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Genetic differentiation in weedy (*O. sativa* f. spontanea) and wild rice (*O. rufipogon*) revealed by DNA sequence in the QTL *SD7-1/Rc* locus

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Abstract

The QTLSD7-1/Rc is a pleiotropic locus that controls seed dormancy and pericarp color in rice. DNA sequence variation in promoter region, exon1, and intron 1 in wild rice from Thailand and Laos, weedy rice accessions from Thailand, as well as cultivated rice were investigated using re-sequencing DNA technique. For promoter region, three types of DNA variation were observed including variation in number of nucleotide $(T)_n$ (n=9,10,11), five SNPs and 19-bp indel. For coding region exon 1, no DNA sequence variation was observed. For non-coding region intron1, weedy rice had the 10-bp insertion in the region, but there was the deletion in wild rice. In addition, deletion of 34-bp in intron1 was observed in all wild rice accessions examined, while weedy rice had the 34-bp insertion. Haplotype analysis of weedy rice indicated that the pairwise nucleotide diversity parameter, P_i (π) and the level of the Watterson estimator (Θ_w) in the DNA sequences were 0.00401 and 0.0048, respectively. This finding enhances knowledge of the DNA sequence

Keywords: QTL SD7-1/Rc, seed dormancy, pericarp color, DNA variation, weedy rice, wild rice.
Abbreviations: QTL_Quantitative trait loci, SD_Seed dormancy, RC_Red pericarp, bp_base pairs, Indel_insertion/deletion

Introduction

In tropical regions, weedy rice (*O. sativa* f. *spontanea*) has been classified as a tropical ecotype that might have originated from natural variants of a wild ancestor, or from hybrids between wild and cultivated rice (Zhang et al., 2014). Weedy rice is commonly found in paddy fields in Thailand, as a result of limiting factors, such as manpower limitation, problematic water management led to changing in rice cultivation practices from traditional puddled rice transplantation to direct seeded rice. This is a common phenomenon occurred in most rice growing countries (Kraehmer et al., 2016). In addition, weedy rice in tropical regions is closely related to local rice cultivars and shows some evidence of hybridization among wild and different cultivated rice cultivars, leading to support for the hypothesis that weedy rice originated through hybridization (Chen et al., 2004). In terms of germplasm, weedy rice has been recognized as a reservoir of natural variation to utilize for crop improvement (Nadir et al., 2017). Further, some beneficial traits were reported in different levels of variation such as weedy rice seed dormancy, seed pericarp color, seed shattering and stress tolerance (Xia et al., 2011; Fogliatto et al., 2012; Thurber et al., 2010). The wild ancestor of cultivated rice, *O. rufipogon* grows in a broad geographic range spanning eastern India, Indochina, southern China and southeastern Asia (Londo et al., 2006), and has been recognized as valuable genetic resources for rice genetic improvement (Tanksley and McCouch 1997; Nadir et al., 2017). Several studies have investigated population genetics, evolution, and agronomical traits of the wild rice species (Barbier , 1989; Grillo et al., 2009; Huang et al., 2012).

Pericarp color is visually observed as red or white of de-hulled grains and results from the accumulation of anthocyanins and proanthocyanidins in the pericarp (Addel-Ala et al., 2006). In previous research, the *Rc* gene was mapped and it was identified that there are two alleles of *Rc* gene, a wild functional allele generating red pericarp, a functional product and non-functional allele carrying a 14-bp frameshift deletion in exon 7 that generated a nonfunctional gene product and non-pigmented (or white pericarp) (Sweeney et al., 2006; Furukawa et al., 2006). DNA sequences of the *Rc* gene have been deposited in GenBank accession no. DQ204735-38. Furthermore, the QTL for seed dormancy and pericarp color located on short arm of chromosome 7 in weedy rice named QTL SD7-1/Rc has been characterized and finely mapped by Gu et al., (2005). Six years later this research team reported that seed dormancy and red pericarp color in weedy rice are associated with each other via pleiotropic effects (Gu et al., 2011). The genomic DNA sequences of this quantitative trait locus consists of eight exons and seven introns (GenBank accession JF303048.1) (Gu et al., 2011). The QTL SD7-1/Rc is equivalent to the *Rc* gene which is a single locus and encodes a bHLH protein that pleiotropically controls the proanthocyanidin pigment in pericarp and abscisic acid-

mediated seed dormancy (Gu et al., 2011). Seed dormancy (SD) is a seed trait and was evaluated by germination percentage for seed and caryopsis (hull-removed seed enclosed by the pericarp). SD is an important attribute that is a target for manipulation by plant breeders to improve preharvest sprouting tolerance in rice and to develop transgene mitigation strategies to reduce the risk of gene flow from genetically modified crops into weed/wild relatives (Langevin et al., 1990; Zhang et al., 2014).

Weedy rice has been considered as part of the primary gene pool for crop breeding and it is assumed that weedy races harbor genes for tolerance to various adverse conditions (Harlan et al., 1973). New genetic and genomic approaches will facilitate discovery of novel gene or genomic DNA variants in non-domesticated germplasm leading to major advances in crop improvement (Tanksley and McCouch 1997). In addition, the pericarp color of rice grains carries anthocyanin, one of the key determinants of rice nutritional quality (Prathepha et al., 2018; Wang et al., 2020). Therefore, the generation of refined genomic DNA resources through the re-sequencing of a wide range of rice genetic materials would be useful for identifying and exploring allelic or haplotype variation of this trait in wild and weedy rice. The genome of rice is divided into the coding and noncoding regions. The coding regions contain DNA sequences which primarily determine the amino acids sequences of the proteins. Non-coding regions generally contain DNA sequences with no known function or possibly of no function whatsoever. Several studies have reported that the noncoding DNA region tends to have more polymorphism that plays an important role in genome structure, evolution, and diversity (Duan et al., 2017; Ruan et al., 2020). InDel (insertion and deletion) and single nucleotide polymorphisms (SNP) are prominent sources of variation in rice genome and serve as excellent DNA markers (Yonemaru et al., 2013). InDel and SNPs are mostly located in noncoding DNA regions and they might have different consequences at the phenotypic level (Duan et al., 2017). The DNA polymorphisms mostly consist of regulatory elements that control gene expression, but these polymorphisms have remained largely unexplored in rice genomes and their different genetic backgrounds or geographically origins have not been discovered. In Thailand, knowledge of genetic diversity of weedy rice along with procedures and mechanisms of its reoccurrence is still limited, and as a result, it is not possible to design effective practical tools and methods for weedy rice management. In order to fill the knowledge gaps, it is necessary to study weedy rice populations occurring in the region with a proper sampling strategy and to compare them with cultivated rice and wild rice from different habitats by using more powerful tools for characterization. This will enable to better understand of the level and distribution of the genetic diversity of weedy rice populations in Thailand and facilitate exploration of the possible origins of weedy rice and support more effective control.

This paper reports on newly discovered DNA sequences which resulted from exploring DNA polymorphism of the QTL/Rc locus in the representatives of wild rice and weedy rice accessions geographically originating in Thailand. DNA polymorphisms were revealed by nucleotide polymorphism of target DNA regions (promoter, exon1, intron 1, and exon 2). It is likely that a mutation discovered in this work is significant to better understanding on relationships at genetic perspectives in agricultural genes among wild, weed and cultivated rice.

Results

SNPs and insertion-deletion (indel) in DNA sequences of the QTL SD7-1/Rc locus

Overall, samples of wild rice and weedy rice examined revealed one types of InDel in the promoter region and two type of indel in intron1 as shown in Fig. 3. In detail, wild rice accessions exhibited variation in monosatellite T (n=9, 10, and 11), whereas, all weedy rice accessions and a cultivated rice (cv. KDML105) had only $(T)_{10}$ in the promoter region. The 10-bp deletion in intron1 was detected in all accessions of wild rice while weedy rice and cultivated rice had the 10-bp insertion. Moreover, deletion of 34-bp in intron1 was observed in all wild rice accession examined. In contrast, weedy rice accessions and a cultivated rice cv. KDML105 had the 34-bp insertion, which result is consistent with prior observations (Gu et al., 2011). Wild rice, *O. rufipogon* and weedy rice accessions examined exhibited five haplotypes (H1–H5). A phylogenetic tree (NJ tree) using five haplotypes was constructed (Fig. 4). Weedy rice accessions and a single cultivated rice (cv. KDML 105) were defined as haplotype 1 (H1) since these rice accessions contained 10-bp insertion in the first intron of the locus, whereas all wild rice accessions carried the 10-bp deletion in the same region. Haplotypes 2–5 (H2–H5) were represented by wild rice accessions from four different locations (PSL:H2, SKN:H3–H4), and Laos: H5). Haplotype 3 (H3) and haplotype 4 (H4) from a large lake (Nong Harn Lake) in Sakon Nakhon province revealed 19-bp deletion and insertion in promoter regions of the locus, respectively. Moreover, H4, a wild rice sample code Rc-ORCon-4. wild SKN, also had 6 SNPs (ACACGT) in the promoter region (Fig. 3). Previously, DNA sequences of this locus of EM93-1 cultivar had shown the 19-bp deletion (Gu et al., 2011; GenBank Acc. number JF303048.1).

DNA sequences of the QTL SD7-1/Rc locus (1375 bp) were analyzed for their nucleotide diversity in weedy rice. In summary, six SNPs and 66 indels (insertion/deletion) were detected in the aligned 1375 bp sequence (promoter, exon1, intron1, and exon2). The pairwise nucleotide diversity parameter, P_i (π) and the level of the Watterson estimator (Θ_w) in the DNA sequences were 0.00401 and 0.0048, respectively. Tajima's value was not a significant negative value (D=-0.71929, $p > 0.10$). Interestingly, the DnaSP analyses indicated that the DNA sequences were conserved in the aligned sequences at nucleotide positions 881–1151, and 1153–1377.

For wild rice, *O. rufipogon*, the values of Pi (π) and Θ_w per site in a combined DNA sequence data (827 bp) of promoter and intron1 were 0.0118 and 0.0177, respectively. Tajima's D values showed a positive value and did not reach a significant level (D=0.3954, P>0.01). However, the combined DNA sequence data of weedy rice and cultivated rice showed both of these nucleotide diversity parameters as zero. The eight weedy rice accessions and one cultivated rice cultivar (KDML105) exhibited one haplotype (H1) clustered within the same clade, as expected, and might have resulted from recent hybridization or gene introgression between weedy rice and cultivated rice.

Table 1. List of rice species, accessions or code, localities and habitats of samples used in this study.

Species	Number of accessions/ Code	Localities	Habitats
O. sativa f. spontanea	MSK (n=30)	N15° 26.684'/E103° 24' (Maha Sarakham province, NE, TH)	Co-existing in paddy fields
	SR (n=19)	N15° 26.684'/E103° 24' (Surin province, NE, TH)	Co-existing in paddy fields
	PCH1	N16° 31.342'/E100° 08' (Pichit province, C, TH)	Co-existing in paddy fields
Oryza rufipogon	LSK1	N18°21.74'/E102° 38' (Savanakhet province, Laos)	Natural pond (large population, >500 m^2)
	SKN-con4, con5	N17°07.218'/E104° 125' (Sakon Nakhon province, NE, TH)	Nong Harn Lake (> 25 km^2)
	BP6	ND (Pitsanuloke province, C, TH)	Natural pond (large population, >500 m^2)
O. sativa	cv. KDML105		

C= central, NE= northeastern, TH=Thailand, ND= no data.

Fig 1. Localities of wild (*Oryza rufipogon*) and weedy rice (*O. sativa* f. spontanea) samples.

Fig 2. Plant of weedy rice shows panicle with long awn from Maha Sarakham province.

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Rc-SP-06.weed MSK    GACGTCGAAAACGACATGTATGAACCGTTTTTTTTTT--CCTTTGTCGACACATGTGGAG   300
Rc-SP-04.weed SR     GACGTCGAAAACGACATGTATGAACCGTTTTTTTTTT--CCTTTGTCGACACATGTGGAG   300
Rc-ML-105.CV         GACGTCGAAAACGACATGTATGAACCGTTTTTTTTTT--CCTTTGTCGACACATGTGGAG   300
Rc-SP-02.weed SR     GACGTCGAAAACGACATGTATGAACCGTTTTTTTTTT--CCTTTGTCGACACATGTGGAG   300
Rc-SP-08.weed MSK    GACGTCGAAAACGACATGTATGAACCGTTTTTTTTTT--CCTTTGTCGACACATGTGGAG   300
Rc-SP-17.weed MSK    GACGTCGAAAACGACATGTATGAACCGTTTTTTTTTT--CCTTTGTCGACACATGTGGAG   300
Rc-SP-21.weed SR     GACGTCGAAAACGACATGTATGAACCGTTTTTTTTTT--CCTTTGTCGACACATGTGGAG   300
Rc-SP-PS.weed PCH    GACGTCGAAAACGACATGTATGAACCGTTTTTTTTTT--CCTTTGTCGACACATGTGGAG   300
Rc-OR-Con-4.wild SKN GACGTCGAAAACGACATGTATGAACCGTTTTTTTTT---CCTTTGTCGAC---------   300   (19-bp
deletion)
Rc-OR-LSK1.wild LAOS GACGTCGAAAACGACATGTATGAACCGTTTTTTTTTT-CCCTTTGTCGACACATGTGGAG  300
Rc-OR-BP6.wild PSL   GACGTCGAAAACGACATGTATGAACCGTTTTTTTTTT-CCCTTTGTCGACACATGTGGAG   300
Rc-OR-Con-5.wild SKN GACGTCGAAAACGACATGTATGAACCGTTTTTTTTTTTCCCTTTGTCGACACATGTGGAG  300
Rc-SP-06.weed MSK    GCCGGGGAGTAGTACGCATAGTAGCTCCTATAGTCCACGTGACCGACCTCGGCATGAGCC    360
Rc-SP-04.weed SR     GCCGGGGAGTAGTACGCATAGTAGCTCCTATAGTCCACGTGACCGACCTCGGCATGAGCC  360
Rc-ML-105.CV         GCCGGGGAGTAGTACGCATAGTAGCTCCTATAGTCCACGTGACCGACCTCGGCATGAGCC   360
Rc-SP-02.weed SR     GCCGGGGAGTAGTACGCATAGTAGCTCCTATAGTCCACGTGACCGACCTCGGCATGAGCC  360
Rc-SP-08.weed MSK    GCCGGGGAGTAGTACGCATAGTAGCTCCTATAGTCCACGTGACCGACCTCGGCATGAGCC    360
Rc-SP-17.weed MSK    GCCGGGGAGTAGTACGCATAGTAGCTCCTATAGTCCACGTGACCGACCTCGGCATGAGCC    360
Rc-SP-21.weed SR     GCCGGGGAGTAGTACGCATAGTAGCTCCTATAGTCCACGTGACCGACCTCGGCATGAGCC  360
Rc-SP-PS.weed PCH    GCCGGGGAGTAGTACGCATAGTAGCTCCTATAGTCCACGTGACCGACCTCGGCATGAGCC    360
Rc-OR-Con-4.wild SKN ---------ACATGTGCATAGTAGCTCCTATAGTCCACGTGACCGACCTCGGCATGAGCC   360
Rc-OR-LSK1.wild LAOS GCCGGGCAGTAGGACGCATAGTAGCCCCTATAGTCCACGTGACCGACCTCGGCATGAGCC    360
Rc-OR-BP6.wild PSL   GCCGGGGAGTAGTACGCATAGTAGCCCCTATAGTCCACGTGACCGACCTCGGCATGAGCC    360
Rc-OR-Con-5.wild SKN GCCGGGGAGTAGGACGCATAGTAGCTCCTATAGTCCACGTGACCGACCTCGGCATGAGCC    360
                     ****** (SNPs)

Rc-SP-06.weed MSK    AAGGGTACCTACCCTACCTACCTACGACACGATGCACAGTGTTCATCCATGGCCGGCCAT 660  (intron1)
Rc-SP-04.weed SR     AAGGGTACCTACCCTACCTACCTACGACACGATGCACAGTGTTCATCCATGGCCGGCCAT  660
Rc-ML-105.CV         AAGGGTACCTACCCTACCTACCTACGACACGATGCACAGTGTTCATCCATGGCCGGCCAT   660
Rc-SP-02.weed SR     AAGGGTACCTACCCTACCTACCTACGACACGATGCACAGTGTTCATCCATGGCCGGCCAT  660
Rc-SP-08.weed MSK    AAGGGTACCTACCCTACCTACCTACGACACGATGCACAGTGTTCATCCATGGCCGGCCAT 660
Rc-SP-17.weed MSK    AAGGGTACCTACCCTACCTACCTACGACACGATGCACAGTGTTCATCCATGGCCGGCCAT 660
Rc-SP-21.weed SR     AAGGGTACCTACCCTACCTACCTACGACACGATGCACAGTGTTCATCCATGGCCGGCCAT  660
Rc-SP-PS.weed PCH    AAGGGTACCTACCCTACCTACCTACGACACGATGCACAGTGTTCATCCATGGCCGGCCAT 660
Rc-OR-Con-4.wild SKN AAGGGTACCTACCCTACCTACCTACGACACGATGCACAGTGTTCATCCAT----------  660
Rc-OR-LSK1.wild LAOS AAGGGTACCTACCCTACCTACCTTCGACACGATGCACAGTGTTCATCCAT----------  660
Rc-OR-BP6.wild PSL   AAGGGTACCTACCCTACCTACCTACGACACGATGCACAGTGTTCATCCAT----------  660
Rc-OR-Con-5.wild SKN AAGGGTACCTACCCTACCTACCTACGACACGATGCACAGTGTTCATCCAT----------  660
                                    10-bp indel
Rc-SP-06.weed MSK    GAATGAGACATATGCTATGCTAGTACTACGAATCTAAAAAGATGTACATATTTTGATTCG 840
Rc-SP-04.weed SR     GAATGAGACATATGCTATGCTAGTACTACGAATCTAAAAAGATGTACATATTTTGATTCG  840
Rc-ML-105.CV         GAATGAGACATATGCTATGCTAGTACTACGAATCTAAAAAGATGTACATATTTTGATTCG   840
Rc-SP-02.weed SR     GAATGAGACATATGCTATGCTAGTACTACGAATCTAAAAAGATGTACATATTTTGATTCG  840
Rc-SP-08.weed MSK    GAATGAGACATATGCTATGCTAGTACTACGAATCTAAAAAGATGTACATATTTTGATTCG 840
Rc-SP-17.weed MSK    GAATGAGACATATGCTATGCTAGTACTACGAATCTAAAAAGATGTACATATTTTGATTCG 840
Rc-SP-21.weed SR     GAATGAGACATATGCTATGCTAGTACTACGAATCTAAAAAGATGTACATATTTTGATTCG  840
Rc-SP-PS.weed PCH    GAATGAGACATATGCTATGCTAGTACTACGAATCTAAAAAGATGTACATATTTTGATTCG  840
Rc-OR-Con-4.wild SKN GAATGAGACATATGCTATGCTAGTAC---------------------------------- 840
Rc-OR-LSK1.wild LAOS GAATGAGACATATGCTATGCTAGTAC---------------------------------- 840
Rc-OR-BP6.wild PSL   GAATGAGACATATGCTATGCTAGTAC----------------------------------  840
Rc-OR-Con-5.wild SKN GAATGAGACATATGCTATGCTAGTAC---------------------------------- 840
                     34-bp indel
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Fig 3. DNA sequence variation of QTLSD7-1/Rc in wild and weedy rice. Multiple alignment of DNA sequence of the QTLSD7-1/Rc of rice accessions in red and blue indicate promoter and intron 1, respectively. Single nucleotide polymorphisms (SNPs, *), insertion and deletion (indel,-) were observed in the locus. Nucleotide position of DNA sequence comparison according to the published reference GenBank accession number JF303048.1.

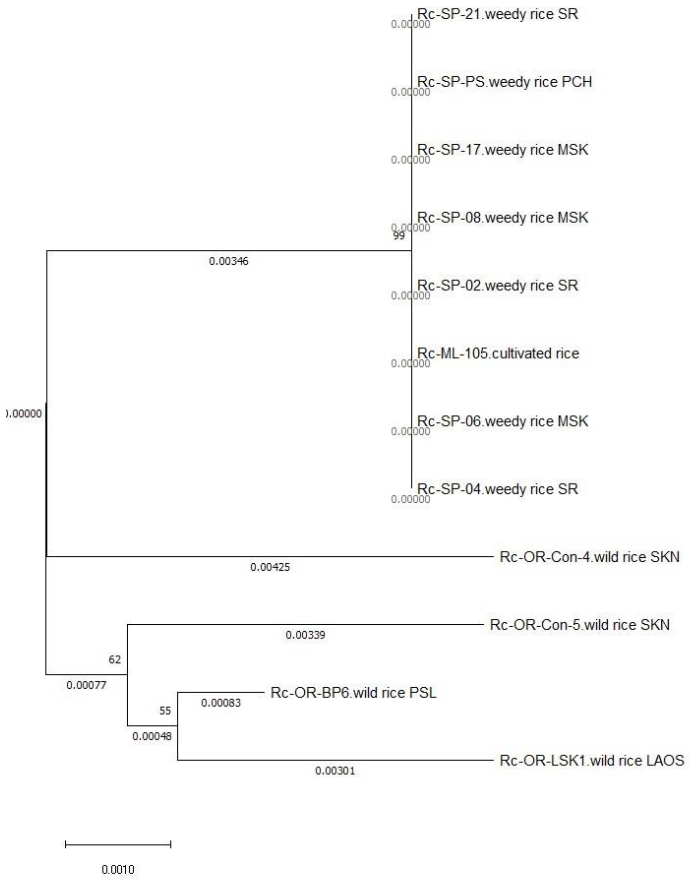

Fig 4. A neighbor-joining (NJ) tree constructed from MEGA program illustrated haplotype relationship based on DNA variations of the QTLSD7-1/Rc locus. A

DNA sequences have been submitted to NCBI Genbank (https://www.ncbi.nlm.nih.gov/genbank/) under accession numbers MW713673–MW713684.

Discussion

Wild rice and weedy rice has been considered in terms of genetic resources of genes that might be used for rice improvement, and provide an opportunity to achieve adaptation to the specific environments that wild and weedy rice have evolved. This study, DNA sequence variation of the QTLSD7-1/Rc in wild and weedy rice were explored. Seed dormancy and pericarp color in rice grains are agronomic traits. The pleiotropic effects of the QTL SD7-1/Rc locus have been detected in research and it has been reported that seed dormancy must contribute most to the adaptation of red rice (Gu et al., 2011). This study focused on DNA sequence variation in the QTLSD7-1/Rc locus, which is responsible for seed dormancy and pericarp color traits in wild and weedy rice. Seed dormancy regarded as the failure of an intact viable seed to complete germination under favorable conditions (Bewley, 1997). Moreover, understanding the DNA sequence variation of seed dormancy is of great interest to plant breeders. A previous study reported the entire DNA sequences of this locus (Gu et al., 2011). To date, it is likely that patterns of DNA sequence diversity have not been explored in the QTL SD7-1/Rc locus. In this report, new SNPs and indels were detected in DNA sequences of promoter and intron1 of the QTL SD7-1/Rc locus in weedy and wild rice accessions from Thailand. As a result, these finding enhances knowledge of the nucleotide polymorphisms in the QTL SD7-1/Rc locus and should lead to further investigation of a probable relationship to seed dormancy traits. Consequently, the new dormancy-associated SNPs will provide real benefits in molecular breeding. In addition, the results will help to address the questions remaining about weedy rice origins in Thailand and others tropical countries. In Thailand, cultivated rice cultivars and weedy rice exhibit overlapping flowering times. Historically, rice landraces grown in wet season in the northern and northeastern region of Thailand (May to October) are photosensitivity with a flowering time during September to October according to each cultivar. However, weedy rice at the margin of paddy fields, shared the flowering time (August to September). This incidence supports the previous study of Wedger et al., (2019) who suggested that most Thai weedy rice strains are not descended from modern rice varieties, but rather, they evolved from red-pericarp rice landraces that predate modern white-pericarp varieties. Interestingly, it is likely that the haplotype types found in this study exist in wild, weedy and cultivated rice samples. The

practical application of these DNA markers is related to germplasm characterization, studies of genetic diversity of the different origins of rice populations, marker-assisted breeding and gene introgression according to these genomic DNA variants.

Materials and Methods

Rice material

A sample of 54 wild and weedy rice accessions including 4 accessions of wild rice and 50 accessions of weedy rice were used in this study. For wild rice, young leaves were collected from mother plants from natural habitats of Savannakhet province, Laos (code Rc-OR-LSK1). In Thailand, collections were from Pitsanuloke province (code RC-OR-BP6), central region and Sakon Nakhon (SKN) province (code Rc-OR-Con-4, code RC-OR-Con-5), northeastern region. Weedy rice samples were collected from Maha Sarakham (MSK) and Surin (SR) provinces, northeastern Thailand (49 accessions) and one accession from Pichit province (PCH), central Thailand (Fig. 1, Table 1)(Supplementary Information 1, SI1). Young leaves of individual of wild rice plant, seeds and flag leaves of weedy rice individuals were collected and brought back to the laboratory. These samples were kept in cool conditions in a refrigerator. Examples of weedy rice plants are shown in Fig. 2.

DNA extraction, PCR amplification

For genomic DNA extraction, approx. 100 mg of young leaves or flag leaves of each individual rice sample was isolated using the CTAB method following the procedures described by Doyle and Doyle (1987). The primers were designed by the author according to GenBank Accession JF393948.1 and were used to amplify the promoter region (the region upstream from the first exon), to exon 2 of the QTL SD7-1/Rc locus: RcF (5'-CATCTTTCAGCTTTCACA-3') and RcR (5'-GCATGCATGCATGCGTGGA-3'). Each 20 mL reaction included 50 ng of DNA, 1.5 mM MgCl2, 0.2 mM of each primer, 0.2 mM dNTPs, 1.5 mL of 10× PCR buffer and 0.75 U of DNA polymerase (Promega, Madison, WI, USA). The PCR conditions were as follows. An initial denaturation step of 3 min at 94 ºC followed by 35 cycles as follows: 45 s at 94 ºC, 2 min at 62 ºC then 1 min 5 s at 72 ºC. After 35 cycles, a final extension of 5 min at 72 ºC was performed. Amplified products were separated by 1.0% agarose gel electrophoresis and visualized with GelRed™ Nucleic Acid Gel Stain (Biotium, Inc., Hayward, CA).

DNA sequencing

The amplified bands were cut and purified using PureDireX PCR Clean-Up&Gel Extraction Kit (The BIO-HELIX Co./ Ltd., Taiwan). For DNA sequencing, PCR products were sequenced using an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Carlsbad, CA, USA). DNA sequencing was performed by the 1st BASE Laboratories, Selangor, Malaysia. New DNA sequences obtained from this report were deposited in GenBank (https://www.ncbi.nlm.nih.gov/) under accession numbers MW713673-MW713684.

DNA sequence analysis

Sequence alignment was conducted on 54 DNA sequences of promoter, exon1, intron1 and exon2 (1375 bp) with ClustalW 2.0.9 (Thompson et al., 1997). Nucleotide diversity was determined by DnaSP ver. 5.0 (Rozas et al., 2003), as follows; theta (Θ_w), the number of segregating (polymorphic) sites and P_i, the average number of nucleotide differences per site between two sequences. The representatives of DNA sequences of 8 out of 50 accessions (haplotype 1, H1) of weedy rice together with 4 accessions of wild rice were used in haplotype analysis with DnaSP ver. 5.0 and, a neighbor–joining cluster was constructed using MEGA4.0, respectively (Tamura et al., 2007). Conclusion: DNA sequence variation of the Quantitative trait loci SD7-1/Rc were explored in wild and weedy rice accessions from Thailand and Laos. Both SNPs and indels were observed in non-coding region: promoter and intron1, whereas no variation of DNA sequence were observed in coding region (exon1) of the locus.

Wild rice and weedy rice has been considered in terms of genetic resources of genes that might be used for rice improvement, and provide an opportunity to achieve adaptation to the specific environments that wild and weedy rice have evolved.

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