Characterization of "Khao Kai Noi", Lao Rice Landrace Group (*Oryza sativa* L.), Derived from Different Collecting Missions and Its Core Collection Development

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Characterization of "Khao Kai Noi", Lao Rice Landrace Group (*Oryza sativa* L.), Derived from Different Collecting Missions and Its Core Collection Development

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ABSTRACT

Rice (*Oryza sativa* L.) is the main food for people in the Lao People's Democratic Republic (Lao PDR or Laos). Lao farmers use traditional and modern varieties. Khao Kai Noi (KKN) is part of the rice landrace group (*O. sativa* L.) mainly grown in two northeastern provinces, Xiengkhouang and Houaphan, of the country. It is preferred by consumers due to its aroma and softness after cooking. It was collected and conserved in the Lao National Genebank. The germplasm maintained in the genebank was described as the "genebank population" for this study. A later investigation was conducted in 2014, and the sample collected at this time was called an "on-farm population." Both the genebank and on-farm population have not yet demonstrated their genetic diversity, which is essential for genetic resources utilization.

The present study included KKN rice landrace survey and sample collection in the northeastern part of Laos, genetic diversity evaluation of both the genebank and on-farm population, and core collection development. Microsatellite or simple sequence repeat (SSR) markers were used to evaluate variation in KKN. The 36 SSR markers that cover the whole genome of rice were first screened for succession of amplification, and then 24 of them were chosen for the study that followed.

The hypotheses were that the genetic diversity of both the Khao Kai Noi genebank and on-farm populations may be high.

The 70 accessions of KKN termed as the genebank population were chosen from the Lao National Genebank to evaluate genetic diversity through the use of 23 polymorphic SSR markers. The markers detected 132 alleles, which ranged from 2 to 17 with an average of 5.7 per locus. The polymorphism information content ranged from 0.036 (RM408) to 0.526 (RM259), with an average of 0.199. Expected heterozygosity ranged from 0.037 (RM408) to 0.574 (RM514). Observed heterozygosity ranged from 0.000 (RM455) to 0.061 (RM447). The total expected heterozygosity or gene diversity of KKN overall was 0.271. Molecular variance was found to be 15%, 79%, and 6% among name subgroups, accessions, and within accessions, respectively. Genetic differentiation among name subgroups was confirmed by *F*st=0.353. Most of the accessions were clustered with temperate japonica type and showed genetic relation to rice from neighboring provinces of Vietnam, thus suggesting a Vietnamese origin.

Survey and sample collection was conducted during harvesting season, from October to November 2014, in two provinces, Xiengkhouang and Houaphan, in Laos. Rice landraces, including KKN and others, were collected. The 60 accessions collected were 49 of KKN and 11 of other rice landraces. In Xiengkhouang Province, 32 accessions (25 KKN and 7 other rice types) were collected. In the province of Houaphan, 28 accessions (24 KKN and 4 other rice types) were collected. All samples were maintained using passport data in the Lao National Genebank, Agriculture Research Center, National Agriculture and Forestry Research Institute, Vientiane, Lao P.D.R

On-farm populations (39 accessions) of KKN were evaluated by using 14 SSR markers, and genetic diversity was 0.314. A phylogenetic tree grouped accessions into three clusters, and population genetic differentiation among them was significant (F_{ST}=0.86, p=0.001). Distance isolation was not a factor in this KKN on-farm population, which was collected in only two different geographical provinces in Laos.

The core collection of the entire KKN collection was developed based on available passport and molecular data. Thirty accessions were chosen for core collection from 109 accessions. This captured all of the alleles and diversity of the entire KKN collection in the Lao National Genebank. It represents all KKN and will be useful for breeders, academic researchers, and genebank managers.

Full, systematic study could explain the diversity of the previous and present population of KKN. This information will be useful for germplasm management, sustainable utilization, and efficiency conservation.

Chapter 1

General Introduction

1.1 Introduction

Rice is one of the most important crops in the world. It is the staple food of more than half the population on our planet. It is grown worldwide, but 90% is grown and consumed in Asia. There are two species of cultivated rice: *Oryza sativa*, known as Asian cultivated rice, and *Oryza graberrima*, known as African cultivated rice.

Lao People's Democratic Republic (Lao P.D.R or Laos) is landlocked and located in Southeast Asia. It is a member of the Asian countries. It is in the center of the "Glutinous Rice Zone" as mentioned in Watabe (1967), cited in Yamanaka et al. (2002) (Fig. 1.1). About 6 million people depend on rice for their main daily carbohydrate. About 80% of them are farmers. In Lao P.D.R, four percent [2.4 million hectares (ha)] of the total land is arable land, of which 63% (0.89 million ha) was cultivated agricultural land in 2010 (USDA, 2011). The total rice growing area was 939,100 ha by 2013 (Department of Agriculture, 2014). This comprised 728,635 ha (78% of total), 92,340 ha (10% of total), and 118,125 ha (12% of total) of rain-fed lowland, irrigated lowland, and upland rice respectively.

1.2 Genus of *Oryza*

The genus of *Oryza* consists of two cultivated species: *Oryza sativa* L. and O. *graberrima* S. (Morishima, 1984). Besides cultivated ones, there are also about 20 species of wild rice in this genus.

1.3. Rice genetic resources collection and conservation in Laos

The great whole country rice survey and collection in Laos was conducted during 1995–2000. It was the joint venture between the Lao Ministry of Agriculture and Forestry and the International Rice Research Institute (IRRI). At that time 13,192 accessions of cultivated rice were collected (Rao et al., 2006a). These rice germplasm are now conserved in the Lao National Genebank at the Agriculture Research Center (ARC), National Agriculture and Forestry Research Institute (NAFRI), Vientiane Capital. There were more minor collections after the major one; for example, KKN collection in 2008 (Bounphanousay et al., 2009) and the collection of rice landraces in northeastern Laos in 2014 (Vilayheuang et al., in press), which is a part of this thesis.

1.4 Problem addressed

KKN is important, so it was collected and conserved in the genebank. There are a number of accessions, but only a few had reported morphological characteristics. However, its variation at a molecular level has not been reported. Full study is needed in order to prove fundamental information.

1.5 Hypothesis

The hypothesis for the current study was that the level of diversity of both the genebank and on-farm populations of KKN may be high.

1.6 Specific objectives

1. To characterize the genetic diversity of the KKN population that is conserved in the Lao National Genebank.
2. To survey, collect, and characterize the cultivated rice in northeastern Laos, where KKN is majorly cultivated.

Figure 1.1. Map showing the "Glutinous Rice Zone" as defined by Watabe (1967) cited in Yamanaka et al. (2002).

Chapter 2

Characterization of the Genebank Population of Khao Kai Noi, Lao Rice Landrace Group (*Oryza sativa* L.)

2.1 Introduction

Rice (*Oryza sativa* L.) is a staple food of nearly half the people on the planet (Mohanty, 2013). In Laos, most people depend on it as their staple food, and rice dominates agricultural production (Eliste & Santos, 2012). Although Lao farmers grow improved cultivars for their high yields, some farmers still grow local landraces because of their adaptation to the local environment and preferred traits such as aroma. Despite their lower yields, those landraces can serve as useful resources for breeding programs.

Khao Kai Noi (KKN) is a rain-fed, lowland glutinous rice grown in northern and northeastern Laos with good grain quality, softness, and aroma (Rao et al., 2006b, c). The name means "small chicken rice" in Lao. Its spelling in English is not standardized and is variously written as Khao Kay Noi or Khao Kai Noi (Rao et al., 2006c) or Khao Kai Noy (Worklivelaos, 2014a). The spelling used in this study is Khao Kai Noi. KKN is grown not just for domestic consumption but also for export to France and Vietnam. It is also used for brewing beer (Vientiane Times Newspaper 2014; Worklivelaos, 2014b).

From 1995 to 2000, the Lao Ministry of Agriculture and Forestry and the International Rice Research Institute (IRRI) collected 13,192 local accessions of rice throughout the country (Rao et al., 2006a). The collection, which includes KKN, is held in the National Genebank of the Agriculture Research Center, National Agriculture and Forestry Research Institute (NAFRI). Another specifically KKN survey and collection were performed in 2008 in the provinces of Houaphan (HP) and Xiengkhouang (XK) (Bounphanousay et al., 2009).

Knowledge of the genetic diversity of KKN is required for the sustainable use of the germplasm, efficient collection management, plant variety rights protection, core collection development, and breeding. Many studies have used simple sequence repeat (SSR) markers to examine the genetic diversity, population structure, and genetic variation within rice landraces (Bajracharya et al., 2006; Das et al., 2013; Pusadee et al., 2009; Roy et al., 2013; Zhang et al., 2013). However, no reports of Lao rice landraces are available, with the exception of an evaluation of black glutinous rice (Bounphanousay et al., 2008). Kanyavong (2013) described the agro-morphological variation within KKN, but its molecular diversity has not been reported.

This study initially evaluated the genetic diversity of accessions of KKN via SSR markers. Next, I studied genetic relatedness among accessions of KKN collected from different geographical locations. Finally, I compared the genetic relatedness between KKN and other landraces within Laos and between Laos and Vietnam.

2.2 Materials and Methods

2.2.1 Plant materials

Seventy accessions of KKN were chosen from the Lao National Genebank (Table 2.1). This collection consists of a variety of different subgroups, some of them having additional descriptors in the varietal name to reflect such special traits as glume color as well as other characteristics such as KKN Deng (red), KKN Khao or KKN Khaw (white), KKN Leuang (yellow), KKN Lai (striped), and KKN Dam (black). These names were given by farmers at the time of collection (Rao et al. 2006b). The following control accessions were added: Thankdokkham11 or TDK11, Nipponbare, and Kasalath, which represent a Lao modern variety, Japonica-type, and Indica-types respectively. Nipponbare and Kasalath were provided by the National Institute of Agrobiological Sciences (NIAS), Japan. The controls included 18 accessions of other landraces from Houaphan Province (HP) and Xiengkhouang Province (XK), where KKN is majorly produced, and a neighboring province of Vietnam (Table 2.2). These materials were provided by IRRI. Eight individual dehulled seeds of each accession were used in this study.

2.2.2 Simple sequence repeat (SSR) assay

2.2.2.1 Screening SSR primers for successive amplification

The first 36 nuclear SSR markers, which covered the whole genome with two to four markers per chromosome, with an average of three markers per chromosome,

were screened for testing compatibility with the samples by using a polymerase chain reaction (PCR). They were chosen from a panel of 50 standard SSR markers, and all primer sequence information is available at http://archive.gramene.org/markers/ microsat/50_ ssr.html. I then found that 12 of them were not well amplified for testing samples; thus, 24 markers, with a distribution of one to three per chromosome, were finally selected for this study (Table 2.3).

2.2.2.2 Genomic DNA extraction

Genomic DNA was extracted from single brown rice seed (dehulled). Seed was put in the tube and first ground by using a multi-bead shocker (YASUI Kikai, Japan) at 2,000 rpm for 1 min; then homogeneous powder was subjected to DNA extraction by using the cetyl trimethyl ammonium bromide (CTAB) method (Doyle & Doyle, 1987). Finally, all samples were quality checked by using 0.8% agarose gel electrophoresis and quantitation by spectrophotometer DU640 (Beckman Coulter, USA).

2.2.2.3 Amplification of DNA

PCR was performed as described in the Multiplex PCR strategy. Twenty-four SSR primer pairs were selected at the screening step, and forward primers were labeled with one of four fluorophores (6FAM, NED, PET and VIC). They were later divided into four groups called “panels” with six markers each (Table 2.3) selected by Multiplex Manager v. 1.0 software to design efficient combinations of primers in a

PCR reaction (Holleley & Geerts, 2009). Each reaction contained 1× Type-it Multiplex PCR Master Mix (Qiagen, Hilden, Germany), 2–5 pmol of the forward and reverse primers of each of the six markers in a panel, and 50–100 ng of genomic DNA in a total volume of 15 µL. PCR amplification was performed in a GeneAmp PCR System 9700 (Applied Biosystems, CA, USA) as follows: 95°C for 5 min; 30 cycles of 95°C for 30 s, 58–60°C (depending on the panel, Table 2.3) for 90 s, and 72°C for 30 s; and a final 60°C for 30 min. After completion, the PCR product was covered with aluminum foil to avoid light exposure and stored at a temperature of 4°C before being subjected to capillary electrophoresis.

2.2.2.4 Fragment DNA separation by capillary electrophoresis

The PCR products were diluted 1:20 with sterilized Milli-Q water (PCR product: Milli-Q water). Then 1 µL of each was added to 9.5 µL of Hi-Di formamide plus 0.5 µL of GeneScan 600-LIZ size standard marker (Applied Biosystems, USA). The mixture was heated at 95°C for 3 min, immediately chilled on ice for 5 min, then run in an ABI 3500xL genetic analyzer (Applied Biosystems, USA). Data were collected with 3500xL data collection v. 1 software (Applied Biosystems, USA). Fragment analysis or allele calling was done in GeneMapper v. 5 software (Applied Biosystems, UAS). Finally, the allele size in base pairs was exported to a text or Excel file to enable its importation into data analysis software.

2.2.3 Data analysis

The fundamental parameters for diversity—expected heterozygosity (H_e), observed heterozygosity (H_o), average expected heterozygosity, and polymorphism information content (*PIC*)—of each locus over all 70 accessions (560 individuals) were calculated in PowerMarker v. 3.25 software (Liu & Muse, 2005). Later, the accessions were grouped based on their name subgroup in the passport information. They were KKN, KKN Leuang, KKN Deng, KKN Lai/Lay, KKN Hay, KKN Dam, and KKN Khao. The average expected heterozygosity (H_S), total expected heterozygosity (H_T), and genetic differentiation (F_{ST}) were calculated for the name subgroups or subpopulations in GenAlEx v. 6.5 software (Peakall & Smouse, 2012). The analysis of molecular variance (AMOVA) was performed in GenAlEx v. 6.5 to determine hierarchical partitioning of genetic variation among name subgroups, accessions within the name subgroup, and within an accession. Phylogenetic reconstruction used the data of 70 accessions of KKN and other control accessions, which each consisted of eight individuals, and was based on the unweighted pair-group method for the arithmetic mean (UPGMA; Nei et al., 1983) in PowerMarker 3.25 with 1,000 bootstrap replications.

The KKN population structure was assessed by a model-based method in STRUCTURE v. 2.3.4 software (Pritchard et al., 2000). The number of populations was tested from K = 1 to 10 with admixture and correlated allele frequencies models. Ten separate runs were performed for each K with a burn-in period of 100,000 and a run of 100,000. The optimum K value was determined from the log probability of data (ln

P(*D*)) and ad hoc statistic Δ*K* of Evanno et al. (2005) by using the STRUCTURE HARVESTER website and software (Earl & vonHoldt, 2012).

2.3 Results

2.3.1 Genetic diversity values of the KKN collection

Of the 24 markers, one (RM133) was monomorphic, so it was excluded from analysis. In total, 132 alleles of the 23 polymorphic markers were detected in 560 seeds of KKN. The number of alleles ranged from 2 (RM171 and RM455) to 17 (RM259), with an average of 5.7 per locus (Table. 2.3). The *PIC* ranged from 0.036 (RM408) to 0.526 (RM259), with an average of 0.199. H_o ranged from 0.033 (RM55) to 0.502 (RM259). H_e ranged from 0.040 (RM408) to 0.623 (RM259; Table 2.3). AMOVA revealed that 79% of the total variation occurred among accessions, 15% among KKN name subgroups, and 6% within accessions (Table 2.4).

2.3.2 Genetic diversity values of individual accessions

The number of alleles detected per accession ranged from 25 (10 accessions) to 62 (LG14117). H_e ranged from 0.01 (LG14125) to 0.49 (LG14117). H_o was highest (0.09) in two accessions: LG12923 and LG5845.

2.3.3 Genetic diversity among KKN named subgroups

Within the 70 KKN accessions, we found 7 KKN name subgroups: Khao Kai Noi (with no descriptor), Khao Kai Noi Leuang (yellow), Khao Kai Noi Deng (red), Khao Kai Noi Lai (red), Khao Kai Noi Hay (upland), Khao Kai Noi Dam (black), and Khao Kai Noi Khaw (white). Among the subgroups, H_o varied from 0.001 (Khao Kai Noi Dam) to 0.038 (Khao Kai Noi Hay). H_e ranged from 0.040 (Khao Kai Noi Khaw) to 0.315 (Khao Kai Noi Deng). H_S among subgroups was 0.175. H_T was 0.271. Genetic differentiation of the KKN name subgroups was confirmed by F_{ST} (0.353; Table 2.5).

2.3.4 Population differentiation between Houaphan and Xiengkhouang Provinces

Of the 70 KKN accessions, 81.4% originated from two provinces—Houaphan (HP, 22) and Xiengkhouang (XK, 35)—where the production of KKN is predominant (Rao et al., 2006b). To study the phylogeography of KKN, we compared the genetic diversity values of HP and XK. In HP, H_e = 0.286 and H_o = 0.014; in XK, H_e = 0.147 and H_o = 0.012 (Table 2.5). H_S was 0.216 and H_T was 0.222 across the two provinces, and F_{ST} between HP and XK was 0.025.

2.3.5 Dendrogram clustering and STRUCTURE analysis

The genetic-distance-based UPGMA method divided the accessions into two major clusters: Indica and Japonica-type (Fig. 2.1). The Indica cluster contained Kasalath, TDK11 (Lao-improved cultivar), six control traditional landraces, and one

KKN accession (LG6644). The Japonica-type cluster was divided into two subclusters. The smaller subcluster consisted of three KKN accessions (LG6746, LG6493, and LG9212) and eight control landraces from Laos and Vietnam, including two placed in the tropical Japonica-type (javanica) cultivar group (IRGC111242 and IRGC111233) according to passport data in the Genesys Gateway to Genetic Resources (www.genesys-pgr.org, accessed March 6, 2015). The larger subcluster consisted of Nipponbare, four traditional landraces from Vietnam, and most KKN accessions. This cluster was further divided into three subpopulations.

In the model-based population structure analysis, I first tested all the 70 accessions of KKN, and one accession was grouped with Indica-type (LG6644). We omitted this accession from the analysis, and then 69 accessions were used for the second analysis. The values of ln $P(D)$ changed greatly, and ΔK were highest when the number of populations was $K = 4$ followed by $K = 7$. In the STRUCTURE results with $K = 4$, accessions assigned to the tropical Japonica-type (javanica-type) clusters in the dendrogram were clearly separated from other KKN accessions (green). Most of the accessions were assigned to three inferred populations (blue, yellow, and red in Fig 2.1). Subsequently, we removed three accessions grouped with tropical Japonica accessions (LG9212, LG6493, and LG6746) and carried out construction of dendrogram and STRUCTURE analysis only with temperate Japonica KKN accessions. Clustering patterns of the dendrogram did not change significantly without three *tropical Japonica* accessions. However, the STRUCTURE results demonstrated a distinct pattern (optimum $K = 3$). Although KKN has been considered to be in between the *Indica* and *tropical Japonica* types (Rao et al., 2006c), our results

showed that most of the KKN had a temperate Japonica background. Studying the effects of tropical and temperate Japonica genetic background is important in revealing the general genetic structure of KKN, and thus we decided to include accessions of both tropical and temperate Japonica in further analysis and discussions.

The overall clustering pattern of the dendrogram coincides with the STRUCTURE results. The inferred populations indicated in red and yellow with $K = 4$ were localized in two clusters of the dendrogram, but these populations were separated into distinct inferred populations with K=7. Accessions LG14117, LG13535, and LG14118, which were in the Japonica cluster but were not grouped with other KKN accessions, contain genetic compositions of tropical Japonica type. It is noteworthy that there were only two accessions, LG10133 and LG14018, that possess three genetic backgrounds (blue, yellow, and red) as inferred by STRUCTURE analysis.

The population of KKN used in this study consists of name subgroups (Table 2.1). The distribution of the name subgroups—KKN Leuang (yellow, Y), KKN Deng (red, R), KKN Khao/Khaw (white, W), KKN Lai/Lay (striped, S), KKN Dam (black, B), are demonstrated in Fig. 2.1. Although accessions of the name group Y are distributed throughout the temperate *Japonica* clusters, most of them coincide with the blue inferred population by STRUCTURE. The other name subgroups did not correspond clearly with any cluster or inferred populations and are present throughout the dendrogram.

2.4 Discussion

2.4.1 Genetic diversity of KKN accessions

The 23 SSR markers revealed relatively high genetic diversity in the 70 KKN accessions, with an average of 5.7 alleles per locus and H_T = 0.271 (Table 2.5). The number of alleles per locus was smaller than that of Indian landraces comprising different cultivar groups (7.9 and 7.8; Das et al., 2013; Jian et al., 2004), similar to that of Indian local aromatic cultivars (5.4; Roy et al., 2013) and greater than those of Indian aromatic rice from Orissa state (2.08; Meti et al., 2013) and black glutinous rice from Laos (3.1; Bounphanousay et al., 2008). H_e was similar to that of Balam, an indigenous cultivar from India (Choudhury et al., 2013).

By analyzing morphology, Rao et al. (2006b) identified nine name subgroups within the KKN group; we found seven of these in the KKN collection. The high level of diversity is consistent with the use of SSR markers. Nevertheless, the collection of additional accessions might be needed to obtain variant forms that are not currently observed.

Within the seven forms of KKN found in this study, the KKN Deng (red) group had the highest genetic diversity (Table 2.5). As the name indicates, the grain is red. A red pericarp is ubiquitous in wild populations and is associated with resistance to biotic stressors (Sweeney & McCouch, 2007). This trait is due to an SNP in the *Rc* gene that occurred during domestication, changing the pericarp from red to white (Sweeney et al., 2007). Since wild rice is common throughout Laos (Kuroda et al., 2006), the red pericarp of KKN Deng may have been caused by gene flow from wild rice. Further

study of the *R*c haplotype of KKN Deng and adjacent wild populations may clarify the issue.

Locus RM259 had the most alleles (17). Interestingly, the same locus had the most alleles in a group of Lao black glutinous rice accessions (7) (Bounphanousay et al., 2008). Pusadee et al. (2014) also reported high diversity at the same locus in Thai landraces. Marker RM259 is mapped on chromosome 1 (Chen et al., 1997) and has been used for quantitative trait locus analysis of early flowering (Thomson et al., 2006) and grain yield under drought stress (Sandhu et al., 2014). Rice germplasm collected from HP, XK, and nearby regions may contain novel alleles for traits linked to this marker.

2.4.2 Heterozygosity of KKN implications for germplasm management

Only 6% of the total variation occurred within accessions (Table 2.4). $H_e > H_o$ when averaged across all loci in almost all accessions. This situation indicates inbreeding. Inbreeding is common in self-pollinated species such as rice (Matsui & Kagata, 2003), as seen in the high inbreeding coefficient (F_{IS}) of Bue Chomee, a Thai landrace (Pusadee et al., 2009), which ranged from 0.859 to 1. This observation reflects strong, but not complete, inbreeding in the KKN populations in farmers' fields.

Germplasm regeneration is a fundamental role of genebanks. Cross and Wallace (1994) recommended that accessions be held as pure lines on the basis of their simulation of allele loss during the regeneration of heterogeneous self-

pollinating accessions. A study of the effect of genetic integrity in bulked and pure line approaches to seed management demonstrated that selection or genetic drift may occur during rejuvenation in the bulked approach (Hirano et al., 2009). Thus, KKN accessions identified as heterogeneous populations—for example, LG14117—should be subdivided into pure lines and preserved to maintain their genetic integrity during rejuvenation and conservation in the Lao National Genebank.

2.4.3 Relations between name subgroups, geographical distribution, and genetic structure of KKN

The most popular name subgroup, KKN Leuang (yellow), shared nearly 40% of the total accession of KKN used in this study, followed by KKN Lai (striped) (14%) and KKN Deng (red) (12%). Accessions of these name subgroups originated from both HP and XK. Name subgroups present in HP are always present in XK, except KKN Hay (upland). Kai Noi Khaw/Khao (white) and KKN Mihang (awn) are present only in XK. Rao et al. (2006b) described eight out of nine name subgroups collected from HP, and only KKN Mihang (awn present) was collected from XK. In this study, the variations in name subgroups in HP and XK were almost same.

Interestingly, names that describe more than two grain traits [e.g., Kai Noi Lai Dam (striped and black)] were only found in XK. These name combinations were not described in previous reports (Rao et al., 2006b). Only one form of KKN name subgroup is grown in most farmers' fields (Rao et al., 2006c). The present study showed that with the presence of heterogeneous accessions, which contain different

genotypes within accessions, gene flow and hybridization is expected. The introduction of new traits through hybridization may have caused the establishment of combined grain morphology and the successive development of a combined name. One should pay attention to XK in order to analyze broader KKN morphological and genetic diversity.

No clear genetic structure was observed in terms of the name subgroups based on the dendrograms and inferred populations of STRUCTURE analysis. Not only the name subgroup but also the genetic background inferred by dendrogram and STRUCTURE analysis did not differentiate HP and XK clearly. Isolation by distance occurs in fragmented populations owing to a lack of gene flow (Wright, 1943, 1946). For example, Bue Chomee, a Thai landrace, was significantly differentiated among villages (Pusadee et al., 2009). Genetic differentiation of KKN accessions from the two main production provinces, HP and XK, was very low (F_{ST} = 0.025). Therefore, accessions from these two provinces share a genetic background, maybe via human-mediated gene flow, such as seed exchange among farmers, as KKN was introduced first into HP and then into XK (Bounphanousay et al., 2009; Rao et al., 2006c). KKN became popular and therefore spread to other villages and provinces because of its good eating quality. HP and XK, which share a border, on account of the promotion of production for industrial uses (Vientiane Times, 2014).

2.4.4 Genetic background of KKN and the origin implications for conservation and genetic improvement

The dendrogram revealed genetic relationships between KKN and other common cultivars and among KKN accessions. Rao et al. (2006c) described KKN as intermediate between the Indica-type and tropical Japonica-type based on their gross morphology. However, most of the KKN accessions were grouped with Japonica Nipponbare, and temperate Japonica background can be assumed for most KKNs. This group, together with the tropical Japonica type, was clearly separated from the *Indica* group in the dendrogram, reflecting the genetic structure and diversity in *O. sativa* reported by Garris et al. (2005). Only one accession, LG6644, was grouped with *Indica* accessions. This clustering pattern was supported by a high bootstrap value, implying that LG6644 has an *Indica* background. Possible explanations are an error during collection and substitution at the time of seed regeneration.

KKN accessions LG6493, LG6746, and LG9212 were grouped with upland tropical Japonica accessions, whereas most KKN accessions are lowland. Since all the traditional Lao rice landraces used in this study were grouped with tropical Japonica type, these accessions may have been influenced by other traditional varieties in the region.

Four traditional Vietnamese landraces were clustered in the temperate Japonica type together with KKN accessions in the dendrogram, yet all the traditional Lao landraces were included in the tropical Japonica group (Fig. 2.1). This result shows that Vietnamese landraces are more closely related to KKN than other Lao

landraces. KKN is believed to have been introduced into HP from Vietnam and later into XK (Rao et al., 2006c). Our results support this origin. The distribution of crop cultivars across borders is common where the local people share either sociocultural backgrounds (Kyndt et al., 2009) or climatic conditions (Forsberg et al., 2015). Rice landraces closely related to KKN in Vietnam may possess promising agromorphological characteristics. Therefore, such landraces should be added to the germplasm collection, either by germplasm exchange between Laos and Vietnam or by joint collection expeditions, to enhance genetic diversity within the collection and for use in breeding programs.

Table 2.1. Passport data of 70 accessions of Khao Kai Noi, a Lao rice landrace group used in this study (data from Lao National Genebank)

No.	Acc. No.	Local name	Meaning	Ec	En	Mt	Pro.	Dist.	GPS data		
									Altitude (m)	Latitude (N)	Longitude (E)
1	LG5845	Khao Kai Noi	Chicken small	L	G	L	XK	XKH	N. A.	N. A.	N. A.
2	LG6493	Khao Kai Noi	Chicken small	U	G	E	LP	LPL	500	20.22359	102.19490
3	LG6795	Khao Kai Noi	Chicken small	L	G	L	XK	XKT	1000	19.31257	103.12900
4	LG7488	Khao Kai Noi	Chicken small	L	G	M	VM	VMN	N. A.	N. A.	N. A.
5	LG9212	Khao Kai Noi	Chicken small	U	G	E	LP	LPB	N. A.	N. A.	N. A.
6	LG10035	Khao Kai Noi	Chicken small	U	G	M	VM	VMN	N. A.	N. A.	N. A.
7	LG10195	Khao Kai Noi	Chicken small	U	G	M	XS	XSL	N. A.	N. A.	N. A.
8	LG10898	Khao Kai Noi	Chicken small	L	G	E	XS	XSL	N. A.	N. A.	N. A.
9	LG12360	Khao Kai Noi	Chicken small	L	G	M	XK	XKP	N. A.	N. A.	N. A.
10	LG12584	Khao Kai Noi	Chicken small	L	G	M	XK	XKM	N. A.	N. A.	N. A.
11	LG12923	Khao Kai Noi	Small chicken	L	G	L	XK	XKN	N. A.	N. A.	N. A.
12	LG13251	Khao Kai Noi	Small chicken	U	G	E	BK	BKV	N. A.	N. A.	N. A.
13	LG13480	Khao Kai Noi	Chicken	L	G	M	BK	BKB	N. A.	N. A.	N. A.
14	LG13535	Khao Kai Noi	Chicken	L	G	L	CS	CSB	N. A.	N. A.	N. A.
15	LG13771	Khao Kai Noi	Chicken	L	G	L	LN	LNV	N. A.	N. A.	N. A.
16	LG13970	Khao Kai Noi	Chicken	L	G	L	XK	XKT	N. A.	N. A.	N. A.
17	LG2755	Khao Kai Noi Dam	Chicken small black	L	G	L	HP	HPS	N. A.	N. A.	N. A.

No.	Acc. No.	Local name	Meaning	Ec	En	Mt	Pro.	Dist.	GPS data		
									Altitude (m)	Latitude (N)	Longitude (E)
18	LG10133	Khao Kai Noi Dam	Chicken small black	L	G	L	XK	XKT	N. A.	N. A.	N. A.
19	LG14112	Khao Kai Noi Dam Lay	N. A.	L	G	L	XK	Koun	1118	19.36235	103.28445
20	LG14024	Khao Kai Noi Dam Mihang	Chicken small with tail	L	G	L	XK	XKX	1020	N. A.	N. A.
21	LG2793	Khao Kai Noi Deng	Chicken small red	L	G	L	HP	HPS	N. A.	N. A.	N. A.
22	LG6644	Khao Kai Noi Deng	Chicken small red	L	G	L	HP	HPS	1400	20.24176	104.28240
23	LG6762	Khao Kai Noi Deng	Chicken small red	L	G	L	XK	XKP	1150	19.28077	103.96650
24	LG14018	Khao Kai Noi Deng	N. A.	L	G	L	XK	XKT	1050	N. A.	N. A.
25	LG14023	Khao Kai Noi Deng	N. A.	L	G	L	XK	XKP	1000	N. A.	N. A.
26	LG14095	Khao Kai Noi Deng	N. A.	L	G	M-L	XK	P	1130	19.51468	103.33508
27	LG14113	Khao Kai Noi Deng	N. A.	L		L	XK	PhK	1087	19.61161	103.11186
28	LG14126	Khao Kai Noi Deng	N. A.	L	G	L	HP	CN	1070	20.27735	104.44020
29	LG6746	Khao Kai Noi Hai	Chicken small upland	U	G	M	HP	HPS	1000	20.18723	104.15610

No.	Acc. No.	Local name	Meaning	Ec	En	Mt	Pro.	Dist.	GPS data		
									Altitude (m)	Latitude (N)	Longitude (E)
30	LG14028	Khao Kai Noi Khaw	N. A.	L	G	L	XK	XKX	1020	N. A.	N. A.
31	LG14027	Khao Kai Noi Khaw Mihang	N. A.	L	G	L	XK	XKP	1000	N. A.	N. A.
32	LG14077	Khao Kai Noi Lai	N. A.	L	G	N. A.	N. A.	N. A.	N. A.	N. A.	N.A
33	LG14016	Khao Kai Noi Lai Dam	N. A.	L	G	L	XK	XKT	1050	N. A.	N. A.
34	LG14020	Khao Kai Noi Lai Dam	N. A.	L	G	L	XK	XKT	1050	N. A.	N. A.
35	LG2790	Khao Kai Noi Lay	Chicken small striped	L	G	L	HP	HPS	N. A.	N. A.	N. A.
36	LG2794	Khao Kai Noi Lay	Chicken small striped	L	G	L	HP	HPS	N. A.	N. A.	N. A.
37	LG2841	Khao Kai Noi Lay	Chicken small striped	L	G	L	HP	HPV	N. A.	N. A.	N. A.
38	LG6665	Khao Kai Noi Lay	Chicken small striped	L	G	L	HP	HPV	900	20.22364	104.17351
39	LG6760	Khao Kai Noi Lay	Chicken small striped	L	G	L	XK	XKP	1150	19.28077	103.96650
40	LG6838	Khao Kai Noi Lay	Chicken small striped	L	G	L	XK	XKX	1100	19.20322	103.22167
41	LG10899	Khao Kai Noi Lay	Chicken small striped	L	G	E	XS	XSL	N. A.	N. A.	N. A.
42	LG14110	Khao Kai Noi Lay	N. A.	L	G	L	XK	Koun	1089	19.35169	103.32763

No.	Acc. No.	Local name	Meaning	Ec	En	Mt	Pro.	Dist.	GPS data		
									Altitude (m)	Latitude (N)	Longitude (E)
43	LG14124	Khao Kai Noi Lay	N. A.	L	G	L	HP	CN	988	20.47555	103.99674
44	LG2746	Khao Kai Noi Leuang	Chicken small yellow	L	G	L	HP	HPS	N. A.	N. A.	N. A.
45	LG2792	Khao Kai Noi Leuang	Chicken small yellow	L	G	L	HP	HPS	N. A.	N. A.	N. A.
46	LG2806	Khao Kai Noi Leuang	Chicken small yellow	L	G	L	HP	HPS	N. A.	N. A.	N. A.
47	LG6732	Khao Kai Noi Leuang	Chicken small yellow	L	G	L	HP	HPV	600	20.15989	104.31190
48	LG14017	Khao Kai Noi Leuang	N. A.	L	G	L	XK	XKT	1050	N. A.	N. A.
49	LG14021	Khao Kai Noi Leuang	N. A.	L	G	L	XK	XKT	1050	N. A.	N. A.
50	LG14022	Khao Kai Noi Leuang	N. A.	L	G	L	XK	XKT	1100	N. A.	N. A.
51	LG14026	Khao Kai Noi Leuang	N. A.	L	G	L	XK	XKP	1000	N. A.	N. A.
52	LG14029	Khao Kai Noi Leuang	N. A.	L	G	L	XK	XKH	1160	N. A.	N. A.
53	LG14030	Khao Kai Noi Leuang	N. A.	L	G	L	XK	XKH	1000	N. A.	N. A.
54	LG14031	Khao Kai Noi Leuang	N. A.	L	G	L	XK	XKH	1001	N. A.	N. A.

No.	Acc. No.	Local name	Meaning	Ec	En	Mt	Pro.	Dist.	GPS data		
									Altitude (m)	Latitude (N)	Longitude (E)
55	LG14033	Khao Kai Noi Leuang	N. A.	L	G	L	XK	XKH	1000	N. A.	N. A.
56	LG14043	Khao Kai Noi Leuang	N. A.	L	G	L	XK	N. A.	N. A.	N. A.	N. A.
57	LG14076	Khao Kai Noi Leuang	N. A.	L	G	N. A.	N. A.	N. A.	N. A.	N. A.	N. A.
58	LG14103	Khao Kai Noi Leuang	N. A.	L	G	L	XK	PhX	1100	19.2993	103.11452
59	LG14107	Khao Kai Noi Leuang	N. A.	L	G	L	XK	PhX	1105	19.3235	103.17975
60	LG14109	Khao Kai Noi Leuang	N. A.	L	G	L	XK	Koun	1088	19.32463	103.37611
61	LG14114	Khao Kai Noi Leuang	N. A.	N. A.	N. A.	N. A.	XK	PhK	1093	19.60373	103.10825
62	LG14115	Khao Kai Noi Leuang	N. A.	L	G	L	XK	PhK	1046	19.52399	103.70930
63	LG14116	Khao Kai Noi Leuang	N. A.	L	G	L	HP	VX	698	20.56699	104.28337
64	LG14117	Khao Kai Noi Leuang	N. A.	L		L	HP	VX	695	20.36757	104.29326
65	LG14118	Khao Kai Noi Leuang	N. A.	L	G	L	HP	VX	663	20.34343	104.34200
66	LG14120	Khao Kai Noi Leuang	N. A.	L	G	L	HP	VX	893	20.42316	104.23027

No.	Acc. No.	Local name	Meaning	Ec	En	Mt	Pro.	Dist.	GPS data		
									Altitude (m)	Latitude (N)	Longitude (E)
67	LG14121	Khao Kai Noi Leuang	N. A.	L	G	L	HP	CN	1016	20.43453	104.8200
68	LG14122	Khao Kai Noi Leuang	N. A.	L	G	L	HP	CN	1002	20.46686	103.97265
69	LG14123	Khao Kai Noi Leuang	N. A.	L	G	L	HP	CN	988	20.47555	103.99674
70	LG14125	Khao Kai Noi Leuang	N. A.	L	G	L	HP	CN	938	20.42412	104.39960

Ec: ecology, L: lowland, U: upland, N. A.: not available, En: endosperm, G: glutinous, Mt: maturity, L: late, M: medium, E: early, BK: Borikhamxay, CS: Champasak, HP: Houaphan, LN: Luang Namtha, LP: Luang Prabang, VM: Vientiane Municipality, XK: Xiengkhouang, XS: Xaisomboun

Table 2.2. List of control accessions used in this study

No.	Accession no.	Local name	Provider	Origin
1	IRGC 89625	Do Keo	IRRI	Laos
2	IRGC 89941	Khen Seua	IRRI	Laos
3	IRGC 92257	Tam	IRRI	Laos
4	IRGC 98381	Muang Souy	IRRI	Laos
5	IRGC 107576	Leuang Kham	IRRI	Laos
6	IRGC 111233	Do Lao Soung	IRRI	Laos
7	IRGC 111242	Hang Ngoua Kom	IRRI	Laos
8	IRGC 56034	Chao	IRRI	Vietnam
9	IRGC 79554	Mac Duoi	IRRI	Vietnam
10	IRGC 82649	Khau Chan Hay	IRRI	Vietnam
11	IRGC 82651	Khau Mo	IRRI	Vietnam
12	IRGC 82653	Khau Non Hay	IRRI	Vietnam
13	IRGC 90679	Khau Tan	IRRI	Vietnam

No.	Accession no.	Local name	Provider	Origin
14	IRGC 90689	Khau Tan Nong	IRRI	Vietnam
15	IRGC 90721	Tan Nhe	IRRI	Vietnam
16	IRGC 96930	Khau Cham Hang	IRRI	Vietnam
17	IRGC 112709	Ngo Gan	IRRI	Vietnam
18	IRGC 113858	Khau Phroung	IRRI	Vietnam
19	Nipponbare	Nipponbare	NIAS	--
20	Kasalath	Kasalath	NIAS	--
21	TDK11	Thadokkham 11	LNGB	Laos

IRRI: International Rice Research Institute, NIAS: National Institute of Agrobiological Sciences, LNGB: Lao National Genebank

Table 2.3. Diversity of 23 polymorphic SSR markers in 70 accessions of Khao Kai Noi

Marker	Chr.	Label	Panel[a]	Tm (°C)[b]	*A*	H_o	H_e	*PIC*
RM1	1	PET	A	60	8	0.314	0.368	0.323
RM259	1	VIC	A	60	17	0.502	0.623	0.526
RM495	1	NED	C	58	7	0.105	0.114	0.132
RM452	2	NED	B	58	3	0.04	0.044	0.044
RM514	3	NED	A	60	6	0.442	0.564	0.482
RM55	3	PET	D	60	4	0.033	0.271	0.082
RM489	3	6FAM	D	60	4	0.273	0.457	0.357
RM307	4	6FAM	D	60	5	0.122	0.141	0.099
RM507	5	VIC	D	60	3	0.126	0.138	0.101
RM162	6	NED	B	58	6	0.159	0.186	0.119
RM11	7	PET	B	58	7	0.45	0.587	0.47
RM455	7	VIC	B	58	2	0.039	0.043	0.044
RM152	8	6FAM	A	60	3	0.258	0.444	0.313
RM408	8	6FAM	B	58	3	0.036	0.04	0.036
RM447	8	PET	C	58	6	0.102	0.11	0.085
RM215	9	VIC	C	58	5	0.138	0.325	0.143
RM316	9	6FAM	C	58	6	0.093	0.305	0.108
RM171	10	NED	D	60	2	0.042	0.045	0.045

Marker	Chr.	Label	Panel[a]	Tm (°C)[b]	*A*	H_o	H_e	*PIC*
RM287	11	NED	B	58	5	0.104	0.336	0.182
RM536	11	PET	C	58	6	0.086	0.092	0.112
RM552	11	NED	D	60	13	0.241	0.424	0.292
RM19	12	PET	A	60	6	0.043	0.047	0.05
RM277	12	NED	A	60	5	0.279	0.522	0.427
Average					5.7			0.199
Total					132			

Chr.: chromosome, [a]: panels managed by Multiplex Manager software, [b]: optimum annealing temperature for each panel in this study, *A*: number of alleles, H_o: observed heterozygosity, H_e: expected heterozygosity, *PIC*: polymorphism information content

Table 2.4. Analysis of molecular variance of seven name subgroups of Khao Kai Noi on the basis of 23 SSR markers

Sources	Degree of freedom	Sum of square	Variance components	Variation (%)
Among KKN subgroups	6	363.707	0.404	15
Among accessions	553	2422.449	2.116	79
Within accessions	560	83.500	0.149	6
Total	1119	2869.645	2.669	100

Table 2.5. Genetic diversity parameters of seven name subgroups of Khao Kai Noi on the basis of 23 SSR markers

	No.	H_e	H_o	H_S	H_T	F_{ST}
Name subgroups	70			0.175	0.271	0.353
Khao Kai Noi	16	0.252	0.023			
Khao Kai Noi Leuang (yellow)	27	0.164	0.008			
Khao Kai Noi Deng (red)	8	0.315	0.017			
Khao Kai Noi Lay/Lai (striped)	12	0.166	0.009			
Khao Kai Noi Hay (upland)	1	0.199	0.038			
Khao Kai Noi Dam (black)	4	0.089	0.001			
Khao Kai Noi Khaw (white)	2	0.040	0.005			
Provinces	57			0.216	0.222	0.025
HP	22	0.286	0.014			
XK	35	0.147	0.012			

No: number of accession, H_e: expected heterozygosity, H_o: observed heterozygosity, H_S: average expected heterozygosity, H_T: total expected heterozygosity, F_{ST}: population genetic differentiation

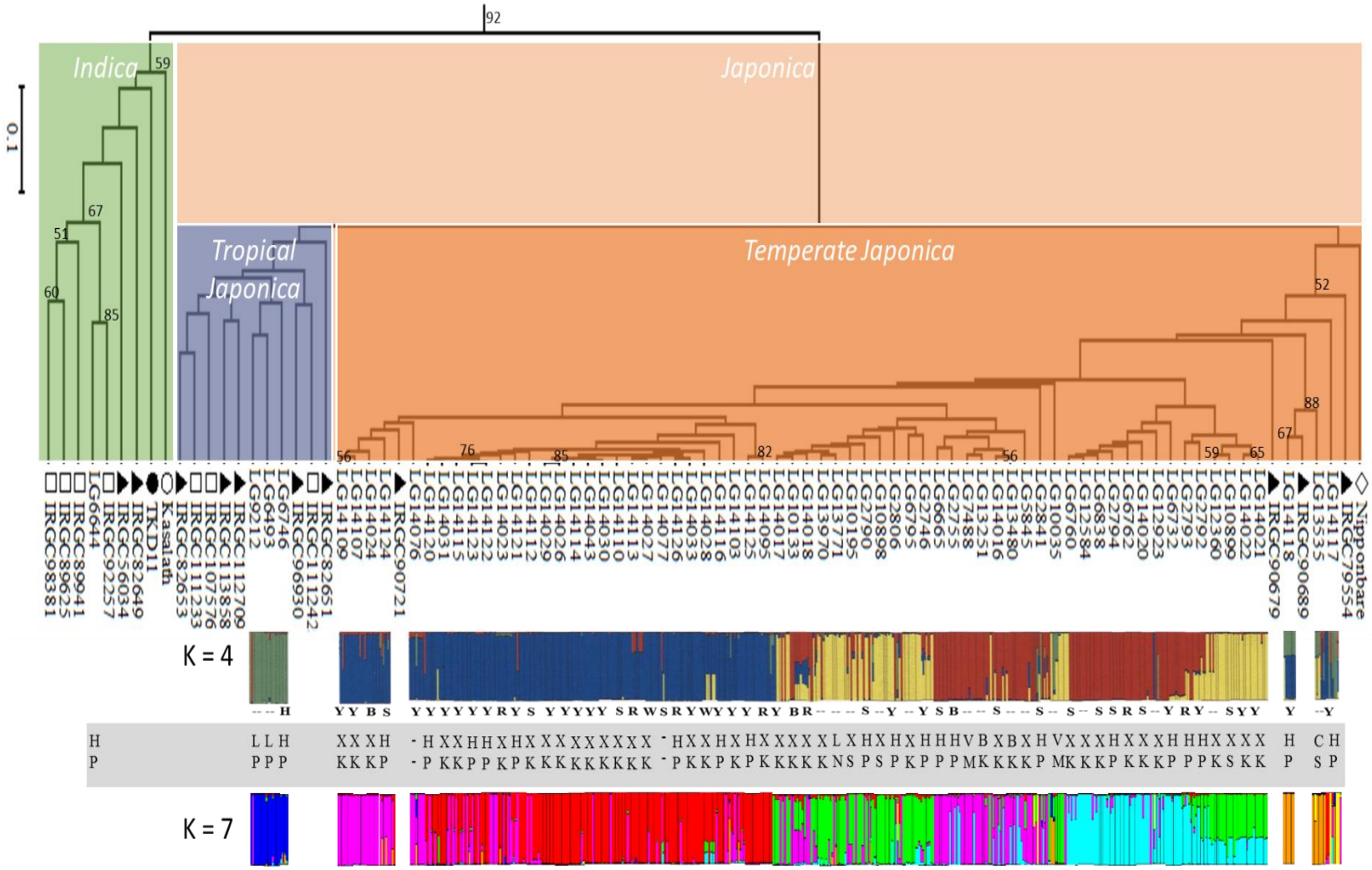

Indica
Japonica
Tropical Japonica
Temperate Japonica
0.1
92
59
67
51
60
85
56
76
85
82
56
59
65
52
88
67
IRGC98381
IRGC89625
IRGC89941
LG6644
IRGC92257
IRGC56034
IRGC82649
TKD11
Kasalath
IRGC82653
IRGC111233
IRGC107576
IRGC113858
IRGC112709
LG9212
LG6493
LG6746
IRGC96930
IRGC111242
IRGC82651
LG14109
LG14107
LG14024
LG14124
IRGC90721
LG14076
LG14120
LG14031
LG14123
LG14122
LG14115
LG14121
LG14112
LG14029
LG14026
LG14114
LG14043
LG14030
LG14110
LG14113
LG14027
LG14077
LG14126
LG14033
LG14028
LG14116
LG14103
LG14125
LG14095
LG14017
LG10133
LG14018
LG13771
LG13970
LG13251
LG14016
LG13480
LG5845
LG2841
LG10035
LG10195
LG2790
LG10898
LG2806
LG6795
LG2746
LG6665
LG2755
LG7488
LG6760
LG12584
LG6838
LG2794
LG14020
LG6762
LG12923
LG6732
LG2793
LG2792
LG12360
LG10899
LG14022
LG14021
IRGC90679
LG14118
IRGC90689
LG13535
LG14117
IRGC79554
Nipponbare
K = 4
K = 7

Figure 2.1. Dendrogram of 70 accessions of Khao Kai Noi (KKN) and 21 control accessions

Clustered by the unweighted pair-group method for calculating the arithmetic mean (UPGMA). Bootstrap values with 1,000 replications are shown in each branch. □ traditional Lao landraces, ○ a representative of Indica-type, ● improved Lao cultivars, and ▲ Vietnamese landraces. Bar plots obtained from STRUCTURE analysis using 69 accessions in Japonica group (K = 4 and 7) are shown beneath the KKN accession. The letters under the bar plot indicate the characteristics of each accession implied by the accession name. H: upland, B: black, R: red, S: striped, Y: yellow, W: white, and –: no implication by accession name. The letters in the grey box are the names of provinces from which samples were collected: HP: Houaphan, XK: Xiengkhouang, CS: Champasak, BK: Bolikhamxay, XS: Xaisomboun, LN: Luang Namtha, LB: Luang Prabang, VM: Vientiane Municipality, and -: no record (unknown)

Chapter 3

Rice Landraces (*Oryza sativa* L.) Survey and Sample Collection

3.1 Introduction

Rice (*Oryza sativa* L.) is the main staple food in Laos, and its production is prioritized over other agricultural processes. Rice genetic resources were surveyed and collected throughout the country from 1995–2000 (Rao et al., 2006a). A total of 13,192 accessions of cultivated rice were collected and conserved in the Lao National Genebank, Agriculture Research Center (ARC), National Agriculture and Forestry Research Institute (NAFRI). The collection included KKN, a landrace with good eating quality due to its softness and aroma. Another KKN collection mission was performed individually in Xiengkhouang and Houaphan Provinces in 2008, and its morphological data were reported (Bounphanousay et al., 2009). Within the KKN group, varieties are distinguished by farmers—for example, KKN Leuang (yellow), KKN Deng (red), and KKN Lai (striped). This germplasm was defined as a "genebank population," and its genetic diversity was examined (Vilayheuang et al., in press).

Recently, KKN has been strongly promoted not only for daily consumption but also as a high-value crop for international trade with neighboring countries and also as a raw material in industry (Worklivelaos, 2014). Genetic diversity and population structure may change with time and human activities. For efficient

conservation of such valuable rice, the recent diversity of its on-farm population should be studied to allow comparison with genebank materials. Therefore, in this survey, KKN and other traditional rice varieties (landraces) in farmers' fields were collected during the harvest season in Laos.

3.2 Materials and Methods

3.2.1 Materials

A number of materials were prepared for the survey. The most important ones are listed in Table 3.1.

3.2.2 Method of survey and sample collection

3.2.2.1 The routes

All members of the research team gathered in Vientiane Capital on October 16, 2015 and headed to Xiengkhouang Province by car. We used 13th South Road from Vientiane Capital to Paksan District, Borikhamxay Province. From there we went to Khoun District, Xiengkhouang Province through Bolikhan, and the Thathom District of Borikhamxay and Xaysomboun Provinces. However, the road number was unknown. The full itinerary is summarized in Table 3.2. In Xiengkhouang, primary survey and collection was done in Khoun District. The team then moved to Houaphan Province the next day. The team completed a full survey in Houaphan Province and then returned to Xiengkhouang Province to resume the surveying and collecting in the other districts. Every district of these two provinces was surveyed. We went to the

provincial borders between Xiengkhouang and Luang Prabang and Houaphan and Luang Prabang. We also came close to the Laos-Vietnam border in Nonghet District, Xiengkhouang Province.

3.2.2.2 Farmer interviews and sample collection

The team went immediately into farmer residences or farms in remote target areas and asked for KKN and other cultivated rice. The seed samples were collected once permission was granted by the owner (farmer) according to the code of conduct of the International Society for Ethnobiology (http://www.ethnobiology.net). This collection mission focused on KKN, but if the farmer grew other rice landraces in the same field, that rice was also collected. However, We did not collect any other rice landraces from the farmers who did not grow any KKN.

Once a farmer agreed to provide us with samples, we selected five panicles from different individual plants (hills), placed them in a paper bag, and assigned them to one accession. The accession numbers were assigned in numeric sequence beginning with "#1," which was written on the paper bags in which samples were kept. These numbers were identical to the numbers 001 and up on the sample collection form (Fig. 3.1). The photo and passport data of each collected sample were recorded according to the collection form (Fig. 3.1) that was provided by the National Institute of Agrobiological Science (NIAS), Japan. The geographic position information or GPS data of the sites were also recorded (GARMIN OREGON 300, USA). Finally, the farmer (owner) was interviewed using the collection form

questions, and additional information—such as usage, growing area, total yield, and application of pesticides and fertilizer—was collected as well. Then numbers were further assigned as R01, R02, ... R(n) as presented in the sample list.

3.3 Results and Discussions

We surveyed in two provinces, Houaphan and Xiengkhouang, to include a total of 15 districts and 40 villages. The geographical data of sampling sites were recorded and plotted on a map (Fig. 3.2). A general observation was that these provinces have large scale production of commercial maize in the upland for exportation to neighboring countries. Rice genetic resource surveying in Laos was done during 1995–2000 and collected cultivated rice throughout the country of Laos (Rao et al., 2006a). In the present collection, we focused on KKN landrace in its major production provinces (Houaphan and Xiengkhouang). In the areas we visited, KKN and other rice landraces could be found and collected in almost every district in both provinces. We collected 32 accessions comprised of 25 KKN and 7 other rice landraces in Xiengkhouang Province. In addition, 28 samples were collected, including 24 KKN and 4 other rice landraces, in Houaphan Province. Therefore, a total of 60 accessions of rice (49 KKN and 11 other rice) was collected (Table 3.3).

The KKN (49 accessions), including eight diverse variant names or subgroups, were: KKN Leuang (yellow), KKN Deng (red), KKN Lai (striped), KKN Khao (white), KKN Dam (black), KKN Met Noi (small grain), KKN Met Yai (big grain), and KKN in this survey. The variation in the grain color of this rice is shown in an

example figure (Fig. 3.3); however, all sample photos were included in another report (Vilayheuang et al., in press). The diversity of the KKN group is still maintained. The previous collection of KKN in 2008 in Xiengkhouang and Houaphan Provinces (Bounphanousay et al., 2009) found four distinct name subgroups: KKN Deng (red), KKN Leuang (yellow), KKN Lai (striped), and KKN Lai-Dam (striped-black). Ten variant names within KKN were reported (Rao et al., 2006c).

Most of farmers grew more than one of them in their single family paddy field because they could be used for different purposes. For example, KKN Lai could be sold for a higher price, but other KKN forms could produce a higher yield (according to a farmer in Xiengkhouang). Some families grew KKN and other rice in separate plots in the same field. We collected all possible samples from those farmers. However, the proportion of other rice types was smaller than that of KKN in both Xiengkhouang and Houaphan Provinces. This indicated that KKN was currently well adapted to this environment.

The samples' passport data (60 accessions) and related information was transferred from a collection form to a table (Table 3.4). In addition, photos were taken of them. After the survey was completed, the samples together with the passport data were maintained in the Lao National Genebank at the Agriculture Research Center, NAFRI, Vientiane, Laos.

Two samples, R88 and R89, were collected at Pu Village, Thathom District, Xaysomboun Province (Table 3.4, Fig. 3.2). This actually is the endpoint of Xienkhouang, a provincial border between Xiengkhouang and Xaysomboun Province. This province was previously a district of Xiengkhouang, and it was then renamed

Xaysomboun about two years before this collecting mission. The farmer, or the owner of these samples, still held a Xiengkhouang ID card and family registration book (according to a farmer, Mr. Khampern). Thus, two provinces—Xiengkhouang and Houaphan were accounted for in our report.

Table 3.1. Important materials list used in the survey

No.	Description	Use
1	Data collection form	Recording sample information, photo numbers, etc.
2	GPS	Recording geographical position
3	Digital camera	Recording sample photos
4	Meter box	Measuring panicle and culm length
5	Ruler	Scaling
6	Field notebook	Noting activities and other observations
7	Pen and pencil	Writing notes and sample data
8	Paper bag (10x20 cm)	Holding the sample
9	Net bag (25x40 cm)	Gathering sample bags and air drying

Table 3.2. Itinerary of the survey

Day	Month	Date		Activities
1	Oct	16	Thu	Traveled from Vientiane to Khoun District, Xiengkhouang
2	Oct	17	Fri	Surveyed in Xiengkhouang
3	Oct	18	Sat	Traveled from Xiengkhouang (Phonsavan District) to Houaphan (Sam Neua District)
4	Oct	19	Sun	Surveyed in Houaphan (Viengxai District)
5	Oct	20	Mon	Surveyed in Houaphan (Sam Neua District – Sop Bao District – Et District)
6	Oct	21	Tue	Surveyed in Houaphan (Et District – Sam Neua District)
7	Oct	22	Wed	Surveyed in Houaphan (Sam Neua District – Sam Tai District)
8	Oct	23	Thu	Surveyed in Houaphan (Sam Tai District – Viengxai District)
9	Oct	24	Fri	Surveyed in Houaphan (Viengxai District – Houameuang District)
10	Oct	25	Sat	Surveyed in Houaphan [Houameuang District – Viengthong (Hiem) District]

Day	Month	Date		Activities
11	Oct	26	Sun	Traveled from Houaphan (Viengthong District) to Xiengkhouang (Kham District)
12	Oct	27	Mon	Surveyed in Xiengkhouang (Kham District – Nonghet District – Phonsavan District)
13	Oct	28	Tue	Surveyed in Xiengkhouang (Phonsavan District)
14	Oct	29	Wed	Surveyed in Xiengkhouang (Phonsavan District – Kham District)
15	Oct	30	Thu	Surveyed in Xiengkhouang (Phonsavan District – Mok)
16	Oct	31	Fri	Surveyed in Xiengkhouang (Phonsavan District – Khoun District)
17	Nov	1	Sat	Traveled back from Xiengkhouang (Phonsavan District) to Vientiane

Table 3.3. Summary of rice samples collected in Xiengkhouang and Houaphan Provinces from October 16 to November 1, 2014

Provinces	Khao Kai Noi (accessions)	Other rice (accessions)	Total (accessions)
Houaphan	24	4	28
Xiengkhouang	25	7	32
Total	49	11	60

Table 3.4. List and passport data of rice (*Oryza sativa* L.) samples collected in Xiengkhouang and Houaphan Provinces, Laos from Oct. 16–Nov. 1, 2014

Ent No	Samp ID	Coll Date	Stat [*1]	Local name	Sam-ple[*2]	Prov	Districts	Villages	Latitude		Longitude		Alt (m)	Condi-tion[*3]	Collec-tion	Remarks
1	R01	16-Oct	4	Khao Kai Noi Dam	In	XKH	Khoun	Na Ou	N	19.21056	E	103.19397	1087	5-1-1-2-3	Seed	Farmer: Mrs. Kham On. Neither fertilizer nor pesticides were applied. Only two Khao Kai Noi grew in this family field (R01 and R02).
2	R02	16-Oct	4	Khao Kai Noi Leuang	In	XKH	Khoun	Na Ou	N	19.21056	E	103.19397	1087	5-1-1-2-3	Seed	Farmer: Mrs. Kham On. No fertilizer is used.
3	R03	17-Oct	4	Khao Kai Noi Deng	In	XKH	Phoukoud	Poung Man	N	19.38054	E	103.07583	1073	5-1-1-2-3	Seed	Farmer: Mrs. Siphan. Sale to Vietnamese business man. Culm length: 90 cm. Neither fertilizer nor pesticides were applied. Some product sold to Beerlao company.
4	R04	17-Oct	4	Khao Kai Noi Leuang	In	XKH	Phoukoud	Poung Man	N	19.38054	E	103.07583	1073	5-1-1-2-3	Seed	Same farmer as R03. Culm length: 120 cm.
5	R05	17-Oct	4	Khao Leuang	In	XKH	Phoukoud	Poung Man	N	19.38054	E	103.07583	1073	5-1-1-2-3	Seed	Same owner as R03 and R04. Culm length: 110 cm.

6	R06	18-Oct	4	Khao Kai Noi Leuang	In	HP	Sam Neua	Phonxay	N	20.14148	E	104.01259	1195	6-1-1-2-3	Seed	Farmer: Mrs. Somphone. Culm length: 130 cm. Yield about 4 ton/ha. This family grew 2 ha. Neither fertilizer nor pesticides were applied.
7	R07	19-Oct	4	Khao Kai Noi Leuang	In	HP	Sam Neua	Sam Nuea	N	20.25382	E	104.02342	956	5-1-1-2-3	Seed	Farmer: Mr. Vieng. Culm length: 120 cm. Grew 2,300 m2. Yield about 4 ton/ha.
8	R08	19-Oct	4	Khao Kai Noi Leuang	In	HP	Sam Neua	Sam Nuea	N	20.25382	E	104.02342	956	5-1-1-2-3	Seed	Same owner as R07. Culm length: 120 cm. Neither fertilizer nor pesticides were applied.
9	R09	20-Oct	4	Khao Kai Noi Leuang	In	HP	Viengxai	Na Poung	N	20.28145	E	104.09060	855	6-1-1-2-3	Seed	Farmer: Mrs. Nam. Culm length: 110 cm. Neither fertilizer nor pesticides were applied.
10	R10	20-Oct	4	Khao Kai Noi Lai	In	HP	Sop Bao	Sop Hao	N	20.33407	E	104.26598	230	5-1-1-2-3	Seed	Farmer: Mr. Phosy. Culm length: 110 cm. Yield about 5 ton/ha. Paddy 4,000 kip/kg. This family planted Khao Kai Noi (KKN) and N79 (Vietnamese rice)—more aromatic than other types of KKN.
11	R11	20-Oct	4	Khao Kai Noi Deng	In	HP	Xiengkhor	Na Meuang	N	20.47108	E	104.09225	261	6-1-1-2-3	Seed	Farmer: Mr. Out. Culm length: 120 cm. Grew 0.5 ha. Yield about 4.7 ton/ha. Neither fertilizer nor pesticides were used.

12	R12	20-Oct	4	Khao Suan Huang	In	HP	Xiengkhor	Na Meuang	N	20.47108	E	104.09225	261	6-1-1-2-3	Seed	Same owner as R11. Culm length: 110 cm.
13	R16	21-Oct	4	Khao Kai Noi Deng	In	HP	Et	Phieng Yang	N	20.48068	E	104.00208	260	6-1-1-2-4	Seed	Farmer: Mr. Kai Yeng. Culm length: 110 cm. Yield about 6 ton/ha. Neither fertilizer nor pesticides were used. There were smaller and bigger grains, but they preferred the big ones. Modern varieties also grew in this family field.
14	R17	21-Oct	4	Khao Kai Noi	In	HP	Et	Dai	N	20.46386	E	103.59358	286	6-1-1-2-4	Seed	Farmer: Mr. Sermchit. Culm length: 120 cm. Neither fertilizer nor pesticides were used. Yield about 3.3 ton/ha. Modern varieties (TDK, Dok Kham Khao) were also grown here.
15	R19	21-Oct	4	Khao Kai Noi Khao	In	HP	Et	Houay Kok	N	20.42345	E	104.01220	708	6-1-1-2-	Seed	Farmer: Mr. Oun. Culm length: 130 cm. Planted 0.3 ha. Khao Pae and Khao Viet also grown.
16	R27	21-Oct	4	Bray, Bet Soua	P	HP	Sam Neua	Phou Jaeng	N	20.41126	E	104.02568	1022	6-2-1-2-4	Seed	Farmer: Mr. Porye. Neither fertilizer nor pesticides were used.
17	R28	21-Oct	4	Khao Kai Noi Leuang	In	HP	Sam Neua	Sa Naen	N	20.39175	E	104.04391	916	6-1-1-2-3	Seed	Farmer: Mr. Bounphone. Culm length: 140 cm. Yield about 5.88 ton/ha. Planted 0.85 ha. Neither fertilizer nor pesticides were used.

18	R29	21-Oct	4	Khao Kai Noi	In	HP	Sam Neua	Nong Khang	N	20.37077	E	104.04366	1042	6-1-1-2-3	Seed	Farmer: Mr. Khen. Culm length: 140 cm. No pesticides used. Nitrogen fertilizer at the seedling stage.
18	R32	22-Oct	4	Khao Meuang Lan	In	HP	Sam Tai	Siengdii	N	19.55585	E	104.37442	648	6-1-1-2-3	Seed	Farmer: Mr. Maisa. Culm length: 110 cm. Planted 1.8 ha. Also grew KKN. Neither pesticides nor fertilizer were applied.
20	R33	22-Oct	4	Khao Kai Noi Lai	In	HP	Sam Tai	Siengdii	N	19.55585	E	104.37442	648	6-1-1-2-3	Seed	Same owner as R32. Culm length: 110 cm. Grew a separate plot in the same field as other traditional rice.
21	R34	22-Oct	4	Khao Kai Noi Deng	In	HP	Sam Tai	Siengdii	N	19.55452	E	104.36535	676	6-1-1-2-3	Seed	Farmer name: Mr. Panit. Culm length: 110 cm.
22	R35	22-Oct	4	Khao Kai Noi Khao	In	HP	Sam Tai	Nala	N	19.56227	E	104.37250	684	6-1-1-2-3	Seed	Farmer: Mr. Thongloun. Culm length: 130 cm. Only KKN planted. Yield 3.6 ton/ha. Planted 0.5 ha. Neither pesticides nor fertilizer were applied.
23	R36	22-Oct	4	Khao Kai Noi Deng	In	HP	Sam Tai	Nala	N	19.56227	E	104.37250	684	6-1-1-2-3	Seed	Same owner as R36. Culm length: 130 cm. Same owner as R35.

24	R37	22-Oct	4	Khao Borng	In	HP	Sam Tai	Nala	N	19.56227	E	104.37250	684	6-1-1-2-3	Seed	Same owner as R35 and R36. Culm length: 130 cm. Had been cultivating this variety for a long time (from their grandparents) before KKN was introduced.
25	R40	22-Oct	4	Khao Kai Noi Leuang	In	HP	Viengxai	Donkhoun	N	20.15569	E	104.31456	416	6-1-1-2-3	Seed	Farmer: Mr.Viengphone. Culm length: 130 cm. Single seedlings planted. This village preferred both KKN Leuang and KKN Lai, but this family grew KKN Leuang (or Khao). Neither pesticides nor fertilizer were applied.
26	R43	24-Oct	4	Khao Kai Noi Leuang	In	HP	Sam Neua	Vaen	N	20.12448	E	104.10205	765	6-1-1-2-3	Seed	Farmer: Mr. Onkham. Culm length: 110 cm. Yield about 5 ton/ha. In this village all farmers grew KKN. Neither pesticides nor fertilizer were applied.
27	R44	24-Oct	4	Khao Kai Noi Leuang	In	HP	Sam Neua	Done	N	20.16371	E	104.02383	1122	6-1-1-2-3	Seed	Farmer: Mr. Pheng. Culm length: 150 cm. Yield about 5.4 ton/ha. In this village all farmers grew KKN. Neither pesticides nor fertilizer were applied.
28	R45	25-Oct	4	Khao Kai Noi Deng	In	HP	Houameuang	Phiengdi	N	20.03334	E	103.38712	562	6-1-1-2-3	Seed	Farmer: Mr. Phanvilay. Culm length: 110 cm. Yield about 2.8 ton/ha. Planted 0.56 ha. Neither pesticides nor fertilizer were applied.
29	R47	25-Oct	4	Khao Kai Noi Deng	In	HP	Viengthong	Thaenhing	N	20.03413	E	103.22093	676	6-1-1-2-3	Seed	Farmer: Mrs. Jaeng. Culm length: 120 cm. Neither pesticides nor fertilizer were applied.

30	R49	25-Oct	4	Khao Nong	In	HP	Viengthong	Kor Kieng	N	20.01145	E	103.20529	643	6-1-1-2-3	Seed	Farmer: Mr. On. Culm length: 120 cm. Yield about 6 ton/ha. Planted 0.2 ha. Neither pesticides nor fertilizer were applied.
31	R50	25-Oct	4	Khao Kai Noi Khao	In	HP	Viengthong	Kor Kieng	N	20.01145	E	103.20529	643	6-1-1-2-3	Seed	Same owner as R49. Culm length: 120 cm. This village and nearby farmers grew KKN Khao and KKN Deng.
32	R51	25-Oct	4	Khao Kai Noi Deng	In	HP	Viengthong	Kor Kieng	N	20.01145	E	103.20529	643	6-1-1-2-3	Seed	Same owner as R49 and R50. Culm length: 120 cm.
33	R53	25-Oct	4	Khao Kai Noi Deng	In	HP	Houameuang	Soplao	N	20.01310	E	103.42558	532	6-1-1-2-3	Seed	Farmer: Mr. Phonema. Culm length: 130 cm. Neither pesticides nor fertilizer were applied.
34	R54	26-Oct	4	Khao Kai Noi Khao	In	XKH	Kham	Jom Thong Tai	N	19.37143	E	103.34054	597	6-1-1-2-3	Seed	Farmer: Mr. Khoun. Culm length: 120 cm. Yield 5.3 ton/ha. Planted 0.15 ha. Neither pesticides nor fertilizer were applied.
35	R56	26-Oct	4	Khao Kai Noi	In	XKH	Nonghet	Khangphannien	N	19.30548	E	103.51234	1187	6-1-1-2-3	Seed	Farmer: Mr.Vasamour. Culm length: 100 cm. Planted just a small plot to conserve the variety. Neither pesticides nor fertilizer were applied.

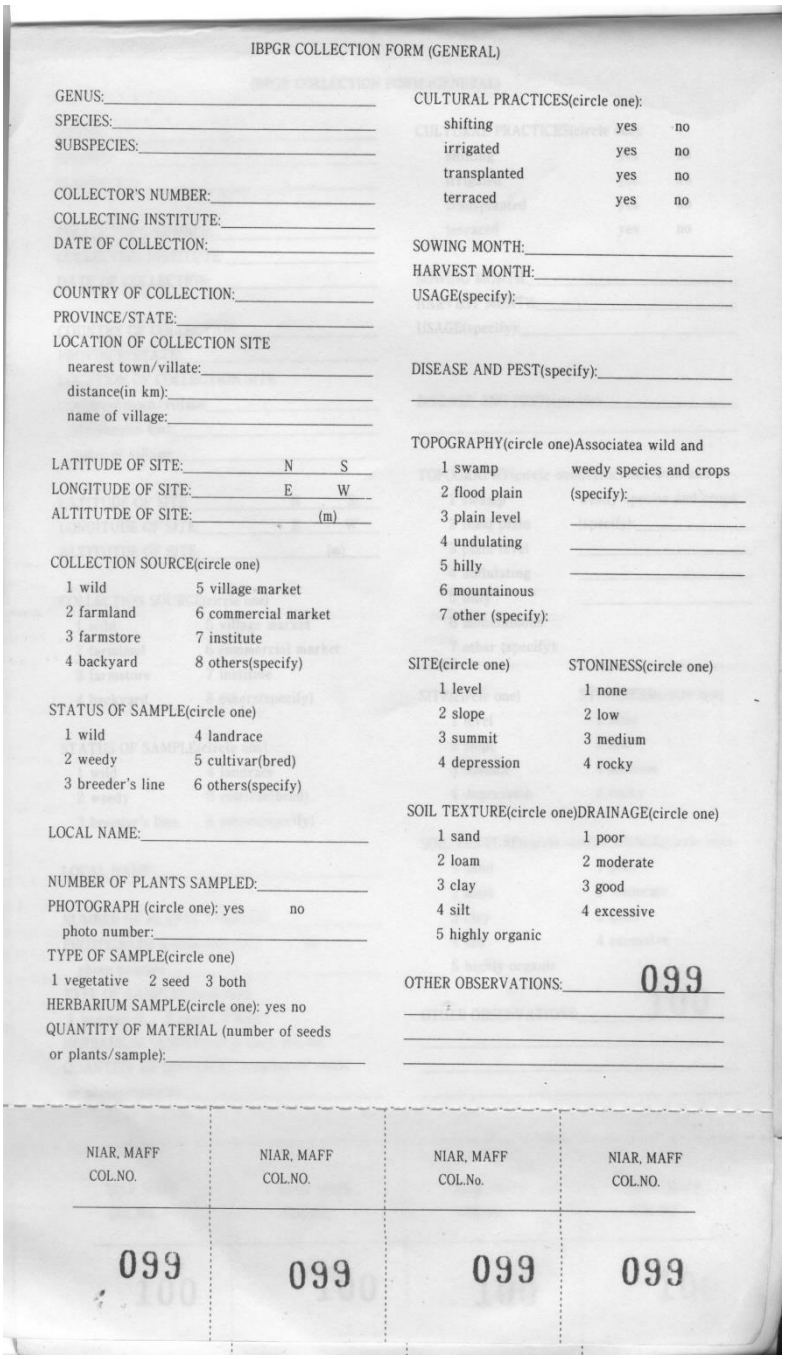
36	R57	26-Oct	4	Khao Lai	In	XKH	Nonghet	Khangphannien	N	19.30548	E	103.51234	1187	6-1-1-2-3	Seed	Same owner as R56. Culm length: 90 cm. Planted in the same field as KKN.
37	R58	27-Oct	4	Khao Kai Noi Khao	In	XKH	Phonsavan	Na O	N	19.25036	E	103.10295	1084	6-1-1-2-3	Seed	Farmer: Mr. Touy. Culm length: 100 cm. Yield about 5.4 ton/ha. Planted 0.7 ha. A little fertilizer used, but no pesticide. They prefer KKN Deng and KKN Khao due to their high yields. No rice other than KKN was grown in this field.
38	R59	27-Oct	4	Khao Kai Noi Deng	In	XKH	Phonsavan	Na O	N	19.25036	E	103.10295	1084	6-1-1-2-3	Seed	Same owner with R58. Culm length: 110 cm.
39	R61	28-Oct	4	Khao Kai Noi (met yai)	In	XKH	Phoukoud	Namchat	N	19.29052	E	102.43215	817	6-1-1-2-3	Seed	Farmer: Mr. Phaivan. Culm length: 150 cm. Only this family planted KKN. Introduced from Nong Tang many years ago. Planted 0.3 ha. Yield about 7.8 ton/ha. Neither fertilizer nor pesticides were applied.
40	R62	28-Oct	4	Khao Kai Noi (met noi)	In	XKH	Phoukoud	Namchat	N	19.29052	E	102.43215	817	6-1-1-2-3	Seed	Same owner as R61. Culm length: 110 cm.

41	R63	28-Oct	4	Khao Kai Noi Khao	In	XKH	Phoukoud	Xiengngna	N	19.29048	E	102.51452	1132	5-1-1-2-3	Seed	Farmer: Mr. Khamban. Culm length: 120 cm. Only this rice was grown. Neither fertilizer nor pesticides were applied.
42	R64	28-Oct	4	Khao Kai Noi Deng	In	XKH	Phoukoud	Xiengngna	N	19.29048	E	102.51452	1132	5-1-1-2-3	Seed	Same owner as R63. Culm length: 110 cm.
43	R65	28-Oct	4	Khao Kai Noi	In	XKH	Phoukoud	Sai	N	19.31057	E	103.01499	1040	5-1-1-2-3	Seed	Farmer: Mr. Jarn Noansy. Culm length: 90 cm. Yield about 4 ton/ha. Planted 0.8 ha. Neither fertilizer nor pesticides were applied. Manure was used. He had grown KKN for 13 years. Not only KKN was grown here.
44	R67	29-Oct	4	Khao Kai Noi Leuang	In	XKH	Kham	Namsai	N	19.45241	E	103.23018	1031	6-1-1-2-3	Seed	Farmer: Mr. Phor Pao. Culm length: 110 cm. This village farmer used KKN and Chao Mali. Threshing machine was used. Neither fertilizer nor pesticides were applied.
45	R68	29-Oct	4	Khao Chao Mali	In	XKH	Kham	Namsai	N	19.45241	E	103.23018	1031	6-1-1-2-3	Seed	Same owner as R67. Culm length: 120 cm.
46	R71	29-Oct	4	Khao Kai Noi Deng	In	XKH	Kham	Naphan	N	19.43293	E	103.27425	874	6-1-1-2-3	Seed	Farmer: Mr. Guxong. Culm length: 120 cm. Yield about 6 ton/ha. Planted 0.7 ha. Planted both KKN Deng and KKN Khao. Some families in the village grew Khao Chao Teer. Neither fertilizer nor pesticides were applied.

																Harvested by hand and threshing machine used.
47	R72	29-Oct	4	Khao Kai Noi Khao	In	XKH	Kham	Naphan	N	19.43293	E	103.27425	874	6-1-1-2-3	Seed	Same owner as R71. Culm length: 110 cm.
48	R74	29-Oct	4	Khao Kai Noi Lai	In	XKH	Kham	Fai	N	19.39466	E	103.35071	587	5-1-1-2-3	Seed	Farmer: Mrs. Mae Tieng. Culm length: 110 cm. Planted 1.8 ha. Yield about 3.3 ton/ha. This KKN Lai was the most expensive among KKNs. KKN Deng was introduced from Sam Neua District, Houaphan Province about four years ago. Fertilizer used but no pesticides.
49	R75	29-Oct	4	Khao Kai Noi Khao	In	XKH	Kham	Fai	N	19.39466	E	103.35071	587	5-1-1-2-3	Seed	Same owner as R74. Culm length: 110 cm.
50	R76	29-Oct	4	Khao Kai Noi Deng	In	XKH	Kham	Fai	N	19.39466	E	103.35071	587	5-1-1-2-3	Seed	Same owner as R74 and R75. Culm length: 120 cm.

51	R77	29-Oct	4	Khao Kai Noi Deng	In	XKH	Phonsavan	Gnod Ngeum	N	19.33283	E	103.22435	1127	5-1-1-2-3	Seed	Farmer: Mr. Ket. Culm length: 90 cm; Yield about 4 ton/ha. Neither fertilizer nor pesticides were applied.
52	R78	29-Oct	4	Khao Kai Noi Khao	In	XKH	Phonsavan	Gnod Ngeum	N	19.33283	E	103.22435	1127	5-1-1-2-3	Seed	Same owner as R77. Culm length: 80 cm.
53	R79	30-Oct	4	Khao Kai Deng	P	XKH	Mok	Na Khae	N	19.04474	E	103.57090	468	6-1-1-2-	Seed	Farmer: Mr. Dourvang. Culm length: 140 cm. Yield 4 ton/ha. Planted 2.5 ha. 50% of their land was used for KKN Deng and the other half for Khao Chao (non-glutinous rice). Neither fertilizer nor pesticides were applied.
54	R80	30-Oct	4	Khao Chao, Khao Sanleuang	P	XKH	Mok	Na Khae	N	19.04474	E	103.57090	468	6-1-1-2-	Seed	Same owner as R79. Culm length: 140 cm.
55	R82	30-Oct	4	Khao Kai Noi Khao	In	XKH	Khoun	Dokmai	N	19.14072	E	103.40066	1345	6-1-1-2-3	Seed	Farmer: Ms. La. Culm length: 110 cm. 1 ha of different landraces were grown in this single field: Khao Pong, KKN Khao and Khao La.
56	R83	30-Oct	4	Khao La	In	XKH	Khoun	Dokmai	N	19.14072	E	103.40066	1345	6-1-1-2-3	Seed	Same owner as R82. Culm length: 110 cm.

57	R84	30-Oct	4	Khao Pong	In	XKH	Khoun	Dokmai	N	19.14072	E	103.40066	1345	6-1-1-2-3	Seed	Same owner as R82. Culm length: 110 cm.
58	R87	31-Oct	4	Khao Kai Noi Leuang	In	XKH	Khoun	Nahor	N	19.16522	E	103.21289	1086	6-1-1-2-3	Seed	Farmer: Mr. Xingxong. Yield about 5 ton/ha. Planted 0.6 ha. Threshing machine used. Neither fertilizer nor pesticide were used.
59	R88	1-Nov	4	Khao Kai Noi Khao	In	Xaisomboun	Thathom	Pu	N	19.01354	E	103.27206	347	5-1-1-2-3	Seed	Farmer: Mr. Khampern. Culm length: 110 cm. This field grew two types of rice: KKN and Khao Saiya (Saya). Seeds were shared by relatives within the village.
60	R89	1-Nov	4	Khao Saiya	In	Xaisomboun	Thathom	Pu	N	19.01354	E	103.27206	347	5-1-1-2-3	Seed	Same owner as R88. Culm length: 140 cm.



IBPGR COLLECTION FORM (GENERAL)

GENUS:_____
SPECIES:_____
SUBSPECIES:_____

COLLECTOR'S NUMBER:_____
COLLECTING INSTITUTE:_____
DATE OF COLLECTION:_____

COUNTRY OF COLLECTION:_____
PROVINCE/STATE:_____
LOCATION OF COLLECTION SITE
nearest town/villate:_____
distance(in km):_____
name of village:_____

LATITUDE OF SITE:_____ N S
LONGITUDE OF SITE:_____ E W
ALTITUTDE OF SITE:_____ (m)

COLLECTION SOURCE(circle one)
1 wild 5 village market
2 farmland 6 commercial market
3 farmstore 7 institute
4 backyard 8 others(specify)

STATUS OF SAMPLE(circle one)
1 wild 4 landrace
2 weedy 5 cultivar(bred)
3 breeder's line 6 others(specify)

LOCAL NAME:_____

NUMBER OF PLANTS SAMPLED:_____
PHOTOGRAPH (circle one): yes no
photo number:_____
TYPE OF SAMPLE(circle one)
1 vegetative 2 seed 3 both
HERBARIUM SAMPLE(circle one): yes no
QUANTITY OF MATERIAL (number of seeds or plants/sample):_____

CULTURAL PRACTICES(circle one):
shifting yes no
irrigated yes no
transplanted yes no
terraced yes no

SOWING MONTH:_____
HARVEST MONTH:_____
USAGE(specify):_____

DISEASE AND PEST(specify):_____

TOPOGRAPHY(circle one)
1 swamp
2 flood plain
3 plain level
4 undulating
5 hilly
6 mountainous
7 other (specify):

Associatea wild and weedy species and crops (specify):_____

SITE(circle one)
1 level
2 slope
3 summit
4 depression

STONINESS(circle one)
1 none
2 low
3 medium
4 rocky

SOIL TEXTURE(circle one)
1 sand
2 loam
3 clay
4 silt
5 highly organic

DRAINAGE(circle one)
1 poor
2 moderate
3 good
4 excessive

OTHER OBSERVATIONS: 099

NIAR, MAFF COL.NO. 099
NIAR, MAFF COL.NO. 099
NIAR, MAFF COL.No. 099
NIAR, MAFF COL.NO. 099

Figure 3.1. Data collection form used in the survey

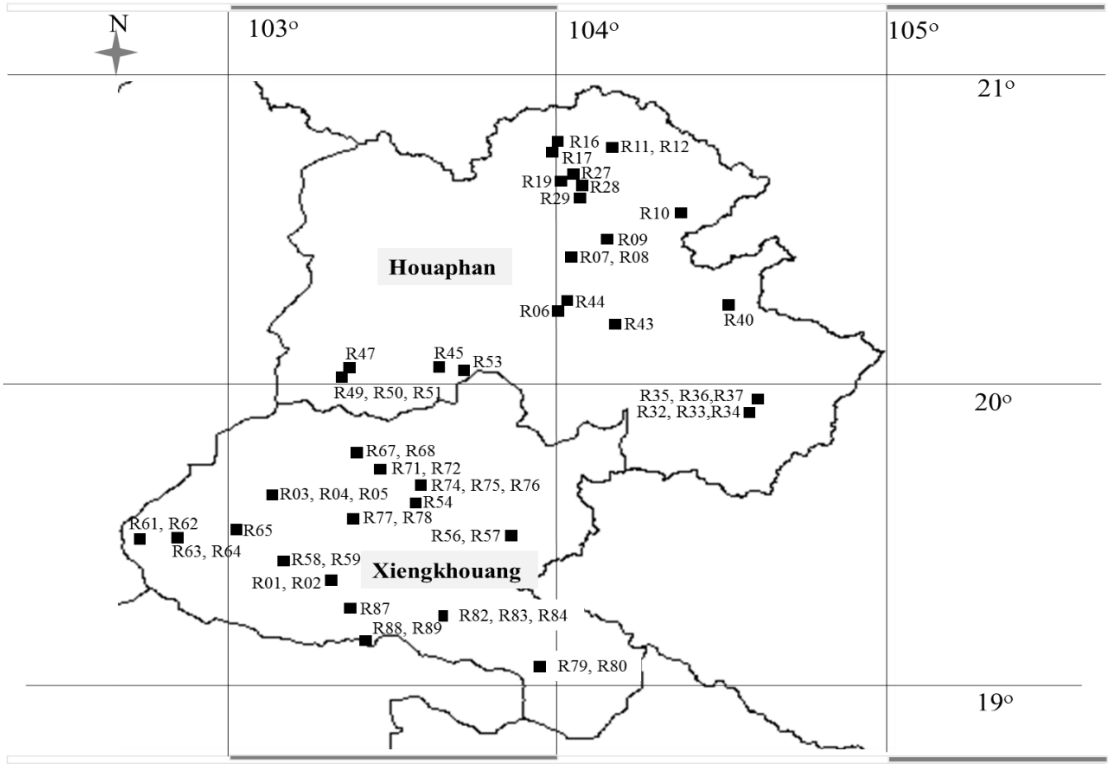


Figure 3.2. Map of Xiengkhouang and Houaphan Provinces showing the collection sites of rice landraces (*O. sativa* L.) in 2014. R: rice, followed by the ID number of the sample in this collection

(A) **(B)**

(C)

Figure 3.3. An example of grain colors reflecting the variant name of Khao Kai Noi: (A) Khao Kai Noi Leuang, (B) Khao Kai Noi Deng, and (C) Khao Kai Noi Dam

Chapter 4

Characterization of the On-Farm Population of Khao Kai Noi

4.1 Introduction

Crop genetic resource conservation is important and is of concern worldwide. On-farm, or *in* situ, and *ex situ* conservation is well defined in terms of conservation. In *ex situ* conservation, genebanks are the most important tools for storing the germplasm, thus a number of genebanks have been developed worldwide. Lao P.D.R has abundant rice genetic resources and is ranked second after India, which has the highest number of rice accessions conserved in the international rice collection at the International Rice Research Institute (Jackson & Lettington, 2003). In addition, the local Lao National Genebank, Agriculture Research Center (ARC), National Agriculture and Forestry Research Institute (NAFRI), Vientiane, was initiated in 1995 and has conserved 13,192 cultivated rice accessions. Khao Kai Noi (KKN), landrace glutinous, which has a good eating quality in terms of its softness and aroma, is included in that collection. Its production is not only for consumption but also for exportation to Vietnam and European countries (Worklivelaos, 2014a). Currently, its production is promoted as a material of industrial production (Worklivelaos, 2014b). To investigate its current on-farm diversity, additional accessions of this rice were collected during October and November of 2014 and added to the collection. The

genetic diversity of new additional on-farm populations is unknown. In this study, the objective was to evaluate the genetic diversity of KKN on-farm populations.

4.2 Materials and methods

4.2.1 Plant material

Thirty-nine accessions of KKN collected in 2014 were used (Table 4.1). They were freshly collected from farmer fields during harvesting season (October to November) in Xiengkhuang, Houaphan, and Xaisomboun Provinces, Lao P.D.R (Fig. 4.1). Nipponbare, Kasalath, and TDK11 representing the Japonica type; Indica type; and Lao improved varieties were added as control varieties.

4.2.2 Microsatellite assay

4.2.2.1 DNA extraction

Brown rice seeds were germinated in petri dishes with wet tissue paper. Eight five-day-old seedlings of each accession were cut and ground separately in liquid nitrogen, and then homogenous powder was used for DNA extraction by using the CTAB method (Doyle & Doyle, 1987). Next, the DNA quantity was measured by using a spectrophotometer (NANODROP 2000), and quality was checked by using 0.8% agarose gel. Finally, the DNA was adjusted to 100 ng/µl by using a 1X TE buffer and stored at -20°C for experimentation.

4.2.2.2 DNA amplification and capillary electrophoresis

Twenty-four SSR primers pairs, with the forward side labelled by fluorophor (6FAM, NED, PET, and VIC), were used. This was the same set as that used for characterizing the genebank population, which was mentioned in Chapter 2. The PCR procedure, electrophoresis, and data collection were conducted as described in Chapter 2.

4.2.3 Data analysis

Population genetic diversity parameters—including expected heterozygosity (H_e), observed heterozygosity (H_o), and polymorphism information content (*PIC*)—for each locus were calculated by using the PowerMarker v3.25 software (Liu & Muse, 2005).

The phylogenetic tree was constructed based on Nei's 1983 genetic distance with 1,000 replication bootstrapping in PowerMarker software v3.25 (Liu & Muse, 2005), and the result output was summarized and obtained by the "consensus" program in the PHYLIP package v3.696 (Felsenstein, 2005). The final tree was then visualized in MEGA6 (Tamura et al., 2013).

Analysis of molecular variance (AMOVA) was conducted in GenAlEx v6.5 (Peakall & Smouse, 2012) to determine hierarchical partitioning of the genetic variation of clusters of 39 KKN on-farm accessions among clusters, among accessions within a cluster, and within accessions.

Genetic differentiation among samples sourced from different provinces was tested. For this comparison, three populations were assigned based on the point of collection of samples: Houaphan, Xiengkhuang, and Xaisomboun Provinces. F-statistics were calculated in GenAlEx v6.5.

The population structure of KKN was assessed by a model-based method using the software STRUCTURE v2.3.4 (Pritchard et al., 2000). The number of the presumed population (*K*) was tested from 2 to 10 with an admixture and correlated allele frequencies model. Five separately repeated runs were performed for each *K* with 100,000 length of burning period, 100,000 MCMC (Markov Chain Monte Carlo) repeats after burning period. The optimum *K* value was determined by two statistical parameters, Ln *P*(*D*) and Δ*K*, using the STRUCTURE HARVESTER (Earl & vonHoldt, 2012) and implementing the parameters of Evanno et al. (2005).

4.3 Results

4.3.1 Marker informativeness

A total of 24 SSR markers were used in this study. Ten of them showed monomorphism over all accessions, so they were excluded from the analysis. Fourteen polymorphism markers generated 38 alleles from 39 accessions (310 individuals) of the KKN on-farm population. It ranged from 2 to 6, with an average of 2.7 alleles per locus (Table 4.2). Polymorphism information content (*PIC*) ranged from 0.003 (RM215 and RM507) to 0.675 (RM259). Diversity of KKN was observed.

Expected herterozygosity (*He*) or gene diversity varied among loci, ranging from 0.003 (RM215 and RM507) to 0.721 (RM259) with an average of 0.314 (Table 4.2).

The dendrogram of KKN can be divided into three main clusters at a distance of 0.15 and assigned to G1, G2, and G3 (Fig. 4.1). The number of accessions in each group was varied. It was 27 (R29, R54, R09, R65, R19, R63, R34, R71, R35, R72, R56, R87, R28, R43, R64, R53, R76, R62, R75, R07, R40, R08, R33, R04, R06, R01, R59), 5 (R02, R88, R45, R44, R47), and 7 accessions (R16, R03, R36, R77, R74, R10, R11) in G1, G2, and G3 respectively.

AMOVA of the three clusters was done to partition the proportion of total variation. The percentage of variation—86%, 13%, and 1%—was due to the differences among clusters, among accessions within a cluster, and within an accession respectively (Table 4.3). The population genetic differentiation among the three clusters was significant [(F_{ST}=0.861); (Table 4.3)]. Pairwise genetic differentiation (F_{ST}) among the three clusters (G1, G2, and G3) was highly significant (p=0.001) for all possible pairs (Table 4.4). The greatest pairwise genetic distance was between G1 and G3 (0.907), while the lowest was between G1 and G2 (0.273).

4.3.2 Isolation by distance testing

KKN was collected from three different provinces: Houaphan, Xiengkhouang, and Xaisomboun. AMOVA results showed that 6% of the variation was due to the differences among provinces (Table 4.5). However, the genetic differentiation (F_{ST}) between these geographical regions was insignificant (F_{ST}=0.055).

4.3.3 Structure analysis

A model based on admixture was carried out by varying K from 2 to 10 using 39 accessions of KKN and 14 SSR polymorphism markers. Population structure showed the highest Δ*K* at *K*=3 (Fig. 4.2A); therefore, the KKN on-farm population was assigned to three subclusters: S1, S2, and S3 (Fig. 4.2B).

4.4 Discussion

Twenty-four SSR markers were used in this study; however, 10 of them showed monomorphism. The same set of markers was used as in previous genebank population, but only one exhibited monomorphism. The total number of alleles and average number of alleles per locus was lower than the genebank population, however—loci RM259 exhibited the highest number of alleles. This result was consistent with the previous genebank population. It is also similar to the results found in a study of the highest number of alleles in a mini core set of Japanese rice (Ebana et al., 2008).

The KKN on-farm population consisted of three clusters, which had significant genetic differentiation between them. However, this clustering did not correspond to the variant names in their passport data. There were, in fact, many variant names in these accessions. The local names of this rice are Khao Kai Noi Lai, Khao Kai Noi Leuang, Khao Kai Noi Deng, Khao Kai Noi Khao, and Khao Kai Noi Dam (Table 4.1). The names begin with the three word phrase “Khao Kai Noi,” which is then followed by “Lai”, “Leuang”, “Deng”, and “Dam,” which mean striped,

yellow, red, and black respectively. These extension words describe the hull color of these rice landraces. The farmers in Laos thus name their traditional rice landraces based on morphology (Rao et al., 2006b). The appearance of the hull color is clearly defined, but they could not be differentiated by using molecular data in this study. These results are similar to those for landraces found in high altitude sites in Nepal (Bajracharya et al., 2006).

The KKN rice on-farm population was collected from three different provinces (Vilayheuang et al., in press). Population genetic differentiation among geographical regions was non-significant (F_{ST}=0.05) (Table 4.5). Genetic differentiation among populations (F_{ST}) ranges from 0 to 1. Silo (0) means no differentiation, and one (1) means differentiation existed. F_{ST} is said to be significant when it is above about 0.15 (Frankham et al., 2002). The degree of differentiation depends upon the gene flow. A greater difference can be observed when gene flow is limited, and little difference is present when gene flow occurs. Gene flow occurs by dispersion to another nearby population. When dispersion cannot take place, gene flow may occur by human-mediated means like seed exchange among farmers. KKN genetic differentiation was insignificant between two geographic provinces (Chapter 2). In this study, KKN was collected in the same geographical areas as those of the genebank population was also non-significant. This indicated that seed exchange is continually practiced in this area.

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Table 4.1. List of Khao Kai Noi, Lao rice landrace (*O. sativa* L.) on-farm populations used in this study

No.	Acc. No.	Local name	Ec	En	Pv	Dt	Coordinates		
							Altitude (m)	Latitude (N)	Longitude (E)
1	R01	Khao Kai Noi Dam	L	G	XK	Khoun	1087	19.2106	103.1940
2	R02	Khao Kai Noi Leuang	L	G	XK	Khoun	1087	19.2106	103.1940
3	R03	Khao Kai Noi Deng	L	G	XK	Phoukoud	1073	19.3805	103.0760
4	R04	Khao Kai Noi Leuang	L	G	XK	Phoukoud	1073	19.3805	103.0760
5	R06	Khao Kai Noi Leuang	L	G	HP	Sam Neua	1195	20.1415	104.0130
6	R07	Khao Kai Noi Leuang	L	G	HP	Sam Neua	956	20.2538	104.0230
7	R08	Khao Kai Noi Leuang	L	G	HP	Sam Neua	956	20.2538	104.0230
8	R09	Khao Kai Noi Leuang	L	G	HP	Viengxai	855	20.2815	104.0906

No.	Acc. No.	Local name	Ec	En	Pv	Dt	Coordinates		
							Altitude (m)	Latitude (N)	Longitude (E)
9	R10	Khao Kai Noi Lai	L	G	HP	Sop Bao	230	20.3341	104.2660
10	R11	Khao Kai Noi Deng	L	G	HP	Xiengkhor	261	20.4711	104.0923
11	R16	Khao Kai Noi Deng	L	G	HP	Et	260	20.4807	104.0021
12	R19	Khao Kai Noi Khao	L	G	HP	Et	708	20.4235	104.0122
13	R28	Khao Kai Noi Leuang	L	G	HP	Sam Neua	916	20.3918	104.0439
14	R29	Khao Kai Noi	L	G	HP	Sam Neua	1042	20.3708	104.0437
15	R33	Khao Kai Noi Lai	L	G	HP	Sam Tai	648	19.5559	104.3744
16	R34	Khao Kai Noi Deng	L	G	HP	Sam Tai	676	19.5545	104.3654
17	R35	Khao Kai Noi Khao	L	G	HP	Sam Tai	684	19.5623	104.3725

No.	Acc. No.	Local name	Ec	En	Pv	Dt	Coordinates		
							Altitude (m)	Latitude (N)	Longitude (E)
18	R36	Khao Kai Noi Deng	L	G	HP	Sam Tai	684	19.5623	104.3725
19	R40	Khao Kai Noi Leuang	L	G	HP	Viengxai	416	20.1557	104.3146
20	R43	Khao Kai Noi Leuang	L	G	HP	Sam Neua	765	20.1245	104.1021
21	R44	Khao Kai Noi Leuang	L	G	HP	Sam Neua	1122	20.1637	104.0238
22	R45	Khao Kai Noi Deng	L	G	HP	Houameuang	562	20.0333	103.3871
23	R47	Khao Kai Noi Deng	L	G	HP	Viengthong	676	20.0341	103.2209
24	R53	Khao Kai Noi Deng	L	G	HP	Houameuang	532	20.0131	103.4256
25	R54	Khao Kai Noi Khao	L	G	XK	Kham	597	19.3714	103.3405
26	R56	Khao Kai Noi	L	G	XK	Nonghet	1187	19.3055	103.5123

No.	Acc. No.	Local name	Ec	En	Pv	Dt	Coordinates		
							Altitude (m)	Latitude (N)	Longitude (E)
27	R59	Khao Kai Noi Deng	L	G	XK	Phonsavan	1084	19.2504	103.1030
28	R62	Khao Kai Noi (Met Noi)	L	G	XK	Phoukoud	817	19.2905	102.4322
29	R63	Khao Kai Noi Khao	L	G	XK	Phoukoud	1132	19.2905	102.5145
30	R64	Khao Kai Noi Deng	L	G	XK	Phoukoud	1132	19.2905	102.5145
31	R65	Khao Kai Noi	L	G	XK	Phoukoud	1040	19.3106	103.0150
32	R71	Khao Kai Noi Deng	L	G	XK	Kham	874	19.4329	103.2743
33	R72	Khao Kai Noi Khao	L	G	XK	Kham	874	19.4329	103.2743
34	R74	Khao Kai Noi Lai	L	G	XK	Kham	587	19.3947	103.3507
35	R75	Khao Kai Noi Khao	L	G	XK	Kham	587	19.3947	103.3507

No.	Acc. No.	Local name	Ec	En	Pv	Dt	Coordinates		
							Altitude (m)	Latitude (N)	Longitude (E)
36	R76	Khao Kai Noi Deng	L	G	XK	Kham	587	19.3947	103.3507
37	R77	Khao Kai Noi Deng	L	G	XK	Phonsavan	1127	19.3328	103.2244
38	R87	Khao Kai Noi Leuang	L	G	XK	Khoun	1086	19.1652	103.2129
39	R88	Khao Kai Noi Khao	L	G	XS	Thathom	347	19.0135	103.2721

L: lowland rice, G: glutinous rice, XK: Xiengkhouang, HP: Houaphan, XS: Xaisomboun, (N): north, (E): east

Table 4.2. Diversity parameters of 39 accessions of Khao Kai Noi

Loci	No. of alleles	*H*e	*H*o	*PIC*
RM1	2	0.302	0.048	0.257
RM11	2	0.430	0.006	0.338
RM133	3	0.293	0.000	0.273
RM152	2	0.275	0.000	0.237
RM215	2	0.003	0.003	0.003
RM259	6	0.721	0.000	0.675
RM287	2	0.257	0.000	0.224
RM489	2	0.476	0.110	0.363
RM495	2	0.500	0.000	0.375
RM507	2	0.003	0.003	0.003
RM514	3	0.332	0.016	0.288
RM552	5	0.450	0.100	0.421
RM277	3	0.222	0.006	0.201
RM452	2	0.126	0.000	0.118
Total	38			
Mean	2.7	0.314	0.021	0.270

No.: number, *H*e: expected heterozygosity, *H*o: observed heterozygosity, *PIC*: polymorphism information content

Table 4.3. Analysis of molecular variance of Khao Kai Noi clustered according to a cluster dendrogram

Source	d.f.	S.S.	Est. Var.	% of total variance	*F*-statistics	*P*-value
Among clusters	2	49356.367	170.028	86	*F*st = 0.861	0.001
Among accessions within cluster	307	16175.168	25.337	13		
Within accessions	310	624.000	2.013	1		
Total	619	66155.535	197.378	100		

d.f: degree of freedom, S.S.: sum of square, Est. Var: estimated variance, F_{ST}: genetic differentiation

Table 4.4. Pairwise genetic distance of three clusters of the Khao Kai Noi on-farm population

	G1	G2	G3
G1	0		
G2	0.273	0	
G3	0.907	0.780	0

Table 4.5. Analysis of molecular variance of Khao Kai Noi collected from different geographical provinces

Sources	d.f.	S.S.	Est. Var.	%	F_{ST}	*P*-value
Between provinces	2	44.371	0.124	6	0.055	0.002
Between accessions within province	617	1317.23	2.135	94		
Total	619	1361.6	2.259	100		

d.f.: degree of freedom, S.S.: sum of square, Est. Var: estimated variance, F_{ST}: genetic differentiation

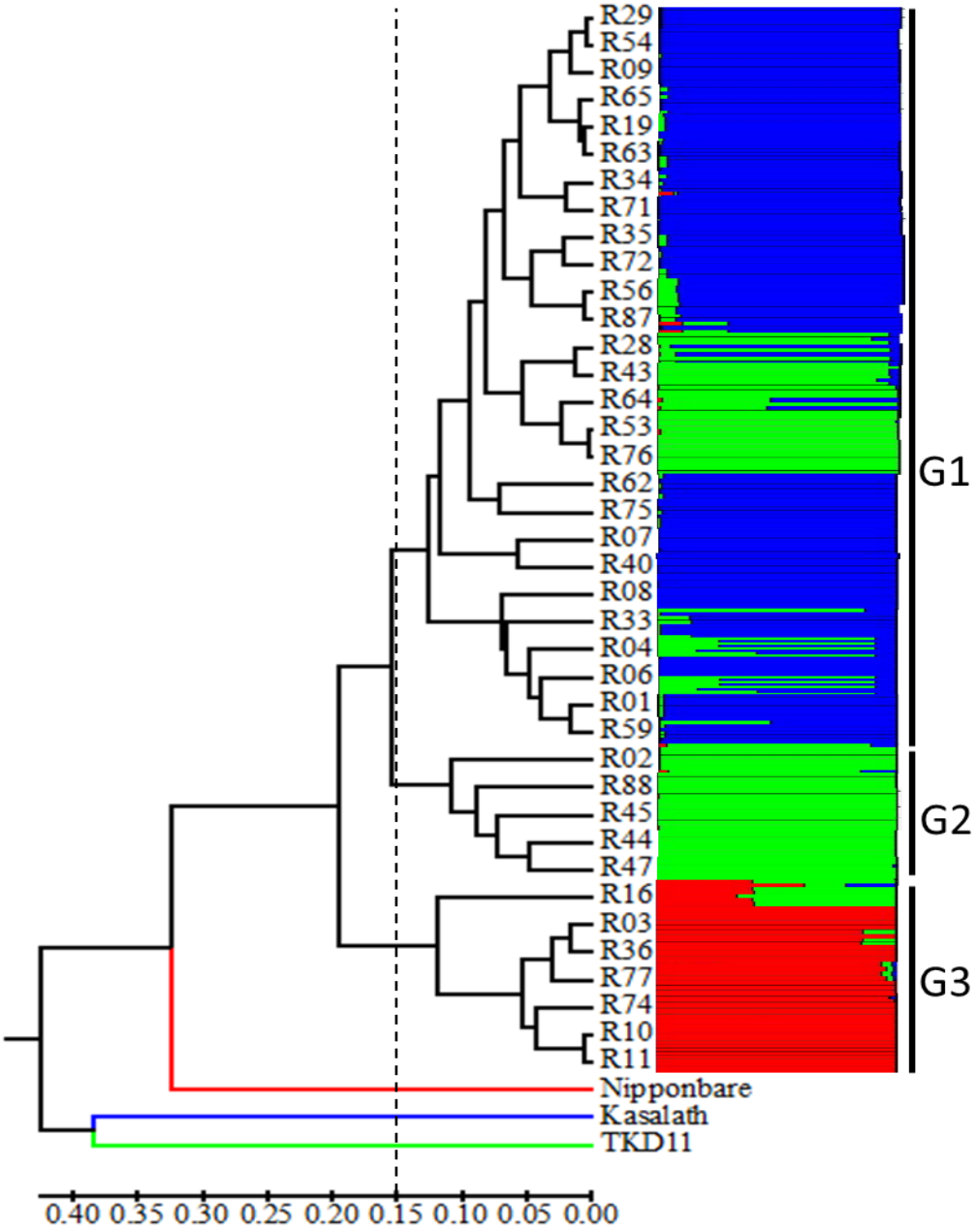
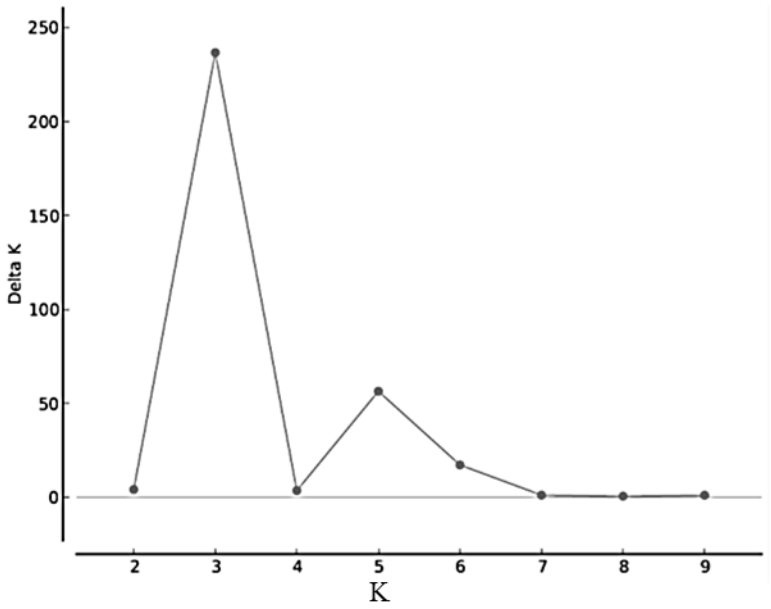


Figure 4.1. UPGMA dendrogram based on Nei's 1983 genetic distance showing relatedness of 39 KKN on-farm population and control varieties. Nipponbare and Kasalath are Japonica-type and Indica-type is the control. TDK11: a Lao improved variety, G: group. Bar plot showing results of population structure analysis

(A)



(B)

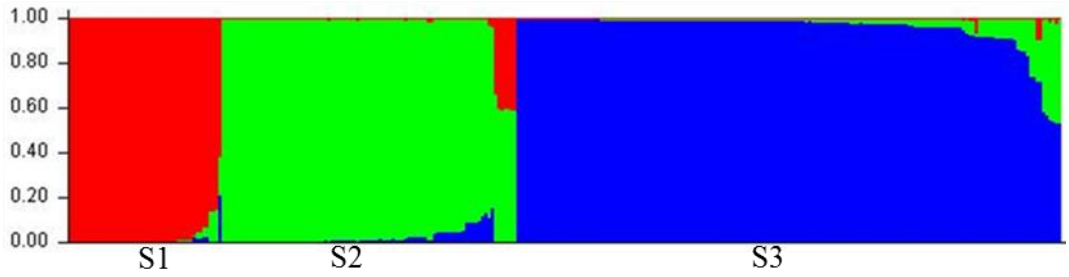


Figure 4.2. Statistics of population structure results—A: Evanno's Delta K, B: Clusters assumed

Chapter 5

Core Collection Development, Overall Conclusions, and Recommendations

5.1 Introduction

"The conservation of the crop varieties of traditional agriculture in the center of genetic diversity is essential to provide genetic resources for plant improvement" (Frankel & Bennett, 1974). Genebanks play a key role in the conservation, availability, and use of a wide range of plant genetic resources for crop improvement to provide food and nutrition security. They help to ensure the continued availability of genetic resources from the past to aid future research and breeding programs (FAO, 2014). Once a genebank is established, the number of germplasms in the collection gradually increases, and, as a result, a genebank will have to deal with a larger number of accessions. In general, it will not be easy for breeders and academic researchers to use a larger number of accessions to meet their needs; therefore, "core collection" is needed. A core collection consists of a limited set of accessions, derived from an existing germplasm collection, chosen to represent the genetic spectrum in the whole collection (Brown, 1995). This is a very useful tool for managing germplasm in genebanks and for users to acquire the appropriate materials. Scientists and breeders may easily access, study, and analyze their diversity. The process of selecting a subset

of accessions from the whole collection is called core collection development and was first proposed by Frankel and Brown (1984). It is very important because it plays a significant role in the management and use of genetic resources (Brown, 1995). Core collection development has been implemented with many crops such as rice (Chung et al., 2009; Ebana et al., 2008; Tiwari et al., 2015), common beans (Zeven et al., 1999), chilis (Mongkolporn et al., 2015), barley (Igatua et al., 1998; Munoz-Amatriain et al., 2014), soybeans (Kaga et al., 2012) and sesame (Xiurong et al., 2000).

The Lao National Genebank is located in the Agriculture Research Center, NAFRI. It provides active and base collection of the germplasm. The active collection is stored in a room at 4°C with 50% relative humidity, while the base collection is stored in a freezer at -18°C. It holds more than 14,000 accessions of rice germplasm including a quality landrace group, KKN.

KKN was chosen as the first group for which to make a core collection because it is important for both domestic consumption and export. Currently, about 200 accessions of KKN germplasm are conserved in the Lao National Genebank. Some of them were collected from 1995–2000 (Rao et al., 2006a) and in 2008 (Bounphanousay et al., 2009), and the rest were collected in 2014 (Vilayheuang et al., in press). Since the number of accessions of KKN is small compared to the whole collection in the genebank, they can all be conserved. Even the Lao National Genebank needs the entire Lao rice core collection, chosen from a total of 14,000 accessions of rice conserved, and specific landraces like KKN need their own core collection because they will be frequently requested by different users such as breeders and researchers. Through core collection development, the genetic diversity

and genetic structure of KKN will be revealed. Also, hotspots of diversity will be determined for promoting on-farm conservation. More importantly, appropriate core collection development procedures will be determined for KKN, which will be helpful for core collection development for the whole Lao rice germplasm in the future.

Many procedures for core collection development are summarized in Table 5.1. Recently, reliable and free software for selecting a core set has been developed (Kim et al., 2007). It could be applied for choosing a core set accurately. Some examples of the application of this software are developing a mini core subset in the USDA rice genebank (Agrama et al., 2009), selecting a core collection of *Oryza sativa* L. in China (Zhang et al., 2011), selecting a chili core collection in Thailand (Mongkolporn et al., 2015), and selecting a preliminary core set of Turkish melon (Frary et al., 2013). In this chapter, I propose to choose core accessions from the whole collection of KKN in the Lao National Genebank, which will be useful for breeding purposes, identifying hotspots of diversity, recommending on-farm conservation sites of this rice landrace, and managing genebank and germplasm samples. In addition, this chapter presents the overall conclusions and recommendations of this study.

5.2 Materials and Methods

5.2.1 Choosing core set accessions from the whole collection

The complete steps of KKN core collection are illustrated in the flow chart in Figure 5.1. One hundred and nine accessions were selected from the Lao National

Genebank and used as the whole collection from which core accessions were chosen in this study (Table 5.2). This was a combination of both the genebank and on-farm populations, which were characterized in Chapter 2 and 4 respectively. Of 109 accessions, 61% (67 accessions) contained coordinate data. Their genotypic data, generated from 24 SSR markers' loci, were prepared and imported to PowerCore v1.0 (Kim et al., 2007) in order to choose core accessions. The details of SSR analysis were described in Chapter 2 and 4. After the software selected core accessions, two parameters—diversity index and number of alleles—were recorded for both the core and whole collection.

5.2.1 Core set evaluation

In order to justify the effectiveness of the core set, I compared the parameters of diversity index and number of alleles between the core set and whole collection. To observe the effect of the reduction in the number of accessions of the core set on allele number and diversity index, I randomly removed five accessions from the core set. They were removed in the following order: LG6644, R74, LG6746, LG2841, and LG5845 (Table 5.3). The allele number and diversity index was observed once an accession was removed.

To observe the representatives of their genetic cluster of core accessions, the UPGMA dendrogram was first constructed in PowerMarker v. 3.25 software (Liu & Muse, 2005) and visualized by MEGA6 (Tamura et al., 2013). Then the core accession was tracked in that dendrogram.

To find their diversity hotspots, some core accessions that had coordinate data available were plotted on a geographic map of the Xiengkhouang and Houaphan Provinces of Laos, where they were collected.

5.3 Results and Discussion

5.3.1 Results

In order to choose representative accessions of KKN for breeding purposes and identify hotspots of diversity to make recommendations for on-farm conservation sites of this rice landrace, 24 SSR allelic data were analyzed for 109 accessions. Thirty accessions (28%) were chosen from the total of 109 and designated the core accessions (Table 5.3). These covered all subgroups: KKN, KKN Deng (red), KKN Hay (upland), KKN Khao (white), KKN Lai (striped), KKN Lai Dam (striped and black), and KKN Leuang (Table 5.3). They also covered both provinces of Houaphan (HP) and Xiengkhouang (XK), which are well known for KKN production (Table 5.3). The diversity index of this subset was slightly greater (0.60) than the whole collection (0.48) (Fig. 5.2 A). This set encompassed 100% (262 alleles) of all the alleles that presented in the whole collection (Fig. 5.2 B).

When accessions were randomly removed one by one, the total number of alleles and Nei diversity index continually decreased (Fig. 5.3). Reducing the number of accessions in the core set from 30 to 25 could reduce the number of alleles from 262 to 238 and the Nei diversity index from 0.60 to 0.55 (Fig. 5.3).

To confirm if an accession was chosen from each different genetic group, a dendrogram was constructed, and then the core accession was tracked on it (Fig. 5.4). The members of the core set are distributed in every subgroup in the dendrogram. The distribution of some accessions of the core set covered provinces where Khao Kai Noi is primarily grown (Fig. 5.5).

5.3.2. Discussion

The core collection consists of a limited set of accessions derived from an existing germplasm collection, chosen to represent the genetic spectrum in the whole collection and should include as much as possible of its genetic diversity (Brown, 1995). In this study, 30 accessions from 109 (28%) of the whole collection were chosen as the core collection of KKN. This set contains all the alleles detected in the original collection. The percentage of the core set in this study does not conform with the recommended percentage mentioned by Frankel and Brown (1984), who indicated that 5% should be in a large and 10% in a small collection. The core accessions captured 100% of the total alleles, and the diversity index was slightly greater than the whole collection from which they were chosen (Fig. 5.2 A and B). This result is similar to that of the core collection of chili germplasm in Thailand (Mongkolporn et al., 2015). This indicates that core collection was completed appropriately. The total number of alleles and diversity index changed when some accessions were removed from the core set (Fig. 5.3). Therefore, the number of accessions in the core set of KKN should not be reduced. Observing their distribution through the dendrogram,

some paired accessions appear to be closely related; for example, R07 and R40, LG13535 and LG14118, and LG2792 and LG2793 (Fig. 5.4). However, they represent different geographical positions and variants. R07 and R40 were collected from different districts in a particular province and at 956 m and 416 m above sea level respectively (Table 5.3). Accession number LG13535 was named Khao Kai Noi, while LG14118 is called Khao Kai Noi Leuang. LG2792 is Khao Kai Noi Leuang, while LG2793 is Khao Kai Noi Deng (Table 5.3). Based on this supporting information, the core set was confirmed to be chosen appropriately and efficiently. It represents the whole range of KKN in the Lao National Genebank. This core set will therefore be useful for breeders, interested researchers, and genebank managers. More importantly, it will be helpful in determining diversity hotspots for on-farm conservation.

Table 5.1. Summary of the general procedures of core collection development from different authors

No.	Procedure description	Source
1	1) Defining the collection to be represented, assembling all the relevant data on the accessions in that collection, and deciding the size of the core 2) Grouping the accession into groups that reflect the major genetic and ecological categories within the whole collection 3) Choosing entries for the core—how many per group and which accessions 4) Managing the core set	Brown and Spillane, 1999
2	1) Data assembly 2) Grouping 3) Handling	Brown, 1995
3	1) Choose accessions from the whole collection 2) Collect molecular and morphological data 3) Choose a mini core set	Ebana et al., 2008
4	1) Choose the core from the whole collection 2) Analyze core set by using phenotypic trait and molecular markers 3) Choose a mini core set from the core based on phonotypic traits and molecular data	Agrama et al., 2009
5	1) Choose some from the whole collection 2) Analyze the chosen accession by microsatellite markers 3) Use software (PowerCore) to select core accession	Monkolporn et al., 2015

No.	Procedure description	Source
6	1) Define the domain 2) Divide the collection into distinct groups 3) Allocate entries over the groups 4) Choose entries	van Hintum, 1999
7	1) Identify the material (collection) that will be represented 2) Decide on the size of the core collection 3) Divide the set of materials used into distinct groups 4) Decide on the number of entries per group 5) Choose the entry from each group that will be included in the core	van Hintum et al., 2000

Table 5.2. Passport data of 109 accessions of Khao Kai Noi used as the original set for core collection development

No.	Acc. No.	Local name	Ec	En	Mt	Pv	Dt	Coordinates		
								Altitude (m)	Latitude (N)	Longitude (E)
1	LG5845	Khao Kai Noi	L	G	L	XK	XKH	N.A.	N.A.	N.A.
2	LG6493	Khao Kai Noi	U	G	E	LP	LPL	500	20.2236	102.1949
3	LG6795	Khao Kai Noi	L	G	L	XK	XKT	1000	19.3126	103.1290
4	LG7488	Khao Kai Noi	L	G	M	VM	VMN	N.A.	N.A.	N.A.
5	LG9212	Khao Kai Noi	U	G	E	LP	LPB	N.A.	N.A.	N.A.
6	LG10035	Khao Kai Noi	U	G	M	VM	VMN	N.A.	N.A.	N.A.
7	LG10195	Khao Kai Noi	U	G	M	XS	Longsan	N.A.	N.A.	N.A.
8	LG10898	Khao Kai Noi	L	G	E	XS	Longsan	N.A.	N.A.	N.A.
9	LG12360	Khao Kai Noi	L	G	M	XK	XKP	N.A.	N.A.	N.A.
10	LG12584	Khao Kai Noi	L	G	M	XK	XKM	N.A.	N.A.	N.A.
11	LG12923	Khao Kai Noi	L	G	L	XK	XKN	N.A.	N.A.	N.A.
12	LG13251	Khao Kai Noi	U	G	E	BK	Veingthong	N.A.	N.A.	N.A.
13	LG13480	Khao Kai Noi	L	G	M	BK	Bolikhan	N.A.	N.A.	N.A.
14	LG13535	Khao Kai Noi	L	G	L	CS	CSB	N.A.	N.A.	N.A.
15	LG13771	Khao Kai Noi	L	G	L	LN	Viengphoukha	N.A.	N.A.	N.A.
16	LG13970	Khao Kai Noi	L	G	L	XK	XKT	N.A.	N.A.	N.A.

No.	Acc. No.	Local name	Ec	En	Mt	Pv	Dt	Coordinates		
								Altitude (m)	Latitude (N)	Longitude (E)
17	LG2755	Khao Kai Noi Dam	L	G	L	HP	Sam Neua	N.A.	N.A.	N.A.
18	LG10133	Khao Kai Noi Dam	L	G	L	XK	XKT	N.A.	N.A.	N.A.
19	LG14112	Khao Kai Noi Dam Lai	L	G	L	XK	Khoun	1118	19.3624	103.2845
20	LG14024	Khao Kai Noi Dam Mihang	L	G	L	XK	XKX	1020	N.A.	N.A.
21	LG2793	Khao Kai Noi Deng	L	G	L	HP	Sam Neua	N.A.	N.A.	N.A.
22	LG6644	Khao Kai Noi Deng	L	G	L	HP	Sam Neua	1400	20.2418	104.2824
23	LG6762	Khao Kai Noi Deng	L	G	L	XK	XKP	1150	19.2808	103.9665
24	LG14018	Khao Kai Noi Deng	L	G	L	XK	XKT	1050	N.A.	N.A.
25	LG14023	Khao Kai Noi Deng	L	G	L	XK	XKP	1000	N.A.	N.A.
26	LG14095	Khao Kai Noi Deng	L	G	M-L	XK	Phonsavan	1130	19.5147	103.3351
27	LG14113	Khao Kai Noi Deng	L		L	XK	Phoukoud	1087	19.6116	103.1119
28	LG14126	Khao Kai Noi Deng	L	G	L	HP	CN	1070	20.2774	104.4402
29	LG6746	Khao Kai Noi Hai	U	G	M	HP	Sam Neua	1000	20.1872	104.1561
30	LG14028	Khao Kai Noi Khao	L	G	L	XK	XKX	1020	N.A.	N.A.
31	LG14027	Khao Kai Noi Khao Mihang	L	G	L	XK	XKP	1000	N.A.	N.A.
32	LG14077	Khao Kai Noi Lai	L	G	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.

No.	Acc. No.	Local name	Ec	En	Mt	Pv	Dt	Coordinates		
								Altitude (m)	Latitude (N)	Longitude (E)
33	LG14016	Khao Kai Noi Lai Dam	L	G	L	XK	XKT	1050	N.A.	N.A.
34	LG14020	Khao Kai Noi Lai Dam	L	G	L	XK	XKT	1050	N.A.	N.A.
35	LG2790	Khao Kai Noi Lai	L	G	L	HP	Sam Neua	N.A.	N.A.	N.A.
36	LG2794	Khao Kai Noi Lai	L	G	L	HP	Sam Neua	N.A.	N.A.	N.A.
37	LG2841	Khao Kai Noi Lai	L	G	L	HP	Viengxai	N.A.	N.A.	N.A.
38	LG6665	Khao Kai Noi Lai	L	G	L	HP	Viengxai	900	20.2236	104.1735
39	LG6760	Khao Kai Noi Lai	L	G	L	XK	XKP	1150	19.2808	103.9665
40	LG6838	Khao Kai Noi Lai	L	G	L	XK	XKX	1100	19.2032	103.2217
41	LG10899	Khao Kai Noi Lai	L	G	E	XS	Longsan	N.A.	N.A.	N.A.
42	LG14110	Khao Kai Noi Lai	L	G	L	XK	Khoun	1089	19.3517	103.3276
43	LG14124	Khao Kai Noi Lai	L	G	L	HP	CN	988	20.4756	103.9967
44	LG2746	Khao Kai Noi Leuang	L	G	L	HP	Sam Neua	N.A.	N.A.	N.A.
45	LG2792	Khao Kai Noi Leuang	L	G	L	HP	Sam Neua	N.A.	N.A.	N.A.
46	LG2806	Khao Kai Noi Leuang	L	G	L	HP	Sam Neua	N.A.	N.A.	N.A.
47	LG6732	Khao Kai Noi Leuang	L	G	L	HP	Viengxai	600	20.1599	104.3119
48	LG14017	Khao Kai Noi Leuang	L	G	L	XK	XKT	1050	N.A.	N.A.

No.	Acc. No.	Local name	Ec	En	Mt	Pv	Dt	Coordinates		
								Altitude (m)	Latitude (N)	Longitude (E)
49	LG14021	Khao Kai Noi Leuang	L	G	L	XK	XKT	1050	N.A.	N.A.
50	LG14022	Khao Kai Noi Leuang	L	G	L	XK	XKT	1100	N.A.	N.A.
51	LG14026	Khao Kai Noi Leuang	L	G	L	XK	XKP	1000	N.A.	N.A.
52	LG14029	Khao Kai Noi Leuang	L	G	L	XK	XKH	1160	N.A.	N.A.
53	LG14030	Khao Kai Noi Leuang	L	G	L	XK	XKH	1000	N.A.	N.A.
54	LG14031	Khao Kai Noi Leuang	L	G	L	XK	XKH	1001	N.A.	N.A.
55	LG14033	Khao Kai Noi Leuang	L	G	L	XK	XKH	1000	N.A.	N.A.
56	LG14043	Khao Kai Noi Leuang	L	G	L	XK	N.A.	N.A.	N.A.	N.A.
57	LG14076	Khao Kai Noi Leuang	L	G	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
58	LG14103	Khao Kai Noi Leuang	L	G	L	XK	PhX	1100	19.2993	103.1145
59	LG14107	Khao Kai Noi Leuang	L	G	L	XK	PhX	1105	19.3235	103.1798
60	LG14109	Khao Kai Noi Leuang	L	G	L	XK	Khoun	1088	19.3246	103.3761
61	LG14114	Khao Kai Noi Leuang	N.A.	N.A.	N.A.	XK	Phoukoud	1093	19.6037	103.1083
62	LG14115	Khao Kai Noi Leuang	L	G	L	XK	Phoukoud	1046	19.5240	103.7093
63	LG14116	Khao Kai Noi Leuang	L	G	L	HP	Viengxai	698	20.5670	104.2834
64	LG14117	Khao Kai Noi Leuang	L		L	HP	Viengxai	695	20.3676	104.2933
65	LG14118	Khao Kai Noi Leuang	L	G	L	HP	Viengxai	663	20.3434	104.3420

No.	Acc. No.	Local name	Ec	En	Mt	Pv	Dt	Coordinates		
								Altitude (m)	Latitude (N)	Longitude (E)
66	LG14120	Khao Kai Noi Leuang	L	G	L	HP	Viengxai	893	20.4232	104.2303
67	LG14121	Khao Kai Noi Leuang	L	G	L	HP	CN	1016	20.4345	104.8200
68	LG14122	Khao Kai Noi Leuang	L	G	L	HP	CN	1002	20.4669	103.9727
69	LG14123	Khao Kai Noi Leuang	L	G	L	HP	CN	988	20.4756	103.9967
70	LG14125	Khao Kai Noi Leuang	L	G	L	HP	CN	938	20.4241	104.3996
71	R01	Khao Kai Noi Dam	L	G	N.A.	XK	Khoun	1087	19.2106	103.1940
72	R02	Khao Kai Noi Leuang	L	G	N.A.	XK	Khoun	1087	19.2106	103.1940
73	R03	Khao Kai Noi Deng	L	G	N.A.	XK	Phoukoud	1073	19.3805	103.0760
74	R04	Khao Kai Noi Leuang	L	G	N.A.	XK	Phoukoud	1073	19.3805	103.0760
75	R06	Khao Kai Noi Leuang	L	G	N.A.	HP	Sam Neua	1195	20.1415	104.0130
76	R07	Khao Kai Noi Leuang	L	G	N.A.	HP	Sam Neua	956	20.2538	104.0230
77	R08	Khao Kai Noi Leuang	L	G	N.A.	HP	Sam Neua	956	20.2538	104.0230
78	R09	Khao Kai Noi Leuang	L	G	N.A.	HP	Viengxai	855	20.2815	104.0906
79	R10	Khao Kai Noi Lai	L	G	N.A.	HP	Sop Bao	230	20.3341	104.2660
80	R11	Khao Kai Noi Deng	L	G	N.A.	HP	Xiengkhor	261	20.4711	104.0923
81	R16	Khao Kai Noi Deng	L	G	N.A.	HP	Et	260	20.4807	104.0021
82	R19	Khao Kai Noi Khao	L	G	N.A.	HP	Et	708	20.4235	104.0122

No.	Acc. No.	Local name	Ec	En	Mt	Pv	Dt	Coordinates		
								Altitude (m)	Latitude (N)	Longitude (E)
83	R28	Khao Kai Noi Leuang	L	G	N.A.	HP	Sam Neua	916	20.3918	104.0439
84	R29	Khao Kai Noi	L	G	N.A.	HP	Sam Neua	1042	20.3708	104.0437
85	R33	Khao Kai Noi Lai	L	G	N.A.	HP	Sam Tai	648	19.5559	104.3744
86	R34	Khao Kai Noi Deng	L	G	N.A.	HP	Sam Tai	676	19.5545	104.3654
87	R35	Khao Kai Noi Khao	L	G	N.A.	HP	Sam Tai	684	19.5623	104.3725
88	R36	Khao Kai Noi Deng	L	G	N.A.	HP	Sam Tai	684	19.5623	104.3725
89	R40	Khao Kai Noi Leuang	L	G	N.A.	HP	Viengxai	416	20.1557	104.3146
90	R43	Khao Kai Noi Leuang	L	G	N.A.	HP	Sam Neua	765	20.1245	104.1021
91	R44	Khao Kai Noi Leuang	L	G	N.A.	HP	Sam Neua	1122	20.1637	104.0238
92	R45	Khao Kai Noi Deng	L	G	N.A.	HP	Houameuang	562	20.0333	103.3871
93	R47	Khao Kai Noi Deng	L	G	N.A.	HP	Viengthong	676	20.0341	103.2209
94	R53	Khao Kai Noi Deng	L	G	N.A.	HP	Houameuang	532	20.0131	103.4256
95	R54	Khao Kai Noi Khao	L	G	N.A.	XK	Kham	597	19.3714	103.3405
96	R56	Khao Kai Noi	L	G	N.A.	XK	Nonghet	1187	19.3055	103.5123
97	R59	Khao Kai Noi Deng	L	G	N.A.	XK	Phonsavan	1084	19.2504	103.1030
98	R62	Khao Kai Noi (met noi)	L	G	N.A.	XK	Phoukoud	817	19.2905	102.4322
99	R63	Khao Kai Noi Khao	L	G	N.A.	XK	Phoukoud	1132	19.2905	102.5145

No.	Acc. No.	Local name	Ec	En	Mt	Pv	Dt	Coordinates		
								Altitude (m)	Latitude (N)	Longitude (E)
100	R64	Khao Kai Noi Deng	L	G	N.A.	XK	Phoukoud	1132	19.2905	102.5145
101	R65	Khao Kai Noi	L	G	N.A.	XK	Phoukoud	1040	19.3106	103.0150
102	R71	Khao Kai Noi Deng	L	G	N.A.	XK	Kham	874	19.4329	103.2743
103	R72	Khao Kai Noi Khao	L	G	N.A.	XK	Kham	874	19.4329	103.2743
104	R74	Khao Kai Noi Lai	L	G	N.A.	XK	Kham	587	19.3947	103.3507
105	R75	Khao Kai Noi Khao	L	G	N.A.	XK	Kham	587	19.3947	103.3507
106	R76	Khao Kai Noi Deng	L	G	N.A.	XK	Kham	587	19.3947	103.3507
107	R77	Khao Kai Noi Deng	L	G	N.A.	XK	Phonsavan	1127	19.3328	103.2244
108	R87	Khao Kai Noi Leuang	L	G	N.A.	XK	Khoun	1086	19.1652	103.2129
109	R88	Khao Kai Noi Khao	L	G	N.A.	XS	Thathom	347	19.0135	103.2721

L: lowland rice, G: glutinous rice, XK: Xiengkhouang, HP: Houaphan, XS: Xaisomboun, (N): north, (E): east

Table 5.3. List of 30 core accessions of Khao Kai Noi developed from this study

No.	Acc. No.	Local name (modified)[1]	Radom removing order	Local name (original spelling in Lao genebank)	Ec	En	Mt	Pv	Dt	Alt (m)
1	LG13535	Khao Kai Noi		Khao Kay Noi	L	G	L	CS	CSB	N.A.
2	LG6493	Khao Kai Noi		Khao Kay Noi	U	G	E	LP	LPL	500
3	LG9212	Khao Kai Noi		Khao Kay Noi	U	G	E	LP	LPB	N.A.
4	LG10035	Khao Kai Noi		Khao Kay Noi	U	G	M	VM	VMN	N.A.
5	LG5845	Khao Kai Noi	5th	Khao Kay Noi	L	G	L	XK	XKH	N.A.
6	LG12923	Khao Kai Noi		Khao Kay Noi	L	G	L	XK	XKN	N.A.
7	LG10898	Khao Kai Noi		Khao Kay Noi	L	G	E	XS	XSL	N.A.
8	LG6644	Khao Kai Noi Deng	1st	Khao Kay Noi Deng	L	G	L	HP	HPS	1400
9	LG2793	Khao Kai Noi Deng		Khao Kay Noi Deng	L	G	L	HP	HPS	N.A.
10	LG14023	Khao Kai Noi Deng		Khao Kay Noi Deng	L	G	L	XK	XKP	1000
11	LG14095	Khao Kai Noi Deng		Khao Kay Noi Deng	L	G	M-L	XK	P	1130
12	LG6746	Khao Kai Noi Hay	3rd	Khao Kay Noi Hay	U	G	M	HP	HPS	1000
13	R75	Khao Kai Noi Khao		Khao Kai Noi Khao	L	G	L	XK	Kham	587

No.	Acc. No.	Local name (modified)[1]	Radom removing order	Local name (original spelling in Lao genebank)	Ec	En	Mt	Pv	Dt	Alt (m)
14	R88	Khao Kai Noi Khao		Khao Kai Noi Khao	L	G	L	XS	Thathom	347
15	LG6665	Khao Kai Noi Lai		Khao Kay Noi Lay	L	G	L	HP	HPV	900
16	LG2790	Khao Kai Noi Lai		Khao Kay Noi Lay	L	G	L	HP	HPS	N.A.
17	LG2841	Khao Kai Noi Lai	4th	Khao Kay Noi Lay	L	G	L	HP	HPV	N.A.
18	R74	Khao Kai Noi Lai	2nd	Khao Kai Noi Lai	L	G	L	XK	Kham	587
19	LG6838	Khao Kai Noi Lai		Khao Kay Noi Lay	L	G	L	XK	XKX	1100
20	LG14020	Khao Kai Noi Lai Dam		Khao Kay Noi Lai Dam	L	G	L	XK	XKT	1050
21	R40	Khao Kai Noi Leuang		Khao Kai Noi Leuang	L	G	L	HP	VX	416
22	LG14118	Khao Kai Noi Leuang		Khao Kay Noi leuang	L	G	L	HP	VX	663
23	LG14117	Khao Kai Noi Leuang		Khao Kay Noi leuang	L		L	HP	VX	695
24	LG14116	Khao Kai Noi Leuang		Khao Kay Noi Leuang	L	G	L	HP	VX	698
25	R07	Khao Kai Noi Leuang		Khao Kai Noi Leuang	L	G	L	HP	Sam Neua	956
26	LG2792	Khao Kai Noi Leuang		Khao Kay Noi Leuang	L	G	L	HP	HPS	N.A.

No.	Acc. No.	Local name (modified)[1]	Radom removing order	Local name (original spelling in Lao genebank)	Ec	En	Mt	Pv	Dt	Alt (m)
27	LG2806	Khao Kai Noi Leuang		Khao Kay Noi Leuang	L	G	L	HP	HPS	N.A.
28	LG14021	Khao Kai Noi Leuang		Khao Kay Noi Leuang	L	G	L	XK	XKT	1050
29	R02	Khao Kai Noi Leuang		Khao Kai Noi Leuang	L	G	L	XK	Khoun	1087
30	LG14109	Khao Kai Noi Leuang		Khao Kay Noi Leuang	L	G	L	XK	Khoun	1088

[1] Local name modified is respelled for standardization without changing the meaning of the local name spelled in the original form of the Lao National Genebank.

No.: number, Acc. No.: accession number, Ec: ecosystem, En: endosperm type, Mt: maturity, Pv: province, Dt: district, Alt: altitude

Column Ec: L: lowland, U: upland; Column Mt: E: early, M: medium, L: late; Column Pv: CS: Chanpasak, LP: Luang Prabang, HP:Houaphan, XK:Xiengkhouang, XS: Xaysomboun.

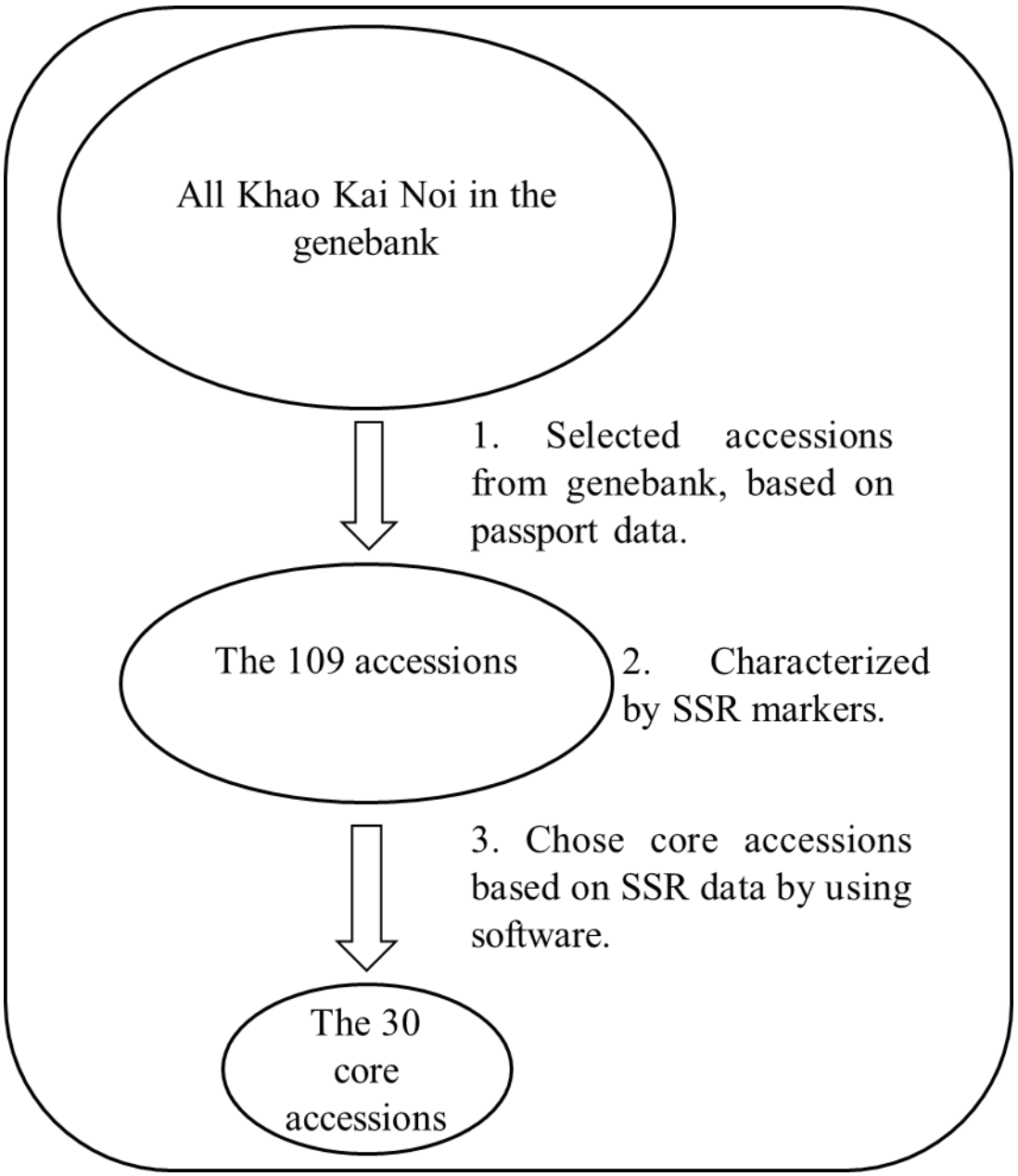
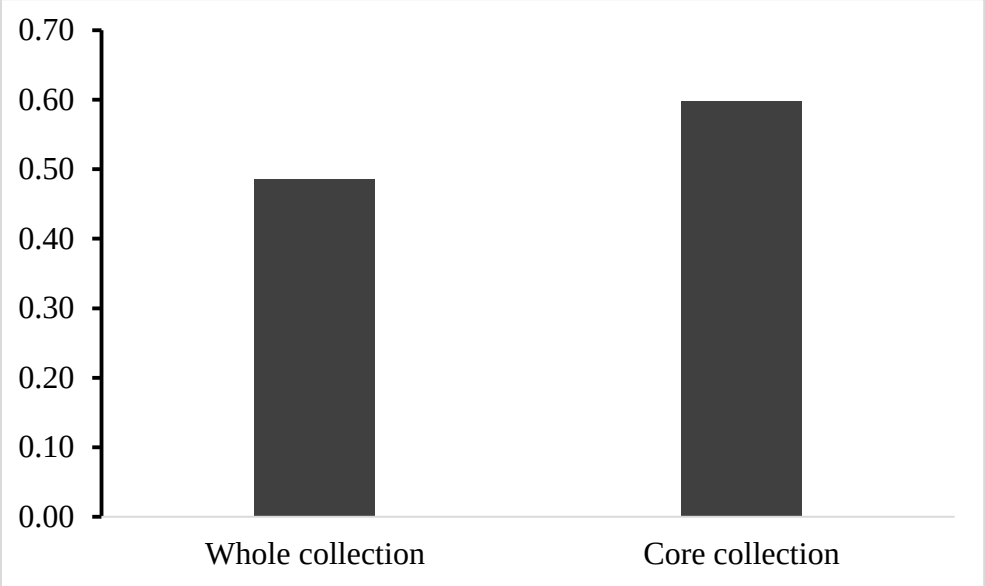


Figure 5.1. Khao Kai Noi core collection development procedure

(A)



(B)

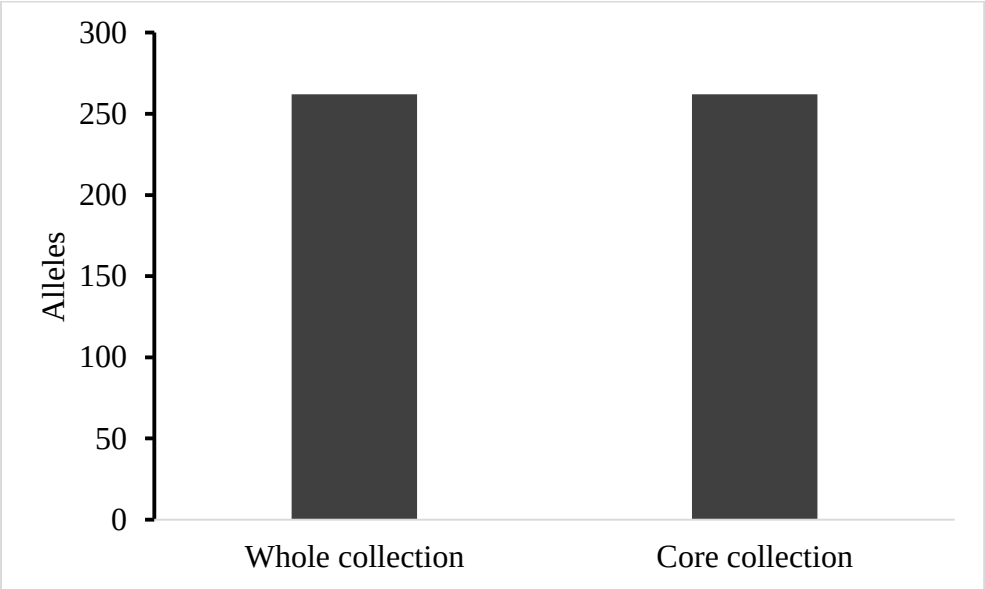


Figure 5.2. Statistical comparison of the core and entire collection of Khao Kai Noi (A) The Nei diversity index of the whole collection and core collection (B) The total of alleles in the whole collection and core collection

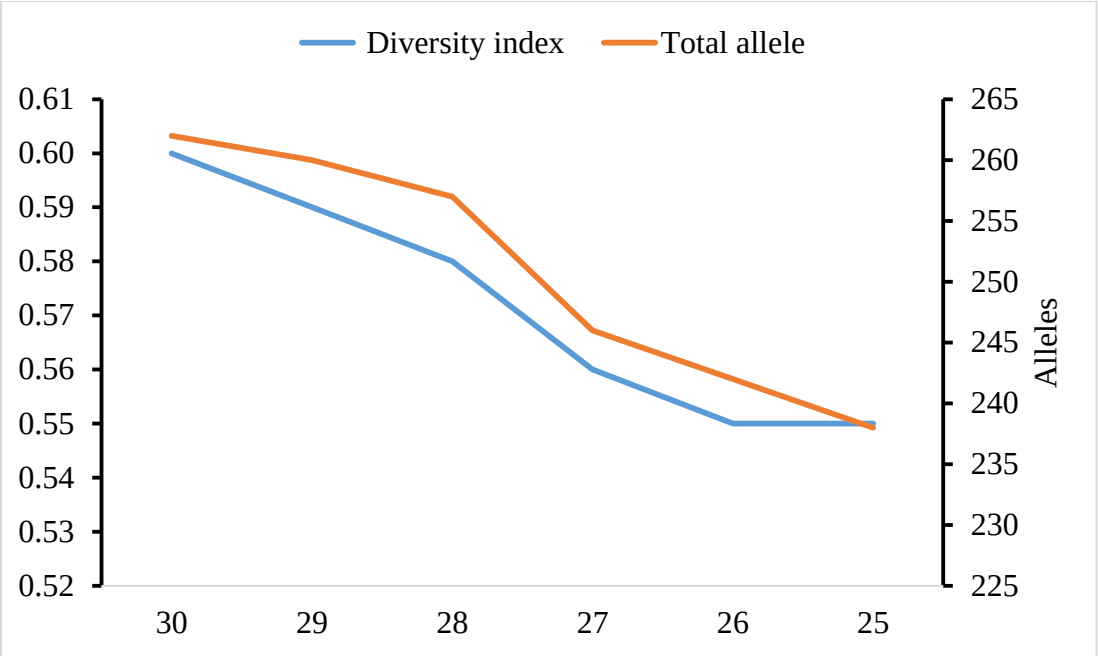


Figure 5.3. Total number of alleles and Nei diversity index of different numbers of accessions in the core set of Khao Kai Noi

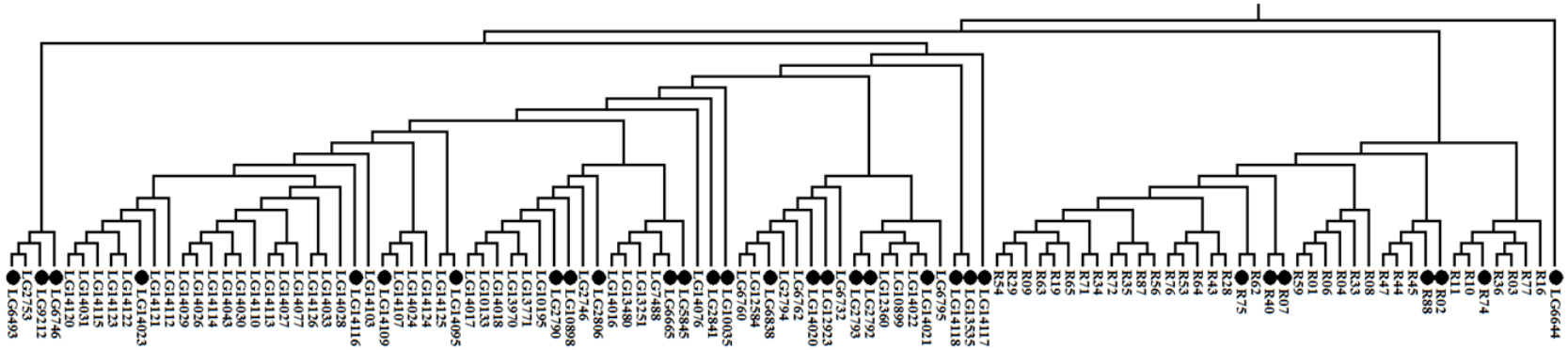


Figure 5.4. UPGMA dendrogram showing the relatedness of 109 accessions of Khao Kai Noi. The black circles represent 30 core accessions

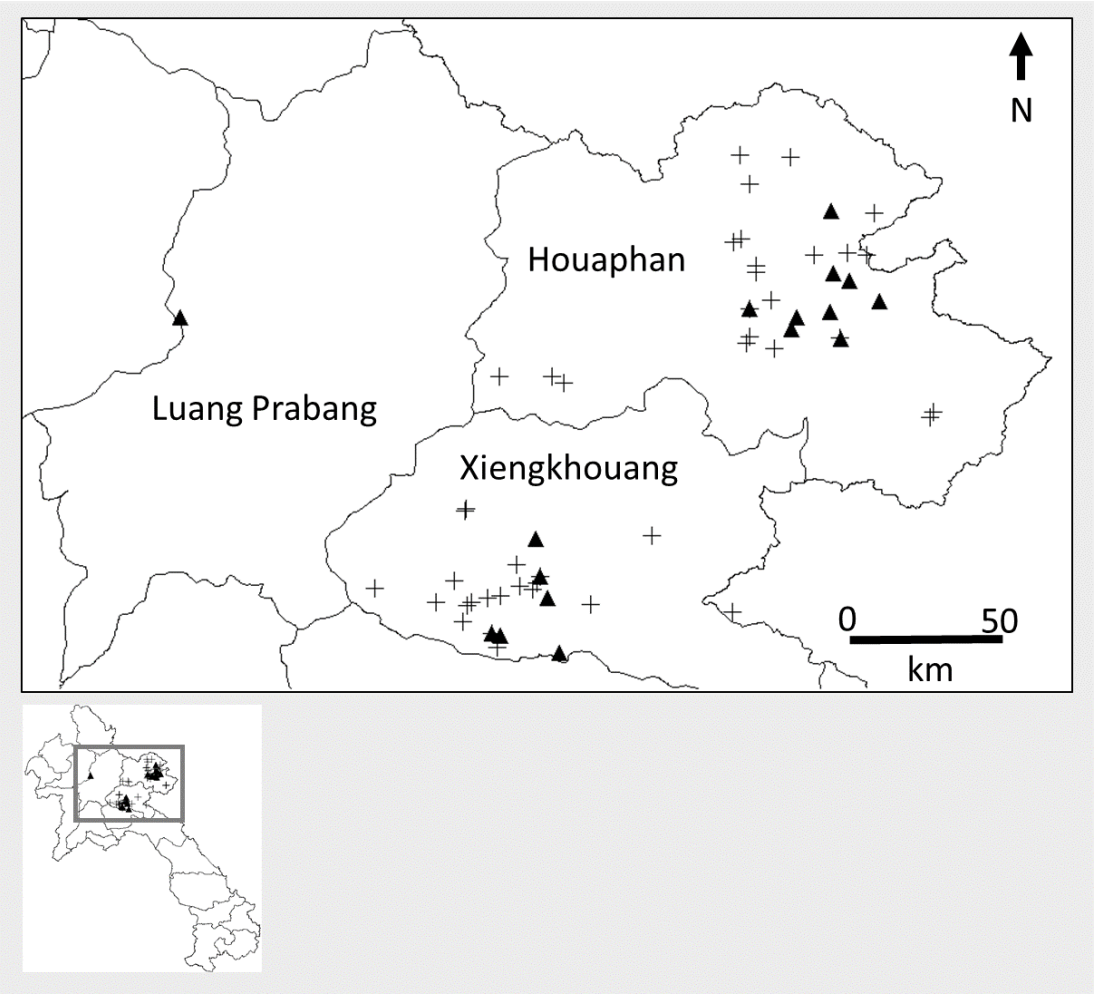


Figure 5.5. Map of Laos, with a focus on Luang Prabang, Xiengkhouang, and Houaphan Provinces, showing the distribution of 67 of 109 accessions of Khao Kai Noi used for collection development. The black triangles represent core accessions. The map was created by using DIVA-GIS v7.5 software (Hijmans et al., 2001)

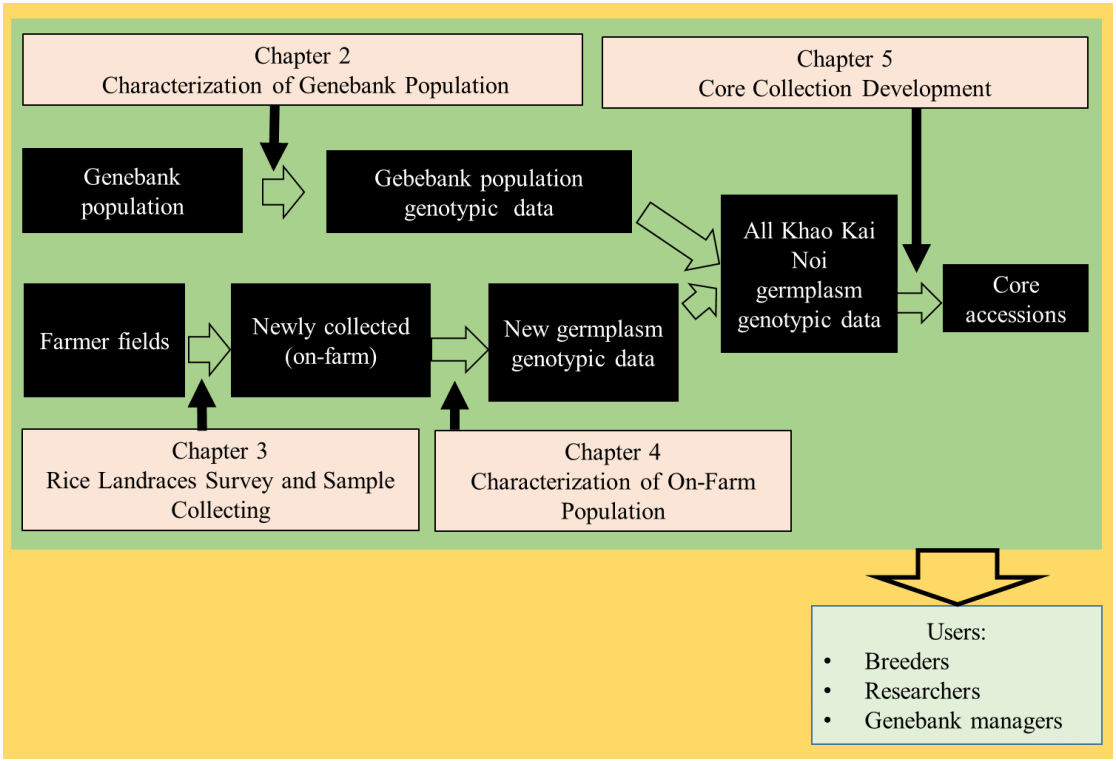


Figure 5.6. Relationships among chapters in the research

5.4 Overall conclusions

KKN is a one important rice landrace because it is good eating quality and economical value. Its genetic diversity has not been reported yet. This study, Chapter 2, Chapter 3, and Chapter 4 reported regarding characterization of KKN genebank population, rice landraces survey and sample collection and KKN on-farm population characterization, respectively. The relatedness among chapters was showed in Fig.-5.6.

5.4.1 Diversity in Khao Kai Noi

KKN genebank population diversity was high. The genetic differentiation among their name subgroup was significant, however genetic differentiation was non-significant between Xiengkhouang and Houaphan Provinces, where there were collected. Most of them were temperate Japonica-type, few were tropical Japonica-type and only one accession was Indica-type. There also had genetically relativeness to landrace of Vietnam, neighboring country.

Rice landraces survey and sample collection in Xiengkhouang and Houaphan Province was performed in 2014 in Laos. I went to every district of these two provinces, collected 60 samples of cultivated rice landrace landraces. Most of them were recorded as KKN and rest were other landraces. Theses samples were then stored in the Lao national genebank for further characterization and utilization.

KKN samples collected in 2014, termed “on-farm population” were characterized by using SSR markers, same set of those were used with genebank

population. The genetic diversity was also high. This population of KKN was temperate Japonica-type. They could be grouped into 3 clusters which it corresponding to its grain color.

5.4.2 Core collection of Khao Kai Noi

Khao Kai Noi collection in Laos's national genebank is about 200 accessions at present, and recognizing availability of the materials for the research, 109 accessions were studied. Of them, 30 accessions chosen to be a proposed core set, which will be useful for users such as breeders and researchers.

5.5 Recommendation

Genebank and germplasm utilization:

The total about 200 accessions of KKN in the Lao national genebank can be conserved all in the base collection. The 30 of them, which chosen for core set can be stored in active collection, because they can be easy to access by users such as breeders, interested researchers and genebank curators.

On-farm conservation promotion:

Base on the distribution of diversity that covers Phonsavan, Kham, and Khoun District of Xiengkhouang Provinces and Sam Neua and Viengxai District of Houaphan Province. Therefore, the promotion of on-farm conservation should be established in mentioned areas.

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List of Publications

Vilayheuang, K., Machida-Hirano, R., Bounphanousay, C., & Watanabe, N. K. (accepted Nov 3rd, 2015) Genetic diversity and population structure of 'Khao Kai Noi', a Lao rice (*Oryza sativa* L.) landrace, revealed by microsatellite DNA markers. *Breeding Science*.

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Yamamoto, S., Vilayheuang, K., & Okuizumi, H. (in press) A market survey of commercial crop and utilized plants in Xiengkhouang and Houaphan Provinces in northern Laos, 2014. *Annual Report an Exploration and Introduction of Plant Genetic*

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List of Poster and Oral Presentations

Vilayheuang, K., Machida-Hirano, R., Bounphanousay, C., & Watanabe, N. K. (2014) Genetic variation of Khao Kai Noi, Lao rice landrace, (*Oryza sativa* L.), examined by simple sequence repeat markers. *The 126th Meeting of the Japanese Society of Breeding*, Minami Kyushu University, Miyakonojo Campus, Miyakonojo City, Miyazaki Prefecture, Sep 27, 2014 (*Poster presentation*).

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