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Research Paper

Genetic diversity of the *wx* flanking region in rice landraces in northern Laos

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A glutinous texture of endosperm is one of the important traits of rice (*Oyza sativa* L.). Northern Laos is known as a center of glutinous rice diversity. We genotyped INDEL, SSR and SNP markers in a sample of 297 rice landraces collected in northern Laos. These glutinous varieties were confirmed to share a loss-of-function mutation in *Granule bound starch synthase I* (*Wx*). INDEL markers revealed a high frequency of recombinant genotypes between *indica* and *japonica*. Principal component analysis using SSR genotypes of *Wx* flanking region revealed that glutinous *indica* landraces were scattered between non-glutinous *indica* and glutinous-*japonica* types. High ratios of heterozygosity were found especially in glutinous *indica*. Haplotype analysis using SNP markers around *Wx* locus revealed that glutinous *indica* landraces would have a few chromosome segments of glutinous *japonica*. Frequent recombinations were confirmed outside of this region in glutinous *indica*. This intricate genetic structure of landraces suggested that glutinous *indica* landraces in Laos were generated through repeated natural crossing with glutinous-*japonica* landraces and severe selection by local farmers.

Key Words: *Wx*, *indica*, rice landrace, selective sweep, outcrossing, Laos.

Introduction

The endosperm of major seed crops such as rice, maize, and wheat is the major organ in which starch is stored. Many genes such as the starch biosynthesis and the color of the aleurone layer have been targeted for artificial selections (Olsen and Purugganan 2002, Palaisa *et al.* 2004, Sweeney *et al.* 2006, Wang *et al.* 1999, Whitt *et al.* 2002). One of the genes, *Granule bound starch synthase I* (*GBSSI* ; *Wx*) regulates amylose content of rice endosperm through three different functional alleles, Wx^a, Wx^b and *wx* (Sano 1984). Wx^a, generally found in non-glutinous *indica* varieties and wild rice, confers relatively high amylose content (Umemoto and Terashima 1999) whereas Wx^b carries a G-T exon 1 substitution resulting in an intron splicing mutation that confers a lower amylose content. The allele has tended to be found in non-glutinous *japonica* varieties (Hirano *et al.* 1998, Isshiki *et al.* 1998). The *wx* allele found in glutinous rice is defined by a complete loss of function of *GBSSI* which confers a lack of amylose synthesis (glutinous phenotype), generally caused by a 23-bp duplication in exon 2 (Inukai *et al.* 2000). Rice with lower amylose content and a glutinous phenotype predominates in hilly areas of Southeast Asia and also in northern Asia. There is molecular evidence of a strong selective sweep in a starch of about ~250 kb flanking the *Wx* locus among landraces cultivated in Asian countries (Olsen *et al.* 2006).

The Lao PDR (Laos) is characterized by consumption of glutinous cereals including rice, foxtail millet, and other millets, and for this reason it is known as the “glutinous zone” (Nakagahra 1986, Sakamoto 1987, Watabe 1967). The glutinous quality of the crops in this area probably reflects the preference of the local people, who constitute 49 independent ethnic groups (Sisouphanthong and Taillard 2000), most of whom consume predominantly glutinous rice as part of their culture (Schiller *et al.* 2006). The rice fields in northern Laos consist mostly of upland fields and

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partly paddy fields. Recently, some modern rice varieties have been introduced to paddy fields as cash crops, but not for private consumption (Ishikawa *et al.* 2002). However, the majority of the fields are cultivated for glutinous landraces mostly on 1.5–2 ha farms in hilly areas that are owned by single families. These landraces are sown as seeds harvested by the farmers themselves. Multiple landraces recognized from their morphological appearance are cultivated in single fields and later identified molecular bases (Ishikawa *et al.* 2002, Muto *et al.* 2010). Local farmers also recognized their phenotypic and agronomic differences created through unconscious selection. These are regarded as valuable genetic resources for improvement of modern varieties to supply stress resistance or novel phenotypes. Intensive efforts to collect these landraces have been made by local research organizations with international collaboration (Ishikawa *et al.* 2002, Muto *et al.* 2010). However, there are several factors that limit the accurate evaluation of these resources: 1) Landraces showing various phenotypes often share a single name, 2) genetically different individuals are cultivated as single landraces, 3) local people frequently exchange their seeds within or among villages, and 4) outcrossing between genetically different individuals is frequent (Ishikawa *et al.* 2002, 2006, Muto *et al.* 2010). Such factors resulted from natural outcrossing and management of heterogeneous seed stocks. These events are largely allowed under the traditional slash and burn system. It acted as genetic buffer to keep diversity. Thus, these landraces have huge potential for future rice breeding.

In the present study, we evaluated the genetic constitutions of these glutinous accessions at the cytoplasmic and nuclear levels and determined their fine structure around the *Wx* locus. These molecular data allowed us to grasp how glutinous landraces have been maintained through slash and burn system in Laos and how frequently outcrossing has occurred inside fields. In this study, we focus on *Wx* locus because the phenotype is a key trait for Lao farmers.

Materials and Methods

Plant materials

Two hundred ninety-seven rice (*Oryza sativa* L.) accessions comprising 276 landraces and 21 modern varieties were collected from 45 villages in six northern provinces of Laos—Luang Prabang, Luang Namtha, Oudom Xai, Phong Sali, Vientiane and Vientiane Municipality—between 2003 and 2005 (**Fig. 1**). The field survey was carried out under a material transfer agreement (MTA) between National Agriculture and Forestry Research Institute (NAFRI, Laos) and Research Institute for Humanity and Nature (RIHN, Japan). Dr. Chay Bouphanousay who was a researcher of NAFRI participated in the exploration. All of the collected materials were conserved in genebank of NAFRI. The duplications of these materials were used in this study. All of the materials had been grown by local farmers belonging to various ethnic groups. These landraces showed various appearances

Fig. 1. Sampling sites of local rice accessions in northern Laos. Rice accessions were collected from 45 local villages in six provinces, Luang Phrabang, Luang Namtha, Oudomxai, Phongsali, Vientiane, and Vientiane Municipality.

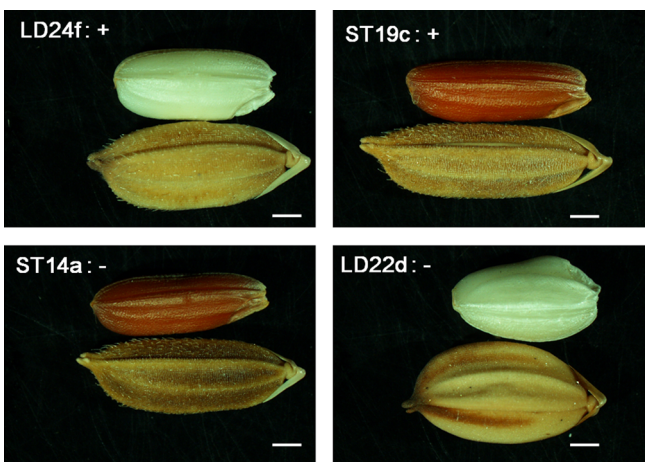

Fig. 2. Various appearances of seeds and hull in glutinous rice landraces collected from northern Laos. Accession No. and genotype of ORF100-INDEL (+; deletion type, –; non-deletion type) were shown in each photo.

(**Fig. 2**). Differentiation between glutinous and non-glutinous endosperm was made on the basis of staining with 0.7% iodine-potassium-iodine (I/KI). After an overview of their genetic characteristics, 75 accessions were selected for further analysis (**Table 1**). DNA from all of these materials was extracted using the CTAB method from young leaves of single individual landrace plants.

INDEL markers for waxy and indica-japonica classification

Information of all primers used in this study is described

Table 1. Information and *indica-japonica* classification of representative accessions judged by the four INDEL markers

Acc.No.	Sample ID	Site	Name	Ethnic	Field condition	Variety type	J/I	ORF100[a]	*Pgi* 1[b]	*Cat* 1[c]	*Acp* 1[d]
LD23d	Jwx105	LD	ngo lamtan	Khmu	Up	L	J	ND	D	ND	INS
LL81b	Jwx131	LL	khao yuak	–	Up	L	J	ND	D	ND	INS
LL81k	Jwx139	LL	no-record	–	Low	L	J	ND	D	ND	INS
LL83f	Jwx149	LL	khao nang	Khmu	Up	L	J	ND	D	ND	INS
LL88e	Jwx172	LL	khao dong	Khmu	Up	L	J	ND	D	ND	INS
LL90d	Jwx180	LL	khao pat hin	Khmu	Up	L	J	ND	D	ND	INS
LL91d	Jwx185	LL	no-record	Khmu	Up	L	J	ND	D	ND	INS
LL93b	Jwx192	LL	khao vieng	Khmu	Up	L	J	ND	D	ND	INS
LL95c	Jwx201	LL	mak kheua khao	Khmu	Up	L	J	ND	D	ND	INS
LL96a	Jwx204	LL	no-record	Khmu	Up	L	J	ND	D	ND	INS
LL97a	Jwx211	LL	no-record	Khmu	Up	L	J	ND	D	ND	INS
LL101b	Jwx222	LL	fo hian	–	Up	L	J	ND	D	ND	INS
LL104d	Jwx240	LL	khao deng du	Tai Lue	Up	L	J	ND	D	ND	INS
LL105f	Jwx250	LL	ngo tam	Lao	Up	L	J	ND	D	ND	INS
LD22d	Jwx264	LL	ngo muong	Khmu	Up	L	J	ND	D	ND	INS
KW1g	Jwx307	LP	mai platiyao	Lanteng	Up	L	J	ND	D	ND	INS
LD26a	Jwx316	LD	ngo yansuang	Khmu	Up	L	J	ND	D	ND	INS
LD32c	Jwx380	LD	ngo iinm	Khmu	Up	L	J	ND	D	ND	INS
LD25h	Jnonwx56	LD	ngo clock	Khmu	Up	L	J	ND	D	ND	INS
LD23e	Jnonwx110	LD	ngo salii	Khmu	Up	L	J	ND	D	ND	INS
LL87j	Jnonwx167	LL	khao chao	Thai Dam	Up	L	J	ND	D	ND	INS
LL90b	Jnonwx178	LL	khao deng	Khmu	Up	L	J	ND	D	ND	INS
LL92b	Jnonwx189	LL	khao ngo	Lao Lum	Up	L	J	ND	D	ND	INS
LL96c	Jnonwx206	LL	no-record	Khmu	Up	L	J	ND	D	ND	INS
LL105h	Jnonwx252	LL	ngo chao	Khmu	Up	L	J	ND	D	ND	INS
LD23a	Jnonwx270	LD	ngo chao	Khmu	Up	L	J	ND	D	ND	INS
KW2a	Jnonwx308	LP	manwa	Lao ku	Up	L	J	ND	D	ND	INS
KW3c	Jnonwx315	LP	hoche	Akha	Up	L	J	ND	D	ND	INS
KW4f	Jnonwx327	LN	cheju	Akha	Up	L	J	ND	D	ND	INS
KW4i	Jnonwx330	LN	lokuu	Akha	Up	L	J	ND	D	ND	INS
LD27g	Jnonwx358	LD	ngo chao clock	Khmu	Up	L	J	ND	D	ND	INS
LD31a	Jnonwx370	LD	ngo hiyan	Khmu	Up	L	J	ND	D	ND	INS
LL107b	Jnonwx389	LL	ngo prai	Khmu	Up	L	J	ND	D	ND	INS
LL107h	Jnonwx395	LL	hoichalyng	Khmu	Up	L	J	ND	D	ND	INS
ST22a	Jnonwx451	LV	khao niipun	–	Low	–	J	ND	D	ND	INS
LD24f	Iwx1	LD	khao kalong	Tai Lue	Low	L	I	D	ND	D	Non-INS
LD25g	Iwx51	LD	ngo ber	Khmu	Low	L	I	D	ND	D	Non-INS
LL82a	Iwx142	LL	khao pee pii	Khmu	Up	L	I	D	ND	D	Non-INS
LL94a	Iwx196	LL	khao leuan	–	Up	L	I	D	ND	D	Non-INS
LL103f	Iwx236	LL	Khao Mea Tor	Lao	Low	L	I	D	ND	D	Non-INS
LD20e	Iwx257	LD	ngo pet	Khmu	Up	L	I	D	ND	D	Non-INS
LL103c	Iwx233	LL	khao Dor Dai	Tai Lue	Low	L	I	D	ND	D	Non-INS
ST02	Iwx400	LV	khao kam	–	Low	L	I	D	ND	D	Non-INS
ST5c	Iwx405	LV	khao angii	Lao	ND	L	I	D	ND	D	Non-INS
ST6c	Iwx409	LV	khao kii tom	Lao Lum	Low	L	I	D	ND	D	Non-INS
ST10b	Iwx424	LV	khao hoom neuang	Lao	Low	L	I	D	ND	D	Non-INS
ST16b	Iwx434	LV	khao Tomburi	–	ND	L	I	D	ND	D	Non-INS
ST17b	Iwx436	LV	khao sai mai	Lao Lum	Low	L	I	D	ND	D	Non-INS
ST20b	Iwx444	LV	khao saam patone do	–	Low	L	I	D	ND	D	Non-INS
LL81i	Inonwx138	LL	khao chao	–	Up	L	I	D	ND	D	Non-INS
LL87i	Inonwx166	LL	khao chao pun	Thai Dam	Up	L	I	D	ND	D	Non-INS
LL93d	Inonwx194	LL	khao chao pun	Khmu	Up	L	I	D	ND	D	Non-INS
LL97i	Inonwx218	LL	no-record	Khmu	Up	L	I	D	ND	D	Non-INS
LL97j	Inonwx219	LL	no-record	Khmu	Up	L	I	D	ND	D	Non-INS
KW4c	Inonwx324	LN	khao lao soon	Akha	Low	L	I	D	ND	D	Non-INS
KW4e	Inonwx326	LN	teche	Akha	Low	L	I	D	ND	D	Non-INS
ST5a	Inonwx403	LV	RD8	Lao	Low	M	I	D	ND	D	Non-INS
ST6b	Inonwx408	LV	khao chao hoom mali	Lao Lum	Low	M	I	D	ND	D	Non-INS
ST7a	Inonwx413	LV	khao ubong deeng	–	Low	M	I	D	ND	D	Non-INS
ST8a	Inonwx414	LV	khao hoom mali	Hmong	Low	M	I	D	ND	D	Non-INS
ST9e	Inonwx420	LV	CR203	–	Low	M	I	D	ND	D	Non-INS
ST16a	Inonwx433	LV	khao chao hoom mali	Dounhien	Low	M	I	D	ND	D	Non-INS
ST20d	Inonwx446	LV	khao chao do	–	Low	L	I	D	ND	D	Non-INS

Table 1. (continued)

Acc.No.	Sample ID	Site	Name	Ethnic	Field condition	Variety type	J/I	ORF100[a]	*Pgi* 1[b]	*Cat* 1[c]	*Acp* 1[d]
LL87a	Jwx158-rec	LL	khao hin	Thai dam	Up	L	Rec	ND	ND	ND	INS
ST14a	Jwx430-rec	ST	khao pee deng	Lao Lum	Up	L	Rec	ND	D	D	INS
LL97h	Iwx217-rec	LL	no-record	–	Up	L	Rec	D	D	ND	Non-INS
LL97k	Iwx220-rec	LL	no-record (hybrid)	–	Up	M	Rec	D	ND	ND	Non-INS
ST9c	Iwx418-rec	LV	RD6	–	Low	M	Rec	D	D	D	Non-INS
ST9g	Iwx422-rec	LV	no-record	–	Low	M	Rec	D	D	D	Non-INS
ST11b	Iwx426-rec	LV	khao pee deng	Lao lum	Up	L	Rec	D	D	D	Non-INS
ST19c	Iwx441-rec	LV	khao phee dian	Lao Lum	Up	L	Rec	D	D	D	Non-INS
KW5d	Jnonwx338-rec	LN	oyof	Mushuda	Up	L	Rec	ND	ND	ND	INS
ST4a	Jnonwx402-rec	LV	khao chao laai	Hmong	Low	L	Rec	ND	ND	ND	INS
ST15a	Jnonwx432-rec	LV	khao chao dam	Hmong	Up	L	Rec	ND	ND	ND	INS
KW6h	Inonwx348-rec	LN	ngo suangungcho	Khmu Khuen	Low	L	Rec	D	D	D	Non-INS

Site: LL; Louang Phrabang, LN; Louang Namtha, LP; Phongsali, LD; Oudomxai, LV; Viengtiane.
Field condition: Up; Upland, Low; Lowland.
Variety type: L; Landrace, M; Modern variety.
J/I: J; *japonica*, I; *indica*, Rec; Recombinant. Judged by four INDEL genotypes. INDEL genotypes were classified by letters, D as deletion, ND as non-deletion, Non-INS as no insertion, INS as insertion.
[a]ORF100: D; I type, ND; J type. [b]Pgi1: D; J type, ND; I type. [c]Cat1: D; I type ND; J type. [d]Acp2: INS; J type, Non-INS; I type.

in **Supplemental Table 1**. The causal mutation of glutinous rice, a 23-bp duplication in exon 2 of *GBSSI*, was confirmed using a primer pair: WaxyA1 and WaxyB1. PCR reactions were carried out as described in previous study (Muto *et al.* 2010). For genotyping, amplicons were electrophoresed on 1.5% agarose gels. Discrimination of accessions into *indica-japonica* types was based on the chloroplast INDEL marker (ORF100; Chen *et al.* 1993, 1994, Kanno *et al.* 1993) and nuclear INDEL markers (*Pgi*1-INDEL, *Cat*1-INDEL and *Acp*1-INDEL) were designed based on the isozymes that were flanked by *indica-japonica* diagnostic isozyme genes (Ishikawa *et al.* 1991, Sano and Morishima 1992). The INDEL patterns of these three nuclear INDEL markers were firstly confirmed by applying to a set of reference rice accessions provided as "Dr.Oka's tester strain" by National Institute of Genetics that previously had been classified into *indica* or *japonica* based on its morphological and physiological characteristics (Oka 1958) and isozyme genotypes (Ishikawa *et al.* 1991). All PCR reactions were performed with 0.25 U rTaq (Takara Co., Japan) per single reaction. The conditions were; 95°C preheating for 5 min, followed by 35 cycles of 96°C for 30 s, 72°C for 1 min, and 75°C for 5 min. All INDEL markers were checked their performance with typical *indica* and *japonica* landraces, 40 accessions collected from Japan, China, and South-east Asian countries were compared for their genetic characteristics (**Supplemental Table 2**).

SSR analysis of the total genome and Wx flanking region

All 297 accessions were analyzed for genotypes at 12 SSR loci, which were randomly selected from 12 chromosomes in order to clarify the general genetic characteristics. In order to focus on polymorphism around the *Wx* locus, other set of 11 SSR markers dispersed at about 100-kb intervals around the *Wx* locus were genotyped for 75 representative accessions from northern Laos (**Fig. 3**). RM190 located in exon 1 of *Wx* is composed of multiple alleles (Temnykh *et al.* 2000). The multiple alleles were tightly linked with 23 bp tandem duplication which was causal mutation of glutinous rice (Bligh *et al.* 1995).

Haplotype analysis around the Wx locus

The Wx^b allele was associated with low amylose resulting from a G to T substitution (hereafter, G-T substitution) at the splicing donor site between exon 1 and intron 1. Nucleotide diversity was determined with SNPs in 12 fragments dispersed at about 50 kb intervals around the *Wx* locus. Six fragments named KN5-0, KN5-2, KN5-4, KN5-6, KN5-8, and KN5-10 were located upstream of the *Wx* locus, and another six fragments named KN3-2, KN3-4, KN3-6, KN3-8, KN3-10, and KN3-12 were located downstream (**Fig. 3**). The PCR reaction consisted of preheating at 95°C for 5 min, followed by 30 cycles of 95°C for 10 s, 55°C for 30 s, 72°C for 30 s, and 75°C for 5 min. Amplicons were sequenced.

Data analysis

SSR data were analyzed using GenAlEx 6.1 (http://www.anu.edu.au/BoZo/GenAlEx/) to determine the number of alleles, allele frequencies, Nei's genetic diversity, and for PCA (principal component analysis). Nucleotide diversity estimated as the Pai value was analyzed using Dna SP 5.10.1 (Librado and Rozas 2009).

Results

Overview of genetic structure of rice landraces in Laos

The landraces comprised 237 upland rice and 60 lowland rice accessions, and on the basis of endosperm traits, 231 were classified as glutinous and 66 as non-glutinous rice accessions. The origins of maternal lineages were identified with cpDNA INDEL (ORF100-INDEL) which was flanked

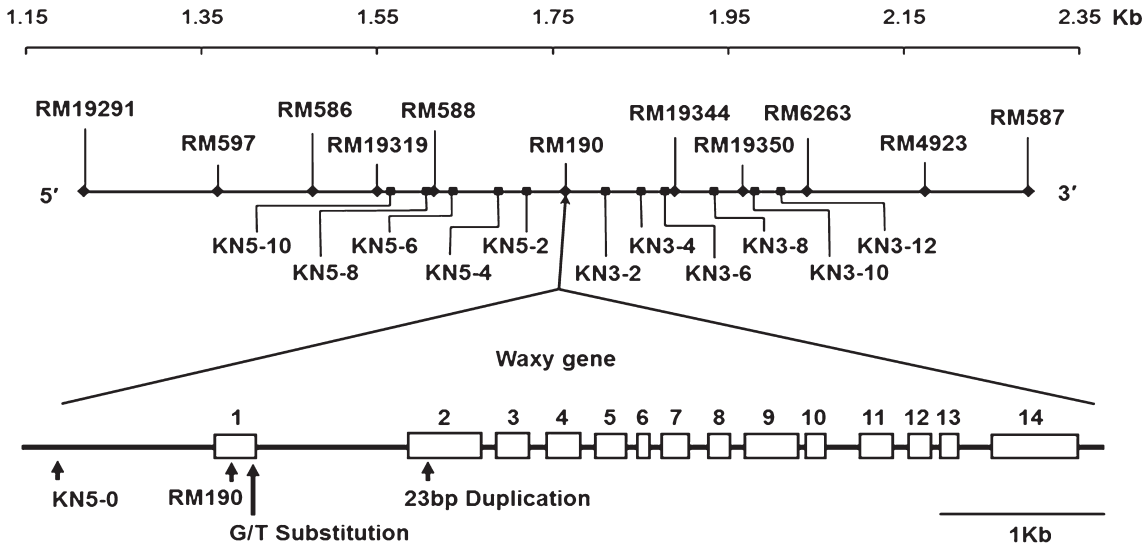

Fig. 3. Location of Wx flanked markers used in this study.

by ORF100 (Chen *et al.* 1993, 1994, Kanno *et al.* 1993). Alternative plastid types, deletion or non-deletion type were defined as indicative of *indica* or *japonica* plastid type, respectively. Seventy-one percent of landraces, i.e. 223 out of 297 accessions, carried the non-deletion type whereas the remaining 74 carried the deletion type. Genomic diversity was surveyed by examining the genotypes of 12 randomly selected SSRs from 12 chromosomes. A general overview of the landraces was obtained using PCA based on the SSR genotypes and ORF100-INDEL (**Fig. 4**). The first and second components explained 41.0% and 17.4% of the total variation. The scatter plot showed that the population consisted of two distinct groups corresponding to *indica* and

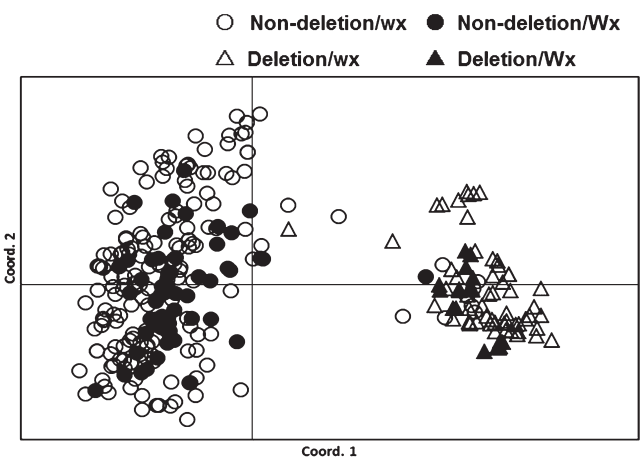

Fig. 4. Principal coordinates analysis by using SSR polymorphisms of 12 random SSR markers on each chromosome among 297 accessions. Horizontal and vertical axes show first and second principal components respectively. Non-deletion type of ORF100-INDEL and deletion type ORF100-INDEL were noted as circles and triangles. Glutinous type was noted as open symbols and non-glutinous type as solid symbols.

japonica group, respectively. Both groups contained glutinous and non-glutinous types. To perform sequence based analysis focused on the *Wx* flanking region, 75 accessions were chosen as representatives depending on endosperm phenotype (glutinous/non-glutinous) determined by I/KI staining and *indica-japonica* type of plastid determined by ORF100-INDEL type. These 75 accessions comprised 20 glutinous/non-deletion, 20 non-glutinous/non-deletion, 20 glutinous/deletion, and 15 non-glutinous/deletion accessions. In the last group, nine of the non-glutinous/deletion accessions were landraces, and the remaining six were modern varieties. Some of these included modern, recently introduced lowland varieties. For more exact *indica-japonica* classification, three INDELs for nuclear DNA—*Acp*1-INDEL, *Cat*1-INDEL, and *Pgi*1-INDEL were applied. These markers were designed tightly linked to each of the isozyme genes, *Acp*1, *Cat*1 and *Pgi*1, respectively. These three isozymes are locating different chromosomes respectively, and it was confirmed that there was no linkage disequilibrium among these three isozymes that have been widely used to know sub-species (Sano and Morishima 1992). Genotypes of these INDELs were first confirmed with *indica-japonica* reference accessions to follow the past result. Another chloroplast-based INDEL has been widely used to identify maternal linage (Garris *et al.* 2005). The results of ORF100-INDEL and three isozyme INDEL markers were consistent with the reference data (Ishikawa *et al.* 1991, Oka 1958, **Supplemental Table 2**). These genotypes were used to know rough classification of sub-species among landraces collected in Laos. Based on the genotypes of four INDEL markers (ORF100-INDEL, *Acp*1-INDEL, *Cat*1-INDEL, and *Pgi*1-INDEL), 18 accessions were classified as *japonica*/glutinous (J/*wx*), 17 as *japonica*/non-glutinous (J/*Wx*), 14 as *indica*/glutinous (I/*wx*), and 14 as *indica*/non-glutinous (I/*Wx*). Some of the accessions carried inconsistent combinations of nuclear INDELs and the ORF100-

INDEL. These accessions were regarded as "recombinant type" (referred to as Rec-type hereafter). In total, eight recombinant/glutinous (Rec/*wx*) and four recombinant/non-glutinous (Rec/*Wx*) accessions were recognized (**Table 1**).

Wx locus is reported as a recombination hotspot in rice by previous studies (Inukai *et al.* 2000). As we had speculated about frequent recombination around the *Wx* locus, 11 SSR markers around the locus were chosen to monitor the compositions and genetic diversity of the chromosomal segments. The SSR polymorphism of 75 representatives were compared between two SSR sets, namely, 12 SSRs independent from *Wx*, and 11 SSRs flanked by *Wx* locus. The average heterozygosity (*He*) of the *Wx* independent SSRs was 0.654. PCA was performed using SSRs and INDEL markers. Two distinct groups corresponding to *indica* or *japonica* were recognized. However, the Rec-type accessions were involved in both groups (**Fig. 5A**). This trend of distribution is similar to that of 295 accessions shown in **Fig. 4**. From this result, it was confirmed that 75 accessions acted properly as representative. On the other hand, the average *He* over *Wx* flanked SSRs was 0.475 (**Table 2**). The RM588 showed lower *He* than its average in three groups, glutinous *japonica*, non-glutinous *japonica* and glutinous *indica*. But the other four SSRs (RM19291, RM597, RM586 and RM 19319) in upstream of *Wx* gene did not show effect of selective sweep because *He* was high in all groups (**Table 2**). PCA analysis revealed that glutinous *indica* accessions were scattered between the non-glutinous *indica* and *japonica* groups (**Fig. 5B**). Recombinant accessions were also scattered randomly. Number of heterozygotes obtained with *Wx* flanked SSRs indicated frequent heterozygote in Laos landraces (**Table 3**). The number of heterozygotes were eight out of 18 (44% of population size) in glutinous *japonica*, two out of 17 (12%) in non-glutinous *japonica*, 12 (86%) in glutinous *indica*, seven (50%) in non-glutinous *indica*, respectively. All of these value are higher than ever observed (Ishikawa *et al.* 2002, 2006), but especially glutinous *indica* showed quite a high value. The existence of heterozygous indicated that outcrossing recently occurred, because it should be replaced into homozygous during the selection. Observed heterozygosity (*Ho*) were calculated in two set of SSRs, Random-SSRs and *Wx*-flanked SSRs (**Supplemental Table 3**). Glutinous *indica* showed heterozygotes at nine SSR sites out of total 23, whereas glutinous *japonica* showed only three.

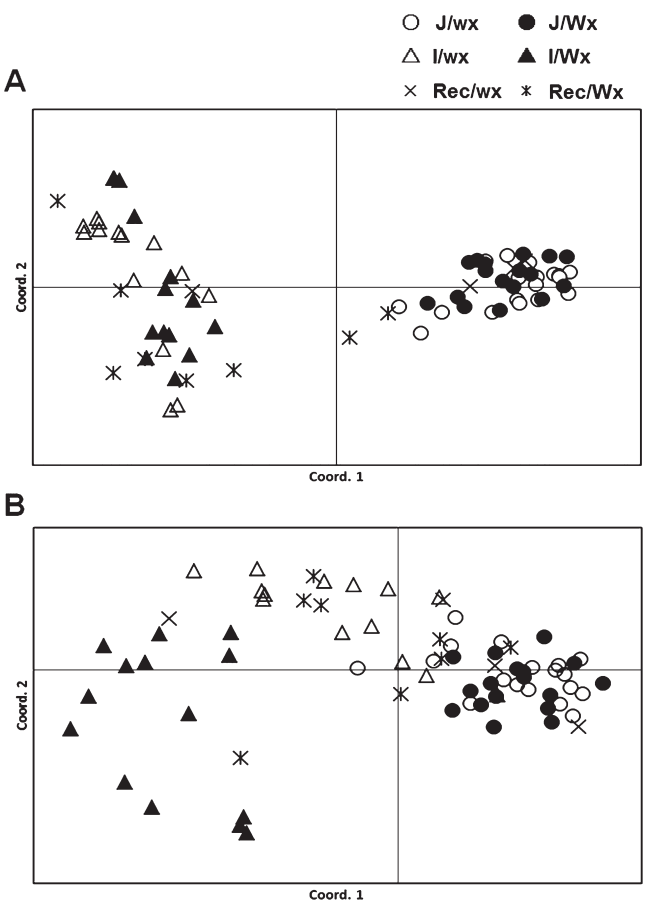

Fig. 5. Principal coordinates analysis by using SSR polymorphisms among 75 representative accessions. Horizontal and vertical axes show first and second principal components respectively. A: Result based on 12 Wx independent SSR markers. B: Result of 11 Wx flanking SSR markers. All accessions were distinguished additional diagnostic nuclear INDELs in order to distinguish accessions into indica or japonica. Recombinant type (Rec-type) carried combinations of the diagnostic INDELs.

Molecular characterization of the Wx locus

All accessions were genotyped for a 23-bp duplication in exon 2 of the *Wx* gene and also for the G-T substitution at the exon1-intron1 junction of the gene. All glutinous accessions were found to carry the 23-bp duplication and G-T substitution at the splice junction. *Ho* was high in the glutinous *indica* group with *Wx* flanked SSRs (**Table 3**).

Table 2. Average heterozygosity (*He*) at 11 *Wx* flanked SSRs

	n	RM19291	RM597	RM586	RM19319	RM588	RM190	RM19344	RM19350	RM6263	RM4923	RM587	Total
J/*wx*	18	0.691	0.424	0.796	0.695	0.000	0.685	0.623	0.364	0.346	0.391	0.648	0.515
J/*Wx*	17	0.791	0.570	0.828	0.742	0.117	0.422	0.758	0.305	0.000	0.648	0.555	0.521
I/*wx*	14	0.698	0.379	0.379	0.379	0.000	0.568	0.594	0.595	0.701	0.473	0.485	0.477
I/*Wx*	14	0.556	0.473	0.379	0.805	0.426	0.663	0.000	0.074	0.524	0.355	0.355	0.419
Rec/*wx*	8	0.694	0.541	0.653	0.667	0.337	0.245	0.571	0.653	0.663	0.245	0.735	0.546
Rec/*Wx*	4	0.444	0.444	0.444	0.444	0.000	0.444	0.000	0.667	0.278	0.444	0.444	0.369
Total	75	0.646*	0.472	0.580	0.622	0.147*	0.505	0.424	0.443	0.419	0.426	0.537	0.475

* Significant difference ($P = 0.05$).

Table 3. Number of heterozygotes of random SSRs and *Wx* flanked SSRs among six groups of 75 represented accessions

Group	n	No. of heterozygotes
J/*wx*	18	8 (44%)
J/*Wx*	17	2 (12%)
I/*wx*	14	12 (86%)
I/*Wx*	14	7 (50%)
Rec/*wx*	8	7 (88%)
Rec/*Wx*	4	2 (50%)
Total	75	38

There were allelic variations at RM190 located inside exon 1. Eight alleles were found among 75 representatives. Glutinous *japonica* carried four alleles and glutinous *indica* shared two of them (**Fig. 6**). These multiple alleles were located quite close to the G-T substitution and the 23-bp duplication in the *Wx* locus, therefore, it may reflect *Wx* selection. Additionally, Japanese glutinous landraces shared a small proportion of these multiple alleles at RM190 (**Supplemental Table 4**). Glutinous landraces in Japan carried narrower ranges of multiple alleles at the locus. It was probably due to a bottle neck effect occurred when glutinous landraces was introduces into Japan.

In order to confirm how such glutinous landraces had arisen, DNA fragments flanking the *Wx* locus were sequenced. Nucleotide diversity (Pai) of silent SNPs was evaluated for 12 DNA fragments within 0.45 Mb of the *Wx* locus (**Table 4**). The *indica* group showed higher diversity (Pai = 0.028 for glutinous *indica*; 0.0039 for non-glutinous *indica*) than the *japonica* group (Pai = 0.002 for glutinous *japonica*; 0.008 for non-glutinous *japonica*) in terms of the Pai score throughout the regions. In KN5-10 and KN5-8, diversities were low in all groups irrespective of whether they were glutinous or non-glutinous suggesting that selection of this region was not for the glutinous phenotype. The Pai score for glutinous *indica* ranged from 0.0000 to 0.0005, and that of glutinous *japonica* was also low. The lower diversity of glutinous *indica* was not due to the small number of *indica* accessions examined because the Pai score for non-glutinous *indica* was higher. Comparative heterozygosity of *Wx* flanked SSRs also showed lower diversity between RM588 and RM19350. Relative to the 5′ region, higher diversity of Pai scores was found from KN3-4 to downstream in the 3′ region. Glutinous *indica* showed quite high diversity at KN3-4, KN3-6 and KN3-8. The major SNPs at each fragment were used to summarize the haplotypes (**Fig. 6**). The haplotypes were described according to their frequencies at each site, for example, most major haplotype was A and minor one was D. Haplotypes of KN3-2 were described with numbers, since there was a different tendency with other regions. Glutinous *japonica* shared single haplotypes except for RM190 and KN3-2 where multiple alleles were detected in all the groups. In the 5′ region, from KN5-6 to KN5-0, alternative haplotypes existed. All glutinous accessions except for one shared the single Haplotype A. Another Haplotype B was shared with non-glutinous *indica* and also Rec-groups. In contrast to the 5′ region, there were multiple haplotypes in the 3′ region. Glutinous *japonica* shared the single Haplotype A. Other groups showed a complex haplotype pattern in the 3′ region. Glutinous *indica* shared Haplotype A along KN3-4 to KN3-12 in 10 out of 14 accessions, but three of the remainder carried different haplotypes (B-B-B-B/C-B) which were typical of non-glutinous *indica*. Only one accession carried C-B-B-C-A. This haplotype could not be found in the other groups, but Haplotype C of KN3-4 appeared to be derived from Haplotype B, based on the compositions of the SNPs inside the haplotype. From these results, Haplotypes A and B were considered to be typical of either *japonica* or *indica*, respectively. Haplotypes C and D were considered to have resulted from recombination between *indica* and *japonica* (**Supplemental Fig. 1**). These complex recombination patterns in the 3′ region indicated that outcrossing had occurred frequently in these regions. Glutinous *indica* shared some haplotypes with glutinous *japonica*, but also with a proportion of non-glutinous *indica*. Lower diversity prevented any estimation of selective sweep in the 5′ region of the *Wx* locus. Higher polymorphism was maintained in the 3′ region in non-glutinous *indica*. Relatively frequent recombinations occurred at 3′ region when compared to its 5′ region. Higher number of haplotypes in glutinous *indica* revealed that multiple events involving to recombinations by outcrossing and under a process of self-fertilization happened after glutinous allele had been introduced into *indica* genetic background.

KN3-2 showed multiple haplotypes, but two haplotypes, Haplotype 2 and Haplotype 3, predominated as was the case for multiple alleles in RM190 in glutinous *indica*. In glutinous *japonica*, although *Wx* flanking region were highly conserved, there are multiple alleles, $(CT)_{12}$, $(CT)_{16}$, $(CT)_{17}$, and $(CT)_{18}$ at RM190 which is located in exon 1 of *Wx* gene. On the other hand, glutinous *indica* also carried multiple alleles, $(CT)_{17}$ and $(CT)_{18}$, at RM190. To elucidate whether these two alleles of glutinous *indica* occurred after *wx* causal allele introduced into *indica* accessions, or introduced independently from glutinous *japonica*, combination patterns of alleles of RM190 and haplotype of KN3-2 fragment were compared between glutinous *indica* and glutinous *japonica* accession (**Supplemental Table 5**). Among glutinous *japonica*, accessions with $(CT)_{17}$ carried Haplotype 1, 2, 3, 4, and 5 at KN3-2 fragment, and accessions with $(CT)_{18}$ carried Haplotype 1, 3, and 5, respectively. All of haplotypes carried by $(CT)_{18}$ were contained haplotypes carried by $(CT)_{17}$. The $(CT)_{17}$ is the dominant among multiple allele of RM190 locus. From these facts, $(CT)_{18}$ allele was considered to be derived from $(CT)_{17}$ allele in glutinous *japonica* population. On the other hand in glutinous *indica*, accessions with $(CT)_{17}$ carried Haplotype 2 and 3, and accessions with $(CT)_{18}$ carried Haplotype 2, 3, and 6, respectively. It is more likely that glutinous *indica* with $(CT)_{18}$ was derived from $(CT)_{17}$ of glutinous *indica* rather than $(CT)_{18}$ of glutinous *japonica*.

Group	Sample ID	KN5-10	KN5-8	KN5-6	KN5-4	KN5-2	KN5-0	RM190	G/T	23bp Dup	KN3-2	KN3-4	KN3-6	KN3-8	KN3-10	KN3-12
		1565535	1606454	1636951	1688662	1720798	1764120	1765770	1765732	1766917	1810724	1850705	1878381	1934288	1980794	2010616
J/*wx*	Jwx105	A	A	A	A	A	A	17	T	+	1	A	A	A	A	A
	Jwx131	A	A	A	N	A	A	17	T	+	2	A	A	A	A	A
	Jwx139	A	A	A	A	A	A	16	T	+	1	N	A	A	A	A
	Jwx149	A	A	A	A	A	A	16	T	+	2	A	A	A	A	A
	Jwx172	A	A	A	A	A	A	17	T	+	3	N	A	A	A	A
	Jwx180	A	A	A	N	A	A	18	T	+	1	A	A	A	A	A
	Jwx185	A	A	A	A	A	A	17	T	+	2	A	A	A	A	N
	Jwx192	A	A	A	A	A	A	16	T	+	2	A	A	A	A	A
	Jwx201	A	A	A	A	A	A	16	T	+	2	A	A	A	A	A
	Jwx204	A	A	A	A	A	A	17	T	+	4	A	A	A	A	A
	Jwx211	A	A	A	A	A	A	17	T	+	5	A	A	A	A	A
	Jwx222	A	A	A	A	A	A	17	T	+	5	A	A	A	A	A
	Jwx240	A	A	A	A	A	A	16	T	+	5	A	A	A	A	A
	Jwx250	A	A	A	A	A	A	16	T	+	3	A	A	A	A	A
	Jwx264	A	A	A	A	A	A	17	T	+	4	A	A	A	A	A
	Jwx307	A	A	A	A	A	A	12	T	+	3	A	A	A	A	A
	Jwx316	A	A	A	A	A	A	18	T	+	3	N	A	A	A	A
	Jwx380	A	A	A	A	A	A	18	T	+	5	A	A	A	A	A
J/*Wx*	Jnonwx56	A	A	A	A	A	A	17	T	-	N	A	A	B	A	A
	Jnonwx110	A	A	A	A	A	A	17	G	-	7	A	A	B	A	A
	Jnonwx167	A	A	A	A	A	A	17	G	-	3	A	A	A	A	A
	Jnonwx178	A	A	A	A	A	A	17	T	-	4	A	A	A	A	A
	Jnonwx189	A	A	A	A	A	A	17	T	-	5	A	A	B	A	A
	Jnonwx206	A	A	A	A	A	A	17	T	-	5	A	A	A	A	A
	Jnonwx252	A	A	A	A	A	A	17	T	-	5	A	A	B	A	A
	Jnonwx270	A	A	A	A	A	A	18	T	-	3	A	A	B	A	A
	Jnonwx308	A	A	A	A	A	A	12	T	-	8	A	A	B	A	A
	Jnonwx315	A	A	A	A	A	A	17	T	-	3	A	A	A	A	A
	Jnonwx327	A	A	A	A	A	A	19	G	-	4	A	N	B	A	A
	Jnonwx330	A	A	A	A	A	A	18	G	-	3	A	A	B	A	A
	Jnonwx358	A	A	A	A	A	A	17	T	-	4	A	A	B	A	A
	Jnonwx370	A	A	A	A	A	A	17	G	-	5	A	A	A	A	A
	Jnonwx389	A	A	A	A	A	A	17	T	-	9	A	A	B	A	A
	Jnonwx395	A	A	A	A	A	A	16	G	-	1	A	A	B	A	A
	Jnonwx451	A	A	A	A	A	A	17	T	-	10	A	A	A	A	A
I/*wx*	Iwx1	A	A	A	A	A	A	18	T	+	6	A	A	A	A	A
	Iwx51	A	A	A	A	A	A	18	T	+	2	A	A	A	A	A
	Iwx142	A	A	A	A	A	N	17	T	+	2	B	B	B	C	B
	Iwx196	A	A	A	A	A	A	17	T	+	3	B	B	B	C	B
	Iwx236	A	A	A	A	A	A	18	T	+	3	A	A	A	A	A
	Iwx257	A	A	A	A	A	A	17	T	+	3	C	B	B	C	A
	Iwx233	A	A	A	A	A	A	18	T	+	3	A	A	A	A	A
	Iwx400	A	A	D	C	A	A	17	T	+	2	A	N	A	A	A
	Iwx405	A	A	A	A	A	A	18	T	+	3	A	A	A	A	A
	Iwx409	A	A	A	A	A	A	18	T	+	2	A	A	A	A	A
	Iwx424	A	A	A	A	A	A	17	T	+	3	B	B	B	B	B
	Iwx434	A	A	A	A	A	A	18	T	+	2	A	A	A	A	N
	Iwx436	A	A	A	A	A	A	18	T	+	3	A	A	A	A	A
	Iwx444	A	A	A	A	A	A	17	T	+	2	A	A	A	A	A
I/*Wx*	Inonwx138	A	A	B	B	B	B	10	G	-	6	B	B	B	C	A
	Inonwx166	A	A	B	B	B	B	10	G	-	3	N	B	B	C	A
	Inonwx194	A	A	B	B	B	B	10	G	-	3	B	B	B	C	A
	Inonwx218	A	A	B	B	B	B	10	G	-	3	B	B	B	C	A
	Inonwx219	A	A	B	B	B	B	10	G	-	3	B	B	B	C	A
	Inonwx324	A	A	B	B	B	B	10	G	-	4	B	B	B	B	B
	Inonwx326	A	A	B	B	B	B	10	G	-	5	B	B	B	B	B
	Inonwx403	A	A	C	B	B	A	17	G	-	5	A	A	A	A	A
	Inonwx408	A	A	A	A	A	A	17	T	-	3	B	B	B	B	B
	Inonwx413	A	A	A	A	A	A	17	T	-	5	B	B	B	B	B
	Inonwx414	A	A	A	A	A	A	17	T	-	2	B	N	B	B	B
	Inonwx420	N	A	B	B	A	A	11	T	-	11	B	N	B	C	N
	Inonwx433	A	A	A	A	A	A	17	T	-	5	B	B	B	B	B
	Inonwx446	A	A	A	A	A	A	16	T	-	5	B	B	B	B	B
Rec/*wx*	Jwx158-rec	N	N	A	A	A	A	18	T	+	N	A	N	A	A	A
	Jwx430-rec	A	A	A	A	A	A	17	T	+	6	N	C	B	C	N
	Iwx217-rec	A	A	A	A	A	A	17	T	+	5	A	A	A	A	A
	Iwx220-rec	A	A	A	A	A	A	17	T	+	4	N	D	C	C	C
	Iwx418-rec	A	A	A	A	A	A	18	T	+	2	A	A	A	A	A
	Iwx422-rec	A	A	B	B	B	A	17	T	+	3	A	A	A	A	A
	Iwx426-rec	A	A	A	A	A	A	17	T	+	5	B	B	B	C	B
	Iwx441-rec	A	A	A	A	A	A	16	T	+	3	B	B	B	C	B
Rec/*Wx*	Jnonwx338-rec	A	A	A	A	A	A	20	G	-	3	A	A	B	A	A
	Jnonwx402-rec	A	A	A	A	A	A	17	T	-	3	A	A	B	A	A
	Jnonwx432-rec	A	A	A	A	A	N	17	T	-	3	A	A	N	A	A
	Inonwx348-rec	N	A	B	B	N	B	11	G	-	11	B	B	B	C	N

Fig. 6. Haplotypes evaluated with SNPs of 12 DNA fragments Wx flanking region. N: not-determined.

Discussion

Introduction of wx causal allele into indica glutinous rice

According to previous studies, glutinous rice has a single origin derived from single causal mutation, 23-bp duplication in exon 2 of *Wx* gene (Wanchana *et al.* 2003), and it was occurred in *japonica* which have a G to T substitution at the splice junction if exon-intron 1 (Yamanaka *et al.*

Table 4. Nucleotide diversity (Pai) of silent sites at 12 fragments *Wx* flanking region

Group	site	KN5-10 103 bp	KN5-8 300 bp	KN5-6*	KN5-4 633 bp	KN5-2 370 bp	KN5-0 449 bp	KN3-2 152 bp	KN3-4 262 bp	KN3-6 308 bp	KN3-8 235 bp	KN3-10 471 bp	KN3-12 569 bp	Total
J/*wx*	Pai	0.0000	0.0000	–	0.0004	0.0000	0.0000	0.0019	0.0000	0.0000	0.0000	0.0000	0.0004	0.0002
	SD	0.0000	0.0000	–	0.0002	0.0000	0.0000	0.0008	0.0000	0.0000	0.0000	0.0000	0.0002	0.0001
J/*Wx*	Pai	0.0000	0.0004	–	0.0005	0.0000	0.0000	0.0017	0.0000	0.0000	0.0103	0.0003	0.0000	0.0008
	SD	0.0000	0.0003	–	0.0002	0.0000	0.0000	0.0010	0.0000	0.0000	0.0017	0.0002	0.0000	0.0001
I/*wx*	Pai	0.0000	0.0000	–	0.0005	0.0000	0.0000	0.0000	0.0087	0.0045	0.0094	0.0025	0.0014	0.0028
	SD	0.0000	0.0000	–	0.0004	0.0000	0.0000	0.0000	0.0024	0.0011	0.0024	0.0008	0.0005	0.0005
I/*Wx*	Pai	0.0000	0.0000	–	0.0057	0.0018	0.0066	0.0000	0.0027	0.0016	0.0043	0.0029	0.0019	0.0039
	SD	0.0000	0.0000	–	0.0008	0.0000	0.0006	0.0000	0.0023	0.0013	0.0027	0.0006	0.0002	0.0004
Total		0.0000	0.0001	–	0.0031	0.0007	0.0025	0.0010	0.0080	0.0038	0.0110	0.0027	0.0011	0.0030

* No silent site in KN5-6 fragment.

2004). However, there is little reference focused on origin of *indica* glutinous rice. In this report, it was found that there was a minor glutinous landraces belonging to the *indica* type rice, although *japonica* was predominated. So we tried to discuss about the origin of *indica wx* allele in rice landraces in northern Laos where is known as the center of glutinous rice zone (Watabe 1967).

All glutinous accession in Laos carried both a 23-bp duplication inside exon 2 and G-T substitution at the splice junction in *Wx* gene. Thus, the glutinous origin was considered as a single. In glutinous *japonica*, although sequence compositions in *Wx* flanking region was highly conserved, there were four alleles at RM190 which is located in exon 1 of *Wx* gene. These alleles derived from a single *wx* allele. Glutinous *japonica* populations in Japan also carried the multiple alleles of RM190 but less than Laos. It implied that Laos has a long history as a center for *japonica* glutinous rice (Watabe 1967). On the other hand, glutinous *indica* also carried multiple alleles, $(CT)_{17}$ and $(CT)_{18}$, at RM190. Allelic frequency suggested that haplotype carrying $(CT)_{17}$ repeat would be the origin (**Supplemental Table 5**). Haplotype data suggested that glutinous *indica* shared same haplotypes with glutinous *japonica* but not with non-glutinous *indica*. From these results, it was newly suggested that the *wx* causal mutation was occurred in *japonica Wx* gene which carried $(CT)_{17}$ at RM190, then this *wx* allele was introduced into *indica* landraces through outcrossing.

Further recombination of Wx franking region of glutinous indica

The previous data indicated the selective sweep window spanning about ~250-kb of *Wx* flanking region. It corresponded with the region spanned from KN5-10 to KN3-2. The comparatively lower diversity around *Wx* locus in particular varietal groups suggested that there might be selection during cultivation. In our materials, two fragments, KN5-8 and KN5-10 in 5′ region of *Wx* gene showed quite low diversity through all groups (**Table 4**, **Fig. 6**). It was probably due to restricted materials collected inside Laos. Successive outcrossing among local landraces could be introduced much diversity not only flanking regions of *Wx* locus but also all genome regions. It was also proved by control sequence used by Olsen *et al.* (2006). The heterozygosity (*He*) of an SSR, RM588 which flanked the 3′ region of KN5-8 showed low diversity, whereas other SSRs such as RM19319 near KN5-10 showed diversity to some extent (**Supplemental Table 6**). It was considered that there could be any other reason to keep low genetic diversity not against for glutinous phenotype for KN5-8 to KN5-10. Therefore, this region should be excluded from sweep window for glutinous phenotype.

There was a gap between the preserved size of *Wx* flanking region of glutinous *indica* and glutinous *japonica* in landraces in Laos. In glutinous *japonica*, all fragments appeared quite low genetic diversity and single haplotype. The preserved region of glutinous *japonica* was estimated spanning about 404~-kb, from the 3′ terminal of KN5-8 (genomic position 1606959) to the 3′ terminal of KN3-12 (genomic position 2011364). It was wider than previously considered. The *He* of glutinous *japonica* was low in RM6263 and RM4923, which located downstream of KN3-12 (**Supplemental Table 6**). The sweep window of glutinous *japonica* possibly contained these regions. In addition to the existence of multiple alleles of RM190, it implies that glutinous *japonica* has been conserved quite long time in this area. On the other hand, many recombinant events were found at *Wx* flanking region in glutinous *indica*. The recombinant points were quite complicated in 5′ flanking region of *Wx* gene. Recombinant was also found in KN5-4 and KN5-6 where were regarded as inside of the selective sweep window in previous study. On the other hand, RM588 close to KN5-8 showed the lowest score in glutinous *indica*, but recover situation at RM19319. Therefore, sweep window of glutinous *indica* considered to be narrower than glutinous *japonica* but the still ~300-kb wide from RM19319 (genomic position 1550199) to KN3-4 (genomic position 1850705). The fragments derived from *japonica* genetic background was preserved to some extent among glutinous *indica* populations. According to Watabe (1967), glutinous *japonica* was cultivated primary in Laos and Thailand, and then non-glutinous *indica* expanded with replacing glutinous *japonica* gradually from Thailand after 10th century. A residual glutinous rise zone was formed after 18th century. It is considered that the glutinous *indica* occurred by outcrossing during this

process, then expanded and stabilized with further recombination. Recombinants would result from high outcrossing rate in farms and on-going selection. In general, local farmers selected only from appearance of panicles. Thus, they allowed such recombinants to escape selection.

Genetic diversity conserved through the slash and burn farming system in northern Laos

In order to classify *indica* and *japonica*, we adopted a set of indicators including cytoplasmic (ORF100) and three nuclear markers (*Acp*1-indel *Cat*1-indel and *Pgi*1-indel). Generally, these four indicators show same genotype in a homozygote plant of *indica* or *japonica*. However, among rice in Laos, recombinant-type accessions were frequently found in glutinous *indica* diagnosed by deletion type ORF100. PCA clearly showed that the group contained scattered *indica* and *japonica*. Additional recombinant accessions were also found when *Wx* flanked SSRs were genotyped, although general indicators identified these accessions as *indica*. The introduction of *wx* allele and erosion of sweep window in glutinous *indica* could be also brought by frequent outcrossing events in slash and burn system.

All of these results indicated that frequent outcrossing and introgression had occurred frequently not only inside the population but also across the various populations. In general, outcrossing between different varietal types such as *indica* and *japonica*, or between cultivars and wild rice, has resulted in weedy types with a high rate of sterility and a tendency for seed shattering (Suh *et al.* 1997, Tang and Morishima 1996). However, no such weedy types with marked seed shattering or sterility were recognized in our field observations in northern Laos. Outcrossing and successive self-crossing may stabilize sterility in the progeny but still maintain diversity through introductions from other varietal groups. Such selection possesses may have resulted in plants with non-shattering seeds. The slash and burn farming system in Laos may have allowed such heterogeneity to exist in populations. From our field survey, with regard to rice seed propagation, local farmers tend to stock and re-use their harvested seeds for the following year, and scrupulous seed selection is performed once in a few years. Local farmers usually grow two or three landraces per a household. They tend to reserve various landraces. In some cases, there are more than 20 landraces in a village. They frequently exchange rice seed between neighbors, relatives and others beyond the ethnic or geographical communities. Thus, once gene flow occurs, heterogeneous gene pools would be inherited from generation to generation, and successive generations would have higher fertility and more stable traits. This system has allowed mixing of multiple crop species, various vegetables, and different rice landraces within a single field. Variation in genetic background may play an important role in the maintenance of stable cultivation in Laos to confer biotic and abiotic stress during upland cultivation. The heterozygous populations present in Laos may work to keep rice populations free of severe pandemic disease or insect damage. On the other hand, the preference of local farmers for glutinous rice may result in severe selection against the *Wx* allele. Only a single causal mutation at the *Wx* locus has been diversified and distributed intricately. This genetic structure of landraces may give us a chance to consider how such diversity can be maintained on farms or how efficiently genetic resources can collected from such farms before they are replaced by modern varieties introduced as cash crops.

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