Variation and dispersal of landraces in northern Laos based on the differentiation of waxy gene in rice (O. sativa L.)

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ABSTRACT We investigated the diversification and diversity in waxy rice cultivars (Oryza sativa L.) in Laos, using three complementary DNA marker systems. We documented three variable DNA markers, including a SSR locus RM190 (number of CT-motif repeat), G-T substitution, and 23 bp duplication. These were analyzed in 389 strains that were collected from northern and central Laos. Variation in cpDNA, presence of absence of a 69 bp deletion at the ORF100, was used for a background classification of all strains as indica or japonica. The three nuclear polymorphisms was all variation within waxy locus. In the RM190 region, nine alleles with different numbers of CT repeats (n=10, 11, 12, 16, 17, 18, 19, 20 and 22) were detected in non-waxy (Wx) alleles, while seven of them (n=12, 16, 17, 18, 19, 20 and 22) were found in waxy (wx) alleles. Compared to previous studies, more allele types were found in northern and central Laos than in neighboring countries. It is considered that the genetic diversity of rice landraces is high in northern and central Laos, and it may be because the high cultural diversity and diverse agricultural systems that persist there. The distribution of landraces with each CT allele type showed neither ethnic bias nor geographical bias. This suggests that there has been frequent exchange of landraces beyond political-borders and between ethnic groups in northern Laos.

Key words: Ethnic group, Genetic diversity, DNA markers, Rice landraces, Waxy

INTRODUCTION

The Waxy gene (Wx) controlling the amylose content in the endosperm has been well analyzed from the viewpoint of molecular genetics of rice (Olsen and Purugganan 2002; Yamanaka et al. 2004), barley (Domon et al. 2002) and foxtail millet (Fukunaga et al. 2002; Kawase et al. 2005). This gene encodes Granule Bound Starch Synthase (GBSS1) that synthesizes amylose in the rice (Oryza sativa L.) endosperm, and is located on chromosome 6 in the rice genome. Generally, two alleles are found in rice: 1) Wx, the wild-type that produces GBSS1 resulting in about 10-30% amylose content and 70-90% amylpectin in the endosperm, and 2) wx, the recessive allele that loses the function of GBSS1 synthesis resulting in almost 100% amylpectin. In rice the difference between these two alleles is determined by the presence or absence of a 23 bp duplication in the second exon of the gene (Inukai et al. 2000; Wanchana et al. 2003). Among non-waxy rice, there are two types that have the G-T polymorphism at the putative 5' leader intron splice site of the waxy gene. The wild-type gene is designated as Wx and this is also found in cultivated rices, especially most indica strains. The gene with reduced function also on the Wx allele is designated as Wx* (Sano, 1984). Although it was reported previously that Wx and Wx* were indica- and japonica-specific, respectively (Hirano et al. 1998; Isshiki et al. 1998), there are exceptions to this, as discussed below.

Nucleotide polymorphisms of the waxy gene of commercial rice varieties, mainly non-waxy rice, collected from a geographically broad area have been analyzed in many studies (e.g. Bligh et al. 1995; Ayres et al. 1997, Bao et al. 2002; Olsen and Purugganan, 2002; Prathepha and Baimai, 2004). A simple sequence repeats (SSR), RM190
is known at region of CT repeats in exon 1 of the waxy gene as the one of these polymorphisms. This is a valuable marker to elucidate the genetic diversity of waxy gene. Because the waxy gene may have been selected intensively for waxy endosperm trait, the polymorphisms in waxy gene can be useful for observing the relationship between human activity and genetic diversity. In addition, among non-waxy rice strains, this marker is also used to classify alleles with different amylose content regardless of the number of CT repeats (Bligh et al. 1995; Ayres et al. 1997; Bao et al. 2006).

There is a useful marker to clarify rice strain into indica and japonica by using a 69 bp deletion at ORF100 region in rice chloroplast DNA. The deletion-type and normal-type are predominant in indica and japonica, respectively (Kanno et al. 1993). This deletion was detected not only in cultivated rice but also in its wild relatives. This is the one reason to consider that the indica–japonica differentiation occurred before domestication (Yamanaka et al. 2003).

The mountainous region throughout the Indochina Peninsula is recognized as the “Glutinous Rice Zone” (Watabe, 1967) where waxy landraces have been grown for daily diet. Lao P.D.R. (The Lao People’s Democratic Republic) is at the center of this “Glutinous Rice Zone” and comprises 47 different official ethnic subgroups with different cultures and languages. This area is important for the study of the origin of waxy rice. The genetics of cultivated rice in this region has been reported previously (Morishima et al. 1984; Sato 1994; Ishikawa et al. 2002; Yamanaka et al. 2002, 2004), but information at the molecular-genetics level is still limited.

To elucidate the diversification and the mechanism of maintaining genetic diversity in rice cultivars (Oryza sativa L.), polymorphisms at three different mutations at the waxy gene: 1) a 23 bp duplication in exon 2 that causes loss of function, 2) G-T polymorphism at the 5’ of intron 1 that causes reduction of function, and 3) SSR polymorphism of exon 1, and the deletion at ORF100 region in chloroplast DNA, were compared with geographic distribution and ethnic distribution.

MATERIALS AND METHODS
Field collection for sampling of the materials
From 2003 to 2005, expeditions were carried out in provinces, Phongsali, Louang Namtha, Louang Phrabang, Oudomxai, in northern Laos and Vientiane Municipality in central Laos. Seed samples of rice landraces (Oryza sativa L. subspp. japonica and subspp. indica) were collected from 71 villages that comprise 11 ethnic groups (Fig. 1). There were some landraces which had the same name but were collected from different villages. Therefore, in this study, we identified each of these landraces as different strains. A total of 389 strains were used in this study. The seed materials are stored in Rice and Commercial Crop Research Center (RCCRC), Vientiane, Lao P.D.R.

We also interviewed farmers about the features of each variety, such as variety name, growing condition (upland or lowland), time of maturity (early, medium or late), and other information for cultivation. All strains were classified as waxy (glutinous) or non-waxy (non-glutinous) by staining with 0.7% iodine-potassium-iodine (1/IKI): waxy endosperm shows brown, and non-waxy endosperm does bluish black. Some genetic characters, such as pericarp color and awn length, were also observed for each sample.

DNA extractions and indica–japonica classification by chloroplast DNA ORF100
Seeds of collected samples were grown at experimental fields in RCCRC. For DNA analysis, whole genome DNA was extracted from 100mg of young leaves from each strain by using a modified cetyltrimethylammonium bromide method (Doyle, 1991).

We classified the strains into two types of rice strain, according to the presence or absence of a 69 bp-deletion in the ORF100 region of chloroplast DNA. Deletion-type is taken to be indica, while non-deletion is taken to be japonica. PCR amplification was followed Kanno et al. (1993) by using primer set (F:5’- GCC TGC AGT CAC TGG ACC TGA CTC C-3’, and R:5’- GCC GAT CCG AGG TCG TGG TAA ATC C-3’).

The PCR reaction was done under the following conditions: a 10μl reaction mixture consisted of 0.25U Ex Taq polymerase (Takara Co.), 1μl 10 × Ex Taq Buffer (Takara Co.), 1μl dNTPs (2.5mM), 0.5μl dimethyl sulfoxide, 2μl of each primer (2.5μM), 2μl DNA and 1.45μl ddH2O. The reaction included pre-heating for 5 min at 95°C, 35 cycles of 30 sec at 96°C, 30 sec at 55°C, 1 min at 72°C, then an extension for 5 min at 72°C. After amplification, 5μl of the product was electrophoresed in 1.5% agarose gel. The bands were stained with ethidium bromide and were visualized by ultraviolet illumination.

Variation in the waxy gene
Variation of a 23 bp duplication in exon2
We detected a 23 bp duplication in exon 2 as polymorphism amplified by primer set Wax-Al (5’-CAC
Analysis of SSR polymorphism in the waxy gene

G-T polymorphism at the 5’ splicing site in intron 1

To detect G-T polymorphism at the putative 5'-leader intron splice site of the waxy gene, analysis of derived cleaved amplified polymorphic sequences (dCAPS; Michaels and Amasino 1998; Neff et al. 1998) followed the procedure of Yamanaka et al. (2004) (Fig. 2). Each digested product (10μl) was electrophoresed in 3.0% agarose gel, and we classified the strains as G-type or T-type.

Analysis of SSR polymorphism in the waxy gene

The SSR in the first exon of the waxy gene was detected by primers fluorescently labeled by fluorescein isothiocyanate (Fig. 2). The primers were designated as RM190 (F: 5'-CTT TGT CTA TCT CAA GAC AC-3', R: 5’-TTG CAG TTC TCT CTG ATG-3', Temnykh et al. 2000). The amplified products were run on 10.0% polyacrylamide gels, including 48% urea for 2.0 hours at 2000V. To determine the sequence of the number of CT repeats for at least one strain of each allele at RM190, we amplified by using PCR primer set wax-ex1F (5’-TAG ACA AAA GTC GGT TTT GCT TTT-3’) and wax-ex1R (5’-ACC AAA CAT AAC GAA CGA AGA TTT-3’), because the PCR products of RM190 were too short for sequencing. The PCR products were cloned by using a topo TA cloning kit (Invitrogen) and then were sequenced using universal primer M13 with a DTCS Quick Start Kit by using a SEQ8000 Genetic Analysis System (Beckman Coulter).

The allelic richness (Rs) at RM190 per population was calculated by using genetic software FSTAT (ver.2.9.3; Goudet 2001). Rs was calculated by using the formula: Rs = Ω [2N-Ni/2n] / (2N/2n).

RESULTS

Classification based on the indel at the ORF100 and waxy gene

Table 1 shows the indica-japonica classification based on the indel in the ORF100 region. The indica and japonica are classifiable based on this indel (Kanno et al. 1993). Out of the 389 strains, 320 (82.3%) were normal-type of ORF100, and 69 (17.7%) were deletion-type. Among 335 upland strains, 307 (91.6%) were normal-type and 28 (8.4%) were deletion-type. Among 54 lowland strains, 13 (24.1%) were normal-type and 41 (75.9%) were deletion-type. The normal-type was high frequency in upland strains, and deletion-type type was higher in lowland strains. These results suggest that upland and lowland strains tended to correspond to japonica and indica, respectively.

As a result of I/KI staining, 316 strains (81.2%) were classified as waxy strains, and 73 strains (18.8%) were classified as non-waxy strains. Among the 71 sampled villages, 35 villages had both of waxy and non-waxy strains and 32 villages had waxy strains only. The remaining 4 villages had non-waxy strains only and all of these villages had the Hmong ethnic group.

Out of 389 strains, 316 (81.2%) strains were waxy strains that had a 23 bp duplication in exon 2 at the waxy locus, and the remaining 73 (18.8%) strains were non-waxy strains. This result agreed completely with the classification by I/KI staining, namely, all waxy strains had this duplication but non-waxy strains had no duplication. This result indicated that the upland /normal-type /waxy type’s strain was predominant (64%) in northern Laos cultivars used in this study.

The low amylose content controlled by Wx’ is caused

Table 1. The relationship of genotypes between waxy gene and ORF100.

<table>
<thead>
<tr>
<th>Genotype of Wx gene</th>
<th>No. of strains (%)</th>
<th>Genotype of ORF100 in each eco-system</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Genotype of ORF100</td>
<td>Upland</td>
<td>Lowland</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>wx</td>
<td></td>
<td>251(74.9)</td>
<td>20 (6.0)</td>
</tr>
<tr>
<td>Wx</td>
<td></td>
<td>56 (16.7)</td>
<td>8 (2.4)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>307 (91.6)</td>
<td>28 (8.4)</td>
</tr>
</tbody>
</table>

The upland or lowland classification was according to the interview to farmer, and the wx-Wx classification was done by iodine steining.

+ and - indicate the normal and deletion types at ORF100 region, respectively.
by a G-to-T substitution in the 5' splice site of the leader intron 1 of Wx in non-waxy rice (Hirano et al. 1998; Isshiki et al. 1998). This single base substitution reduces the efficiency of the first intron splicing, and consequently decreases the function of GBSS, making rice slightly sticky but not fully waxy. Among waxy strains, the T-type was dominant in both normal-type (%) and deletion-type (%) (Table 2). Among non-waxy strains, the frequency of the G-type was (%) in normal-type, but it was (%) in deletion-type, while T-type was (%) in normal-type and T-type was (%) in deletion-type. The T-type seemed to be dominant in normal-type and the G-type dominant in deletion-type (Table 2).

Nine alleles were found at RM190 among the 389 strains. We deduced that the length polymorphism was due to the difference in number of CT repeats, which was confirmed by sequence analysis (Fig. 3). Table 3 shows the frequency of each allele. Among waxy strains were 7 alleles (No. of repeats = 12, 16, 17, 18, 19, 20 and 22) and among non-waxy strains were all 9 alleles (No. of repeats = 10, 11, 12, 16, 17, 18, 19, 20 and 22). (CT)α and (CT)β were only among non-waxy strains, and (CT)γ and (CT)δ were only among deletion-type of ORF100 strains. Among 260 normal-type waxy strains, 151 strains had (CT)γ allele, 55 strains had (CT)δ allele, and 42 strains had (CT)ε, indicating that these three alleles were dominant. (CT)α, (CT)β, (CT)δ and (CT)ε were considered to be rare because their allele frequencies were lower than 2% of the total number of CT alleles. Among 60 normal-type /non-waxy strains, 32 strains had (CT)β and this allele was dominant. Among 56 deletion-type /waxy strains, 26 strains had (CT)α and 24 strains had (CT)δ, indicating that these two alleles were dominant. Especially among strains of (CT)α, strains were lowland rice and strains were upland rice. Among 22 strains were lowland rice and 2 strains were upland rice. Among 13 deletion-type /non-waxy strain, 7 strains had (CT)δ and this allele was dominant.

Geographic and ethnic distributions of (CT)n variations of waxy strains
Table 4 shows the number of alleles and number of strains among 316 waxy strains and 73 non-waxy strains, respectively. Among waxy strains from 67 villages, the number of strains grown in a village ranged from 1 to 20 with an average of 4.1. The number of alleles ranged from 1 to 4 with an average of 1.7. Among non-waxy strains from 38 villages, the number of strains grown in a village ranged from 1 to 8 with an average of 2.0, and the number of alleles ranged from 1 to 5, with an at average of 1.6.
Table 4. No. of strains and allelic variation of SSR marker, RM190, in waxy and non-waxy strains collected from five provinces.

a) Waxy strains

<table>
<thead>
<tr>
<th>Province</th>
<th>No. of villages</th>
<th>Total No. of strains</th>
<th>Range of No. of strains per village</th>
<th>Average No. of strains per village</th>
<th>Average No. of alleles per village</th>
</tr>
</thead>
<tbody>
<tr>
<td>LP</td>
<td>3</td>
<td>7</td>
<td>2-3</td>
<td>2.3</td>
<td>1</td>
</tr>
<tr>
<td>LN</td>
<td>8</td>
<td>115</td>
<td>1-11</td>
<td>5.4</td>
<td>1-3</td>
</tr>
<tr>
<td>LL</td>
<td>24</td>
<td>43</td>
<td>2-10</td>
<td>5.4</td>
<td>2-4</td>
</tr>
<tr>
<td>LD</td>
<td>21</td>
<td>129</td>
<td>1-20</td>
<td>5.5</td>
<td>1-3</td>
</tr>
<tr>
<td>LV</td>
<td>11</td>
<td>22</td>
<td>1-4</td>
<td>2.0</td>
<td>1-4</td>
</tr>
<tr>
<td>All</td>
<td>67</td>
<td>316</td>
<td>1-20</td>
<td>4.1</td>
<td>1-4</td>
</tr>
</tbody>
</table>

b) Non-waxy strains

<table>
<thead>
<tr>
<th>Province</th>
<th>No. of villages</th>
<th>Total No. of strains</th>
<th>Range of No. of strains per village</th>
<th>Average No. of strains per village</th>
<th>Average No. of alleles per village</th>
</tr>
</thead>
<tbody>
<tr>
<td>LP</td>
<td>3</td>
<td>7</td>
<td>1-3</td>
<td>2.3</td>
<td>1-3</td>
</tr>
<tr>
<td>LN</td>
<td>6</td>
<td>21</td>
<td>1-3</td>
<td>1.5</td>
<td>1-2</td>
</tr>
<tr>
<td>LL</td>
<td>14</td>
<td>19</td>
<td>1-8</td>
<td>3.2</td>
<td>1-5</td>
</tr>
<tr>
<td>LD</td>
<td>11</td>
<td>22</td>
<td>1-4</td>
<td>2.0</td>
<td>1-3</td>
</tr>
<tr>
<td>LV</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>1.0</td>
<td>1</td>
</tr>
<tr>
<td>All</td>
<td>38</td>
<td>73</td>
<td>1-8</td>
<td>2.0</td>
<td>1-5</td>
</tr>
</tbody>
</table>

LP: Phongsali  
LN: Louang Namtha  
LL: Louang Phrabang  
LD: Oudomxai  
LV: Vientiane Municipality

Table 5 shows the variation of CT alleles among ethnic groups in each province. No geographic cline was found with these nine CT alleles among both waxy and non-waxy strains, except (CT)\(_{12}\), which we discuss below. Three major alleles (CT)\(_{11}\), (CT)\(_{17}\), and (CT)\(_{10}\) were distributed at similar proportions in Louang Namtha, Louang Phrabang, Oudomxai, and Vientiane Municipality. (CT)\(_{12}\) were found in Khamu and Akha in Louang Namtha, and Thai Leu in Louang Phrabang. (CT)\(_{17}\) were found in Lamet groups in Louang Namtha, and Lao group in Vientiane. (CT)\(_{10}\) was found only in one strain in the Khamu group in Louang Namtha. The most frequent allele (CT)\(_{17}\), was found in 10 of 11 ethnic groups, represented by 94 strains in the Khamu group, 21 strains in the Lao group, and 12 strains in the Konsart group. Other major types showed a similar tendency. (CT)\(_{10}\) was distributed in ethnic groups Khamu, Akha and Thai Lue. The remaining minor alleles (CT)\(_{11}\) and (CT)\(_{12}\) were also found in some ethnic groups. (CT)\(_{10}\) was found in Khamu but only one strain. No cline was found in CT type distribution among ethnic groups.

Exceptionally, 9 out of 10 strains that had (CT)\(_{12}\) allele that were collected from 3 villages in Phongsali province. One of these villages grew only strains with (CT)\(_{12}\). This village had the Lanten group and was close to the border of Phongsali province and China.

**DISCUSSION**

Out of the total 389 strains collected, 335 strains (86.1%) were upland rice and 54 (13.9%) were lowland rice. The 389 strains contained 32 black rice (purple pericarp) (8.2%) and 47 red rice (red pericarp) (12.0%). People used black rice landraces frequently for rituals but did not use red rice. Based on the interviews, some strains classified as upland strains in this study were grown in both upland and lowland fields.

Generally, the 23 bp duplication in exon 2 of the waxy gene is considered to be the cause of the waxy trait in
rice (Wanchana et al. 2003). For non-waxy rice, the view is widely accepted that Wx² (G-type) is dominant in deletion-type, while Wx³ (T-type) is dominant in normal-type. Because the wild ancestors of cultivated rice also had only the G-type, the waxy mutation was considered to be originated from japonica (Yamanaka et al. 2004). Among waxy rice in this study, the T-type was dominant in both indica and japonica, and the G-type was rare, but, every waxy strain had the same bp duplication in exon 2, which appears to be the more important causative mutation. Whether G-types could indicate back mutation or are the product of recombination is unclear. There are differences in the allele distribution at RM190 between deletion-type and non-deletion type in non-waxy strains, but no difference in waxy strains (Table 3). This may indicate a reduction in the diversity within the wx gene by comparison to the ancestral Wx gene pool in the case of japonica (non-deletion) strains. However, a role for variable genetic backgrounds within which wx has been introgressed and selected should also be considered especially when comparing the deletion-type /waxy and deletion-type (indica) /non-waxy strains.

### Table 5. Variation of CT alleles of SSR marker RM190 in rice strains in 11 ethnic groups.

<table>
<thead>
<tr>
<th>Province</th>
<th>Ethnic group</th>
<th>No. of villages</th>
<th>No. of strains (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lao</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>Akka</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>Laren</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>Lao</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>Akka</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>Laren</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
</tbody>
</table>

**Table 5. Variation of CT alleles of SSR marker RM190 in rice strains in 11 ethnic groups.**

<table>
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<th>No. of villages</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lao</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>Akka</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>Laren</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>Lao</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>Akka</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>Laren</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
</tbody>
</table>

**Table 5. Variation of CT alleles of SSR marker RM190 in rice strains in 11 ethnic groups.**
agree with the suggestion of previous studies that the waxy endosperm was derived from one mutation, a 23 bp mutation, in the \textit{Wx}\textsuperscript{a} strains (Wanchana et al. 2003). Further study is needed to reveal the origin of waxy rice.

In this study, seven alleles (n=12, 16, 17, 18, 19, 20, 22) were among waxy strains in northern Laos. Four alleles (n=16, 17, 18, 19) were reported for waxy strains in China (Bao et al. 2002) and Thailand (Prathepha and Baimai, 2004), and three alleles (n=16, 17, 18) were found in waxy strains in Japan (Muto unpublished data). The \textit{Rs} was calculated as 4.8 in northern Laos, 4.0 in China and Thailand and 2.9 in Japan (calculated from the data in Bao et al. 2002; Prathepha and Baimai, 2004 and Muto unpublished data, respectively); it was also highest in waxy strains in northern Laos. Because \textit{Rs} is a measure of the number of alleles independent of sample size, it allows comparison between different sample sizes. This study clearly suggests that waxy landraces in northern Laos have quite a high genetic diversity and it may be considered that random drift due to a smaller population size than Laos caused a lower genetic diversity in China, Thailand and Japan.

The high genetic diversity in northern Laos is considered to be derived from two factors. One is ethnicity and the other is a scale of family labor power. To expand upon ethnicity, the various ethnic groups have different habits, palatability, and cultivation methods. For instance, there were mainly three different ways of harvesting, correlated with each ethnic group, and we found that farmers chose the strains with the right degree of seed shattering habits for their way of harvesting. We also found that some black rice strains were grown for ritual purposes by some ethnic groups. Farmers need strains with different maturation periods such as early, middle and late, to harvest gradually at the scale of family labor. Landforms and field conditions are various in upland fields, and therefore people grow a range of rice strains with degrees of resistance to cold, drought and disease.

Genetic diversity is further kept high by the fact that people do not control seed contamination. The genetic diversity shown in Table 3 is considered to be the result of all these human activities.

The most important finding in this study is the genetic effect of seed exchange. Generally it has been considered that ethnic groups do not exchange rice seeds because each ethnic group has its own independent culture and cultivation methods. But in Laos, ethnic groups contribute to the formation of a unique composite society from cultural and agricultural aspects, through exchanges of materials and culture, with the preservation of their respective production systems (Sonoe et al. 2004). Contrary to previous views, the results of this study show neither an apparent geographic cline nor an ethnic difference in number of CT repeats among the ethnic groups. Our field work found that farmers bring rice landraces from not only their relatives and neighboring villages but also from different ethnic groups in other districts, provinces and countries, i.e. rice landraces have been dispersed beyond political-borders and ethnic groups in northern Laos by seed exchange between farmers.

The exception that (CT)\textsubscript{12} has a limited distribution may be because, (CT)\textsubscript{n} is distributed mainly in China, and farmers from there are less likely to exchange their rice landraces. The role of the political boundary in reducing exchange of germplasm might also be considered. Further study is needed to solve this problem.

The seed exchange systems by farmers have been studied anthropologically (e.g. Richards, 1996) and genetic variation and geographical distributions have been studied by using molecular genetics for many cereals. But few studies have shown the relation between ethnic group and seed dissemination at the molecular level. This study provides a new viewpoint for discussion by using DNA polymorphism. Seed exchange by farmers across long distances and cross-pollination have contributed to maintain and increase genetic diversity (Cox and Wood, 1999). Genetic diversity of the waxy rice gene pool indicates that various waxy strains have been kept in effectively large populations by farmers in northern Laos. Reduced population size may lead to reduced allelic frequencies due to genetic drift. Rapid losses of landraces due to displacement by modern cultivars have become a serious problem in the last few decades. Collection and evaluation of rice genetic resources in Laos is urgently needed.

Recently, domestication-related genes of not only waxy rice, but also shattering (\textit{qSH1}: Konishi et al. 2006; \textit{SHA1}: Lin et al. 2007) and pericarp color (\textit{Re Rd}: Furukawa et al. 2007), have been studied in detail. All these studies clearly indicate that domestication-related characters were derived from a single mutation, and these beneficial mutations have been transferred between rice strains, sometimes between \textit{japonica} and \textit{indica}, by seed exchange and cross-pollination. However, it is still unknown whether mutations occurred more often, but that many of them have been eliminated by natural or human selection. The evolutionary process of a particular
gene may be simple, but the evolutionary process of strains is more complicated as shown in this study.

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