terminator reactions and the amplification primer on an automated sequencer (ABI 310; Applied Biosystems, Foster City, CA, USA). DNA sequence alignment of all rice strains examined within the exon 6 region was compared to the published sequence for the wild rice *O. rufipogon* (DQ204737) which lacked the 14 bp deletion and the cultivar Jefferson (DQ204736) which carried the 14 bp deletion and the cultivar H75 (DQ204735) which produced brown pericarp. DNA sequence alignment was performed with SeqMan II (DNASTAR®, Madison, WI).

## RESULTS AND DISCUSSION

Most rice strains have white pericarp, but rice can also produce grains with brown and purple pericarp. To determine the association of the 14 bp deletion and the C→A SNP in the coding region of exon 6 of the Rc gene with pericarp color in a set of 32 strains of cultivated and weedy rice by sequencing exon 6 of the Rc locus. The maternal plants were selected to represent the range of pericarp color of progeny grains (white, light red, red, brown and light green) (Fig 1). Rice with white, light green and brown pericarp contained an identical 14 bp deletion in the Rc gene; this deletion was not found in any of those with red and light red pericarp (Fig. 2a, b). The results of this study agree with the reports of earlier studies that showed that white- and brown-grained rice contained the 14 bp deletion (Furukawa *et al.*, 2007; Sweeney *et al.*, 2007). Furthermore, the 14 bp deletion was also found in weedy rice strains with light green pericarp. It is suggested that these weedy rice strains which had light green, brown and white pericarp, a 14 bp deletion

would have altered the reading frame of the Rc gene resulting in a light green, brown or white pericarp. Theoretically, if a rice strain lacks the 14 bp deletion, the red pigment in rice grain (proanthocyanidin) would be synthesized.

It has been reported that some *aus* and aromatic rice varieties, which lacked pigment (or white pericarp), did not contain the 14 bp deletion, these rice varieties showed a single nucleotide polymorphism (C→A SNP) in the coding region of exon 6, which (SNP) causes the lack of pigment in these rice grains (Sweeney *et al.*, 2007). All 32 strains of cultivated and weedy rice used in this study did not express the SNP, suggesting that the SNP is not predictive of white, brown and light green pericarp for weedy rice strains. Sweeney *et al.* (2007) suggested that the 14 bp deletion conferring white pericarp in rice occurred once in the *japonica* gene pool and was widely introgressed into *indica* and *aus* landraces. Based on this proposition, it could be deduced that weedy rice strains with white rice pericarp arose by gene flow. The flow being from landraces and modern rice varieties with white pericarp, common in this area, into established gene pools of wild rice. The flow resulted in weedy rice with a white-pericarp allele that is maintained in weedy rice gene pool till today.

Remarkably, the seed progeny produced by a maternal plant (code Spontanea 20) showed white and red pericarp grains. As previously reported, either the 14 bp deletion or a SNP (C→A) in exon 6 of the Rc gene causes white pericarp of rice (Sweeney *et al.* 2007). The DNA sequence of exon 6 of the Rc locus of the maternal plant showed a lack of the 14 bp deletion. This implied that the maternal plant would have been a red-pericarp rice, producing a progeny seed with white pericarp. However, seed progeny with red-pericarp, produced by the maternal plant, might have resulted from cross pollination by red-pericarp rice. Since, the pericarp is a



Fig 1: Seed morphology and pericarp color of the nine representative groups of weedy rice



Fig. 2: Comparison of the DNA sequences of exon 6 of red and white rice pericarps. Rice strain with red pericarp, (a) lack the 14 bp deletion (5'-ACGCGAAAAGTCGG-3') while rice strain with white pericarp, (b) contains the 14 bp deletion which is indicated by arrows. The SNP (TAC→TAA) is not observed in the DNA sequences. The TAC sequence is shown in a circle

maternal tissue, the pericarp color is dependent on the maternal genotype (Sweeney *et al.*, 2006). Hence, seed progeny produced by the white-pericarp maternal plant can have both white and red-pericarp. The red-pericarp seed resulted from hybridization with a red-rice plant

acting as pollen donor. This is strongly supported by the analysis of the 8-seed progeny rice plant, produced by a light green pericarp maternal plant (code SP-HYB-RD15). When the grain No. 2 was dehulled, progeny No. 2 showed white pericarp, but the F<sub>1</sub> plant grown from this

Table 1: Pericarp color and DNA sequences and SNP (C→A), of exon 6 of Rc gene and chloroplast subtype of 32 accessions of cultivated and weedy rice (*Oryza sativa* f. spontanea) used in this study

Code of maternal plant/grain pericarp color of progeny	14-base pair deletion* and C/A-SNP	Chloroplast subtype of maternal plant**
<i>Oryza sativa</i>		
cv. KDML 105/white Carried/C		8C8A
cv. RD 15/white Carried/C		8C8A
<i>Oryza sativa</i> f. spontanea		
SPUD-1/red	Lacked/C	6C8A
SPUD-2/green	Carried/C	6C8A
SPUD-3/green	Carried/C	6C8A
SPUD-4/green	Carried/C	6C8A
SPUD-5/green	Carried/C	6C8A
SPUD-6/green	Carried/C	6C8A
SP-Pluak Sung/white	Carried/C	ND***
Spontea I /white	Carried/C	ND
Spontanea 24/white	Carried/C	ND
Spontanea 22/white	Carried/C	ND
Spontanea 20/white or red	Lacked/C	ND
Spontanea 19/red	Lacked/C	ND
Spontanea 18/brown	Carried/C	6C8A
Spontanea 17/white	Carried/C	ND
Spontanea 3/white	Carried/C	6C8A
Spontanea 4/white	Carried/C	ND
SP-S2-7/white	Carried/C	6C8A
SP-S2-6/white	Carried/C	ND
NB-2/brown	Carried/C	7C7A
NB-3/white	Carried/C	6C8A
NB-8/red or light red	Lacked/C	6C8A
NB-9/white	Carried/C	6C8A
SP-HYB-RD15 (one panicle from maternal parent plant)/light green	Carried/C	8C8A
SP-HYB-RD15-1 (plant grown from seed No. 1)	Carried/C	6C7A
SP-HYB-RD15-2 (plant grown from seed No. 2)	Lacked/C	ND
SP-HYB-RD15-3 (plant grown from seed No. 3)	Carried/C	ND
SP-HYB-RD15-4 (plant grown from seed No. 4)	Carried/C	ND
SP-HYB-RD15-5 (plant grown from seed No. 5)	Carried/C	ND
SP-HYB-RD15-6 (plant grown from seed No. 6)	Carried/C	ND
SP-HYB-RD15-7 (plant grown from seed No. 7)	Carried/C	ND

\* 14-bp (5'-acggcaaaagtcgg-3'), \*\* Results according to Prathepha (2009), \*\*\* Not Detected

seed showed a DNA sequence characteristic of red-pericarp rice (Table 1). Thus, outcrossing had occurred between the red- and white-rice pericarp. In addition, evidence on gene flow among weedy rice has been reported from the same rice field which the weedy rice samples used in this study were collected (Prathepha, 2009).

The results from the previous studies provided molecular evidence of natural hybridization, showing that the pattern of gene flow is mostly from the cultivated rice to its wild relative, *O. rufipogon* (Prathepha, 2009). The weedy rice strain showed the chloroplast subtype, 6C8A which was characteristic of wild rice (Table 1). A possible explanation for the origin of the weedy rice strain is hereby proposed that weedy rice originated from a hybridization event, where a wild relative served as the maternal parent, while a cultivated rice was the parental pollen donor.

In summary, observations from this study serves as additional data providing an understanding of pericarp diversity and DNA sequence polymorphism of weedy rice. In addition, this study used the 14 bp nucleotide polymorphism within the Rc locus to detect gene flow

between rice strains of red and white pericarp and/or wild and cultivated rice.

#### ACKNOWLEDGMENTS

This research was supported in part by a grant from the TRF/BIOTEC Special Program for Biodiversity Research and Training (BRT R\_250005). The author extends thanks to V. Pilap for contributions to this study.

#### REFERENCES

- 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. Applied Genet., 117: 575-580.
- Doyle, J.J. and J.L. Doyle, 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull., 19: 11-15.
- Furukawa, T., M. Maekawa, T. Oki, I. Suda and S. Lida *et al.*, 2007. The Rc and Rd genes are involved in proanthocyanidin synthesis in rice pericarp. Plant J., 49: 91-102.

- Maneechote, C., 2004. Invasion of weedy rice in rice fields in Thailand. *Rice Genet. Newsl.*, 29: 20-22.
- Olofsdotter, M., B.E. Valverde and K.H. Madsen, 2000. Herbicide resistant rice (*Oryza sativa* L.): Global implications for weedy rice and weed management. *Ann. Appl. Biol.*, 137: 279-295.
- Prathepha, P., 2009. Seed morphological traits and genotypic diversity of weedy rice (*Oryza sativa* f. *spontanea*) populations found in the Thai Hom Mali rice fields of north-eastern Thailand. *Weed Biol. Manage.*, 9: 1-9.
- 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.
- 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 Gene.*, 3: 1418-1424.
- Weller, S.C., R.A. Bressan, P.B. Goldsbrough, T.B. Fredenburg and P.M. Hasegawa, 2001. The effect of genomics on weed management in the 21st century. *Weed Sci.*, 49: 282-289.