Diversification and Differentiation of Indonesian Rice as Revealed by Morphological Traits and DNA Markers

April 2017

Muhamad KADAPI

Diversification and Differentiation of Indonesian Rice as Revealed by Morphological Traits and DNA Markers

A Dissertation Submitted to
the Graduate School of Life and Environmental Sciences,
the University of Tsukuba
in Partial Fulfillment of the Requirements
for Degree of Doctoral of Philosophy in Agricultural Science
(Doctoral Program in Biosphere Resource Science and Technology)

Muhamad KADAPI

Contents

List of figures

List of Tables

1. General Introduction

1.1 Ancestor of *Oryza sativa* L.

Oryza sativa was domesticated from its progenitor *O. rufipogon* and differentiated into two subspecies groups, *indica* and *japonica* (Morishima et al., 1963; Oka, 1974; Zhu et al., 2007). On the other hand, Chang (1976) reported that *O. sativa* was divided into three groups, *indica*, *javanica* and *japonica*. And it probably occurred between 8,000 and 10,000 years ago (Diamond et al., 2003; Normile, 2004).

There are two hypotheses on how rice has been domesticated. Phylogenetic data revealed that there were genetic differences between *indica* and *japonica*, suggesting multiple domestication events which came from different populations of *O. rufipogon* (Second 1982; Londo et al. 2006; Sweeney and McCouch 2007). However, recent studies using single nucleotide polymorphisms (SNP) markers suggested that the two subspecies originated from a single population of *O. rufipogon* (Molina et al. 2011; Huang et al., 2012).

Dispersal and characteristics of *Oryza rufipogon* and *O. sativa*

Oryza rufipogon grows in tropical Asia (southern China, South and Southeast Asia, and Papua New Guinea) and the north part of Australia, (Vaughan, 1994). It was no surprise that some researchers believed that ancient *O. sativa* came from South China and the mainland of Southeast Asia (Li, 1970; Tanaka, 1995; Londo et al., 2006). Konishi et al. (2006) suggested that *japonica* originated from southern part of Indochina, Indonesia or the Philippines.

Oryza rufipogon has characteristics such as perennial type, photoperiod sensitivity and deep water habitat. The other characteristics of this wild species are tufted and scrambling herb with nodal tillering, spikelets usually 8-9 mm, anther usually >3 mm, reaching 7 mm or more; awn usually 6-10 cm long. It is diploid (2*n*=24) with AA genome (Vaughan, 1994; OECD, 1999).

Oryza sativa became an important crop as staple food for more than half the population in the world (OECD, 1999; UNCTAD, 2011) due to the adaptability to different environmental conditions in nature and human efforts such as immigration and selection. It grows at various altitudes in tropical and temperate climate conditions (latitude) (Chang, 1976). Four major ecosystems of rice are generally recognized as follows: irrigated, rain-fed lowland, upland and flood-prone (Khush, 1984). In the terms of soil condition, rice can grow in a wide range of soil types including saline, alkaline and acid-sulfur soils (Oka, 1988;

OECD, 1999). And characteristics of *O. sativa* could be divided based on three traditional groups (Vaughan, 1994; OECD, 1999):

1. *Indica* varieties with usually slender, awnless grains, light green leaves and many tillers;
2. Temperate *japonica* varieties with usually roundish pubescent grains, dark green leaves and few tillers;
3. Tropical *japonica* (*javanica*) with usually large, rounded, awned, pubescent spikelets; low shattering and few tillers.

Classification of *Oryza sativa*

Glaszmann (1987) classified Asian cultivated rice into 6 groups and showed that *javanica* group (bulu and gundil types) were involved in *japonica* group and are now named as tropical *japonica* based on isozyme polymorphism. Among these six groups, group I, II, III, IV and V belonged to the *indica* and group VI belonged to *japonica* consisting temperate and tropical *japonica* in comparison with conventional groups.

Group II corresponds to *aus* which is characterized by very early maturing and drought-tolerance, and the *aus* varieties are growing in March–June in Bangladesh and West Bengal state in India. Floating rice in Bangladesh and India called Ashinas and Rayadas belongs to Groups III and IV, respectively. Group V consists of aromatic rice in the Indian subcontinent.

Selection for desirable genes on domestication

During domestication, some genes were selected naturally and artificially including *qsh1* and *sh4* genes, which are genes controlling the level of seed shattering (Konishi et al., 2006; Lin et al., 2007), *wx* gene controlling amylose synthesis in endosperm and pollens (Sano et al., 1986; Olsen et al., 2006), grain size (Shomura et al., 2008) and *Hd1* and *GHD7* genes for heading date (Yano et al., 2000; Xue et al., 2008). The selection of these genes during domestication was not recorded on history, but it reveals in the DNA changes of the crop by the molecular markers in the recent century (Sweeney and McCouch, 2007; Izawa et al., 2009).

1.2 Landrace in Indonesian rice germplasm

There was limited study on history of Indonesian landrace as well as puzzle on revealing origin of rice itself. However, Tanaka (1998) revealed some possibilities for the origin of

Southeast Asian rice including Indonesia based on archeological study. He suggested that Indonesian rice is probably one of ancient Southest Asian rice as well as rice from Southwest China. Refer to old classification of rice; he also suggested that Indonesian *indica* (tjereh) was introduced by Indian when there has been cultivated *javanica* (bulu and gundil).

Commonly, traditional rice in Indonesia is cultivated in upland and highland by swidden farming system (Marten 1990; Tanaka 1995; Iskandar and Ellen 1999). Iskandar and Ellen (1999) reported that cultural practices of tribe in Java Island as representative tribe in Indonesia served to maintain diversity in traditional rice landrace because of a traditional system, a religious obligation and a form of cultural identity. Rice landrace is mainly used for performing rituals and in the custom accompanying them. For example, glutinous (pare ketan) landrace is generally superior for culinary purpose because cooked glutinous rice has a pleasant smell and stickiness. It is predominantly used for making traditional cakes and is consumed in rituals and ceremonies. Thomson et al. (2007) using SSR markers clarified that genetic diversity of Indonesian rice has been maintained. Glutinous rice is commonly classified as tropical *japonica* group as reported by Thomson et al (2009) using glutinous landrace in Kalimantan, Indonesia.

1.3. History of rice breeding in Indonesia

International Rice Research Institute (IRRI) was established in 1960 in cooperation with the Government of the Philippines to improve rice production and productivity. In 1966 IR8 was released as the first variety with excellent yield potential in the tropics (Khush, 1997). A recessive semi-dwarf1 gene (*sd1*) was used to develop IR8 (Sasaki et al., 2002). This variety was introduced to many Asian countries and it was used for developing new cultivars. Indonesia is one of Asian countries that have collaborative research projects with IRRI to improve Indonesian varieties. Rice breeding history in Indonesia is divided into three stages according to this collaboration (Susanto et al., 2003):

(1) The first stage (1943-1969); Varieties at this stage were bred using Cina variety, originally from China, Latisail variety from India and Benoang variety from Indonesia (before collaboration with IRRI).

(2) The second stage (1969-1985); In this stage, Indonesia released two famous varieties, PB5 in 1968 and PB8 in 1967 and PB5 related varieties, Pelita I-1 and Pelita I-2. Several collaborative programs with IRRI were performed as activities in the International Network for the Genetic Evaluation of Rice (INGER), International Rice

Tungro Nursery (IRTN), International Rice Blast Nursery (IRBN), International Rice Brown Planthopper Nursery (IRBPHN), and International Rice Bacterial Blight Nursery (IRBBN).

(3) The third stage (1986 ~ presently); A representative variety in this stage was IR64 as one of parents in developing new varieties, which possessed a wider genetic background than IR8 and had genes for resistance to insects and diseases.

In fact, most of the varieties which have been released in Indonesia during 1969 to presently are the result from crosses among the varieties which are closely relative. Besides, the IRRI varieties have been extensively used as breeding materials to breed new varieties (Susanto et al., 2003). Hence, these programs may give negative effect on genetic diversity of Indonesian rice germplasm. The continuous use of elite varieties in breeding caused the stagnation of rice yield, due to genetic uniformity (Tanksley and McCouch, 1997). They also argued that many beneficial alleles have been left due to the bottleneck effect during domestication and selection in modern breeding in the recent century

1.4 Objectives

Indonesia is an archipelagic country that comprises five main islands: Sumatra, Java, Kalimantan, Sulawesi and Irian Jaya (now Papua), two major archipelagos Nusa Tenggara and the Maluku Islands, and 50 smaller archipelagos (FRD, 2011). This condition is ideal to reveal micro-evolutionary forces in the differentiation of Indonesian rice germplasm. Understanding the source of diversity for adaptation could give information on the factors required to maintain genetic diversity in natural populations (Barton and Keightley, 2002).

The distribution of genetic diversity in populations is strongly affected by micro-evolutionary forces such as gene flow, selection, phylogenetic history of populations (Schaal et al., 2003) and mutation (Fisher, 1930). In diversity study of rice germplasm, some researchers compared the diversity of rice (*O. sativa*) with that of wild relatives (*O. rufipogon*), and they concluded that the bottleneck effect was the reason why caused the loss of diversity in *O. rufipogon* (Sun et al., 2001; Zhu et al., 2007). In particular, Sun et al. (2001) used Indonesian wild relatives and reported that *O. rufipogon* from the archipelago of Indonesia and the Philippines is still higher diversity than improved varieties from all Asian countries and it was due to bottleneck effect. On the other hand, the genetic diversity of this species was also affected by hybridization within and among species. Vaughan et al. (2008) suggested that the current genetic diversity of *O. sativa* was a product of gene flow within

and among species and artificial selection by farmers. The evidence of hybridization between this species was reported in Laos (Kuroda et al., 2005) and in China (Song et al., 2006). Furthermore, gene flow from *O. sativa* may reduce the genetic diversity of *O. rufipogon* and lead to population extinctions (Kiang et al., 1979).

The information on the mechanism how Indonesian rice was diversified and differentiated is currently limited. This study was carried out to answer the question "are there in Indonesian rice germplasm, mutation, hybridization through gene flow, natural selection and artificial selection?"

2. Diversity analysis of Indonesian rice landrace and improved varieties as revealed by morphological traits

2.1 Introduction

Selection is one of the ways to understand the genetic diversity that is a major source of varieties' improvement (Harlan, 1992). Selection for plant architecture was essential for rice domestication (Jin et al., 2008). Since 1966, the modification of plant architecture (semidwarf) has succeeded in increase of the yield potential in rice and wheat, for example, plants were selected for reduced plant height, increased number of tillers (shoot), and erect plant type instead of droopy leaves (IRRI, 2001). Rice plant architecture is mainly determined by related traits to plant height, leaf shape and yield traits (Yang and Hwa, 2008)

Within *O. sativa*, a wide range of morphological, ecological and physiological variation exists. As a result of selection for adaptations to different habitats and growing conditions across the globe, *O. sativa* includes about 120,000 different varieties, ranging from traditional varieties preserved by local farmers to modern varieties developed during the green revolution (Khush, 1997).

Diversity study in rice landrace in many countries using morphological traits reported that variation still exists: for example West Java landrace in Indonesia (Iskandar and Ellen 1999), Japanese upland rice in Brazil (do Nascimento et al., 2011); Pokhara valley landrace in Nepal (Tripathi et al., 2013); West Bengal landrace in India (Sinha and Mishra, 2013); and Vrihi landrace in India (Ray et al., 2013). Furthermore, in Vietnam, it was reported that the use of a morphological grouping could not provide convincing discriminatory evidence in the classification of rice (Fukuoka et al., 2006). It only provided a sort of minimum distance among groups of varieties; it was also reported in Philippines archipelago by Rabara et al. (2014). However, improved rice varieties in Indonesia was reported to derived from more than 2000 ancestors in the pedigree, IRRI cultivars such as IR8 and IR64 showed as the largest part of the genetic background (>50%) (Yoshida et al., 2009) and they could cause low diversity of rice as reported in Philippine rice (Caldo et al., 1996). Similar results were reported for improved varieties of Cuba (Fuentes et al. 2005), Africa (Ogunbayo et al. (2005), and India (Kunusoth et al. 2015).

According to the facts, in several countries, there has been a reduction in the diversity of improved rice varieties. The study of the genetic relationships among Indonesian improved rice varieties is important and, thus far, few studies have used morphological traits to

examine relationship between Indonesian rice landrace and improved varieties. This information could be used for future rice improvement, particularly in Indonesia.

2.2 Materials and methods

Plant materials and cultivation

A total of 200 Indonesian rice varieties consist of 100 landrace and 100 improved cultivars (Table 2. 1, 2. 2 and Figure 2. 1). The Indonesian varieties were chosen from the rice germplasm collections at the Indonesian Center for Rice Research (ICRR Subang, Indonesia).

The plant materials were grown at a paddy field at the University of Tsukuba from May to November in 2009 and 2010. The seeds were sown in a nursery box before 30-day-old seedlings were transplanted, with a 15 x 30 cm spacing, with 25 plants per variety. Seventy-eight of one hundred improved varieties were recorded at different growth stages according to the IRRI (1980) recommendations for *O. sativa* L. (1980) (Table 2. 3). Twenty-two of improved varieties and forty eight out of 100 landrace in 2009 and fifty two out of 100 landrace in 2010 could not reach to heading and they were discarded from the evaluation.

Statistical analysis

A statistical analysis of morphological data was conducted using two kinds of software: JMP 5.1 (JMP 2000) and Modicos software (Carvajal-Rodríguez, Rodríguez, 2005). JMP 5.1 was used to create clusters within improved varieties by Ward's hierarchical methods. Furthermore, this software was also used to observe the contribution of morphological traits with standardized data on clustering by principal component analysis (PCA) and bi-plot analysis. Besides, Levene's test, coefficient of variation (CV) and mean test were used (Tukey-Kramer HSD). Modicos software was used to calculate phenotypic divergence among different source locations of landrace (*Qst*), $Qst = V_{pop} / (V_{pop}+2V_{ind})$, in which V_{pop} was the variance among population and V_{ind} was the variance within population. We used data recorded in 2009 and 2010 as replication.

2.3 Results

The dendrogram of Ward's hirarchical in 2009 was divided overall into two clusters, in which the first cluster mostly consisted of landrace, and was divided into two sub-clusters. The second cluster was divided into two sub-clusters and the majority in this cluster was the improved variety (Figure 2. 2). The dendrogram in 2010 (Figure 2. 3) displayed a similar pattern to that of 2009. However, some varieties were misclassified between the two years

based on 130 varieties and 10 traits in 2009, and based on 126 varieties and 10 traits in 2010 (Figs. 2, 3). We found that some varieties released in the first stage (1943-1969) were characterized by consistent appearance together with landrace group, Bengawan, Dewi Ratih, Kartuna and Synta variety (Table 2.1).

The sum of three PCAs based on 10 traits of the 130 Indonesian varieties used in 2009 explained the total variation at 70.49 %, involving 42.59 % in PC1, 15.04 % in PC2 and 12.85 % in PC3 (Table 2. 4). Three traits showed high contributions and positive values on PC1: plant height, culm length and length of leaf. On PC2, two traits width of leaf and width of flag leaf showed positive and high contribution, while length of leaf, length of flag leaf and heading period showed high contribution and negative values. On PC3, high and positive values were shown for panicle length, width of leaf, width of flag leaf and heading period. The grouping based on ten morphological traits using ward hierarchical method analysis was shown in PCA, and the bi-plot analysis could divide into two groups. The characteristics of varieties in group B (improved varieties) were smaller in plant height, culm and panicle length, width of leaf and flag leaf. Group A, in which the majority was landrace showed characteristics of large size in leaf length, flag leaf, sheath leaf, angle of flag leaf and late flowering. Most of the IRRI varieties belong to group B (Figure 2. 4) in which indicated smaller size in both years.

A total of three PCs indicated that 10 traits using 126 Indonesian rice varieties in 2010 explained 78.19% of total variation in which PC1 is 54.49 %, PC2 is 14.76 % and PC3 is 8.46 %, as shown in Table 2. 5. The three traits that have high contribution and positive values on PC1 were plant height, culm length and length of sheath leaf. Meanwhile on PC2, the highest positive contributions were length of flag leaf and heading period while angle of flag leaf showed high contribution in negative value. On PC3, the highest contribution was panicle length. As in 2009, this PCA consisted of two groups based on ten morphological traits using ward hierarchical method analysis. Group B consisting of improved varieties as majority had characteristics of smaller size in plant height, panicle length, width of flag leaf and erectness of flag leaf, and early flowering, while the group A had longer size in plant height, panicle length, length of leaf, flag leaf, sheath leaf, greater width of leaf and flag, late flowering and a non-erect flag leaf and this group consisted of landrace as majority (Figure 2. 5).

Landrace indicated larger in size in six of 10 traits than improved varieties in 2009 (Table 2. 6). In particular, landrace showed higher mean values for plant height, culm length, length of leaf, length of sheath leaf and angle of flag leaf.. The highest significant coefficient

variation (CV) was observed in plant height, length of flag leaf and angle of flag leaf. In 2010, nine out of 10 traits showed significance in mean values between landrace and improved varieties (Table 2. 7), and indicated that landrace was larger in size and all of CV were higher than improved varieties, indicating significant difference between them in plant height, culm length, length of leaf, width of leaf, length of sheath leaf, length of flag leaf, width of flag leaf and angle of flag leaf.

To analyze the level of the selection in each island, we divided the landrace into six groups. The result of Levene's test showed that in 2009 four traits were significant: length of leaf, length of sheath leaf, angle of flag leaf and heading period (Table 2. 8). In 2010, we found seven traits were significant: plant height, culm length, length of leaf, length of sheath leaf, length of flag leaf, angle of flag leaf and heading period (Table 2. 9). Based on CV in 2009, we found high CV on angle of flag leaf (91.08 %) in Sulawesi landrace, and the lowest CV was in angel of flag leaf in Bali Nusa landrace (0.00 %). In 2010, length of flag leaf in Kalimantan landrace showed the highest CV (41.04 %), and the lowest CV was on angle of flag leaf (0.00%) in Kalimantan landrace (Table 2. 9).

Most of phenotypic divergence (*Qst*) showed low level even negative value of differentiation among landrace from different source locations. However we found the most divergent and positive value of *Qst* at least one pairwise between different landrace places in four traits, such as pairwise between landrace from West-Java and Bali-Nusa, between landrace from West-Java and Kalimantan, between landrace from West-Java and Sulawesi and between landrace from Sumatra and Sulawesi (Table 2. 10).

2.4 Discussion

In this study, we revealed that landrace was discriminated from improved varieties and it had larger sizes of vegetative traits than improved varieties. These phenomena might occur because of stronger artificial selection for improved varieties compared with landrace. In modification of plant architecture (morphology), rice varieties were selected for reduced plant height, increased tillers (shoot) and erect plant type (IRRI, 2001; Yang and Hwa, 2008). It seems that a semi-dwarf variety affects Indonesian rice varieties using IRRI varieties. In our study it was showed by tall stature of improved varieties before introducing IRRI varieties as breeding material, Bengawan, Dewi Ratih, Kartuna and Synta variety. Reduced plant stature was a target trait of improved rice varieties, and most of the released varieties had a semi-dwarf1 (*sd1*) gene that came from the Dee-gee-woo-gen variety (Hargrove et al.,

1988) and IR8 (Sasaki et al. 2002). . However, the traits varied in 2009 and 2010 due to environmental changes. Micro-environment and field management influenced clustering of varieties based on morphological traits (Fukuoka et al., 2006; Mokuwa et al., 2014). Furthermore, the high value of coefficient of variation (CV) in particular traits of landrace from each island indicated that Indonesian farmers have maintained the diversity of rice landrace in islands.

The differentiation using dendogram by Wards method and PCA showed that most of rice landrace were grouped in group A in 2009 and 2010. For example, more than 80% of the landrace from West Java were grouped in group A. This result indicated that rice landrace had big in size in most of morphological traits as explained in bi-plot analysis. Iskandar and Ellen (1999) reported that Baduy tribe in West Java has been maintained. They recorded 89 varieties which have been maintained from generation to generation. These varieties are important for the customary tradition and each rice variety has a purpose for obligation in their tradition. In this study, the result of the morphological traits revealed that there was no strong selection while improved varieties were strongly selected as explained above. The varieties released before collaboration with IRRI were big in size compare with the varieties released after collaboration with IRRI.

IRRI varieties that were used in this study showed the distribution in each subgroup of group B in both years. However, these varieties distributed in a different place in each year and IR65 variety was in group A in 2010. These results indicated that IRRI varieties had favorite plant type by rice breeders in Indonesia in developing new varieties. . The change of micro-environmental in the field affected instability of phenotypic performance of IRRI varieties in both years, suggesting that IRRI varieties are sensitive to environmental change. Jagadish et al. (2014) reported that IR64 is sensitive to fertilizer and heat temperature. According to the temperature data that recorded in the field, the daily temperature in summer ranged from 27 to 36^{o}C.

Phenotypic divergence (*Qst*) showed that lack of selection occurred in each trait in pairwise between different landrace, however landrace from West Java showed high positive value in at least 3 pairwises in more than three traits

Figure 2. 1. Map of Indonesia generated by DIVA-gis software and source locations of landrace. We divided our materials into 7 groups; Sumatra Island into two groups (North-Sumatra and South-Sumatra), Java Island into two groups (West-Java and Central-Java), Bali-Nusa group, Kalimantan group and Sulawesi group on calculation of diversification and differentiation of Indonesian rice landrace.

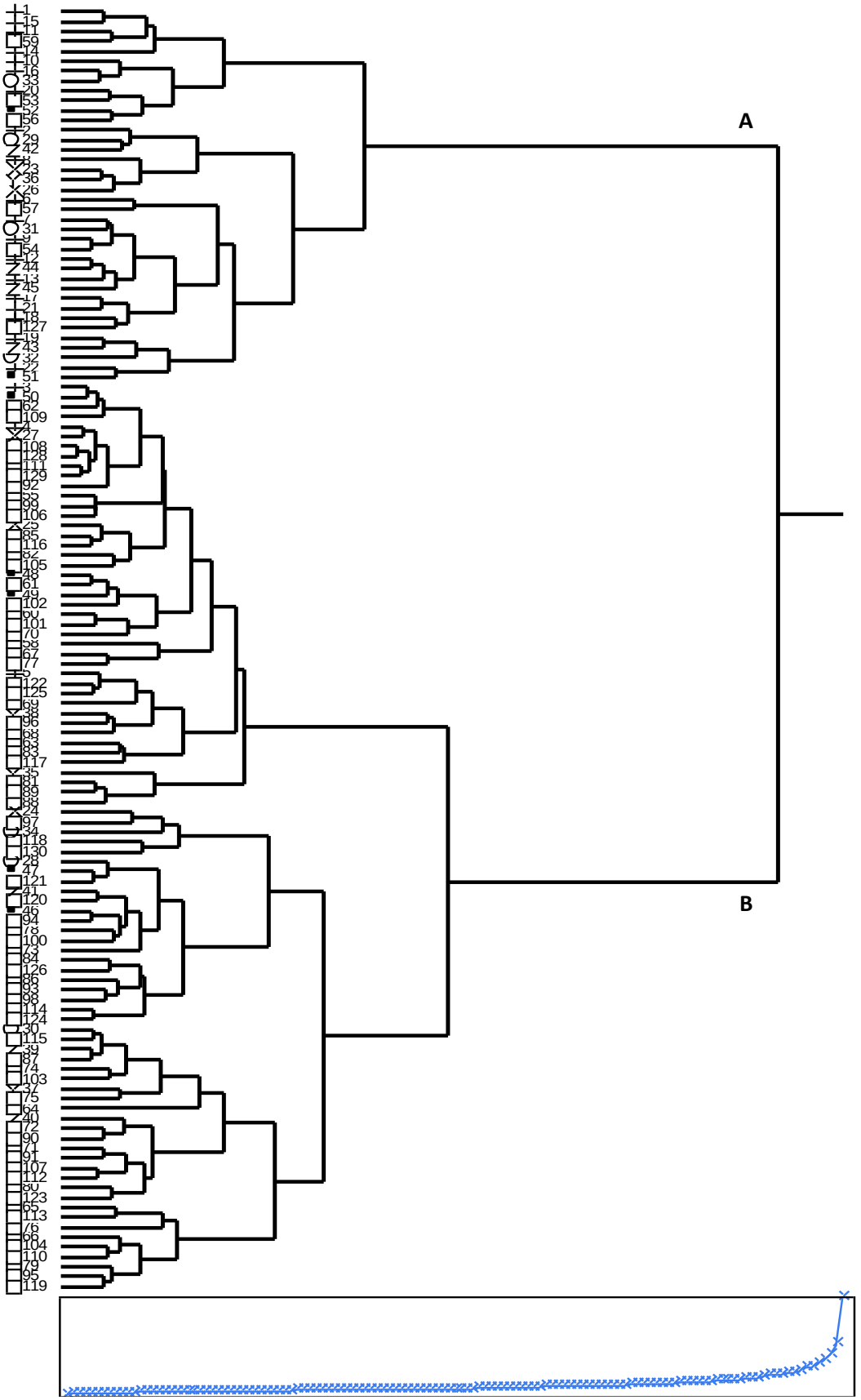

Figure 2. 2. Dendrogram using Ward's method based on 10 morphological traits in 2009.

The different markers on landrace indicate different islands: + is West Java, x is Bali Nusa, ○ is Central Java, Y is Kalimantan, z is Sumatra, and ■ is Sulawesi. Improved varieties indicated by □.

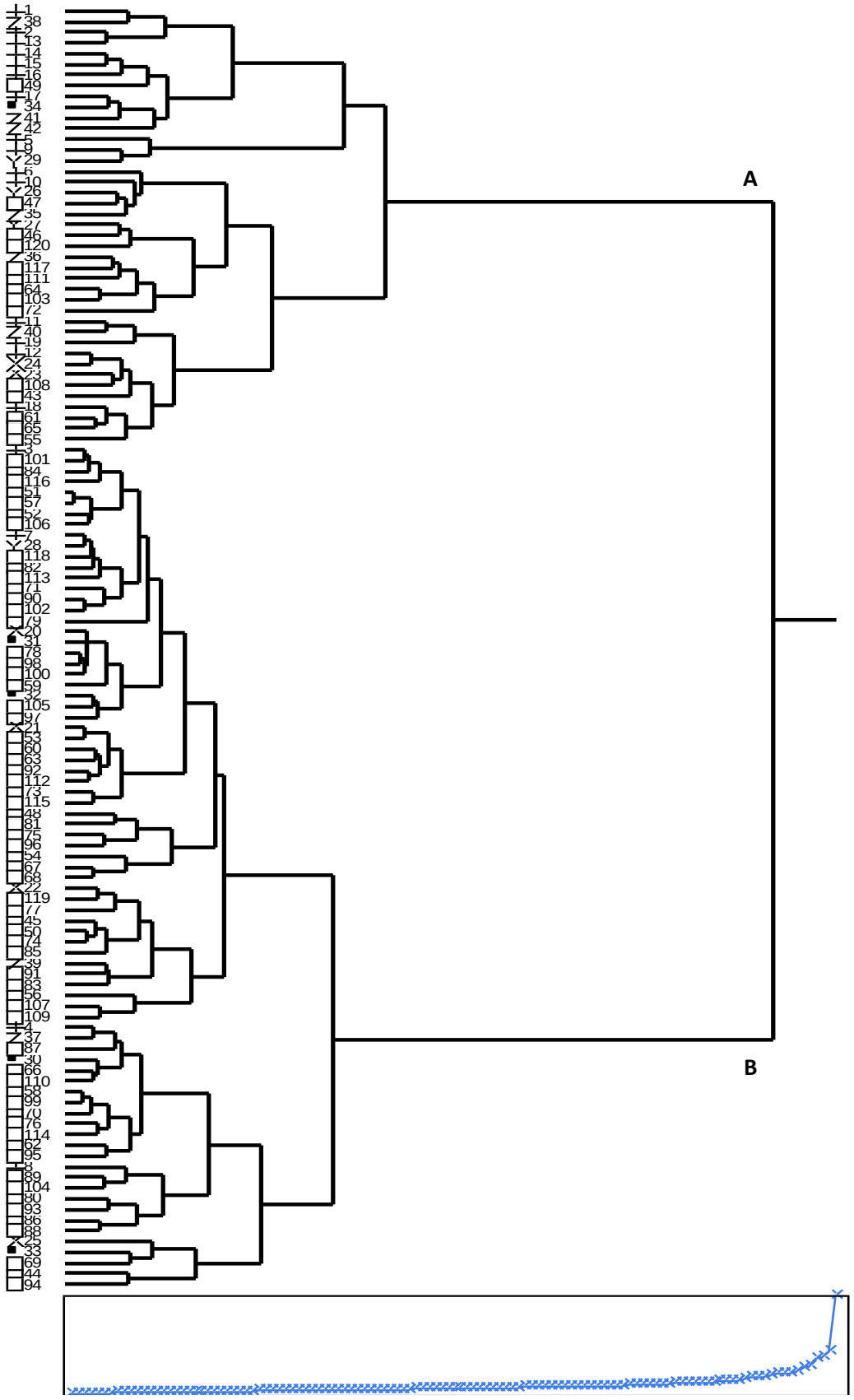

Figure 2. 3. Dendrogram using Ward's method based on 10 morphological traits in 2010.

The different markers on landrace indicate different islands: + is West Java, x is Bali Nusa, Y is Kalimantan, z is Sumatra, and ▪ is Sulawesi. Improved varieties indicated by ☐.

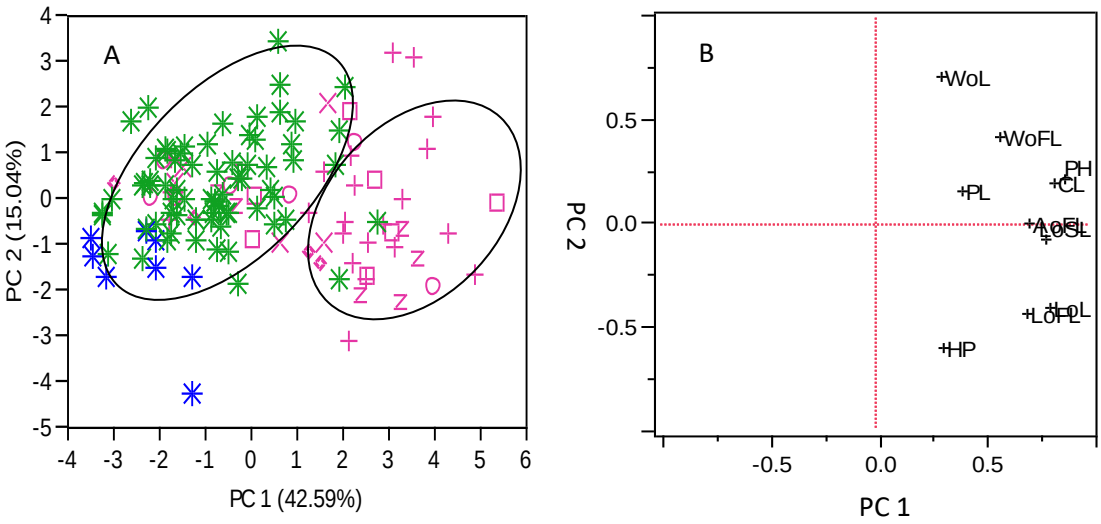

Figure 2. 4. Principal component analysis (PCA) (A) and bi-plot analysis (B) based on 10 morphological traits in 2009.

Purple color indicates landrace, green color indicates improved varieties, and blue color indicates IRRI varieties. The different markers on landrace indicate different islands: + is West Java, x is Bali Nusa, □ indicates Central Java, z indicates Sumatra, and ○ indicates Sulawesi. Abbreviations of the traits are seen in Table 2.5.

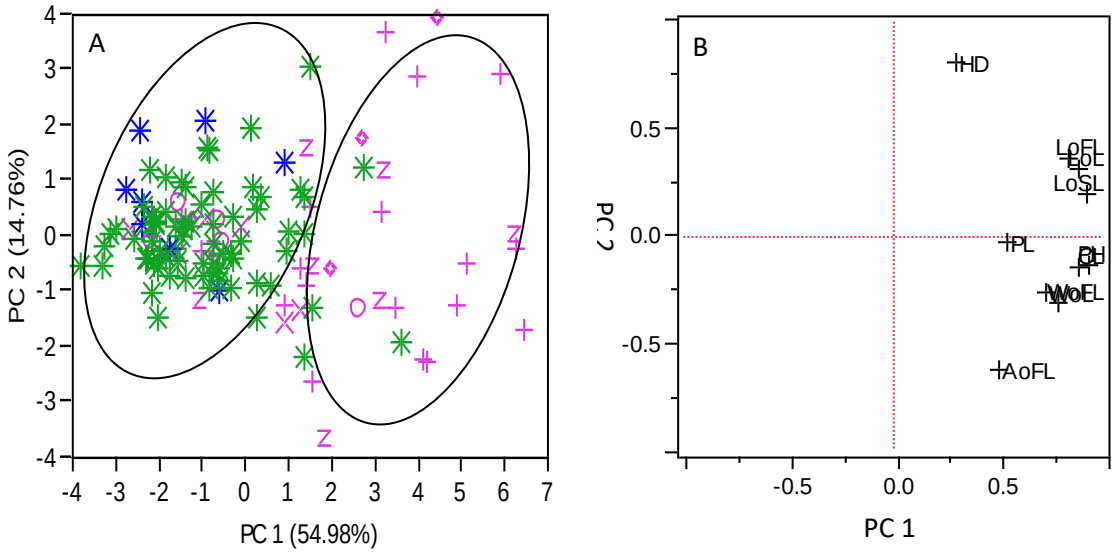

Figure 2. 5. Principal component analysis (PCA) (A) and bi-plot analysis (B) based on 10 morphological traits in 2010.

Purple color indicates landrace, green color indicates improved varieties, and blue color indicates IRRI varieties. The different markers on landrace indicate different islands: + is West Java, x is Bali Nusa, □ indicates Central Java, ◊Kalimantan, z indicates Sumatra, and ○ indicates Sulawesi. Abbreviations of the traits are seen in Table 2.5.

Table 2. 1. Name of improved variety, year of release and classification based on morphological traits in 2009 and 2010.

No.	Name of variety	Year of release	2009	2010	No.	Name of variety	Year of release	2009	2010
1	Bengawan	1943	A	A	51	Way Rarem	1994		
2	Remaja	1954	A	B	52	Cibodas	1995		
3	Seratus Malam	1960	B	B	53	Memberamo	1995	B	B
4	Kartuna	1963	A	A	54	Cilamaya Muncul	1996		
5	Synta	1963	A	A	55	Cilosari	1996	B	B
6	PB 5	1967	B	B	56	Cirata	1996	B	B
7	Dewi Ratih	1969	A	A	57	Digul	1996	B	B
8	Pelita I-1	1971			58	Banyuasin	1997	B	B
9	Gata	1976	B	B	59	Lalan	1997	B	B
10	Gemar	1976	B	B	60	Way Apo Buru	1998	B	B
11	Asahan	1978	B	B	61	Batanghari	1999	B	B
12	IR36	1978	B	B	62	Dendang	1999	B	B
13	Cisadane	1980			63	Ketonggo	1999	B	B
14	IR42	1980	B	B	64	Limboto	1999	B	B
15	Barito	1981	B	A	65	Towuti	1999	B	B
16	Cipunagegara	1981	B	B	66	Widas	1999		
17	Krueng Aceh	1981	B	B	67	Ciherang	2000	B	B
18	Atomita 2	1983	B	B	68	Cisantana	2000	B	B
19	Bahbolon	1983	B	B	69	Indaragiri	2000	B	A
20	Citanduy	1983	B	B	70	Punggur	2000	B	B
21	Mahakam	1983	B	A	71	Tukad Balian	2000	B	B
22	Sadang	1983	B	B	72	Tukad Petanu	2000	B	B
23	Singkarak	1983	B	B	73	Tukad Unda	2000		
24	Batang Ombilin	1984	B	A	74	Angke	2001	B	B
25	Kapuas	1984	B	A	75	Batutegi	2001	B	A
26	Bahbutong	1985	B	B	76	Cimelati	2001		
27	Cisanggarung	1985	B	B	77	Ciujung	2001	B	B
28	Cisokan	1985	B	B	78	Conde	2001	B	B
29	Maninjau	1985	B	B	79	Danau Gaung	2001	B	A
30	IR48	1986	B	B	80	Konawe	2001	B	B
31	IR64	1986	B	B	81	Lambur	2001	B	B
32	IR65	1986	B	A	82	Mendawak	2001		
33	Dodokan	1987	B	B	83	Silugoggo	2001		
34	Batur	1988	B	B	84	Sintanur	2001		
35	Ciliwung	1988	B	B	85	Wera	2001		
36	Danau Atas	1988	B	B	86	Woyla	2001		
37	Batang Sumani	1989	B	B	87	Setail	2002		
38	IR66	1989	B	B	88	Situ Patenggang	2002	B	B
39	IR70	1989	B	B	89	Situbagendit	2002	B	B
40	Lusi	1989	B	B	90	Sunggal	2002		
41	Walanai	1989			91	Batang Piaman	2003	B	B
42	Way Seputih	1989	B	B	92	Ciapus	2003	A	A
43	Barumun	1991	B	B	93	Cibogo	2003	B	B
44	Cenranae	1991	B	B	94	Cigeulis	2003	B	B
45	Danau Tempe	1991			95	Diah Suci	2003		
46	Sutugintung	1992	B	B	96	Fatmawati	2003	B	A
47	Bengawan Solo	1993	B	B	97	Rojolele	2003		
48	Gajah Mungkur	1994	B	B	98	Winongo	2003		
49	Jatiluhur	1994	B	B	99	Mekongga	2004		
50	Kalimutu	1994	B	B	100	Sarinah	2006		

Table 2. 2. Accessions number, name, origin of landrace and classification based on morphological traits in 2009 and 2010.

No	Accession number	Name of Variety	Province	District	2009	2010
1	2213	Abang	Jawa Tengah	Pekalongan (Kab)		
2	498	Aceh-Aceh	Riau	Kepulauan Riau (Kab)		
3	2420	Angsa Jeletuk	Bali	Karangasem (Kab)		
4	3779	Are Sera	Nusatenggara Timur	Ende (Kab)		
5	4676	Ase Puteh	Sulawesi Selatan	Pinrang (Kab)	B	B
6	503	Asemandi	Sulawesi Selatan	Gowa (Kab)	B	
7	3882	Badik/Gadih Kabalai	Sumatera Barat	Bukit Tinggi (Kodya)	B	A
8	4669	Bandang Bujur	Sumatra Barat	Agam (Kab)		
9	2860	Beton	Nusatenggara Barat	Lombok Tengah (Kab)		
10	1047	Beurgeum Dadapan	Jawa Barat	Sukabumi (Kab)		
11	3145	Bintang Landang	Jawa Timur	Malang (Kab)	B	B
12	538	Bujang Inai	Kalimantan Tengah	Kotawaringin Timur (Kab)		
13	3027	Bulang	Sulawesi Selatan	Gowa (Kab)	B	B
14	PN06-17	Bulu Bodas	Jawa Barat	Garut (Kab)	A	
15	3833	Burung Kuning	Nusatenggara Barat	Sumbawa (Kab)		B
16	2600	Cempo Abang Ner	Jawa Barat	Cirebon (Kab)	A	A
17	2386	Cempo Beluluk	Jawa Tengah	Karanganyar (Kab)	A	
18	2368	Cempo Telouluk	Jawa Tengah	Kebumen (Kab)		
19	3389	Cere Beureum	Jawa Barat	Sukabumi (Kab)	A	A
20	2247	Cere Mentik	Jawa Barat	Purwakarta (Kab)	A	A
21	2347	Cere Welut Merah	Jawa Tengah	Banjarnegara (Kab)	A	A
22	PN06-20	Ciburuy 1	Jawa Barat	Garut (Kab)	B	B
23	2548	Cicih Ijo Gading	Bali	Gianyar (Kab)		
24	4707	Daliah Putih	Jawa Barat	Indramayu (Kab)		
25	2450	Deli	Jawa Tengah	Wonosobo (Kab)		
26	2352	Dusel	Jawa Tengah	Batang (Kab)	B	B
27	3385	Enud	Jawa Barat	Sukabumi (Kab)		B
28	1375	Genjah Emer	Jawa Barat	Kuningan (Kab)		
29	2365	Genjah Welut	Jawa Tengah	Pekalongan (Kab)	A	A
30	561	Gonggoi	Sulawesi Tengah	Poso (Kab)	A	A
31	2625	Jaran Mas	Sumatra Utara	Labuhan Batu (Kab)		
32	3935	Jemadi	Jambi	Batang Hari (Kab)	A	A
33	1372	Jidah Bodas	Jawa Barat	Kuningan (Kab)	A	A
34	2779	Jimbruk Joloworo	Jawa Tengah	Boyolali (Kab)		
35	1827	Kalingga Rara	Nusatenggara Timur	Sumba Barat (Kab)	A	A
36	2381	Kangkungan	Jawa Tengah	Banjarnegara (Kab)	A	A
37	PN06-4	Kapas	Jawa Barat	Garut (Kab)	A	A
38	3720	Katik Taram	Sumatera Barat	Tanah Datar (Kab)		
39	2813	Kaya Merah	Kalimantan Barat	Kapuas Hulu (Kab)		
40	2812	Kaya Terabah	Kalimantan Barat	Kapuas Hulu (Kab)		
41	2239	Ketan Bayong	Banten	Serang (Kab)		
42	PN06-39	Ketan Bodas	Jawa Barat	Garut (Kab)	A	A
43	2415	Ketan Bulu Putih	Bali	Karangasem (Kab)		
44	PN06-16	Ketan Gajih	Jawa Barat	Garut (Kab)	A	A
45	3986	Ketan Gondil	Lampung	Lampung Selatan (Kab)		
46	4636	Ketan Huma	Jawa Barat	Garut (Kab)	A	
47	1250	Ketan Keuyeup	Jawa Barat	Kuningan (Kab)	A	
48	4637	Ketan Langgar Sari	Banten	Lebak (Kab)	A	A
49	PN06-14	Ketan Wuluh	Jawa Barat	Garut (Kab)		
50	1388	Kewal	Jawa Barat	Ciamis (Kab)		

No	Accession number	Name of Variety	Province	District	2009	2010
51	3978	Kuntu Kuranyi	Sumatra Barat	Tanah Datar (Kab)	B	B
52	611	Laka	Nusatenggara Timur		B	A
53	613	Laka Tesan A	Nusatenggara Timur	Manggarai (Kab)	B	B
54	3100	Lantiak	Sumatra Barat	Lima Puluh Kota (Kab)	A	A
55	3629	Lapang	Sulawesi Selatan	Maros (Kab)		
56	3945	Markos	Jawa Barat	Tasikmalaya (Kab)	B	
57	4754	Mencrit Beureum	Jawa Barat	Kuningan (Kab)	A	A
58	638	Mentik Sleman	Yogyakarta (DI)	Sleman (Kab)	B	B
59	1829	Mete Kawicho	Nusatenggara Timur	Sumba Barat (Kab)	A	B
60	1816	Nobu Bisara	Nusatenggara Timur	Flores Timur (Kab)	B	B
61	1418	Omad	Jawa Barat	Garut (Kab)		
62	PN06-28	Osog	Jawa Barat	Garut (Kab)	A	A
63	3031	Padi Elo	Sulawesi Selatan	Gowa (Kab)	B	B
64	2287	Padi Rabig	Jawa Barat	Cianjur (Kab)		
65	2733	Padi Serai	Kalimantan Timur	Kutai (Kab)		
66	3741	Padi Sibur	Sumatra Selatan	Ogan Komering Ilir (Kab)		
67	670	Pae Daya Indobye	Sulawesi Tenggara	Kendarai (Kab)	A	
68	4714	Pajar	Lampung	Lampung Utara (Kab)		
69	2776	Pala Idang merah	Aceh (DI)	Aceh Utara (Kab)	A	A
70	1596	Pandan Wangi (leher II)	Jawa Barat	Cianjur (Kab)	A	A
71	668	Pandan Wangi Cianjur	Jawa Barat	Cianjur (Kab)	A	B
72	2876	Pangraman	Aceh (DI)	Aceh Barat (Kab)		
73	1001	Pare Mota	Jawa Barat	Garut (Kab)	A	
74	4614	Plastik	Kalimantan Timur	Kutai (Kab)	B	A
75	2865	Pulut Gaca	Aceh (DI)	Aceh Barat (Kab)		
76	4616	Pulut Kutai	Kalimantan Timur	Kutai (Kab)	B	A
77	3030	Pulut Pagae	Sulawesi Selatan	Gowa (Kab)	A	B
78	683	Pulut Tomene	Sulawesi Tengah	Palu (Kodya)		
79	3934	Raden Kuning	Jambi	Batang Hari (Kab)		
80	3958	Ranggong	Kalimantan Selatan	Kotabaru (Kab)	B	B
81	1319	Rauk Neya	Jawa Barat	Bandung (Kab)	A	A
82	2596	Remaja	Jawa Barat	Majalengka (Kab)		
83	2859	Rembang	Nusatenggara Barat	Lombok Timur (Kab)		
84	4609	Sabai Kecil	Kalimantan Timur	Kutai (Kab)	A	A
85	3466	Saigon	Jawa Tengah	Pati (Kab)		
86	3890	samek	Sumatra barat		B	A
87	1015	Sari Kuning	Jawa Barat	Subang (Kab)		
88	1430	Segon Nyonya	Jawa Barat	Sumedang (Kab)		
89	1541	Segon Saga	Jawa Barat	Garut (Kab)	A	B
90	4010	Sekemiling	Lampung	Lampung Tengah (Kab)		
91	4018	Sepadan	Lampung	Lampung Tengah (Kab)		A
92	3928	Serepet Tinggi	Jambi	Batang Hari (Kab)	A	A
93	4635	Seuweu	Jawa Barat	Garut (Kab)	A	A
94	2318	Si Ampera	Sumatra Utara	Deli Serdang (Kab)		
95	4685	Si Awak	Sumatra Selatan	Bangka (Kab)		A
96	1800	Sintang Pulau Pisau	Kalimantan Tengah			
97	1130	Sri Putih	Jawa Tengah	Sukoharjo (Kab)		
98	2188	Tampai	Jambi	Tanjung Jabung (Kab)		
99	2971	Teratai	Kalimantan Barat	Ketapang (Kab)		
100	1549	Torondol Kuning	Jawa Barat	Garut (Kab)	A	A

Note; A and B are the classification of varieties could grow and measured for morphological traits according to dendogram Figure 2.2 and 2.3, Utara is North, Selatan is South, Timur is East, Barat is West and Tengah is Central

Table 2. 3. Description of 10 morphological traits (IBPGR-IRRI, 1980).

No	Traits	Description	No	Traits	Description
1	(PH) plant height	Measured from ground level to the top of the panicle (n = 5) after heading in centimeters	6	(LoSL) length of sheath leaf	Measured from the basal of blade leaf to the basal of sheath leaf in centimeter
2	(CL) culm length	Measured in centimeters from ground level to the base of the panicle (n = 5) after heading	7	(LoFL) length of flag leaf	Measure length of the flag leaf, from the ligule to the tip of the blade, on five representative plants. Calculate average to nearest cm. Stage: 7 days after anthesis in centimeter
3	(PL) panicle length	Measured in centimeters from the base to the tip of the panicle on near maturity in centimeter	8	(WoFL) width of flag leaf	Measure width at the widest portion of the flag leaf on five representative plants. Calculate average to nearest cm. Stage: 7 days after anthesis in centimeter
4	(LoL) length of leaf	Measured from the top most leaf blade below the flag leaf on the main culm (n = 5) on late vegetative stage in centimeter	9	(AoFL) angle of flag leaf	Measured near collar as the angle of attachment between the flag leaf blade and the main panicle axis (n = 5); 1 (erect), 3 (intermediate), 5 (horizontal), 7 (desedence)
5	(WoL) width of leaf	Measured at the widest portion of the blade on the leaf below the flag leaf (n = 5) on late vegetative stage in centimeter	10	(HD) Heading date	Number of days from sowing to 50% heading.

Table 2. 4. Three principal components, eigenvalue and cumulative of variation in 10 traits in 2009.

Traits	**Eigenvectors**		
	PC 1	**PC 2**	**PC 3**
PH (cm)	0.417	0.179	-0.328
CL (cm)	0.394	0.159	-0.420
PL (cm)	0.191	0.133	0.377
LoL (cm)	0.387	-0.331	0.096
WoL (cm)	0.142	0.583	0.404
LoSL (cm)	0.377	-0.062	-0.128
LoFL (cm)	0.333	-0.349	0.179
WoFL (cm)	0.272	0.343	0.390
HD (day)	0.150	-0.483	0.428
AoFL (^{0})	0.339	0.003	-0.135
Eigenvalue	4.259	1.504	1.285
Cumulative of variation (%)	42.591	57.636	70.487

Note: PH (plant height), CL (culm length), PL (panicle length), LoL (length of leaf), WoL (width of leaf), LoSL (length of sheath leaf), LoFL (length of flag leaf), WoFL (width of flag leaf), HD (heading Date), and AoFL (angel of flag leaf).

Table 2. 5. Three principal components, eigenvalue and cumulative of variation in 10 traits in 2010.

Traits	**Eigenvectors**		
	PC 1	**PC 2**	**PC 3**
PH (cm)	0.389	-0.109	-0.190
CL (cm)	0.369	-0.112	-0.394
PL (cm)	0.224	-0.023	0.821
LoL (cm)	0.367	0.257	-0.018
WoL (cm)	0.302	-0.209	0.136
LoSL (cm)	0.385	0.160	-0.053
LoFL (cm)	0.349	0.295	0.056
WoFL (cm)	0.328	-0.250	0.201
HD (day)	0.121	0.662	-0.131
AoFL (^{0})	0.208	-0.502	-0.230
Eigenvalue	5.498	1.476	0.846
Cumulative of variation (%)	54.976	69.731	78.188

Note: PH (plant height), CL (culm length), PL (panicle length), LoL (length of leaf), WoL (width of leaf), LoSL (length of sheath leaf), LoFL (length of flag leaf), WoFL (width of flag leaf), HD (heading Date), and AoFL (angel of flag leaf).

Table 2. 6. Mean and coefficient of variation (CV) within improved variety and landrace, and homogeneity test between them for 10 morphological traits in 2009.

Population	**Improved variety**		**Landrace**		**Levene's test**	**Tukey-Kramer HSD (5%)**
Ni	**78**		**52**			
Traits	**Mean**	**CV (%)**	**Mean**	**CV (%)**		
PH (cm)	112.651	17.143	147.232	17.777	*	*
CL (cm)	87.037	21.777	119.964	20.83	*	*
PL (cm)	25.614	23.816	27.267	16.782	*	ns
LoL (cm)	41.283	12.082	52.879	12.9	ns	*
WoL (cm)	1.454	15.507	1.439	13.734	ns	ns
LoSL (cm)	25.547	18.943	29.874	20.09	ns	*
LoFL (cm)	28.086	24.12	35.689	25.278	*	*
WoFL (cm)	1.655	13.229	1.717	11.326	ns	ns
HD(day)	123.782	12.082	127.788	12.9	ns	ns
AoFL (^{0})	1.457	23.907	1.780	28.641	**	*

Note; Ni is number of individual within population, ns is non-significant, * is significant level at $p<0.05$, and ** is significant level at $P<0.01$(Levene's test). Abbreviations of the traits are seen in Table 2.5.

Table 2. 7. Mean, coefficient of variation (CV) within improved variety and landrace and homogeneity test between them on 10 morphological traits in 2010.

Population	**Improved variety**		**Landrace**		**Levene's test**	**Tukey-Kramer HSD (5%)**
Ni	**78**		**48**			
Traits	**Mean**	**CV (%)**	**Mean**	**CV (%)**		
PH (cm)	109.350	12.820	135.417	19.251	**	*
CL (cm)	83.635	16.354	106.573	22.717	**	*
PL (cm)	25.715	19.249	28.844	14.934	ns	*
LoL (cm)	39.178	17.952	49.523	24.951	**	*
WoL (cm)	1.288	12.522	1.395	16.011	*	*
LoSL (cm)	25.451	13.026	29.793	17.505	**	*
LoFL (cm)	26.732	23.577	36.345	33.967	**	*
WoFL (cm)	1.422	14.682	1.635	17.715	*	*
HD (day)	128.731	10.880	130.500	12.144	ns	ns
AoFL (^{0})	1.402	22.687	1.667	30.985	**	*

Note; Ni is number of individual, ns is non-significant and ** is significant level at P<0.01 (Levene's test)
Abbreviations of the traits are seen in Table 2.5.

Table 2. 8. Mean, coefficient of variation (CV) within origin of landrace and homogeneity test among them on 10 morphological traits in 2009.

Population	Improved variety		West Java		Central Java		Bali-Nusa		Kalimantan		North Sumatra		Sulawesi		F test
Ni	78		22		7		5		4		7		7		
Traits	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	
PH (cm)	112.651	17.143	155.136	16.140	157.186	18.488	141.600	18.934	128.000	21.847	143.679	13.922	131.000	18.289	ns
CL (cm)	87.037	21.777	127.345	18.644	126.786	22.689	115.320	21.623	105.750	27.346	116.350	17.299	105.000	23.061	ns
PL (cm)	25.614	23.816	27.791	16.879	30.400	14.907	26.280	16.185	22.250	12.909	27.329	14.008	26.000	16.166	ns
LoL (cm)	41.283	19.052	53.500	19.685	54.914	27.888	49.500	23.224	49.325	30.502	56.514	25.173	49.700	27.872	*
WoL (cm)	15.507	0.225	1.483	15.001	1.457	10.376	1.340	23.362	1.300	10.879	1.414	6.362	1.457	10.376	ns
LoSL (cm)	25.547	18.943	31.938	14.434	30.029	12.332	27.470	13.990	22.850	62.732	30.271	17.884	28.571	15.929	**
LoFL (cm)	28.086	24.120	36.693	26.753	40.614	12.442	30.310	21.102	33.450	17.482	36.886	16.972	31.534	40.633	ns
WoFL (cm)	1.655	13.229	1.768	11.855	1.814	8.673	1.685	8.099	1.600	11.411	1.664	8.623	1.600	13.010	ns
HD (day)	123.782	12.082	131.090	13.105	131.286	13.431	113.600	2.210	123.000	7.798	137.140	14.821	117.429	5.246	**
AoFL (^{0})	1.744	66.933	3.636	46.130	3.000	66.667	1.000	0.000	2.000	57.735	3.286	54.767	2.140	91.084	**

Note; Ni is number of individual within population, ns is non-significant, ** is significant level at P<0.01 (Levene's test) and the different letter are significantly different by Tukey-Kramer HSD test. Abbreviations of the traits are seen in Table 2.5.

Table 2. 9. Mean, coefficient of variation (CV) within origin of landrace and homogeneity test among them on 10 morphological traits in 2010.

Population	Improved variety		West Java		Central- Java		Bali-Nusa		Kalimantan		North Sumatra		Sulawesi		F test
Ni	78		19		6		6		4		8		5		
Traits	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	
PH (cm)	109.350	12.820	145.520	18.029	132.160	28.860	116.080	13.698	132.000	15.101	139.380	14.609	120.500	15.442	**
CL (cm)	83.635	16.354	115.263	20.598	103.000	34.930	90.083	20.179	102.000	18.060	111.250	16.306	93.800	22.228	**
PL (cm)	25.715	19.249	30.263	13.695	29.167	10.267	26.000	22.328	30.000	7.201	28.125	12.669	26.700	21.087	ns
LoL (cm)	39.178	17.952	53.305	25.290	50.617	29.253	40.400	9.022	54.300	29.091	49.013	19.063	41.780	17.396	**
WoL (cm)	1.288	12.522	1.486	15.109	1.317	10.095	1.222	9.552	1.475	15.033	1.413	16.684	1.260	19.920	ns
LoSL (cm)	25.451	13.026	32.156	14.455	29.583	22.407	25.367	11.975	30.800	16.367	29.438	19.122	26.140	10.440	**
LoFL (cm)	26.732	23.577	38.379	36.447	37.123	39.626	29.450	14.693	42.300	41.040	38.663	23.579	27.480	10.642	**
WoFL (cm)	1.422	14.682	1.724	17.575	1.600	19.764	1.450	22.559	1.600	15.309	1.731	12.630	1.440	11.620	ns
HD (day)	128.731	10.880	132.680	11.616	129.170	8.696	124.170	12.748	144.250	18.844	127.380	13.296	125.400	4.242	*
AoFL (^{0})	1.402	22.687	1.824	29.820	1.411	32.407	1.735	25.109	1.225	0.000	1.796	31.109	1.449	34.585	**

Note; Ni is number of individual within population, ns is non-significant, ** is significant level at P<0.01 (Levene's test) and the different letter are significantly different by Tukey-Kramer HSD test. Abbreviations of the traits are seen in Table 2.5.

Table 2. 10. Mean of phenotypic divergence (*Qst*).

Pairwise	Traits									
	PH	**CL**	**PL**	**LoL**	**WoL**	**LoSL**	**LoFL**	**WoFL**	**HD**	**AoFL**
WJ-BN	**0.13**	**0.105**	0.099	**0.104**	**0.168**	**0.24**	**0.193**	**0.122**	**0.162**	-0.411
WJ-CJ	-0.007	0.001	-0.274	-0.038	0.039	0.006	-0.103	-0.181	-0.031	-0.033
WJ-KM	**0.15**	**0.115**	**7.83**	-0.066	**0.106**	**0.109**	-0.095	0.044	-0.062	-0.107
WJ-Sum	-0.012	-0.015	-0.15	-0.032	0.001	-0.036	-0.166	-0.033	-0.013	0.006
WJ-SLS	**0.29**	**0.291**	0.081	0.048	-0.031	**0.204**	**0.219**	0.291	0.053	-0.212
BN-CJ	-0.03	-0.048	0.062	-0.016	0.008	0.016	0.267	-0.014	0.109	-1.29
BN-KM	-0.077	-0.08	-0.095	-0.014	-0.18	-0.095	0.096	-0.153	**1.48**	-0.278
BN-Sum	0.059	0.025	-0.009	-5.51	0.057	0.146	-5.69	-0.012	0.178	-0.378
BN-SLS	-0.025	-0.018	-0.057	-0.09	0.046	-0.06	-0.097	-0.033	-2.39	-0.055
CJ-KM	-0.018	-0.041	**6.67**	-0.059	0.027	-0.05	-0.102	**0.279**	-0.089	**0.208**
CJ-Sum	-0.057	-0.05	-0.02	-0.014	0.063	-0.04	0.1	**2.81**	-0.01	0.037
CJ-SLS	0.068	0.04	0.056	-0.029	0.028	-0.001	**0.232**	**1.91**	0.004	-0.186
KM-Sum	0.081	0.038	-1.45	-0.039	0.031	0.000	-0.071	-0.038	-0.035	-0.034
KM-SLS	-0.053	-0.048	-0.095	-0.04	0.019	-0.095	0.066	-0.019	**0.268**	-0.235
Sum-SLS	**0.353**	**0.362**	-0.021	**0.318**	0.05	0.109	**2.28**	0.141	0.089	-0.161

Note: the bold font indicated value of Qst is positive. Abbreviation for source location of landrace; *WJ* is West-Java, *BN* is Bali-Nusa, *CJ* is Central-Java, *KM* is Kalimantan, *Sum* is Sumatra and *SLS* is Sulawesi Abbreviations of the traits are seen in Table 2.5.

3. Diversification and differentiation of Indonesian rice landrace and improved varieties as revealed by SSR markers

3.1 Introduction

Numerous researchers in an effort to classify rice using several methods reported that the presence of intermediate type was observed between two subspecies of *O. sativa* using isozymes (Second, 1982; Sie and Ghesquiere, 1992), morphological traits (Ahmadi et al., 1991) and molecular markers (Mather et al., 2010). They believed that the intermediate type was caused by hybridization between *indica* and *japonica*. Mather et al. (2010) found evidence of asymmetric migration and gene flow between *indica* and tropical *japonica* in Madagascar using molecular markers These results clarified that intermediate type (hybrid) in grain shape between *indica* and tropical *japonica* which previously reported by Ahmadi et al. (1991) was caused by asymmetric gene flow between two subspecies. Asymmetric gene flow could affect the diversity in the recipient crops because of the recurrent gene flow from donor (Bartsch et al. 1999; Song et al., 2006). Also, the level of gene flow within and among species affects differentiation of crops (Schaal et al., 1998; Schaal et al., 2003). This fact caused inconsistency of genetic structure in rice and is called as gene tree discordance (Yang et al., 2012).

Diversity and differentiation analysis based on morphological traits is generally affected by environments. Current DNA markers allow the analysis of a large number of loci which widely distribute throughout the entire genome of crops. Molecular markers are powerful tools for the assessment of genetic variation and the elucidation of genetic relationships within and among species. The diversity of the genus *Oryza* has been analyzed using *random amplified polymorphic* DNA (RAPD) (Martin et al., 1997) and *restriction fragment length polymorphism* (RFLP) (Sun et al., 2001). *simple sequence repeats* (SSR) have been more frequently used to analyze genetic diversity and differentiation in rice (Mackill, et al., 1995; Powell et al., 1996; Ravi et al., 2003). Furthermore, SSR markers that could identify some alleles were derived from *O. sativa* in hybrid populations from the cross between *O. sativa* and *O. rufipogon* in China (Song et al., 2006).

The existence of intermediate type in Indonesian rice germplasm was reported by Khush et al. (2003), indicating 3% of intermediate group in addition to *indica* (69%) and *japonica* (28%) based on isozyme polymorphism. Also, Thomson et al. (2009) reported that there was 2.7% of admixture between indica and *japonica* in Kalimantan landrace. However,

the role of gene flow in diversification of rice germplasm in Indonesia has not been clarified and in this study we focused on analyzing gene flow and other mechanisms to diversify and differentiate rice germplasm in Indonesia using SSR markers. We assume that one of the mechanisms generating intermediate varieties in Indonesian rice is gene flow between subspecies.

3.2 Materials and methods

Plant materials

A total of 200 Indonesian rice accessions involved 100 Indonesian landrace and 100 improved cultivars (Table 2. 1 and 2. 2). Materials were chosen from the rice germplasm collections at the Indonesia Center for Rice Research (ICRR Subang, Indonesia).

DNA extraction and SSR genotyping

Plants were grown in nursery boxes in a greenhouse at the University of Tsukuba, Japan, and leaf samples were harvested from a single 30-day-old plant of each sample. Leaf samples were crushed in a crusher with a zirconium ball in micro-tubes (2 mL), and DNA was isolated using a modified cetyl trimethylammonium bromide (CTAB) method (McCouch et al., 1988).

SSR markers in this study consisted of SSRs used in previous study of Indonesian rice (Thomshon et al., 2007) and Chinese rice (Zhang et al. 2011). These SSRs were reported to effectively discriminate rice varieties into two groups, *indica* and *japonica*. We screened 148 SSR markers mapped across all 12 chromosomes and screened 32 polymorphic markers. The sequences of SSR primers were taken from the Gramene database (http://www.gramene.org/) (Table 3. 1). The PCR solution per tube consisted of 1 µL of 10x buffer, 1 µL of dNTPs, 0.1 µL of *Taq* polymerase (TOYOBO, Japan), 1 µL primer (created from R and F SSR sequences) (Invitrogen, Carlsbad, CA, USA), 1 µL (5 ng) of DNA and 5.9 µL of distilled water. The PCR conditions were 5 min at 94°C; 35 cycles of 30 s at 94°C, 30 s at the primer-specific annealing temperature, 90 s at 72°C; and a final extension at 72°C for 10 min. These SSR markers were genotyped in 200 varieties following electrophoresis in 10% polyacrylamide gels at (250 V, 500 mA); genotypes were determined visually from gel images. Of the 200 DNA samples, 161 genotypes (78 improved varieties and 83 landrace) provided enough data to use.

Data analysis

The molecular marker data were analysed in DARwin 5 (Perrier and Jacquemoud-Collet, 2006), GenAlEx 6.5 (Peakall and Peter, 2006), Migrate-n (Beerli and Palczewski, 2010) and Bayescan v 2.1 (Foll and Gaggiotti, 2008) software. DARwin 5 was used to calculate NJ trees based on genetic distance using 1000 bootstrap. GenAlEx 6.5 was used to calculate the number of alleles per locus (*Na*), number of effective alleles (*Ne*), percentage of polymorphic (P), gene diversity (*H*; Shannon, 1948), allelic richness (Pettit et al. 1998), significance of the inbreeding coefficients within subpopulation (Fis) and among subpopulations (*F*st) per locus were tested using 999 permutations (Wright, 1978). The gene flow (Nm) by formula (1 – FST)/4 (Wright, 1951), genetic distance (*D*) between populations (*indica* and *japonica*) (Nei, 1972), and genetic differentiation (F_{ST}; Nei, 1973) was also tested using 999 permutations.

Migrate-n was used to calculate direction pattern of gene flow (Nm) by estimating posterior distribution mean ($\theta = 4 Ne\mu$, Ne is the effective population size and μ is the mutation rate per site per generation), migration rate ($M = m/\mu$, where m is the rate of migration for each locus) and formula ($Nm = M \theta/4$) was used to estimate gene flow (number of migrants per generation). We run this software for SSR markers with uniform priors which were placed on θ from 0 to 30 and M from 0 to 40 by Bayesian inference strategy for Brownian motion (microsatellite model) and starting parameters for migrant values and θ were generated from several trials. For Markov chain setting, 5000 steps were recorded and followed by 20 sample increments and 10000 burns-in for each chain.

Bayescan v.2.1 software was used to calculate differentiation among neutral locus (FST) and also this software was used to detect candidate loci for divergence based on NJ tree, in which outlier loci generated by this software is signs of natural selection. The evidence of selection in the range $0.5 < \log10 (PO) < 1$ is substantial, strong if $1< \log10 (PO) <1.5$ and very strong if $1.5 < \log10 (PO) < 2$ as explained on manual of this software. In this study, we calculated Fis, *F*st, outlier loci (FST), direction of migration and gene flow using landrace. Improved rice varieties were excluded from calculation using this method because of the complexity in crossings in breeding history to develop new varieties. .

In this study, landrace in Indonesia could be divided by source locations. We calculated mean values of differentiation among neutral loci (FST) in pairwise between different source locations of landrace using Bayescan v. 2.1. These results were compared with mean values of phenotypic divergence based on morphological traits (*Qst*).

3.3 Results

Genetic structure of Indonesian rice germplasm

Genetic structure analysis using 32 SSR markers, Indonesian rice germplasm was divided into two major groups. We call the group involving PB5 and IR36 as *indica* and the group involving Kartuna as tropical *japonica*. According to the NJ tree, we identified some of varieties as admixture varieties and we call these varieties as C type (intermediate). In overall, 82.61 % of varieties used was classified as *indica*, 15.53% as *japonica* and 1.86 % as intermediate (Figure 3. 1, Table 4. 13), and it seems that *indica* landrace was separated from improved varieties. The clustering showed that improved varieties differentiated from landrace and they were divided into five groups, IR36, IR48, IR5, IR64 and *japonica* groups (Figure 3. 2). The landrace also showed the differentiation into three groups, and we call them Indonesian *indica* landrace type I, type II and *japonica* group (Figure 3. 3).

The differentiation (F_{ST}) and genetic distance (D) based on Nei's calculation showed a significant value of F_{ST} in all pairwise comparisons and a high value of D. F_{ST} and D between improved varieties and landrace indicated significant values of F_{ST} and high value D in all pairwise comparisons (Table 3. 2). F_{ST} and D between *indica* landrace and *japonica* landrace were the highest (0.24; 0.22) and F_{ST} and D between *indica* landrace and *indica* improved varieties were the lowest (0.05; 0.06) (Table 3. 2).

Genome scan analysis to detect outliers loci based on differentiation was conducted between *indica* and *japonica* landrace, and we found one of 32 SSR marker loci with Log10 (PO) <1 (0.95) in value of FST (0.09) (Figure 3. 4, Table 3. 3). Inbreeding coefficients derived from inbreeding within sub-populations (Fis) and inbreeding coefficient from differentiation between sub-populations (*F*st) were significantly distant from 0 by SSR markers (Table 3.4) and the gene flow (*N*m) between *indica* and *japonica* is 0.59 (Table 3. 4). The mode of migration rates (M) based on direction ranged from 1.613 (from *japonica* to *indica*) to 6.653 (from *indica* to *japonica*) (Table 3.5). The level of gene flow (N_m) based on direction ranged from 0.254 (from *japonica* to *indica*) to 0.948 (from *indica* to *japonica*). It seems that the pattern of gene flow between *O. sativa* subspecies is asymmetric (Table 3. 5).

Significant values of differentiation (F_{ST}) and genetic distance (D) were found in all pairwise comparisons between improved varieties and landrace derived from seven source locations (Table 3. 6). F_{ST} between improved varieties and landrace from South-Sumatra was the highest (0.117) and F_{ST} between improved varieties and landrace from West-Java was the lowest (0.055). D between improved varieties and landrace from South-Sumatra was the

highest (0.163) and *D* between improved varieties and landrace from Bali-Nusa was the lowest (0.076). Within landrace from different source location, F_{ST} and *D* between landrace from Sulawesi and South-Sumatra were the highest (0.100; 0.157) and F_{ST} and *D* between Kalimantan and West-Java were the lowest (0.036; 0.056). We found a wide range of mean values of differentiation among neutral loci (FST) among landrace from different source locations using Bayesian method (Table 3. 7). The mean FST values ranged from 0.062 (pairwise between West Java and Central Java) to 0.133 (pairwise between Bali-Nusa and Kalimantan).

Diversity of Indonesian rice germplasm

The polymorphism in Indonesian rice germplasm using 32 SSR markers showed 100% and the diversity index Shannon's *H*, allelic richness (AR) and private allele (PA) were 0.78, 3.41 and 1.41, respectively. Within *O. sativa*, *H*, AR and PA of landrace (0.82; 3.11; 0.31) were higher than *H*, AR and PA of improved varieties (0.65; 2.87; 0.16) (Table 3. 8). We found that *H*, AR and PA of *japonica* (0.56; 1.97; 0.06) were lower than *H*, AR and PA of *indica* (0.70; 2.85; 0.19). In contrast, *H* of in improved *japonica* varieties (0.62) was higher than *H* of *indica* varieties (0.60), however AR and PA of improved *japonica* varieties (2.01; 0.00) was lower than AR and PA of *indica* varieties (2.73; 0.09) (Table 3. 9).

Diversity analysis showed that *H* and AR in landrace from West Java (0.76; 2.57) was the highest and *H* and AR in landrace from South Sumatra (0.61; 1.94) was the lowest (Table 3. 10). We found no private allele in rice germplasm from four source locations; Bali-Nusa, Kalimantan, Sulawesi and South Sumatra (Table 3. 10). The consisting *japonica* affected diversity, allelic richness and private allele as shown by *H*, AR and PA of landrace from West Java but it was not the highest (0.55; 2.12; 0.031) when we excluded the *japonica* from calculation. We found no private allele in rice germplasm from five source locations; Bali-Nusa, Central-Java, Kalimantan, Sulawesi, and South-Sumatra (Table 3. 11). We found *japonica* in each source location except North-Sumatra (Figure 3. 5).

3.4 Discussion

This study presented that in our materials of Asian cultivated rice, *indica* dominated over *japonica*. This study also revealed that landrace was more diverse than improved varieties in agreement with previous studies by Thomson et al. (2007) who discussed that Indonesian farmers has maintained diversity of landrace. Besides, Iskandar and Ellen (1999) reported that cultural practices by people in Java Island served to maintain landrace for traditional,

religious and cultural uses. Similar results were reported that the genetic diversity of landrace has been maintained by cultural practices in Kalimantan (Thomson et al. 2009).

The following three possible mechanisms have changed the diversity and differentiation in *O. sativa* in Indonesia, (1) direction of gene flow between two subspecies, (2) artificial selection for rice germplasm *and* (3) natural selection for improved varieties .

In this study, the inbreeding coefficient through inbreeding within sub-population (Fis) and inbreeding coefficient through differentiation between sub-population (*F*st) statistically deviated from zero. Even though we found high value of Fis, we revealed various values of *F*st and it was caused by gene flow (Nm) between sub-population which took place in Indonesian rice germplasm. According to Govindajaru classification, the ranges of Nm in this study were intermediate (0.59). This study revealed that the pattern of migration and gene flow was asymmetric between *indica* and *japonica*. Asymmetric gene flow from donor population to recipient population occurred for long period, in particular alleles of the recipient population were displaced and alleles of the donor population were replaced, unless there was strong selection for donor alleles (Papa and Gepts, 2004). In the continuous asymmetric gene flow, the diversity of recipient population is affected by replacing the gene (Haygood et al., 2003). The presence of C type (intermediate varieties) in this study gave evidence that hybridization has occurred in Indonesian rice between these two subspecies aspresented in J-tree. The probable reason of the limited asymmetric gene flow between *indica* and *japonica* is that two subspecies adapt to different ecological environments, in Indonesia; *japonica* rice involving glutinous rice (*Beras Ketan*) is commonly cultivated in highlands (>500m from sea level) by swidden practice, whereas *indica* rice is cultivated in lowlands (Marten, 1990; Tanaka, 1997; Iskandar, Ellen, 1999). In this study we suggested that these conditions were associated with the differentiation between *indica* and *japonica* in Indonesia.

The finding of numerous *indica* compare to *japonica* in this study corresponded well to previous studies (Khush et al., 2003; Thomson et al., 2007), in which showed that *japonica* was less than *indica* in Indonesian rice germplasm. Glutinous and awned rice varieties were involved in *japonica* by SSR markers, indicating the same result as reported by Thomson et al. (2009).

The diversity in *japonica* was lower than *indica* in this study, in which this result was different from previous study as reported by Thomson et al. (2007) and in agreement with

Glaszmann (1987) and Li and Rutger (2000). In addition to the asymmetric gene flow, we suggested that the lower diversity, less number of effective alleles and fewer private alleles in *japonica* than *indica* might be affected by selection for particular traits. For example, our previous study indicated that selection for seed shattering contributed to differentiation between *indica* and *japonica*, in which *japonica* showed poorer seed shattering (Muhamad and Okuno, 2013). This selection contributed to allelic difference as revealed by positive correlation between differentiation using shattering traits and using SSR markers. The other mechanism decreasing diversity in *japonica* is bottleneck effects as reported in rice germplasm in Madagascar (Mather et al., 2010) and in Asia including Indonesia (Molina et al., 2011). Sun et al. (2001) suggested that effective alleles have been lost by natural and human selection and genetic diversity in cultivated rice has been gradually decreased. In addition, the assessment of allelic richness and private allele in this study supported our result in diversity analysis when the sample size of reference is small and unbalanced among populations as suggested by Foulley and Olliver (2006). The result showed that the pattern of *H* index corresponded well to allelic richness (AR) and indicated that our results were reliable.

We found one outlier locus by sustainable evidence of selection between *indica* and *japonica* using Bayesian method. This locus was identified as *qDSR*11-2 underlying dead seedling rate under alkaline stress by QTL analysis using SSR markers (Qi et al., 2008). This result suggested that natural variations caused by mutation affected the differentiation in Indonesian rice. In rice, it was reported that agronomically important genes have been selected among upland rice (Lyu et al., 2013). The natural selection is one of important mechanisms for evolution of population as suggested by Hardy (1908) and Weinberg (1908) and is popular as Hardy-Weinberg principle. In addition, this study discovered that most of phenotypic divergence (*Qst*) values were lower than differentiation among loci under neutral molecular markers (FST). Our result indicated that the same phenotypes were favoured in different populations due to stabilizing selection in the most of pairwise comparison between landrace from different source location. However, we found higher *Qst* than FST in pairwise landrace from West-Java and Bali-Nusa in the most of traits which were evaluated in this study. These results indicated that the directional selection occurred between these populations. Three scenarios are possible to explain the relation between *Qst* and FST on natural selection. First, a higher divergence in quantitative traits compared with neutral molecular markers (*Qst*> FST) indicated directional selection among populations. Second, the opposite scenario (*Qst* < FST) suggested that the same genotypes were favoured in

different populations due to stabilizing selection. Third, if the two measures do not differ significantly, the possibilities of genetic drift versus selection cannot be disentangled (Pertoldi et al., 2012). These results supported that landrace in this study could not differentiate according to different source locations. This study suggested non-significant differentiation and low genetic distance among pairwise landrace from different source locations.

The possible reason why the diversity decreases in improved varieties is the extensive use of IRRI varieties as breeding materials. Artificial selection causes the lower level of diversity in improved varieties due to the continuous use of elite varieties in breeding program causing genetic uniformity (Tanksley and McCouch, 1997). This evidence showed differentiation of improved varieties into five groups based on IRRI varieties in agreement with 41.4% according to pedigree information (crossing history). The lower diversity in improved *indica* varieties compared with improved *japonica* varieties was supported by the fact that Indonesian breeders have focused on improving *indica* rather than *japonica*, and it was also shown by lower effective number of alleles in *indica* than *japonica*. Changes in allelic frequency occur in response to selection (Vieira et al., 2013). However, allelic richness in improved *indica* varieties is still higher than *japonica* because *indica* has more private alleles than *japonica*. This indicated that improved *indica* varieties have similar alleles. On the other hand, the result is not strange because *japonica* variety group has been used as breeding materials in Indonesian breeding program such as Bengawan and Peta varieties, which were resulted from crossing between Cina and Latisail varieties that were important for developing IR8 varieties. Besides, the varieties classified as *japonica* were developed using IRRI varieties such as Banyuasin and Situ Patenggang varieties (http://www.iris.irri.org/). The rice landrace differentiated into three major groups, landrace type I, II and *japonica*. Seven percentage of landrace was not involved in IR5/PB5 and IR36 (type II). This result indicated that the landrace has its potential for future rice breeding programs in Indonesia.

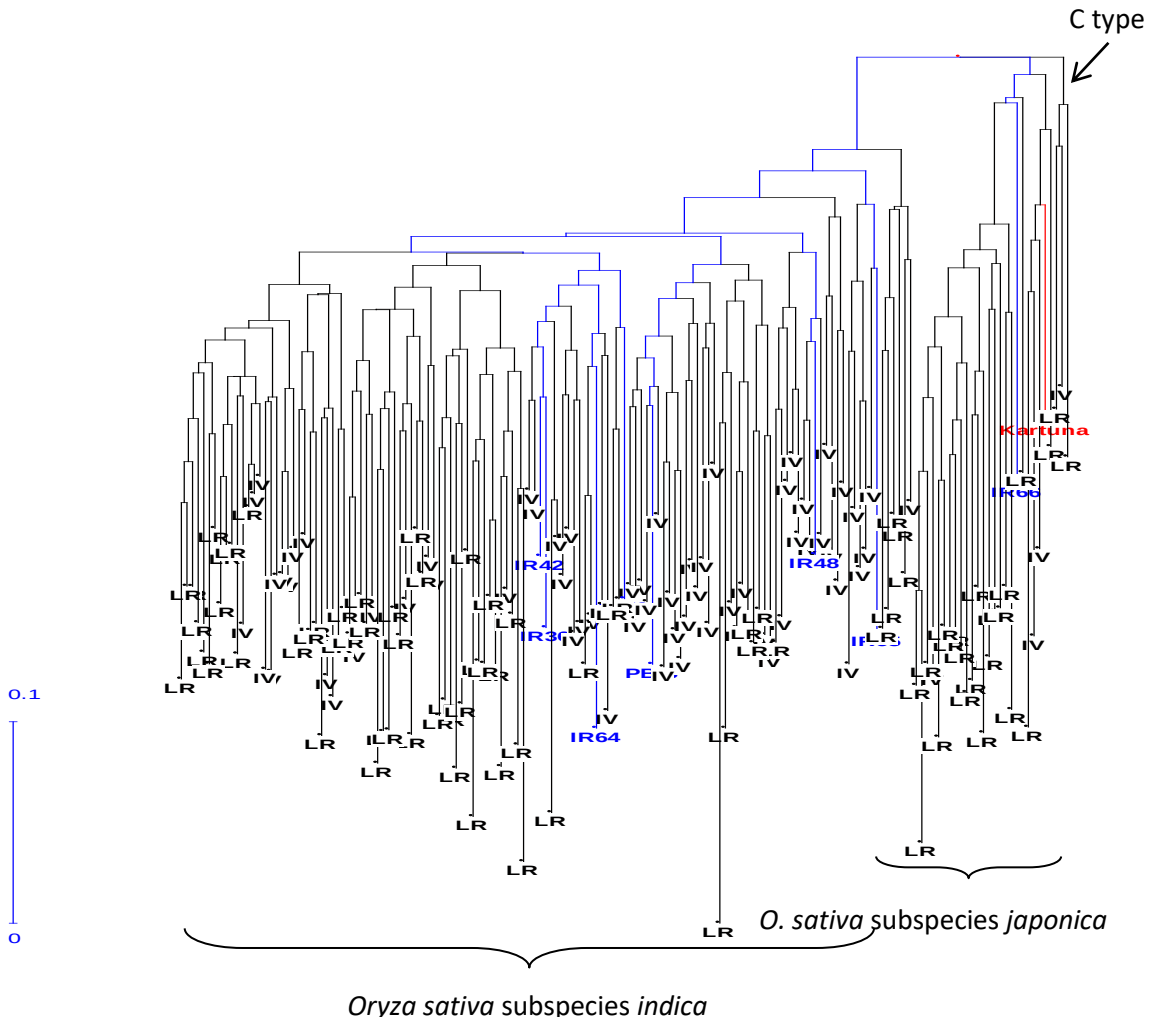

Figure 3. 1. Neighbor joining tree of Indonesian rice germplasm based on 32 SSR markers.

LR is landrace and IV is improved varieties. Blue color indicated IRRI varieties and red color is Kartuna variety, varietal check for tropical *japonica*.

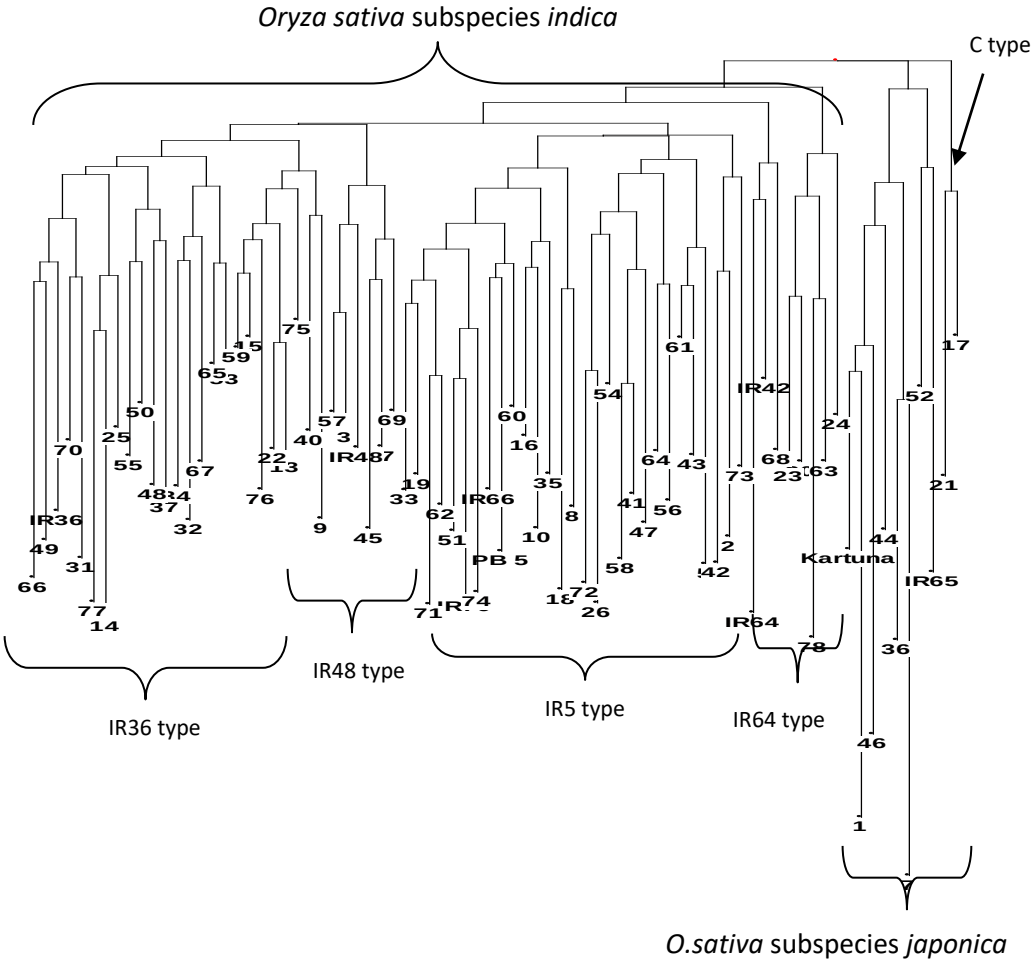

Figure 3. 2. Neighbor-joining tree of 78 Indonesian improved rice varieties based on 32 SSR markers

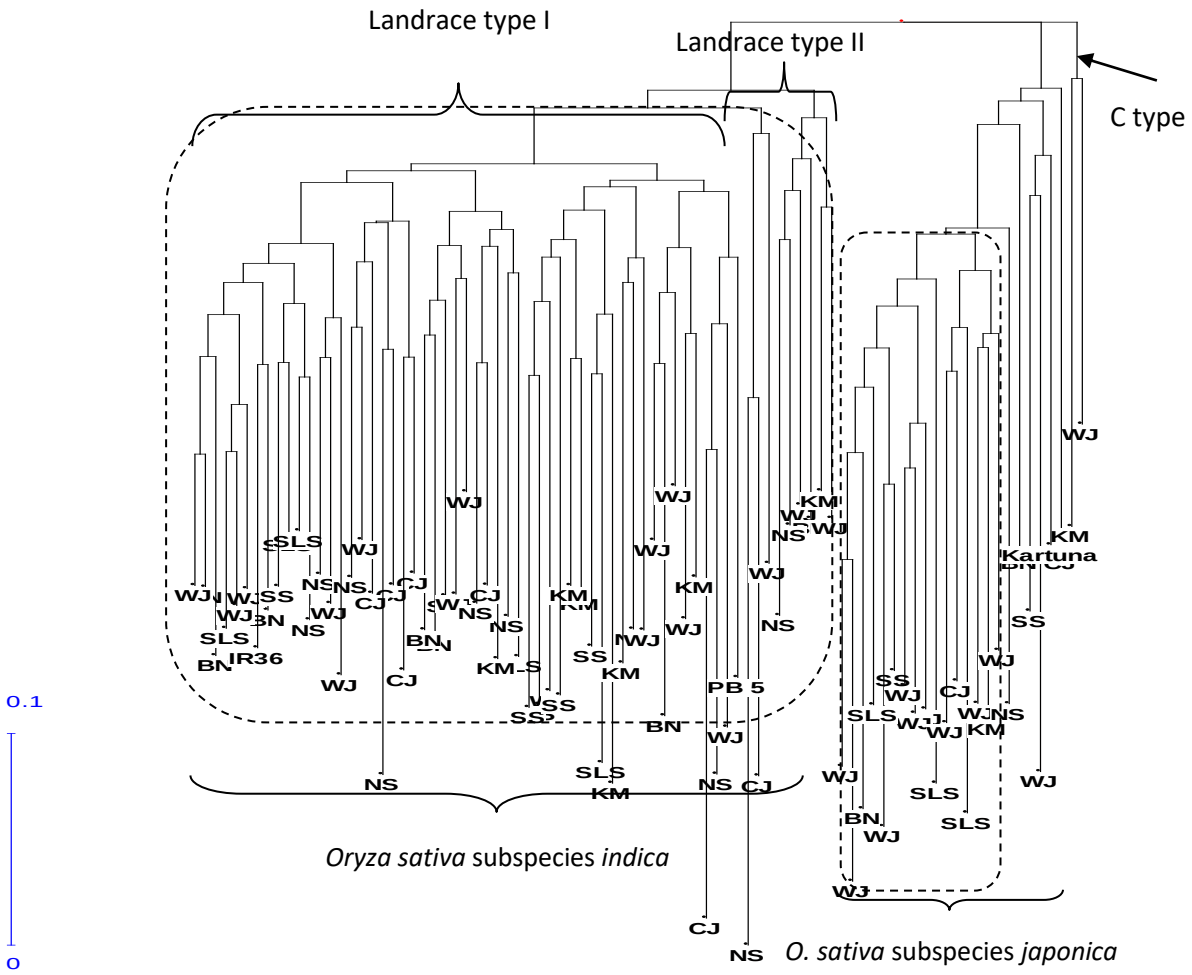

Figure 3. 3. Neighbor-joining tree of 83 Indonesian rice landrace based on 32 SSR markers.

Circle of dashed line indicates two groups, group *indica* and *japonica* which calculated in this study. Abbreviations; *WJ* is West-Java, *CJ* is Central-Java, *BN* is Bali-Nusa, *KM* is Kalimantan, *NS* is North-Sumatra, *SS* is South Sumatra and *SLS* is Sulawesi.

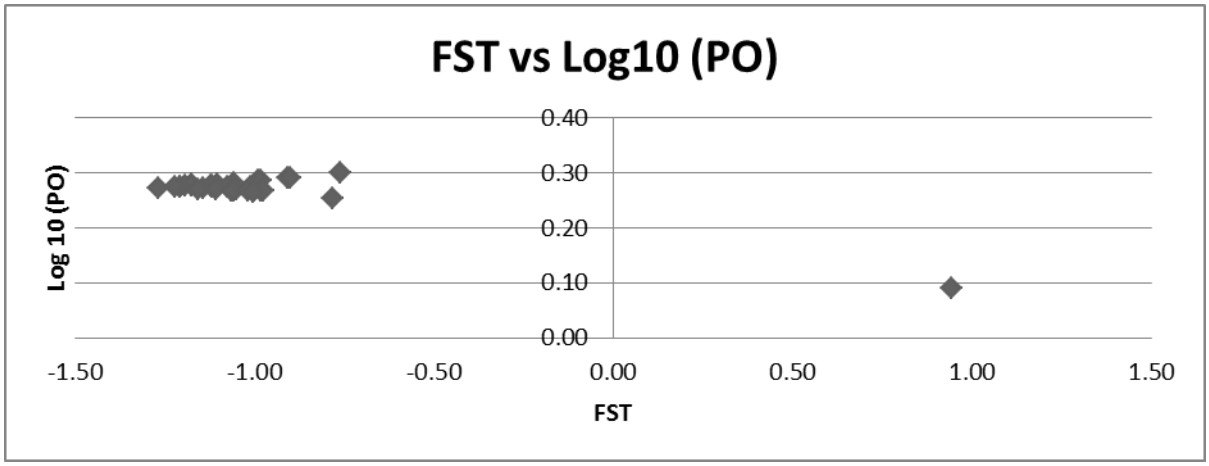

Figure 3. 4. FST vs Log 10 (PO) displayed outlier loci based on SSR markers.

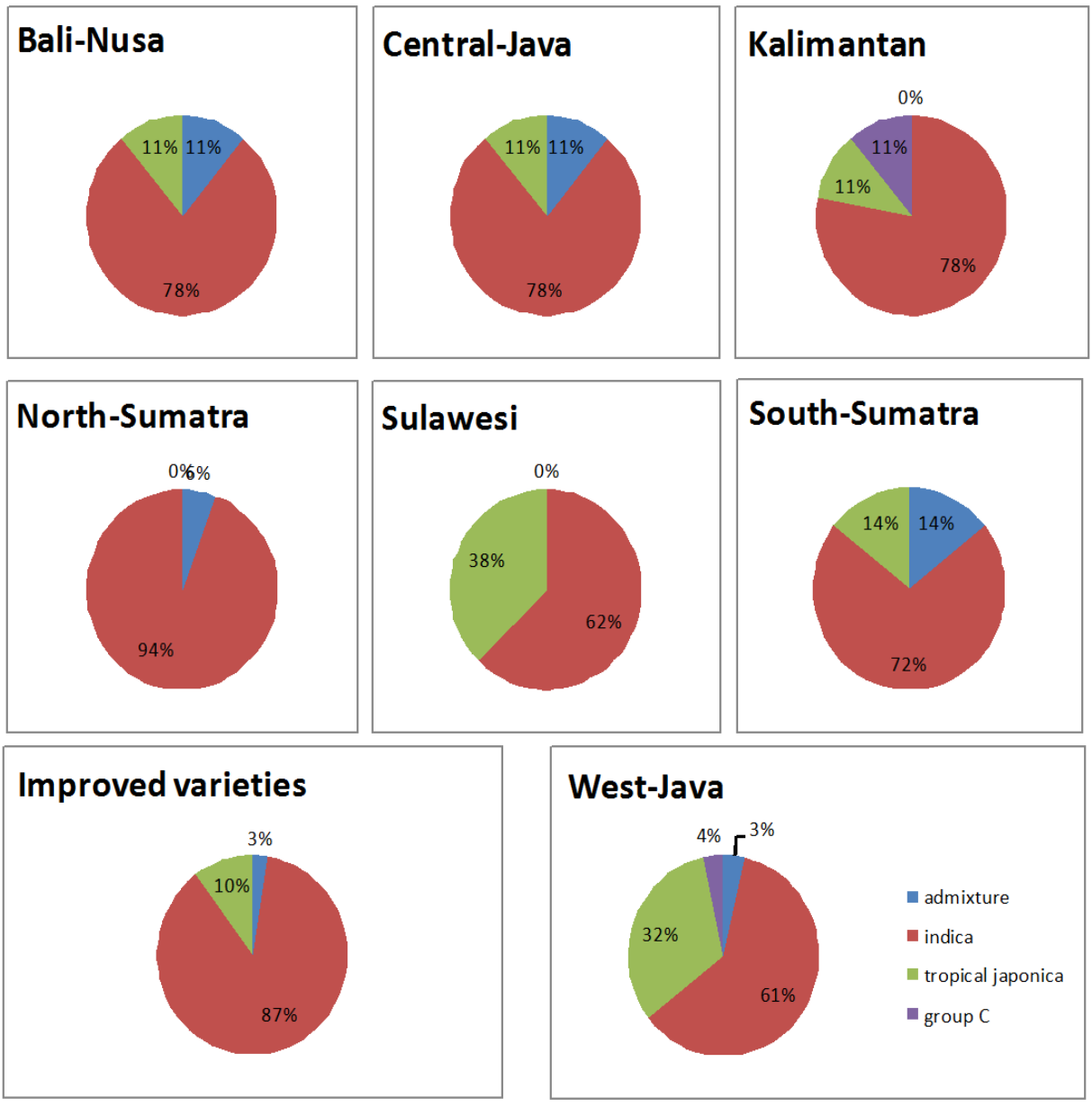

Figure 3. 5. Percentage of composition varietal group of *O. sativa* in Indonesian rice germplasm based on SSR marker.

Table 3. 1. List of SSR markers used in this study.

Locus	Chr.	Repeat motif	PCR primer (5' to 3')		Annealing temp. (^{0}C)
RM431	1	(AG)16	f	TCCTGCGAACTGAAGAGTTG	55
			r	AGAGCAAAACCCTGGTTCAC	
RM312	1	(ATTT)4(GT)9	f	GTATGCATATTTGATAAGAG	55
			r	AAGTCACCGAGTTTACCTTC	
RM583	1	(CTT)20	f	AGATCCATCCCTGTGGAGAG	55
			r	GCGAACTCGCGTTGTAATC	
RM259	1	(CT)17	f	TGGAGTTTGAGAGGAGGG	55
			r	CTTGTTGCATGGTGCCATGT	
RM154	2	(GA)21	f	ACCCTCTCCGCCTCGCCTCCTC	60
			r	CTCCTCCTCCTGCGACCGCTCC	
RM452	2	(GTC)9	f	CTGATCGAGAGCGTTAAGGG	55
			r	GGGATCAAACCACGTTTCTG	
RM71	2	(ATT)10T(ATT)4	f	CTAGAGGCGAAAACGAGATG	55
			r	GGGTGGGCGAGGTAATAATG	
RM240	2	(CT)21	f	CCTTAATGGGTAGTGTGCAC	55
			r	TGTAACCATTCCTTCCATCC	
RM450	2	(AG)17	f	AAACCACAGTAGTACGCCGG	55
			r	TCCATCCACATCTCCCTCTC	
RM489	3	(ATA)8	f	ACTTGAGACGATCGGACACC	55
			r	TCACCCATGGATGTTGTCAG	
RM55	3	(GA)17	f	CCGTCGCCGTAGTAGAGAAG	55
			r	TCCCGGTTATTTTAAGGCG	
RM251	3	(CT)29	f	GAATGGCAATGGCGCTAG	55
			r	ATGCGGTTCAAGATTCGATC	
RM16	3	(TCG)5(GA)16	f	CGCTAGGGCAGCATCTAAA	55
			r	AACACAGCAGGTACGCGC	
RM307	4	(AT)14(GT)21	f	GTACTACCGACCTACCGTTCAC	55
			r	CTGCTATGCATGAACTGCTC	
RM124	4	(TC)10	f	ATCGTCTGCGTTGCGGCTGCTG	67
			r	CATGGATCACCGAGCTCCCCCC	
RM334	5	(CTT)20	f	GTTCAGTGTTCAGTGCCACC	55
			r	GACTTTGATCTTTGGTGGACG	

Locus	Chr.	Repeat motif	PCR primer (5' to 3')		Annealing temp. (^{0}C)
RM161	5	(AG)20	f	TGCAGATGAGAAGCGGCGCCTC	61
			r	TGTGTCATCAGACGGCGCTCCG	
RM162	6	(AC)20	f	GCCAGCAAAACCAGGGATCCGG	61
			r	CAAGGTCTTGTGCGGCTTGCGG	
RM454	6	(GCT)8	f	CTCAAGCTTAGCTGCTGCTG	55
			r	GTGATCAGTGCACCATAGCG	
RM402	6	(ATA)7	f	GAGCCATGGAAAGATGCATG	55
			r	TCAGCTGGCCTATGACAATG	
RM586	6	(CT)23	f	ACCTCGCGTTATTAGGTACCC	55
			r	GAGATACGCCAACGAGATACC	
RM11	7	(GA)17	f	TCTCCTCTTCCCCCGATC	55
			r	ATAGCGGGCGAGGCTTAG	
RM118	7	(GA)8	f	CCAATCGGAGCCACCGGAGAGC	67
			r	CACATCCTCCAGCGACGCCGAG	
RM408	8	(CT)13	f	CAACGAGCTAACTTCCGTCC	55
			r	ACTGCTACTTGGGTAGCTGACC	
RM284	8	(GA)8	f	ATCTCTGATACTCCATCCATCC	55
			r	CCTGTACGTTGATCCGAAGC	
RM404	8	(GA)33	f	CCAATCATTAACCCCTGAGC	55
			r	GCCTTCATGCTTCAGAAGAC	
RM215	9	(CT)16	f	CAAAATGGAGCAGCAAGAGC	55
			r	TGAGCACCTCCTTCTCTGTAG	
RM171	10	(GATG)5	f	AACGCGAGGACACGTACTTAC	55
			r	ACGAGATACGTACGCCTTTG	
RM552	11	(TAT)13	f	CGCAGTTGTGGATTTCAGTG	55
			r	TGCTCAACGTTTGACTGTCC	
RM536	11	(CT)16	f	TCTCTCCTCTTGTTTGGCTC	55
			r	ACACACCAACACGACCACAC	
RM19	12	(ATC)10	f	CAAAAACAGAGCAGATGAC	55
			r	CTCAAGATGGACGCCAAGA	
RM277	12	(GA)11	f	CGGTCAAATCATCACCTGAC	55
			r	CAAGGCTTGCAAGGGAAG	

Table 3. 2. Genetic differentiation (F_{ST}) and genetic distance (D) between *Oryza sativa* subspecies.

Between *O. sativa* subspecies			*F*st	*D*
indica		***japonica***	0.161**	0.294
landrace		landrace	0.242**	0.218
improved variety		improved variety	0.134**	0.186
indica	vs	***indica***		
improved variety		landrace	0.048**	0.057
japonica		***japonica***		
improved variety		landrace	0.173**	0.268

Note: ** indicated significant level at P< 0.001

Table 3. 3. FST (differentiation), log10 (PO) and Qtl of 4 out of 32 locus detected under *indica* and *japonica* by Bayescan v 2.1

No	Locus name	Chr.	FST	Log10 (PO)	Qtl/function	References
1	RM536	11	0.091	0.945	*qDSR*11-2 (dead seedling rate)	Qi et al. (2008)
2	RM408	6	0.291	-0.759	*qShB*6 (sheath bliight resistance)	Jia et al. (2012)
3	RM552	11	0.29	-0.781	*qPS*11 (pollen streility)	Jing et al. (2006)
4	RM454	6	0.286	-0.899	*qRsn*6 (relative number of spikelets per panicle)	Yue et al. (2006)

Table 3. 4. Inbreeding coefficient (Fis and Fst) and gene flow (*N*m) between *Oryza sativa* subspecies

Population	**Fis**	**Fst**	***N*m**
Indica and *japonica*	1*	0.299**	0.585

Note: ** indicated significant level at P< 0.001, three levels of gene flow in self-pollination crops, high, Nm > 1, intermediate, 0.250 < Nm < 0.999; and low, Nm < 0.249 (Govindajaru, 1989)

Table 3. 5. Bayesian estimates of posterior (θ), rate of migration (M) and level of gene flow (*N*m) calculated by Migrate-n from SSR markers.

Theta (θ) and direction of migration (M)	Mode	Range	Nm=M.Θ/4
θ (*indica*)	0.63	0.12-0.65	
θ (*japonica*)	0.57	0.06-0.59	
M (*japonica→indica)*	1.613	0.8-1.64	0.254
M (*indica→ japonica*)	6.653	5.867-6.92	0.948

Note; three levels of gene flow in self-pollination crops, high, Nm > 1, intermediate, 0.250 < Nm < 0.999; and low, Nm < 0.249 (Govindajaru, 1989)

Table 3. 6. Genetic differentiation (F_{ST}) and genetic distance (D) among landrace from different source locations in Indonesian rice germplasm.

Populations			F_{ST}	D
Improved varieties	VS	Bali-Nusa	0.059*	0.076
		Central-Java	0.082*	0.11
		Kalimantan	0.066*	0.089
		North-Sumatra	0.058*	0.078
		South Sulawesi	0.082*	0.119
		South Sumatra	0.117*	0.163
		West Java	0.055*	0.079
Bali-Nusa		Central-Java	0.076	0.109
		Kalimantan	0.071	0.104
		North-Sumatra	0.046	0.066
		South Sulawesi	0.077	0.12
		South Sumatra	0.086	0.121
		West Java	0.044	0.067
Central-Java		Kalimantan	0.066	0.095
		North-Sumatra	0.048	0.068
		South Sulawesi	0.082	0.128
		South Sumatra	0.087	0.122
		West Java	0.051	0.079
Kalimantan		North-Sumatra	0.055	0.082
		South Sulawesi	0.086	0.141
		South Sumatra	0.086	0.125
		West Java	0.036	0.056
North-Sumatra		South Sulawesi	0.065	0.104
		South Sumatra	0.082	0.12
		West Java	0.043	0.069
South Sulawesi		South Sumatra	0.1	0.157
		West Java	0.049	0.083
South Sumatra		West Java	0.068	0.106

Note: ** indicated significant level at P< 0.001, * indicated siginificant at level P< 0.01.

Table 3. 7. Mean of differentiation among neutral loci (FST) among landrace from different source locations.

Pairwise			**FST**
West-Java	VS	Bali-Nusa	0.075
		Central-Java	0.062
		Kalimantan	0.074
		Sumatra	0.065
		Sulawesi	0.097
Bali-Nusa		Central-Java	0.104
		Kalimantan	0.133
		Sumatra	0.105
		Sulawesi	0.118
Central-Java		Kalimantan	0.100
		Sumatra	0.079
		Sulawesi	0.114
Kalimantan		Sumatra	0.093
		Sulawesi	0.128
Sumatra		Sulawesi	0.103

Table 3. 8. Diversity of improved varieties and landrace of Indonesian rice.

Population		**N**	**Na**	**H**	**AR**	**PA**	**Percentage of polymorphism (%)**
Oryza sativa	improved variety	78	3.125	0.647	2.870	0.156	100.00
	landrace	83	3.406	0.816	3.111	0.313	96.88
O. sativa		161	3.688	0.777	3.413	1.406	100.00

Note: N = number of cultivars, Na = number of alleles, H = Shannon's diversity index, AR = allelic richness, PA = private allele

Table 3. 9. Diversity of improved varieties and landrace in comparison between subspecies of Indonesian rice.

Population		**N**	**Na**	**H**	**AR**	**PA**	**Percentage of polymorphism (%)**
Oryza sativa	*indica* improved varieties	68	3.031	0.596	2.726	0.094	100.00
	indica landrace	60	3.156	0.695	2.853	0.188	93.75
	japonica improved varieties	8	2.281	0.623	2.009	0.000	81.25
	japonica landrace	16	2.313	0.556	1.972	0.063	87.50
O. sativa		152	3.625	0.765	3.360	1.375	100

Note: N = number of cultivars, Na = number of alleles, H = Shannon's diversity index, AR = allelic richness, PA = private allele

Table 3. 10. Diversity among taxa of Indonesian rice and in comparison between from different source locations.

Population		**N**	**Na**	**H**	**AR**	**PA**	**Percentage of polymorphism (%)**
Oryza sativa	Improved variety	76	3.125	0.646	2.114	0.156	100.00
	Bali-Nusa	8	2.469	0.655	2.020	0.000	87.50
	Central-Java	8	2.281	0.619	1.869	0.031	87.50
	Kalimantan	8	2.406	0.662	2.081	0.000	87.50
	North-Sumatra	12	2.656	0.694	2.274	0.125	84.38
	Sulawesi	8	2.313	0.673	2.057	0.000	90.63
	South Sumatra	6	2.219	0.605	1.943	0.000	78.13
	West Java	26	2.844	0.763	2.568	0.063	96.88%

Note: N = number of cultivars, Na = number of alleles, H = Shannon's diversity index, AR = allelic richness, PA = private allele

Table 3. 11. Diversity of Indonesian germplasm in comparison between landrace from different source locations (*indica* only).

Population	N	Na	H	AR	PA	Percentage of polymorphism
Bali-Nusa	7	2.219	0.565	1.884	0.000	0.781
Central-Java	7	2.000	0.508	1.640	0.000	0.688
Kalimantan	7	2.219	0.590	1.914	0.000	0.781
North-Sumatra	12	2.656	0.694	2.274	0.125	0.844
Sulawesi	5	1.844	0.451	1.652	0.000	0.625
South Sumatra	5	1.938	0.500	1.724	0.000	0.656
West Java	17	2.375	0.552	2.115	0.031	0.813

Note: N = number of cultivars, Na = number of alleles, H = Shannon's diversity index, AR = allelic richness, PA = private allele

4. Diversification and differentiation of Indonesian rice landrace and improved varieties as revealed by SNP markers

4.1 Introduction

Single nucleotide polymorphism (SNP) markers have been more frequently used for genetic analysis instead of SSR markers (Jenkins and Gibson, 2002). Even though SNPs are less polymorphic than SSR markers because of their biallelic nature, scientists and breeders have used SNPs due to the potential for each SNP, in which a single copy DNA is a potentially useful marker and is efficient to monitor genetic diversity over the whole genome (Ganal et al., 2009).

In recent studies in rice, SNP is used to analyze the origin of domesticated rice. The study suggested that *O. rufipogon* is an ancestor of Asian cultivated rice, *O. sativa* and has high diversity. Also, SNP revealed the diversity and relationships between landrace and modern varieties (McNally et al., 2009). Japanese scientists in National Institute of Agrobiological Sciences (NIAS), Japan have developed SNP marker sets to successfully differentiate 76 rice varieties into four groups: *aus*, *indica*, tropical *japonica*, and temperate *japonica*. Subspecies *japonica* comprises temperate *japonica* and tropical *japonica*, while subspecies *indica* contains *indica* and *aus* as revealed using SSR markers (Garris et al. 2005). These SNP markers have been validated by SNP genotyping arrays and the results corresponded well to McNally et al., (2009). The sequences, as well as additional information such as chromosome and position within chromosome of these SNP markers can be found in their study (Yonemaru et al., 2014).

In this study, we used these SNP markers because of the advantages for SNP markers compared with SSR markers and to clarify the results using SSR markers in Chapter 3. SSR markers have the high mutation rate, while less mutation rate in SNP markers. It might cause inaccuracies and lack of understanding of gene flow among populations. Also, a high mutation rate among polymorphic loci could (mistake) to underestimation of differentiation among subpopulation. The numerous alleles and high mutation rate of SSR markers have the effect on F_{ST} value; even there is no gene flow among populations (Balloux et al. 2000). These advantages could also provide an opportunity to identify numerous 'outliers' loci under selection. Xu et al. (2012) reported that SNP markers could identify a lot of loci between wild relatives and rice landrace population. Polymorphic loci have been selected during the process of domestication of rice. The result could provide valuable information for breeding

program. Wild relatives and landrace are becoming important due to genes conferring resistance to biotic and abiotic stresses and adaptability (Hawkes, 1991). This fact suggested that conserving the genetic resource was important. In this study, we used rice landrace in a broad range of locations in Indonesia. Indonesia is an archipelago country in Asia in which rice landrace has been maintained and *O. rufipogon* grows in the main islands. The objective of this study is to reveal the differentiation and the main mechanism associated with differentiation of Indonesian rice germplasm

4.2 Materials and methods

Plant materials

The total of 200 Indonesian rice varieties consisted of 100 Indonesian landrace and 100 Indonesian improved cultivars from Indonesia Center for Rice Research, Subang, Indonesia (Table 2. 1 and 2. 2).

DNA isolation and SNP genotyping

Plants were grown in nursery boxes in a greenhouse at the University of Tsukuba, Japan, and leaf samples were harvested from a single 30-day-old plant of each cultivar. Leaf samples were crushed in a crusher with a zirconium ball in micro-tubes (2 mL), and DNA was isolated using a modified cetyl trimethylammonium bromide (CTAB) method (McCouch et al., 1988).

Total genomic DNA isolated from fresh leaf tissue of one plant per genotype was quantified and diluted to 50 ng/μL in sterile 10 mM Tris-HCl, pH 8.0, 1.0 mM EDTA, pH 8.0 (TE). Allele-specific oligonucleotide hybridization used 5 μL of single-use template genomic DNA (50 ng/μL) of each genotype. In this study, we used 768 SNP markers. These SNP markers were designed by Yonemaru et al. (2014). Template DNA and a negative control (water) were mixed with 768 different SNPs. The PCR conditions were 10 min at 37°C, then 3 min at 95°C, followed by 34 cycles of 35 s at 95°C, 35 s at 56°C, and 2 min at 72°C, followed by a final extension at 72°C for 10 min. These SNP markers were genotyped in 200 genotypes using the Golden Gate Bead Array technology platform (Illumina, San Diego, CA) and we followed the manufacturer's instructions in all experimental procedures for SNP genotyping. Of the 200 DNA samples, 83 landrace and 78 improved varieties provided enough data (<15% missing) to use. Admixtures varieties (C type) were omitted from further calculation (Table 4. 12 and 4. 13).

Data analysis

The molecular marker data were analysed in DARwin 5 (Perrier and Jacquemoud-Collet, 2006), GenAlEx 6.5 (Peakall and Smouse, 2006), Migrate-n (Beerli and Palczewski, 2010) and Bayescan v 2.1 (Foll and Gaggiotti, 2008) software.

DARwin 5 was used to calculate NJ trees based on genetic distance using 1000 bootstrap. GenAlEx 6.5 was used to calculate the number of alleles per locus (*Na*), number of effective alleles (*Ne*), percentage of polymorphism (P), gene diversity (*H*; Shannon, 1948) and alleleic richness (Pettitt et al. 1998). Significance of the inbreeding coefficients within subpopulation (Fis) and among subpopulations (Fst) per locus was tested using 999 permutations (Wright, 1978), gene flow (Nm) by formula (1 – FST)/4 (Wright, 1951), genetic distance (*D*) between populations (*indica* and *japonica*) (Nei, 1972), and genetic differentiation (F_{ST}; Nei, 1973) using 999 permutations.

Migrate-n was used to calculate direction pattern of gene flow (Nm) by estimating posterior distribution mean ($\theta = 4 Ne\mu$, Ne is the effective population size and μ is the mutation rate per site per generation), migration rate ($M = m/\mu$, where m is the rate of migration for each locus) and formula ($Nm = M \theta/4$) was used to estimate gene flow (number of migrants per generation). We run this software for SNP markers, the uniform priors were placed on θ from 0 to 0.006 and M from 0 to 5000 by Bayesian inference strategy for Hapmap data (SNP model) and starting parameters for migrant value and θ were generated from few trials. For Markov chain setting, 1000 steps were recorded and followed by 20 sample increments and 10000 burns-in for each chain.

Bayescan v.2.1 software was used to calculate differentiation among neutral locus (FST) and also this software was used to detect candidate loci for divergence based on NJ tree, in which outlier loci generated by this software were signs of natural selection. The evidence of selection in the range $0.5 < \log10 (PO) < 1$ is substantial, strong if $1< \log10 (PO) <1.5$ and very strong if $1.5 < \log10 (PO) < 2$ as explained on manual of this software. In this study, we calculated Fis, Fst, neutral locus (FST), direction of migration and gene flow using *O. sativa* (landrace). Improved varieties were excluded from calculation because of complexity in crossing during breeding history to develop new varieties. .

As landrace in Indonesia could be divided by source locations, we calculated mean of differentiation among neutral locus (FST) in pairwise between different source locations of landrace using Bayescan v. 2.1. These results were compared with mean of phenotypic divergence based on morphological traits (*Qst*).

4.3 Results

Genetic structure of Indonesian rice

Genetic structure analysis in Indonesian rice germplasm using 768 SNP markers divided Indonesian rice into two major groups, *indica* and *japonica* as refer to varieties control. We call the group involving PB5 and IR36 as *indica* and the group involving Kartuna as *japonica*. One variety was located in the centre of NJ tree and we call this variety as C type (Figure 4. 1). SNP markers categorized 88.5% as *indica*, 11% as *japonica* and 0.5% as C type (Figure 4. 1). Indonesian landrace was divided into three groups, *indica* landrace type I, II and *japonica* group (Figure 4. 2, Table 4. 12). The clustering showed that improved varieties differentiated from landrace and were divided into 6 groups, IR36 type, IR48 type, IR5 type, IR64 type, IR66 type, x type and *japonica* groups (Figure 4. 3, Table 4. 13). In addition, we found C type involving one landrace and two improved varieties when we performed clustering using each population

The significant differentiation (F_{ST}) and high value of genetic distance (D) based on Nei's calculation were observed between *indica* and *japonica* and among all pairwise comparisons. The F_{ST} and D between improved *indica* variety and *indica* landrace were the lowest (0.054; 0.020), while F_{ST} and D between *indica* landrace and improved *japonica* varieties were the highest (0.517; 0.444) (Table 4. 1).

Outliers based on the differentiation of *indica* and *japonica* by NJ tree have identified 4 loci with Log10 (PO) >1 (strong) in the range of FST (0.25-0.89). The pattern of haplotype mutation in the three loci under natural selection between *indica* and *japonica* with the evidences of these loci in the range FST > 0.8 and Log10 (PO) >1 was generated by Bayesian method and these loci had different function (Figure 4. 4, Table 4. 2 and Table 4. 3).

Inbreeding coefficients derived from inbreeding within sub-populations (Fis) and inbreeding coefficient from differentiation between sub-populations (Fst) were significantly distant from 0 (Table 4. 4). We found the low level of gene flow (Nm) between *indica* and *japonica* (0.12) according to the level Nm of Govindajaru classification. Govindajaru (1989) classified three levels of gene flow in self-pollination crops, high, Nm > 1, intermediate, 0.250 < Nm < 0.999; and low, Nm < 0.249). We found that the mode of migration rates (M) and gene flow (Nm) based on direction from *japonica* to *indica* (283.3, 0.123) were lower than M classified as low gene flow and Nm from *indica* to *japonica* (3045, 0.990) was classified as intermediate gene flow (Table 4. 5). It seems that the pattern of gene flow within cultivated rice was asymmetric.

The significant F_{ST} and various *D* values were found in all pairwise comparison between improved varieties and landrace from different source locations except between improved varieties and landrace from Sulawesi (0.047, 0.024) (Table 4. 6). We found significant F_{ST} and the highest value of *D* between landrace from Bali-Nusa and landrace from Central-Java (0.141; 0.086) and between Central-Java and North-Sumatra (0.111; 0.060), while other pairwise comparison showed non-significance for FST (Table 4. 6).

We found a wide range of mean values of differentiation among neutral loci (FST) in comparison with landrace from different source locations using Bayesian method (Table 4. 7). The FST values ranged from 0.0005 to 0.247. The comparison between morphological and molecular divergence could explain the pattern of natural selection such as directional selection (*Qst*>FST), stabilizing selection and if the *Qst*=FST, the genetic drift and selection could not separate. The result of phenotypic divergence was previously described in Chapter 2.

Diversity of Indonesian rice germplasm

Diversity analysis revealed the percentage of SNP polymorphism (P) was 80% *(*Table 4.8). Shannon's *H* and alelleic richness (AR) of landrace (0.39; 1.88) was higher than *H* and AR of improved varieties (0.32; 1.86), while private allele (PA) in improved varieties was higher than PA in landrace (0.096; 0.056) (Table 4. 8). Within landrace, *H*, AR and PA of *japonica* (0.214; 1.378; 0.012) were lower than *H*, AR and PA of *indica* (0.233; 1.555; 0.034). In contrast, within improved varieties *H* of *japonica* (0.360) showed higher than *H* of *indica* when AR and PA of *japonica* (1.363; 0.012) showed lower than AR and PA of *indica* (1.858; 0.057) (Table 4. 9).

Diversity analysis according to source locations of landrace showed *H* in landrace from Bali-Nusa (0.41) was the highest and *H* in landrace from Central-Java (0.21) was the lowest. Allelic richness (AR) in landrace from West-Java (1.74) was the highest and AR in landrace from Central-Java (1.34) was the lowest. We found similar value of private allele on three source locations; Kalimantan, North Sumatra and Sulawesi (Table 4. 10). The consisting *japonica* affects diversity, allelic richness and private allele. *H*, AR and PA of landrace from Bali-Nusa did not show the highest value (0.14) when we excluded the *japonica* from calculation (Table 4. 11). The *japonica* in each source location was involved (Figure 4. 5).

4.4 Discussion

In Indonesian rice landrace, diversity of *japonica* was lower than *indica.* This result was different from previous study as reported by Thomson et al. (2007) and in agreement with Glaszmann (1987) and Li and Rutger (2000). In contrast, *japonica* was more diverse than

indica in improved varieties. The different result between our study and previous studies is due to excluding admixture varieties in *japonica* group from calculation in this study and including them in calculation in previous study by Thomson et al. (2007). Here, we suggested that the lower diversity in *japonica* landrace than *indica* landrace might be affected by selection for particular traits. The other mechanism on decreasing diversity in *japonica* is bottle-neck effects as reported in rice germplasm in Madagascar (Mather et al., 2010) and in Asia including Indonesia (Molina et al., 2011). Sun et al. (2001) suggested that effective alleles have been lost by natural and human selection and genetic diversity in cultivated rice have been gradually decreased. This phenomenon was supported in improved varieties group, in which *indica* was lower diversity than *japonica*. It indicated that breeding program in Indonesia focused on improving *indica* varieties. However, alleleic richness of *indica* in improved varieties was increased and it was related to increasing number of private allele. This indicated that breeding program generated more alleles but did not affect diversity or less number of effective alleles. The result of allelic richness in this study showed the pattern of *H* index corresponded well to allelic richness (AR), suggesting that our results were reliable. For example, the pattern of *H* index and AR in improved *indica* varieties were 0.25 and 1.86, while *H* index and AR in *indica* landrace were 0.23 and 1.56. These results showed that when the *H* index was high, it (What??) was shown in AR and also that our results were reliable even though we used small number of individuals and unbalanced number of individuals among population. The allelic richness and private allele analysis is useful to discover diversity when the sample size is small and unbalanced among populations (Foulley and Olliver, 2006).

The tendency on selection pressure was revealed by molecular markers. The result indicated that the continuous use of IRRI varieties as breeding materials caused genetic similarities of rice germplasm in Indonesia and it could be clarified by relationship between breeding materials and sub-clustering of variety group into 6 groups. The extensive use of elite varieties as breeding materials might cause the decrease of genetic diversity as similarly reported in Indian rice by Choudhary et al. (2013) and in Italian rice by Mantegazza et al. (2008).

Inbreeding coefficient through inbreeding within sub-population (Fis) and inbreeding coefficient through differentiation between sub-population (Fst) were statistically deviated from zero. Even though we found high value of Fis, we also revealed various values of Fst which was caused by gene flow (Nm) between sub-population which took place in

Indonesian rice germplasm. These results indicated that these sub-populations were deviated from the Hardy-Weinberg equilibrium. According to Govindajaru classification, the ranges of Nm in this study were low to intermediate. This study revealed that the pattern of migration and gene flow was asymmetric between *indica* and *japonica* and *japonica* received more genes from *indica*. Asymmetric gene flow from donor population to recipient population occurred for long period, in particular alleles of the recipient population were displaced and alleles of the donor population were replaced, unless there was strong selection for donor alleles (Papa and Gepts, 2004). This evidence supported that appearance of admixture varieties in *japonica* group in this study was due to hybridization. Besides, possible reason that might take place low diversity in *japonica* in Indonesia is due to the gene flow. Haygood et al. (2003) reported that the recurrent gene flow could fast replace genes even for disfavored crop genes and affect the diversity of recipient crops.

Two subspecies of *Oryza sativa* in this study indicated that Indonesian farmers have maintained a broad range of *O. sativa* (landrace germplasm) and extended its diversity during the history of rice cropping. The study of the staple food culture in Southeast Asia reported that Indonesian people in Sumatra, Java, Borneo and Celebes has maintained and cultivated upland and lowland rice due to very importance of rice for traditional custom and religious obligation (Matsuyama, 2009).

In this study, we identified SNP in *Os*03t0429800-01 which is a homolog to Xanthine dehydrogenase 1 (*XDH 1*) associated with metabolism under drought in *Arabidopsis* (Watanabe et al., 2010) and SNP in *Os*04t0504500-01 which is a homolog to protein BABY BOOM 1 (*BBM 1)* that preferentially expresses in developing embryos and seeds in *Arabidopsis* and *Brassica* (Boutilier et al., 2002). These results suggested that natural variations of SNP in these genes might be related to differentiation between *indica* and *japonica* in Indonesia by mutation and/or recombination event. Recent study in rice reported that agronomically important genes have been selected between cultivated rice and wild rice using Bayesian method to detect potential genes (Xu et al., 2012). Moreover, the presence of these genes has increased genetic diversity in Indonesian rice landrace. For example, the haplotypes A and G in *japonica* were detected, while only the G haplotype in *indica* at locus ad03009571 wherein this SNP is the homolog to *XDH* 1. The presence of haplotype A/T in *japonica*, while only T haplotype in *indica* at locus ad04009074 wherein no information yet about the function of this SNP and the haplotype C and T in *japonica*, while only C haplotype in *indica* at locus P0724 wherein this SNP is homolog to *BBM* 1.

One of other mechanisms on evolution of population as suggested by Hardy-Weinberg (1908) is natural selection. This study discovered that most of phenotypic divergence (*Qst*) values were lower than differentiation among loci under neutral molecular markers (FST). Our result indicated that the same genotype was favoured in different populations due to stabilizing selection in most of pairwise between landrace from different source locations. However, we found higher *Qst* than FST in pairwise landrace from West-Java and Bali-Nusa in most of traits. These results indicated directional selection between these populations. Three scenarios are possible to explain the relation between *Qst* and FST. First, a higher divergence in quantitative traits compared with neutral molecular markers (*Qst*> FST) indicates directional selection among populations. Second, the opposite scenario (*Qst* < FST) suggests that the same genotypes are favoured in different populations due to stabilizing selection. Third, if the two measures do not significantly differ, the possibilities of genetic drift versus selection cannot be separated (Pertoldi et al., 2012). These results supported that landrace in this study was not differentiated among different source locations. In addition, the differentiation of landrace into three groups, landrace type I, II and *japonica* indicated that the selection pressure caused the appearance of landrace type II without IRRI varieties (IR5/PB5 and IR36), suggesting that these varieties are useful for future rice breeding program because these landrace showed wide dissimilarity in within group and among the landrace type (Figure 4.2).

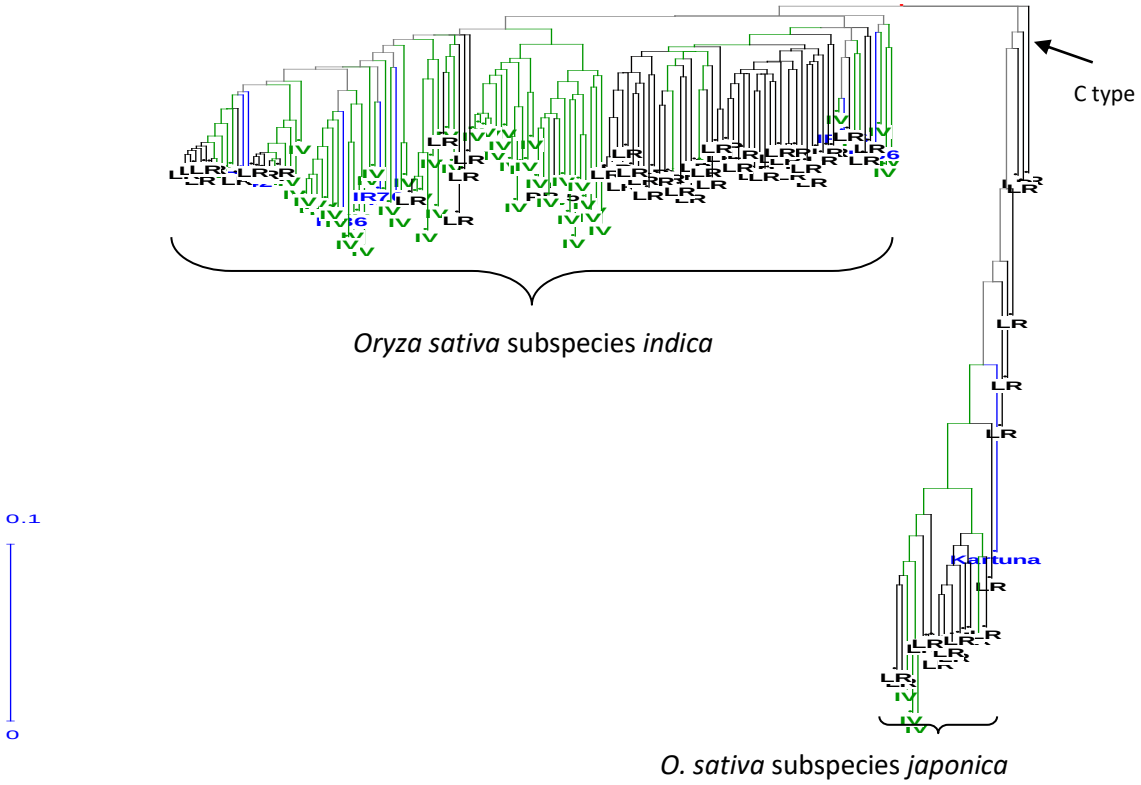

Figure 4. 1. Neighbor- joining tree of Indonesian rice germplasm based on 768 SNP markers. LR is landrace and IV is improved varieties

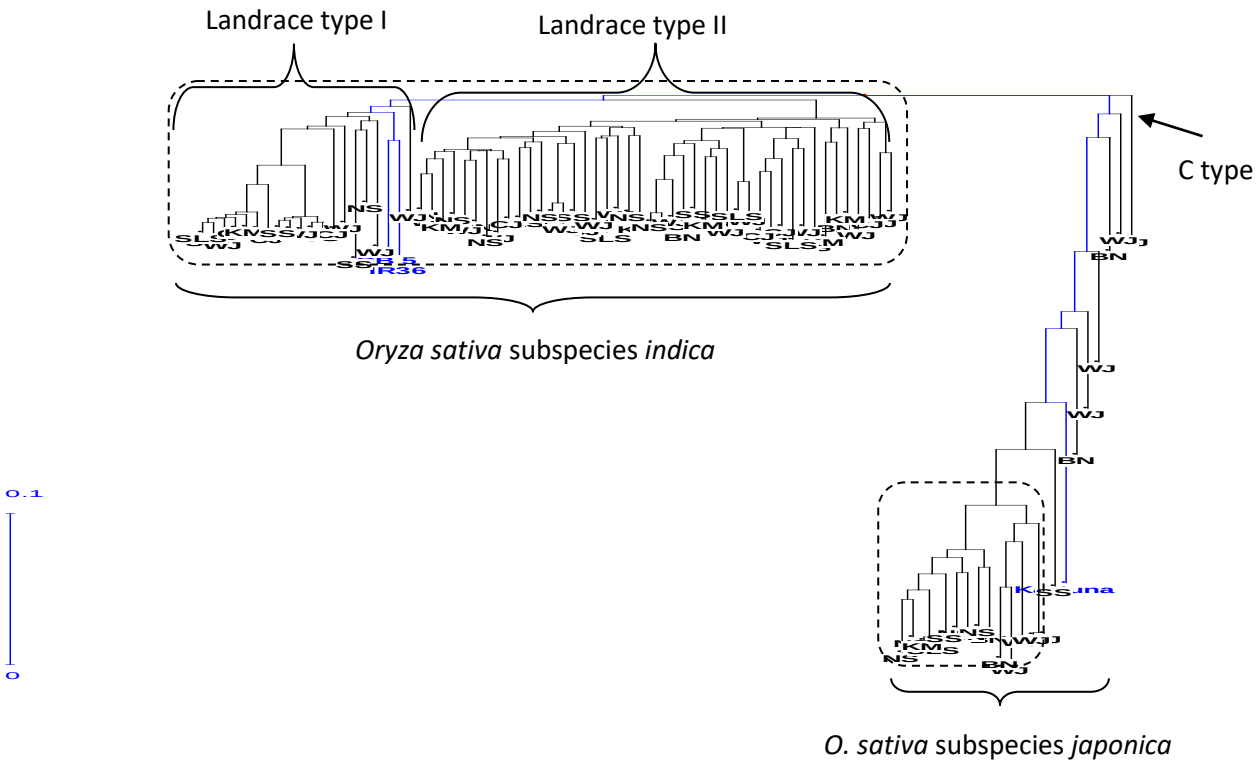

Figure 4. 2. Neighbor-joining tree of 83 rice landrace based on 768 SNP markers.

Circle of dashed line indicates two groups, group *indica* and *japonica* which calculated in this study. Abbreviations; *WJ* is West-Java, *CJ* is Central-Java, *BN* is Bali-Nusa, *KM* is Kalimantan, *NS* is North-Sumatra, *SS* is South Sumatra and *SLS* is Sulawesi.

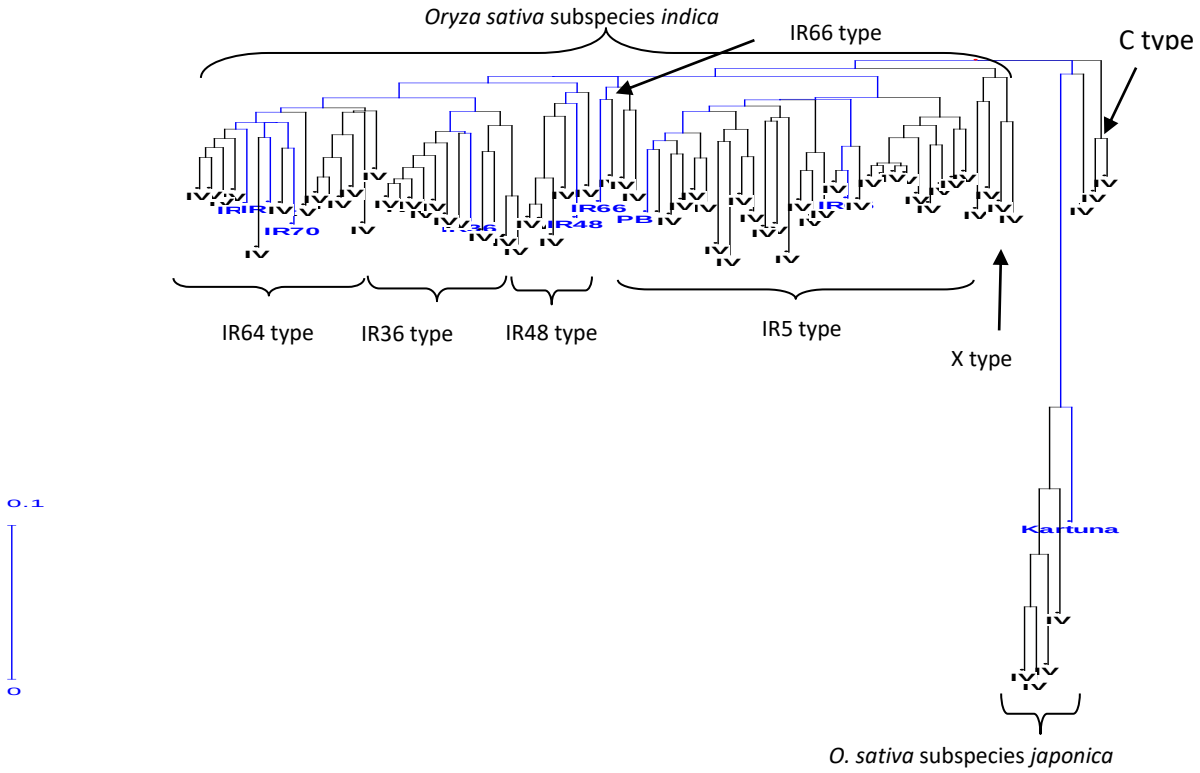

Figure 4. 3. Neighbor-joining tree of 78 Indonesian improved varieties based on 768 SNP markers.

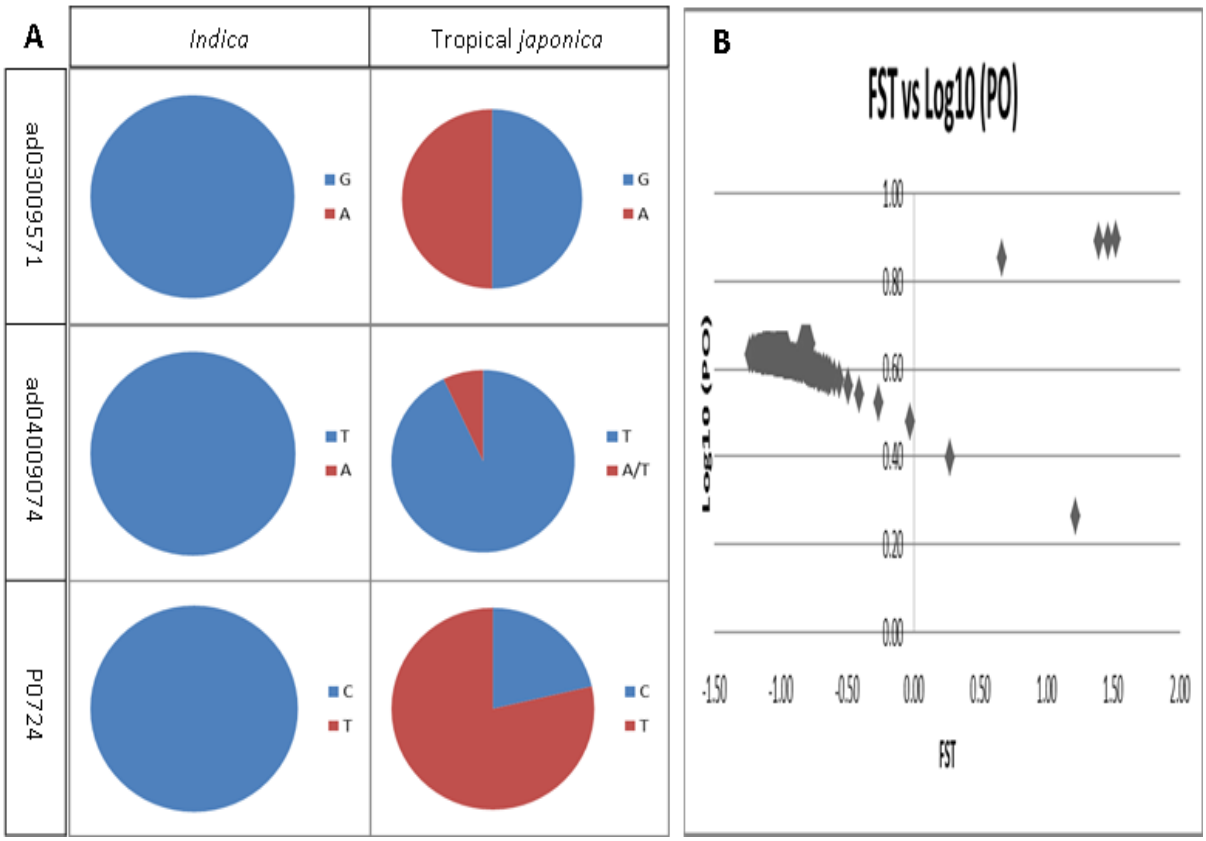

Figure 4. 4. Comparison of SNP haplotypes patterns at 3 loci (Log10 (PO)>0.5) under natural selection between *indica* and *japonica* (A) and FST vs Log10 (PO) displayed outlier loci based on SNP markers (B).

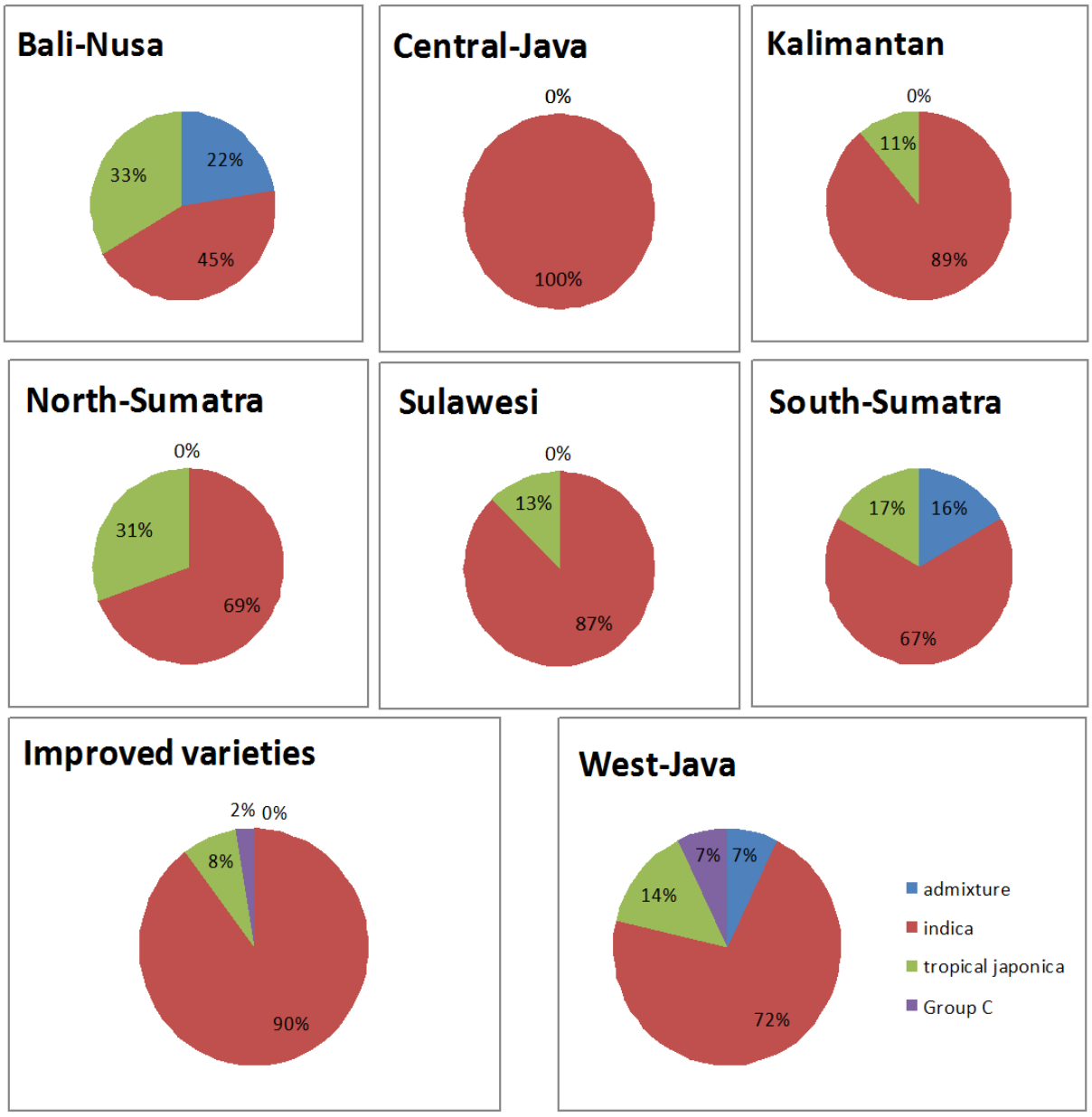

Figure 4. 5. Percentage of composition varietal group of *O. sativa* in Indonesian rice germplasm based on SNP marker.

Table 4. 1. Genetic differentiation (F_{ST}) and genetic distance (D) within and among taxa in Indonesian rice germplasm.

Between *O. sativa* subspecies			F_{ST}	D
indica	vs	***japonica***	0.438	0.398
landrace		landrace	0.517	0.444
improved variety		improved variety	0.364	0.325
indica	vs	***indica***		
improved variety		landrace	0.054	0.02
japonica	vs	***japonica***		
improved variety		landrace	0.112	0.059

Table 4. 2. List of outlier loci detected under *indica* and *japonica* by Bayescan v 2.1 software.

No	Name of locus	Chr.	FST	Log10 (PO)	Function	Reference
1	ad03009571	3	0.894	1.519	Similar to *Xanthine dehydrogenase 1* (EC 1.1.1.204) (Os03t0429800-01) (chr03:18617619..18630481)	http://rapdb.dna.affrc.go.jp/
2	ad04009074	4	0.893	1.461	No	
3	P0724	4	0.891	1.392	Similar to protein *BABY BOOM 1* (Os04t0504500-01) (chr04:25681455..25685718)	
4	ad04003958	4	0.2644	1.215	No	

Table 4. 3. Patterns of SNPs showing different haplotype between *indica* and *japonica* in Indonesian rice landrace according to the high value of FST and Log10 (PO)>1 generated by Bayescan v. 2.1 software.

Name of variety	Group	Name of Locus		
		ad03009571	ad04009074	P0724
		(Chr. 3) 18.628.536bp	(Chr. 4) 24.867.463bp	(Chr. 4) 25.682.427bp
Aceh-Aceh	*Indica*	G	T	C
Angsa Jeletuk		G	T	C
Are Sera		G	T	C
Ase Puteh		G	T	C
Asemandi		G	T	C
Bandang Bujur		G	T	C
Beton		G	T	C
Beurgeum Dadapan		G	T	C
Bujang Inai		G	T	C
Bulang		G	T	C
Bulu Bodas		G	T	C
Cempo Abang Ner		G	T	C
Cempo Beluluk		G	T	C
Cempo Telouluk		G	T	C
Cere Beureum		G	T	C
Cere Welut Merah		G	T	C
Ciburuy 1		G	T	C
Daliah Putih		G	T	C
Deli		G	T	C
Dusel		G	T	C
Enud		G	T	C
Genjah Emer		G	T	C
Genjah Welut		G	T	C
Gonggoi		G	T	C
Jaran Mas		G	T	C
Jimbruk Joloworo		G	T	C
Kalingga Rara		G	T	C
Katik Taram		G	T	C
Kaya Merah		G	T	C
Kaya Terabah		G	T	C
Ketan Bodas		G	T	C
Ketan Gondil		G	T	C
Ketan Huma		G	T	C
Ketan Langgar Sari		G	T	C
Ketan Wuluh		G	T	C
Lantiak		G	T	C
Markos		G	T	C

Name of variety	Group	Name of Locus		
		ad03009571	ad04009074	P0724
		(Chr. 3) 18.628.536bp	(Chr. 4) 24.867.463bp	(Chr. 4) 25.682.427bp
Mentik Sleman	*indica*	G	T	C
Mete Kawicho		G	T	C
Omad		G	T	C
Osog		G	T	C
Padi Rabig		G	T	C
Padi Sibur		G	T	C
Pandan Wangi Cianjur		G	T	C
Pangraman		G	T	C
Pulut Gaca		G	T	C
Pulut Kutai		G	T	C
Pulut Pagae		G	T	C
Pulut Tomene		G	T	C
Raden Kuning		G	T	C
Ranggong		G	T	C
Rauk Neya		G	T	C
Remaja		G	T	C
Sabai Kecil		G	T	C
Saigon		G	T	C
samek		G	T	C
Segon Saga		G	T	C
Sekemiling		G	T	C
Serepet Tinggi		G	T	C
Seuweu		G	T	C
Si Awak		G	T	C
Sintang Pulau Pisau		G	T	C
Badik/Gadih Kabalai	*japonica*	G	A	T
Cicih Ijo Gading		A	A	C
Jidah Bodas		G	A	T
Ketan Gajih		A	A	T
Ketan Keuyeup		A	T/A	T
Kuntu Kuranyi		A	A	C
Laka		A	A	C
Nobu Bisara		A	A	T
Padi Elo		G	A	T
Pala Idang merah		A	A	T
Plastik		G	A	T
Sari Kuning		G	A	T
Segon Nyonya		G	A	T
Sepadan		G	A	T

Table 4. 4. Inbreeding coefficient (Fis and Fst) and gene flow (*N*m) between subspecies of Indonesian rice (*Oryza sativa* L.).

Pairwise	**Fis**	**Fst**	***N*m**
Indica and *japonica*	0.804*	0.669*	0.124

Note: * indicated significant level at P< 0.01, Note; three levels of gene flow in self-pollination crops, high, Nm > 1, intermediate, 0.250 < Nm < 0.999; and low, Nm < 0.249 (Govindajaru, 1989)

Table 4. 5. Bayesian estimates of posterior (θ), rate of migration (M) and level of gene flow (*N*m) calculated by Migrate-n from SNP markers.

Theta (θ) and direction of migration (M)	**Mode**	**Range**	**Nm=M. θ/4**
θ (*indica*)	0.0017	0.0008-0.00096	
θ (*japonica*)	0.0013	0.0001-0.00030	
M (*japonica* →*indica*)	288.3	203.3-373.3	0.123
M (*indica* → *japonica*)	3045	2956.7-3126.7	0.990

Note; three levels of gene flow in self-pollination crops, high, Nm > 1, intermediate, 0.250 < Nm < 0.999; and low, Nm < 0.249 (Govindajaru, 1989)

Table 4. 6. Genetic differentiation (F_{ST}) and genetic distance (D) among landrace from different source locations in Indonesian rice germplasm.

Populations			***F*ST**	***D***
Improved varieties	VS	Bali-Nusa	0.105**	0.075
		Central-Java	0.057**	0.023
		Kalimantan	0.049**	0.025
		North-Sumatra	0.087**	0.056
		South Sulawesi	0.047	0.024
		South Sumatra	0.064**	0.039
		West Java	0.047**	0.027
Bali-Nusa		Central-Java	0.141**	0.086
		Kalimantan	0.073	0.049
		North-Sumatra	0.026	0.019
		South Sulawesi	0.071	0.049
		South Sumatra	0.049	0.038
		West Java	0.044	0.033
Central-Java		Kalimantan	0.055	0.023
		North-Sumatra	0.111**	0.06
		South Sulawesi	0.051	0.021
		South Sumatra	0.093	0.047
		West Java	0.054	0.024
Kalimantan		North-Sumatra	0.048	0.029
		South Sulawesi	0.024	0.012
		South Sumatra	0.047	0.028
		West Java	0.024	0.013
North-Sumatra		South Sulawesi	0.051	0.032
		South Sumatra	0.04	0.029
		West Java	0.03	0.02
South Sulawesi		South Sumatra	0.049	0.03
		West Java	0.026	0.015
South Sumatra		West Java	0.033	0.022

Table 4. 7. Mean of differentiation among neutral loci (FST) among landrace from different source locations based on SNP markers.

Pairwise			**FST**
West-Java	VS	Bali-Nusa	0.002
		Central-Java	0.111
		Kalimantan	0.000
		Sumatra	0.002
		Sulawesi	0.001
Bali-Nusa		Central-Java	0.247
		Kalimantan	0.001
		Sumatra	0.001
		Sulawesi	0.039
Central-Java		Kalimantan	0.001
		Sumatra	0.008
		Sulawesi	0.118
Kalimantan		Sumatra	0.001
		Sulawesi	0.001
Sumatra		Sulawesi	0.007

Table 4. 8. Diversity in improved varieties and landrace in Indonesian rice.

Population		N	Na	H	AR	PA	Percentage of polymorphism
Oryza sativa	improved variety	78	1.952	0.320	1.864	0.096	81.38%
	landrace	83	1.935	0.388	1.875	0.059	80.73%
O. sativa		161	2.046	0.375	1.982	0.969	86.59%

Note: N = number of cultivars, Na = number of alleles, H = Shannon's diversity index, AR = allelic richness, PA = private allele

Table 4. 9. Diversity between subspecies in comparison landrace and improved varieties.

Population		**N**	**Na**	**H**	**AR**	**PA**	**Percentage of polymorphism**
Oryza sativa	*indica* improved varieties	70	1.680	0.247	1.859	0.057	59.38%
	indica landrace	62	1.628	0.233	1.555	0.034	55.86%
	japonica improved varieties	6	1.685	0.360	1.363	0.012	64.06%
	japonica landrace	14	1.435	0.214	1.379	0.012	40.76%
O. sativa		152	2.025	0.368	3.360	0.956	85.68%

Note: N = number of cultivars, Na = number of alleles, H = Shannon's diversity index, AR = allelic richness, PA = private allele

Table 4. 10. Diversity between improved varieties and in comparison between from different source location.

Population		N	Na	H	AR	PA	Percentage of polymorphism
Oryza sativa	Improved variety	76	1.949	0.320	1.864	0.098	81.25%
	Bali-Nusa	7	1.706	0.407	1.615	0.003	66.28%
	Central-Java	9	1.406	0.205	1.341	0.004	38.67%
	Kalimantan	9	1.669	0.308	1.544	0.001	63.80%
	North-Sumatra	13	1.716	0.385	1.639	0.001	66.67%
	Sulawesi	8	1.659	0.316	1.538	0.001	61.98%
	South Sumatra	6	1.633	0.326	1.516	0.005	60.55%
	West Java	24	1.828	0.365	1.738	0.010	74.87%

Note: N = number of cultivars, Na = number of alleles, H = Shannon's diversity index, AR = allelic richness, PA = private allele

Table 4. 11. Diversity between improved varieties and in comparison between from different source location (*indica* only).

Population	**N**	**Na**	**H**	**AR**	**PA**	**Percentage of polymorphism**
Bali-Nusa	4	1.240	0.142	1.200	0.001	23.18%
Central-Java	9	1.406	0.205	1.341	0.004	38.67%
Kalimantan	8	1.392	0.203	1.330	0.003	37.37%
North-Sumatra	9	1.392	0.191	1.323	0.003	37.89%
Sulawesi	7	1.358	0.199	1.307	0.001	34.11%
South Sumatra	5	1.339	0.191	1.283	0.005	32.16%
West Java	20	1.516	0.227	1.447	0.009	47.27%

Note: N = number of cultivars, Na = number of alleles, H = Shannon's diversity index, AR = allelic richness, PA = private allele

Table 4. 12. Placement of Indonesian rice landrace based on each neighbor-joining tree using SSR and SNP markers.

No	Accession number	Name of varieties	SSR			SNP		
			Indica	*japonica*	C type	*Indica*	*japonica*	C type
1	498	Aceh-Aceh	x			x		
2	2420	Angsa Jeletuk	x			x		
3	3779	Are Sera	x			x		
4	4676	Ase Puteh*	x			x		
5	503	Asemandi		x		x		
6	3882	Badik/Gadih Kabalai*	x				x	
7	4669	Bandang Bujur	x			x		
8	2860	Beton	x			x		
9	1047	Beurgeum Dadapan	x			x		
10	538	Bujang Inai	x			x		
11	3027	Bulang*	x			x		
12	PN06-17	Bulu Bodas	x	x		x		
13	2600*	Cempo Abang Ner*	x			x		
14	2386	Cempo Beluluk	x			x		
15	2368	Cempo Telouluk	ex			x		
16	3389*	Cere Beureum*	x			x		
17	2247	Cere Mentik*	x			ex		
18	2347	Cere Welut Merah*	x			x		
19	PN06-20	Ciburuy 1*			x	x		
20	2548	Cicih Ijo Gading	x				x	
21	4707	Daliah Putih	ex			x		
22	2450	Deli	x			x		
23	2352	Dusel*	x			x		
24	3385	Enud	x			x		
25	1375	Genjah Emer	x			x		
26	2365	Genjah Welut*	x			x		
27	561	Gonggoi*		x		x		
28	2625	Jaran Mas	x			x		
29	1372	Jidah Bodas*		x			x	
30	2779	Jimbruk Joloworo	x			x		
31	1827	Kalingga Rara*	x			x		
32	2381	Kangkungan*	x			ex		
33	3720	Katik Taram*	x			x		
34	2813	Kaya Merah	x			x		
35	2812	Kaya Terabah	x			x		
36	2239	Ketan Bayong	x			ex		
37	PN06-39	Ketan Bodas*		x		x		
38	PN06-16	Ketan Gajih*		x			x	
39	3986	Ketan Gondil*		x				
40	4636	Ketan Huma		x		ex		
41	1250	Ketan Keuyeup	x				x	
42	4637	Ketan Langgar Sari*		x		x		
43	3978	Kuntu Kuranyi*	x			x		
44	611	Laka*		x			x	
45	613	Laka Tesan A*	x			x		
46	3100	Lantiak*	x				*x*	
47	3629	Lapang		x		x		
48	3945	Markos	x			x		
49	4754	Mencrit Beureum*	x			x		

No	Accession number	Name of varieties	SSR			SNP		
			Indica	*japonica*	C type	*Indica*	*japonica*	C type
50	638	Mentik Sleman*	ex			x		
51	1829	Mete Kawicho*	ex			x		
52	1816	Nobu Bisara*	x				x	
53	1418	Omad	x			x		
54	PN06-28	Osog*		x		x		
55	3031	Padi Elo*	x				x	
56	2287	Padi Rabig	x			x		
57	3741	Padi Sibur	x			x		
58	4714	Pajar	x			ex		
59	2776	Pala Idang merah*	ex			x		
60	1596	Pandan Wangi (leher II)*		x				x
61	668	Pandan Wangi Cianjur*	x			x		
62	2876	Pangraman	x			x		
63	4614	Plastik*		x			x	
64	2865	Pulut Gaca	x			x		
65	4616	Pulut Kutai	x			x		
66	3030	Pulut Pagae*	x			x		
67	683	Pulut Tomene	x			x		
68	3934	Raden Kuning	ex			x		
69	3958	Ranggong*	x			x		
70	1319	Rauk Neya*	x			x		
71	2596	Remaja	x			x		
72	4609	Sabai Kecil*	x			x		
73	3466	Saigon	x			x		
74	3890	Samek*	x			x		
75	1015	Sari Kuning		x			x	
76	1430	Segon Nyonya	x				x	
77	1541	Segon Saga*	x			x		
78	4010	Sekemiling	x			x		
79	4018	Sepadan	x				x	
80	3928	Serepet Tinggi*	ex			x		
81	4635	Seuweu*		x		x		
82	4685	Si Awak	ex			x		
83	1800	Sintang Pulau Pisau			x	x		

Note: “ex” = excluded from calculation, * = varieties could reach reproductive phase in 2009 and 2010

Table 4. 13. Placement of varieties in relationship between cross combinations, breeding materials and clustering based on morphological traits and DNA markers.

No	Name of varieties	Cross combinations	Breeding materials	Year of release	Morphology		DNA markers	
					2009	2010	SSR	SNP
1	Bengawan	Cina/Latisail		1943	A	A	*japonica*	*japonica*
2	Remaja	Baiang/Cina//Cina/ Latisail		1954	A	A	IR5, IR66 and IR70	IR5 and IR65
3	Seratus Malam	Lampung		1960	B	B	IR48	IR5 and IR65
4	Kartuna	Introduction from Philipine		1963	A	A	*japonica*	*japonica*
5	Synta	Sigadis/Bengawan		1963	A	A	IR5, IR66 and IR70	IR5 and IR65
6	PB5/IR5	Tangkai Rotan/Peta		1967	B	B		
7	Dewi Ratih	22-BC III-20-2 / Randah Cupak	DGWG	1969	A	A	*japonica*	*japonica*
8	Gata	Shorth Sigadis/Syntha	DGWG	1976	B	B	IR5, IR66 and IR70	IR5 and IR65
9	Gemar	Jerak/IR8	IR8	1976	B	B	IR36	IR36
10	Asahan	IR 2042/CR 94-13	IR8	1978	B	B	IR5, IR66 and IR70	IR5 and IR65
11	IR36	IR 2042/CR 94-13	IR8	1978	B	B		
12	IR42	IR 2042/CR 94-13	IR8	1980	A	B		
13	Barito	Pelita I-1/B 2393	IR5 and IR8	1981	B	A	IR36	Other group II
14	Cipunagara	Unknown	Unknown	1981	B	B	IR36	IR5 and IR65
15	Krueng Aceh	Pelita I-1/B2709	IR5 and IR8	1981	B	B	IR36	IR36
16	Atomita 2	mutation of Pelita I-1	IR5	1983	B	B	IR5, IR66 and IR70	IR5 and IR65
17	Bahbolon	IR 14431/IR 2307-64-22	IR8	1983	B	B	Other group	IR36
18	Citanduy	IR 5236/IR 5338	IR8	1983	B	B	IR5, IR66 and IR70	IR36
19	Mahakam	Pelita I-2/t442-36	IR5	1983	B	A	IR5, IR66 and IR70	IR5 and IR65
20	Sadang	B 459 B-PN-132-9-3/IR 2071-588-5-6	IR8	1983	A	B	IR36	IR64, IR42, IR70
21	Singkarak	C22/IR36	IR36 and IR8	1983	A	B	Other group	IR36
22	Batang Ombilin	Kuning Galung/IR28	IR8	1984	A	A	IR36	IR5 and IR65
23	Kapuas	Pelita I-1/B 2709	IR5 and IR8	1984	A	A	IR64 and IR42	IR64, IR42, IR70
24	Bahbutong	C 4-63 GB/PTB 33		1985	B	B	IR64 and IR42	IR5 and IR65

No	Name of varieties	Cross combinations	Breeding materials	Year of release	Morphology		DNA markers	
					2009	2010	SSR	SNP
25	Cisanggarung	Pelita I-1/B 3063-2/Pelita I-2//IR36	IR5 and IR8	1985	B	B	IR36	IR64, IR42, IR70
26	Cisokan	IR36/Pelita I-1	IR5 and IR8	1985	A	B	IR5, IR66 and IR70	IR64, IR42, IR70
27	Maninjau	B 173 C-PN-24/B 57 C-MD-3-3/Tabente Mainti	IR8 and Syntha	1985	B	A	IR48	IR5 and IR65
28	IR48	IR 4125/IR 2055-481-2	IR8	1986	B	B		
29	IR64	IR 5236/IR 5657-33-2-1	IR8	1986	B	B		
30	IR65	IR 17584/IR 52	IR36 and IR8	1986	B	A		
31	Dodokan	IR 25509/IR 9129-209-2-2-2-1	IR36 and IR8	1987	B	B	IR36	IR5 and IR65
32	Batur	IR 3380-13-17/IR 5853-162-1-2-3	IR8	1988	A	B	IR36	IR66
33	Ciliwung	IR 38/2+Pelita I-1/IR 4744	IR5 and IR8	1988	B	B	IR5, IR66 and IR70	IR66
34	Danau Atas	Lampung		1988	A	A	IR36	*japonica*
35	Batang Sumani	IR 22657/IR36	IR36 and IR8	1989	A	B	IR5, IR66 and IR70	IR5 and IR65
36	IR66	IR 13240-108-2-2-3/IR 9129-209-2-2-2-1	IR36 and IR8	1989	B	B	*japonica*	
37	IR70	IR 25604/IR9828-36-3	IR36 and IR8	1989	B	B	IR36	
38	Lusi	Pelita I-1//IR 4744-128-4-1-2/PelitaI-1/IR38	IR5 and IR8	1989	A	B	IR5, IR66 and IR70	IR66
39	Way Seputih	Cisadane/IR36	IR5 and IR8	1989	B	B	IR5, IR66 and IR70	IR5 and IR65
40	Barumun	IR 17496/IR 3403-267-1	IR8	1991	B	B	IR36	IR36
41	Cenranae	IR5/BR 3	IR5 and IR8	1991	A	B	IR5, IR66 and IR70	IR64, IR42, IR70
42	Situgintung	mutation of Seratus Malam		1992	A	B	IR5, IR66 and IR70	Other group
43	Bengawan Solo	Bahbutong/Bogowonto	IR5, IR8 and IR36	1993	B	B	IR5, IR66 and IR70	IR64, IR42, IR70
44	Gajah Mungkur	IRAT 13/ Dourado Precoce	Zaire variety	1994	B	B	*japonica*	IR5 and IR65
No	**Name of**	**Cross**	**Breeding**	**Year of**	**Morphology**		**DNA markers**	

	varieties	combinations	materials	release	2009	2010	SSR	SNP
45	Jatiluhur	Tox1011/Ranau	IR8	1994	A	A	IR48	IR5 and IR65
46	Kalimutu	IAC 1246/Tainan 5	Japanese rice	1994	A	B	*japonica*	IR64, IR42, IR70
47	Memberamo	B 6555 B-199-40/Barumun	IR8	1995	B	A	IR5, IR66 and IR70	IR64, IR42, IR70
48	Cilosari	SM 268-PSJ/IR36	IR8 and IR36	1996	A	B	IR36	IR48
49	Cirata	IR 9129-159-3/2+IR5975-Sel/IR5975-Sel	IR8	1996	B	B	IR36	IR48
50	Digul	IR 19661-131-1-3-1-3/IR 19661/IR64	IR64	1996	B	B	IR36	IR5 and IR65
51	Banyuasin	Kelara/Cisadane	IR5 and IR8	1997	A	B	IR5, IR66 and IR70	*japonica*
52	Lalan	Barito/IR54/IR 9575/IR 54	IR5 and IR8	1997	A	A	*japonica*	IR5 and IR65
53	Way Apo Buru	IR64/IR 18349-53-1-3-1-3/2+IR 19661-131-3-1-3	IR8, IR42, and IR64	1998	B	B	IR36	IR36
54	Batanghari	Cisadane/IR 19661-131-1-3-1-3	IR5 and IR8	1999	B	B	IR5, IR66 and IR70	Other group
55	Dendang	Osok/IR 5657-33-2	IR8	1999	B	B	IR36	IR5 and IR65
56	Ketonggo	B 4183 E-KP-1/B 4183 E-KP-1/IR 28224-Sel	IR8	1999	B	B	IR5, IR66 and IR70	IR64, IR42, IR70
57	Limboto	Papah Aren/IR36/Dogo	IR8 and IR36	1999	B	B	IR48	IR5 and IR65
58	Towuti	S 499 B-28/Carreon//IR64/IR64	IR64 and IR8	1999	B	B	IR5, IR66 and IR70	Other group
59	Ciherang	IR64/IR 18349-53-1-3-1-3/2+IR 19661-131-3-1-3	IR8 and IR64	2000	B	B	IR36	IR64, IR42, IR70
60	Cisantana	IR64/IR 54742-1-19-11-8	IR8 and IR64	2000	B	B	IR5, IR66 and IR70	IR36
61	Indaragiri	B 6256-MR-3-5/Barumun//Rojolele/IR 68	IR8	2000	B	A	IR5, IR66 and IR70	IR64, IR42, IR70
62	Punggur	BKNFR 76106-16/Kapuas	IR5 and IR8	2000	A	A	IR5, IR66 and IR70	IR5 and IR65
63	Tukad Balian	IR 48613-54-3-3-1/IR 28239-94-2-3-6-2	IR36 and IR8	2000	A	B	IR64 and IR42	IR36
64	Tukad Unda	IR 66701/IR64	IR64 and IR8	2000	B	B	IR5, IR66 and IR70	IR64, IR42, IR70
No	**Name of**	**Cross**	**Breeding**	**Year of**	**Morphology**		**DNA markers**	

	varieties	combinations	materials	release	2009	2010	SSR	SNP
65	Angke	IR 64(6)/IRBB5	IR64 and IR8	2001	B	B	IR36	IR36
66	Batutegi	B 6876 B-MR10/B 6128 B-TB-15	Unknown	2001	A	A	IR36	IR48
67	Ciujung	IR64/RP 1837-715-3-2	IR8 and IR64	2001	B	B	IR36	IR64, IR42, IR70
68	Conde	IR64+3/IRBB 7/IR64	IR8 and IR64	2001	B	B	IR64 and IR42	Other group
69	Danau Gaung	ARC 10372/B 6135/Way Rarem	IR8	2001	A	A	IR48	IR5 and IR65
70	Konawe	S 487 B-75/IR 19661//IR-131-3-1///IR64	IR64 and IR8	2001	B	B	IR36	IR5 and IR65
71	Lambur	Cisadane/IR 9884-54-3	IR8 and IR5	2001	B	B	IR5, IR66 and IR70	IR5 and IR65
72	Situ Patenggang	Kartuna/TB 47 H-MR-10	IR36 and IR8	2002	A	B	IR5, IR66 and IR70	*japonica*
73	Situbagendit	Batur/S 2823 A	IR64 and IR8	2002	B	B	IR5, IR66 and IR70	IR36
74	Batang Piaman	IR25393-57/RD203//IR27316-96/// SPLR7735/SPLR2792	IR8 and IR20	2003	A	B	IR5, IR66 and IR70	IR48
75	Ciapus	Memberamo/IR 66154-52-1-2-2/Memberamo	IR8 and IR24	2003	A	A	IR36	IR5 and IR65
76	Cibogo	S 487 B-75/IR 19661//IR 19661-131-3-1///IR64/IR64	IR8 and IR64	2003	B	B	IR36	IR64, IR42, IR70
77	Cigeulis	Ciliiwung/Cikapundung/IR64	IR5, IR8 and IR64	2003	B	B	IR36	IR5 and IR65
78	Fatmawati	Maros/BP68-MR-4-3-2	IR64 and IR8	2003	A	B	IR64 and IR42	Other group

Note: Font A and B indicated group by morphological traits (Figs 2. 2 and 2. 3) and clustering of DNA markers (SSRs and SNPs) based on NJ-tree (Figs 3. 1 and 4. 3). The cross combination and breeding material in this table are according to the online data provided by IRRI ((http://www.iris.irri.org/).

5. General discussion

In the present study, landrace showed more diverse than improved varieties in some particular traits, indicating Indonesian farmers have maintained landrace diversity. However, according to two groups based on hierarchical and PCA, most of the improved varieties were smaller in size than landrace. The varieties developed after the introduction of the IRRI varieties as breeding materials belong to this group. It seems that a semi-dwarf variety with high yield potential became common for Indonesian rice varieties. Reduced plant stature is a target trait of improved rice cultivars, and most of the released varieties had a semi-dwarf1 (*sd1*) gene that came from the Dee-gee-woo-gen variety (Hargrove et al., 1988). A recessive semi dwarf1 (*sd1*) gene was used for IR8 (Sasaki et al. 2002). However, we still found some varieties that were large in size (tall stature) and these varieties were released in the first stage of breeding program without use of IRRI varieties. However, some varieties developed using IRRI varieties as breeding materials showed tall stature in the different time of cultivation and strong sensitivity to micro-environmental changes.

The results of diversification and differentiation in Indonesian rice (*O. sativa* L.) using morphological traits corresponded well to the results using DNA markers, in which the results indicated that Indonesian farmers have maintained a broad range of rice landrace (*O. sativa* L.) germplasm and extended its diversity during the history of rice cropping. The most accessions were *indica*, followed by *japonica* and C type as unclassified into two major groups. This result corresponded well to previous studies (Khush et al., 2003; Thomson et al., 2007), in which showed that *japonica* was less than *indica* in Indonesian rice germplasm. Glutinous and awned rice varieties were involved in *japonica* by SSR markers as the same results as those reported by Thomson et al. (2009). Iskandar and Ellen (1999) reported that cultural practices by people in Java Island served to maintain landrace for traditional, religious and cultural uses. Similar results reported that the genetic diversity of landrace has also maintained by cultural practices in Kalimantan (Thomson et al. 2009). In this study, lower diversity in *japonica* than *indica* was confirmed by two kinds of markers, SSR and SNP. This result was different from previous study as reported by Thomson et al. (2007) and in agreement with Glaszmann (1987) and Li and Rutger (2000). The disagreement with previous study is due to admixture varieties excluded from *japonica* diversity calculation. We suggested that the lower diversity and less number of effective alleles (*Ne*) in *japonica* than *indica* might be affected by selection for particular traits in rice landrace. For example, our previous study indicated that selection for seed shattering contributed to differentiation between *indica* and *japonica*, in which *japonica* was poorer (Muhamad and Okuno 2013).

This selection contributed to allelic difference as revealed by positive correlation between differentiation using shattering traits and SSR markers. The revealed limited asymmetric gene flow between *indica* and *japonica* in this study is one of the evidence that gene flow may play a role in diversification and differentiation between these two subspecies and probably this mechanism was caused by the presence of few admixture varieties as C type. The raw data of SNP marker at SNP ad01011668 on chromosome 1 showed that the C type has the heterozygous allele (A/G) while the other varieties have either allele A or G. The probable reason of limited gene flow between two subspecies is due to the different ecological environments in Indonesia; *japonica* rice involving glutinous rice (Beras Ketan) is commonly cultivated in highlands (>500m from sea level) by swidden practice, whereas *indica* rice is cultivated in lowlands (Marten, 1990; Tanaka, 1997; Iskandar and Ellen, 1999). In this study we suggested that these conditions were associated with the differentiation between *indica* and *japonica* in Indonesia. In addition, the outlier's loci found by both markers suggested that natural variations might affect differentiation between *indica* and *japonica* in Indonesia and be caused by mutation and/or recombination in haplotype, probably due to the cultivation under different environments between *indica* and *japonica* as explained above.

The other mechanism decreasing diversity in *japonica* is bottle-neck effects as reported in Madagascar (Mather et al., 2010) and Asia including Indonesia (Molina et al., 2011). Sun et al. (2001) suggested that effective alleles have been lost by natural and human selection and genetic diversity in cultivated rice have been gradually decreased.

Hardy-Weinberg suggested that one of important mechanisms on evolution in the term of differentiation among population is natural selection. Our result indicated that the same genotype are favoured in different populations due to stabilizing selection in most of pairwise between landrace from different source locations by value of Qst<FST. This explained the reason why Indonesian landrace was not differentiated based on source locations of landrace.

Molecular analysis provided evidence that a few improved varieties are *japonica*, but previous study in Indonesian rice germplasm reported that *japonica* varieties were not found in their samples (Thomson et al., 2007). Our results indicated that Indonesian breeding program focused on high-yielding irrigated rice varieties, which are largely *indica*, rather than upland varieties, which tend to be *japonica.* This assumption was supported by extensive use of IRRI varieties as breeding materials and was shown by clustering in the *indica* group, 41.4% by SSRs and 31.7% by SNPs based on pedigree information. In improved varieties,

the lower diversity in *indica* compared with *japonica* was revealed by both markers and also supported by the fact that Indonesian breeders focused on improving *indica* rather than *japonica*. Changes in allelic frequency occur in response to selection (Vieira et al., 2013). When a gene is subjected to selection pressure by breeder, its frequency changes from parent to progeny, thus allelic frequency in the progeny is variable depending on the differential effect of selection on parental allelic frequency (Staub, 1994)

Correlation between morphological traits in two years, between two kinds of DNA markers and between morphological traits and DNA markers

Disagreement between SSR and SNP markers was attributed to the greater information content of SSR markers and characteristics of markers. Even though we changed the missing allele into the third allele in this study, the percentage of polymorphism based on SNP markers was lower than SSR markers. The greater diversification using SSR markers than SNP markers was also reported in maize (Hamblin et al., 2007), rice (Singh et al., 2013) and grape (Emanuelli et al., 2013) and they required to increase number of SNP markers to make equal information provided by SSR marker. The reason is that SNP marker is bialleleic and SSR marker is multialleic (Rafalski, 2002).

This evidence was showed by slightly positive correlations r (0.13) between SSR and SNP markers (Figure 5. 1 and Table 5. 1). Furthermore, the positive correlation r (0.35 v. 0.06 in 2009 and 0.19 v. 0.18) between morphological and molecular data depended on the kinds of molecular markers. Also, this fact could be also due to the different properties of these two markers, although we changed the missing allele in the SNP data to the third allele. The slightly positive correlation in this study could be also caused by morphological traits associated with a relatively small number of loci, thus the potential difference could be lost in the analysis of large amounts of molecular data as suggested by Diederichsen, (2009). Besides, the weak correlation in 2 years indicated that evaluation of genetic relationships using molecular markers includes difficulties with respect to the effects of different management practices and different environments and it was shown by intermediate correlation r (0. 58) between morphological traits caused by environmental changes.

We concluded that gene flow between *Oryza sativa* subspecies caused the presence of type C varieties which could not be grouped into two major groups of cultivated rice. This phenomenon may play roles in diversification and differentiation between populations in Indonesian rice. Besides, the same genotypes are favoured in different populations due to

stabilizing selection during adaptation and this evidence may explain the pattern of differentiation in Indonesian rice germplasm. Furthermore, in this study we identified several candidate genes that may have been selected during the adaptation of the two cultivated subspecies. The further research on these genes in rice is needed to clarify the association with adaptation.

Consequences for maintaining germplasm, genomics and plant breeding

These findings have consequences for applied maintenance of germplasm, plant breeding and genomics approaches. Isolation during maintenance of *Oryza sativa*, within subspecies of *O. sativa* is important to avoid loss of diversity. Genetic relationships are often the basis of the choice of breeding materials for crop improvement strategies and for the design of experimental cross combinations. Most of useful landrace were distant from improved varieties in rice breeding. The breeders may select for lines to be crossed based on molecular marker data and data on important morphological traits. Therefore, additional researches are required to develop and assess the better marker type on captures of the variation in each subspecies. So in the end, the molecular markers could be used to track loci which are associated with the important agronomic traits as marker assisted selection (MAS). To be effective, the markers closely linked to the target locus should be developed based on the association between genotying and phenotyping data. For example, through association mapping, marker sets by scanning whole genome regions are important. Often, association mapping required a large number of markers for genotyping and the number of markers depends on large part of the genome size and the expected linkage disequilibrium decay.

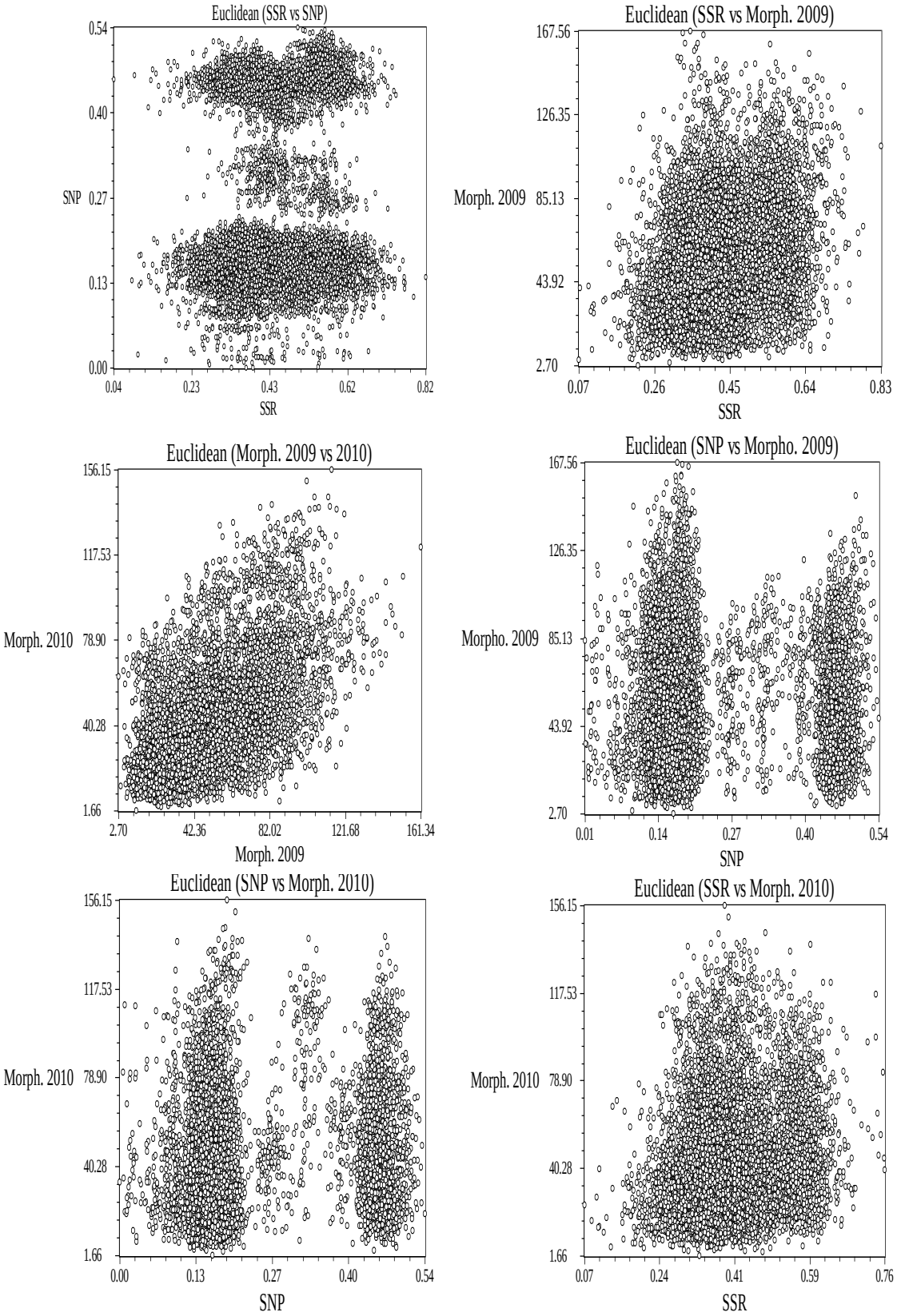

Figure 5. 1. Correlation between Euclidean distance (molecular vs molecular, morphological vs morphological and molecular vs morphological) in Indonesian varieties.

Table 5. 1. Correlation (r) between genetic similarities using different approach.

Pairwise of genetic similarity	**Mantel's test (r)**
Morphology vs Morphology	
2009 vs 2010	0.577
Morphology vs molecular	
2009	
Morphology vs SSR	0.349
Morphology vs SNP	0.062
2010	
Morphology vs SSR	0.185
Morphology vs SNP	0.177
Molecular vs molecular	
SSR vs SNP marker	0.125

Summary

The previous study of diversity in Indonesian rice revealed that Indonesian rice landrace is higher than improved variety. However, there is no study to clarify how Indonesian rice germplasm has diversified and differentiated. Therefore, the objectives of the present study were to reveal diversification and differentiation of Indonesian rice germplasm and the possible mechanism of diversification and differentiation of Indonesian rice germplasm using morphological traits and DNA markers. The ten important morphological traits were measured such as plant height, culm length, panicle length, length and width of leaf, length and width of flag leaf, length of sheath leaf, angel of flag leaf and heading date. F test, coefficient variation (CV), dendrogram by Wards methods, PCA and bi-plot PCA were calculated using JMP ver. 5.1 software and phenotypic divergence (*Qst*) using Modicos software. The 32 SSR and 768 SNP markers as DNA marker was used to examine the diversity index (Shannon *H*), differentiation (FST) and genetic distance (*D*) gene flow (*N*m) and direction of *N*m, neighbour joining tree and also differentiation among neutral loci (FST) using several software such DARwin 5, Migrate-n and Bayescan v. 2.1.

インドネシア米の多様性に関するこれまでの研究は、インドネシアの米の土地は改良品種よりも高いことを明らかにした。しかし、インドネシアの米生殖質がどのように多様化・差別化しているかを明らかにする研究はない。したがって、本研究の目的は、形態学的形質および DNA マーカーを用いたインドネシアのイネ生殖質の多様化および分化ならびにインドネシアのイネ生殖細胞の多様化および分化の可能なメカニズムを明らかにすることであった。植物の高さ、稈長、穂の長さ、葉の長さと幅、葉の長さと幅、葉の葉の長さ、葉の葉の葉の長さと幅などの 10 の重要な形態学的形質を測定した。 F テスト、係数変動（CV）、Wards 法による樹状図、PCA およびバイプロット PCA を JMP ver を用いて算出した。

多様性指数（Shannon H）、分化（FST）および遺伝子距離（D）遺伝子フロー（Nm）および Nm の方向、隣接する樹木の樹木および中性細胞の分化を調べるために、32 個の SSR マーカーおよび 768 個の SNP マーカーを DNA マーカーとして用いた DARwin 5、Migrate-n および Bayescan v.2.1 のようないくつかのソフトウェアを使用して、遺伝子座（FST）

In the present study, landrace showed more diverse than improved varieties in some of particular traits, suggesting that Indonesian farmers have maintained landrace diversity. However, hierarchical and PCA analysis indicated that most of the improved varieties were smaller in size than landrace. The varieties released after the introduction of the IRRI as breeding materials belong to this group. It seems that a semi-dwarf variety with high yield potential became common for Indonesian rice varieties. The finding showed that some varieties have been developed using IRRI varieties as breeding materials and preferential selection for tall stature has been conducted in the different time of cultivation. These varieties were very sensitive to micro-environmental changes.

現在の研究では、土地棚は特定の形質のいくつかの品種改良より多様性を示し、インドネシアの農家が土地の多様性を維持していることを示唆している。 しかしながら、階層的および PCA 分析は、改善された品種の大部分が土地面積よりもサイズが小さいことを示した。 育種材料としての IRRI の導入後に発表された品種はこのグループに属する。 インドネシアのイネ品種では、収量の高いセミドワーフ品種が一般的になったようです。 この発見は、育種材料として IRRI 品種を使用していくつかの品種が開発され、背の高い品種の優先選択が異なる栽培時間に行われたことを示した。 これらの品種は微環境変化に非常に敏感であった。

As well as morphological analysis, molecular analysis also provided evidence that Indonesian rice landrace was more diverse than improved varieties. The results indicated that Indonesian breeding program focused on high-yielding irrigated rice varieties, which are largely *indica* rather than upland varieties which tend to be *japonic*a. This assumption was supported by the extensive use of IRRI varieties as breeding materials and also supported by clustering in the *indica* group, 41.4% by SSRs and 31.7% by SNPs based on pedigree information. The lower diversity in *indica* compared with *japonica* was revealed by both markers and also supported by the fact that Indonesian breeders focused on improving *indica* rather than *japonica.* These results suggested that the lower diversity in improved rice varieties was caused by the extensive use of elite varieties as breeding materials, while the Indonesian farmers have maintained Indonesian rice landrace due to the custom and religion obligation.

In this study, diversity in *japonica* landrace was confirmed to be lower than *indica* landrace using two kinds of markers, SSR and SNP. We suggested that the lower diversity

and less allelic richness (AR) in *japonica* than *indica* might be affected by selection in particular traits. Besides, the limited asymmetric gene flow between *indica* and *japonica* as revealed in this study is one of the evidence that gene flow may play a role in diversification and differentiation between these two subspecies and probably this mechanism might be caused by the presence of few intermediate varieties named as C type.

The finding of outlier's loci by both markers suggested that natural variations might affect differentiation between *indica* and *japonica* in Indonesia and may cause by mutation and/or recombination in haplotype. Probably, the different environment of cultivation between *indica* and *japonica* in Indonesia causes different frequency of the mutation at specific loci/locus. Most of *japonica* rice varieties are cultivated in upland and high altitude >500 m above sea level, while the *indica* rice varieties in wetland and low altitude.

Other mechanisms on evolution of population as suggested Hardy-Weinberg is natural selection. Our result indicated that the same genotype is favoured in different populations due to stabilizing selection in most of pairwise between landrace from different source locations by value of *Qst*<FST. This explained the reason why Indonesian landrace was not differentiated based on source locations of landrace.

. Disagreement between SSR and SNP markers in this study was attributed to the greater information content of SSR markers and characteristic of markers. The weak correlation between phenotypic relationships in 2 years indicated that evaluation of genetic relationships using molecular markers includes difficulties with respect to the effects of different management practices and different environments.

The further researches on candidate genes underlying adaptation in Indonesian rice are needed. Our finding also provides a chance to identify particular genes which have been lost during adaptation. We suggested that landrace used in this study and the varieties released before 1966 could use as valuable sources to broaden the variability of Indonesian rice germpalsm and are useful materials for future rice breeding programs in Indonesia.

Acknowledgements

Firstly, I would like to express my sincere gratitude to my advisor Prof. Kazutoshi OKUNO for the continuous support of my PhD study and related research, for his patience, motivation, and immense knowledge. His guidance helped me in all the time of research and writing of this dissertation and publication. I could not have imagined having a better advisor and mentor for my PhD study.

Besides my advisor, I would like to thank the rest of my thesis committee: Prof. Ryo OHSAWA, Prof. Sachio MARUYAMA, Dr. Junichi TANAKA and Dr. Takashi HARA, for their insightful comments and encouragement, in which helped me to widen my research from various perspectives. Also, thank to Dr. Duncan A. Vaughan for his suggestion as genetic resources expert at National Institute of Agro-biological Sciences (NIAS) where he also was one of the team who collected wild rice in Indonesia (IRRI project).

My sincere thanks also goes to Dr. Kaworu Ebana and Dr. Shuichi Fukuoka who provided me an opportunity to join their team as intern, and who gave access to the laboratory as research facilities and time for discussion, in which they have given me precious support and insightful comments on writing for publication particularly.

My thank also goes to Plant Genetics and Breeding Science Laboratory members since 2008-2015, which there are not enough words to describe their all excellent helps.

I would like to thank my mom and all my family members for their wise counsel, sympathetic ear supporting me spiritually throughout my life. You all are always there for me.

Thanks also to my lovely wife, I owe over to her for being unselfishly let her intelligence, passions, and ambitions collide with me

Words fail me to express my gratitude to my god “AllaH”, whose give me this destiny as one of his good plan for me in order to improve my worship quality to him.

References

Ahmadi N, Glaszmann JC, Rabary E (1991) Traditional highland rices originating from intersubspecific recombination in Madagascar. In: Rice Genetics II: Proceedings of the Second International Rice Genetics Symposium 14–18 May 1990. pp. 67–79, IRRI Press, Manila

Balloux F, Brunner H, Lugon-Moulin N, Hausser J, Goudet J. 2000. Microsatellites can be misleading: an empirical and simulation study. Evolution. 54:1414–1422

Barton NH, Keightley PD (2002) Understanding quantitative genetic variation. Nature reviews Genetics, 3:11–21

Beerli P, Palczewski M (2010) Unified framework to evaluate panmixia and migration direction among multiple sampling locations, Genetics, 185:313-326

Boutilier K, Offringa R, Sharma VK, Kieft H, Ouellet T, Zhang L, Hattori J, Liu C-M, van Lammeren AA, Miki BLA, Custers JBM, van Lookeren Campagnea MM (2002) Ectopic expression of baby boom triggers a conversion from vegetative to embryonic growth. The Plant Cell, 14: 1737-1749

Caldo RA, Sebastian LS, Hernandez, JE (1996) Multivarieate analysis of phenotypic diversity of improved rice varieties. Philippine Journal of Crop Science. 21: 76-85

Carvajal-Rodríguez A, Rodríguez MG (2005) Morphometric and distance computation software oriented for evolutionary studies. Online Journal of Bioinformatics, 6: 34-41

Chang T-T (1976) The origin, evolution, cultivation, dissemination, and diversification of asian and african rices. Euphytica, 25: 425-466

Choudhary G, Ranjitkumar N, Surapaneni M, Deborah DA, Vipparla A, Anuradha G, Siddiq EA, Vemireddy LR (2013) Molecular genetic diversity of major Indian rice cultivars over decadal periods molecular genetic diversity of major Indian rice cultivars over decadal periods. PLOS One 8:e66197

do Nascimento WF, da Silva EF, Veasey EA (2011) Agro-morphological characterization of upland rice accessions. Scienta Agricola. (Piracicaba, Brazilia.), 68: 652-660

Diamond J, Bellwood P (2003). Farmers and their languages: the first expansions. Science, 300: 597-603

Emanuelli F, Lorenzi S, Grzeskowiak L, Catalano V, Stefanini M, Troggio M, Myles S, Martinez-Zapater JM, Zyprian E, Moreira FM, Grando MS (2013) Genetic diversity and population structure assessed by SSR and SNP markers in a large germplasm collection of grape BMC Plant Biology, 13: 1-17

Federal Research Division (FRD) (2011) Indonesia a country study (Sixth Edition). In William H F and Robert L W (Eds). Retrieved from http://www.loc.gov/rr/frd/cs/pdf/CS_Indonesia.pdf

Fisher RA (1930) The Genetical Theory of Natural Selection. Clarendon Press, Oxford. 2nd edition Dover Publications, New York. pp272

Foll M, Gaggiotti OE (2008) A genome scan method to identify selected loci appropriate for both dominant and codominant markers: A Bayesian perspective. Genetics, 180: 977-993

Foulley J-L, Ollivier L (2006) Estimating allelic richness and its diversity. Livestock Science. 102: 150–158

Fuentes JL, Cornide MT, Alvarez A, Suarez E, Borges E (2005) Genetic diversity analysis of rice varieties (*Oryza sativa* L.) based on morphological, pedigree and DNA polymorphism data. Plant Genetic Resources, 3: 353–359

Fukuoka S, Tran DS, Ebana K, Luu NT, Nagamine T, Okuno K (2006) Diversity in phenotypic profiles in landrace populations of Vietnamese rice: A case study of agronomic characters for conserving crop genetic diversity on farm. Genetic Resources and Crop Evolution, 53: 753-761

Ganal MW, Altmann T, Röder, MS (2009) SNP identification in crop plants. Current Opinion in Plant Biology, 12: 211-217

Garris AJ, Tai TH, Coburn J, Kresovich S, McCouch SR (2005) Genetic structure and diversity in *Oryza sativa* L. Genetics, 169:1631–1638

Glaszmann JC (1987) Isozymes and classification of Asian rice varieties. Theoretical and Applied Genetics, 74: 21-30

Govindajaru RD (1989) Variation in gene flow levels among predominantly self-pollinated plants. Journal of Evolution Biology, 2:173-181

Gramene Markers Database (2005). retrieved on January 2008 from http://www.gramene.org/

Hamblin MT, Warburton ML, Buckler ES (2007) Empirical comparison of simple sequence repeats and single nucleotide polymorphisms in assessment of maize diversity and relatedness. PLoS One, 2: e1367

Hardy, G. H., 1908 Mendelian proportions in a mixed population. Science 28: 49–50 (reprinted in Jameson 1977) Hargrove TR, Cabanilla VL, Coffman WR. (1988) Twenty years of rice breeding. Bioscience. 38: 675-681

Harlan JR (1992) Crops and man. 2nd ed. Am. Soc. Agronomy, Madison, WI: American Society of Agronomy, pp 284

Hargrove TR, Cabanilla VL, Coffman WR (1988) Twenty years of rice breeding. BioScience 38:675-681

Hawkes JG (1991) The importance of genetic resources in plant breeding. Biological journal of the linnean society, 43: 3-10

Haygood R, Ives AR, Andow DA (2003) Consequences of recurrent gene flow from crops to wild relatives. Proc R Soc B, 270: 1879–1886

Huang P, Molina J, Flowers JM, Rubinstein S, Jackson SA (2012) Phylogeography of Asian wild rice, *Oryza rufipogon*: a genome-wide view. Molecular Ecology, 21: 4593–4604

Iskandar J, Ellen RF (1999) In situ conservation of rice landrace among the Baduy of West Java. Journal of Ethnobiology, 19: 97-125

Izawa T, Konishi S, Shomura A, Yano M (2009) DNA changes tell us about rice domestication. Current Opinion in Plant Biology, 12: 185-192

IBPGR-IRRI (1980) Descriptor for rice *Oryza sativa* L. pp: 6-21 IRRI, Manila

International Rice Information System (IRIS)-IRRI. Retrived on February 2012 from http://iris.irri.org/

International Rice Research Institute (2001) Rice Genetics IV. by Khush GS, Brar DS, and Hardy B. (Eds). Science Publishers, New Hampshire. United States of America

Jagadish SVK., Craufurd PQ, Shi W and Oane R (2014) A phenotypic marker for quantifying heat stress impact during microsporogenesis in rice (*Oryza sativa* L.). Functional Plant Biology 41: 48-55

Jenkins S, Gibson N (2002) High-throughput SNP genotyping. Comparative Functional Genomics 3:57-66

Jia L, Yan W, Zhu C, Agrama HA, Jackson A, Yeater K, Li X, Huang B, Hu B, McClung A, Wuet D (2012) Allelic analysis of sheath blight resistance with association mapping in rice. PLoS ONE 7: e32703

Jin J, Huang W, Gao J-P, Yang J, Shi M, Zhu M-Z, Luo D, and Lin H-X (2008) Genetic control of rice plant architecture under domestication. Nature Genetics, 40: 1365-1369

Jing W, Liu L, Jiang, Wan JM (2006) Mapping of S33 (t) and S34 (t) for pollen sterility in hybrids between a weedy strain and a *japonica* variety in rice. Rice Genetics Newsletter, 23: 16-19

Khush GS (1984) Terminology for rice growing environments. In Terminology of rice growing environments. International Rice Research Institute, Los Banos, Laguna, Philippines. pp 5-10

Khush GS (1997) Origin, dispersal, cultivation and variation of rice. Plant Molecular Biology, 35*:* 25-34

Khush GS (1997) Origin, dispersal, cultivation and variation of rice. Plant Molecular Biology, 35: 25–34

Khush GS, Brar DS, Virk PS, Tang SX, Mallik SS, Busto GA (2003) *Classifying* rice germplasm by isozyme polymorphism and origin of cultivated rice. In IRRI (ed) Discussion Paper, No. 46. pp 1-13

Kiang YT, Antonovics J, Wu L (1979) The extinction of wild rice (*Oryza perenis formosana*) in Taiwan. Journal of Asian Ecology, 1: 1-9

Konishi S, Izawa T, Lin S-Y, Ebana K, Fukuta Y, Sasaki T, Yano M (2006) An SNP caused loss of seed shattering during rice domestication. Science, 312: 1392–1396

Kovach MJ, Sweeney MT, McCouch SR (2007) New insights into the history of rice domestication. Trends in Genetics, 23: 578-587

Kunusoth K , Vadivel K, Sundaram RM, Sultana R, Rajendrakumar P, Maganti S, Subbarao LV, Chin-Wo SR (2015) Assessment of genetic diversity of elite indian rice varieties using agro-morphological traits and SSR markers. American Journal of Experimental Agriculture, 6: 384-401

Kuroda Y, Sato YI, Bounphanousay C, Kono Y, Tanaka K (2005) Gene flow from cultivated rice (*Oryza sativa* L.) to wild *Oryza* species (*O. rufipogon* Griff. and *O. nivara* Sharma et Shastry) on the Vientiane Plain of Laos. Euphytica 142:75–83

Li HL (1970) The origin of cultivated plants in Southeast Asia. Economic Botany, 24: 3-19

Li Z, Rutger JN (2000) Geographic distribution and multilocus organization of isozyme variation of rice (*Oryza sativa* L.). Theoretical and Applied Genetics, 101:379-387

Lin Z, Griffith ME, Li X, Zhu Z, Tan L, Fu Y, Zhang W, Wang X Xie D, Sun C (2007) Origin of seed shattering in rice (*Oryza sativa* L.). Planta, 226: 11–20

Londo JP, Chiang YC, Hung KH, Chiang TY, Schaal BA (2006) Phylogeography of Asian wild rice, *Oryza rufipogon*, reveals multiple independent domestications of cultivated rice, *Oryza sativa*. Proceedings of the National Academy of Sciences, 103: 9578–9583

Lyu J, Zhang SL, Dong Y, Weiming H, Zhang J, Deng X, Zhang Y, Li X, Li B, Huang W, Wan W, Yu Y, Li Q, Li J, Liu X, Wang B, Tao D, Zhang G, Wang J, Xu X, Hu F, Wang W (2013) Analysis of elite variety tag SNPs reveals an important allele in upland rice. Nature Communications, 4: 2138-2148

Mackill DJ, Zhang Z, Redona ED (1995) Comparison of AFLP, microsatellite and RAPD marker polymorphism in rice. Rice Genetics Newsletter, 12: 245-246

Marten GG (1990) Agroecology and small farm development. In Altieri MA, Hecht S (eds.). CRC Press, pp. 177-194

Martin C, Juliano A, Newbury HJ, Lu BR, Jackson MT, Ford-Lloyd BV (1997) The use of RAPD markers to facilitate the identification of Oryza species within a germplasm collection. Genetic Resources and Crop Evolution, 44: 175-183

Mather KA, Molina J, Flowers JM, Rubinstein S, Rauh BL, Lawton-Rauh A, Caicedo AL, Mcnally KL, Purugganan MD (2010) Migration, isolation and hybridization in island crop populations: the case of Madagascar rice. Molecular Ecology, 19: 4892–4905

McCouch SR, Kochert G, Yu ZH, Wang ZY, Khush GS, Coffman WR, Tanksley SD (1988) Molecular mapping of rice chromosomes. Theoretical and Applied Genetics, 76: 815-829

McNally KL, Childs KL, Bohnert R, Davidson RM, Zhao K, Ulat VJ, Zeller G, Clark RM, Hoen DR, Bureau TE. Stokowskih R, Ballingerh D G, Frazerh K. A, Coxh DR, Padhukasahasrame B, Bustamantee CD, Weigelf D, Mackilla DJ, Bruskiewicha RM, Ratschc G, Buellb CR, Leunga H, Leach JE (2009) Genomewide SNP variation reveals relationships among landrace and modern varieties of rice. Proceeding of the National Academy of Sciences, 106: 12273-12278

Mokuwa A, Nuijten E, Okry F, Teeken B, Maat H (2014) Processes Underpinning Development and Maintenance of Diversity in Rice in West Africa: Evidence from Combining Morphological and Molecular Markers. PLoS ONE, 9: e85953

Molina J, Sikora M, Garud N (2011) Molecular evidence for a single evolutionary origin of domesticated rice. Proceedings of the National Academy of Sciences, 108: 8351–8356

Morishima H, Hinata K, Oka HI (1963) Comparison of modes of evolution of cultivated forms from two wild rice species, *Oryza breviligulata* and *O. perennis*. Evolution, 17:170-181

Muhamad K., Okuno K (2013) Differentiation between tropical *japonica* and *indica* varieties of Indonesian rice landrace as revealed by SSR markers and adaptive traits. Poster presented at International Symposium on "Diversifying Biological Resources", Tsukuba , Japan

Nei M (1972) Genetic distance between populations. The American Naturalist, 106: 283-292.

Nei M (1973) Analysis of gene diversity in subdivided populations (population structure/ genetic variability/heterozygosity/gene differentiation). Proceedings of the National Academy of Sciences, 70: 3321-3323

Normile D (2004) Yangtze seen as earliest rice site. Science, 275:309

OECD (1999) Consensus document on the biology of *Oryza sativa* (rice). OECD Environmental health and Safety Publications, Paris. Report No. env/jm/mono (99)26

Ogunbayo SA, Ojo DK, Guei RG, Oyelakin OO, Sanni KA (2005) Phylogenetic diversity and relationships among 40 rice accessions using morphological and RAPDs techniques. African Journal of Biotechnology, 4:1234-1244

Oka HI (1974) Experimental studies on the origin of cultivated rice. Genetics, *78: 475-486.*

Oka HI (1988) Origin of cultivated rice. Japan Science Society Press/Elsevier. Tokyo/Amsterdam

Olsen KM, Caicedo AL, Polato N, McClung A, McCouch SR, Purugganan MD (2006) Selection under domestication: Evidence for a sweep in the rice waxy genomic region. Genetics, 173:975–983

Papa R, Gepts P (2004) Asymmetric gene flow and introgression between domesticated and wild populations. In: den Nijs HCM, Bartsch D, Sweet J (eds), Introgression from genetically modified plant into wild relatives. (CAB International: Wallingford, UK), pp125-138

Peakall R, Peter ES (2006) GENALEX 6: Genetic analysis in excel.population genetic software for teaching and research. Molecular Ecology, 6: 288–295

Perrier X, Jacquemoud-Collet, JP 2006. DARwin software

Pertoldi C, Jørgensen HBH, Randi E, Jensen LF, Kjærsgaard A, Loeschcke V, Faurby S (2012) Implementation of mixture analysis on quantitative traits in studies of neutral versus selective divergence. Evolutionary Ecology Research, 14: 881–895

Pettit RJ, El Mousadik A, Pons O (1998) Identifying populations for conservation on the basic of genetic markers. Conservation Biology, 12: 844-855

Powell W, Machray GC, Provan J (1996) Polymorphism revealed by simple sequence repeats. Trends in Plant Science, 1: 215-222

Powell W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey S, Rafalski A (1996) Thecomparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Molecular Breeding, 2: 225-238

Qi D, Guo G, Lee M-c, Zhang J, Cao G, Zhang S, Suh S-c, Zhou Q, Han L (2008) Identification of quantitative trait loci for the dead leaf rate and the seedling dead rate under alkaline stress in rice J. Genetic and Genomics, 35: 299−305

Rabara RC, Ferrer MC, Diaz CL, Newingham Ma-CV, Romero GO 2014, Phenotypic diversity of farmers' traditional rice varieties in the Philippines. Agronomy, 4: 217-241

Rafalski A (2002) Applications of single nucleotide polymorphisms in crop genetics. Current Opinion in Plant Biology, 5: 94-100

Ravi M, Geethanjali S, Sameeyafarheen F, Maheswaran M 2003. Molecular marker based genetic diversity analysis in rice (*Oryza sativa* L.) using RAPD and SSR markers. Euphytica, 133: 243–252

Ray A, Deb D, Ray R, Chattopadhayay B (2013) Phenotypic characters of rice landrace reveal independent lineages of short-grain aromatic *indica* rice. AoB PLANTS 5: plt032

Sano Y, Katsumata M, Okuno K (1986) Genetic studies of speciation in cultivated rice. 5. Inter- and intraspecific differentiation in the waxy gene expression of rice. Euphytica, 35: 1-9

Sasaki A, Ashikari M, Tanaka MU, Itoh H, Nishimura A, Swapan D, Ishiyama K, Saito T, Kobayashi M, Khush GS, Kitano H, Matsuoka M. (2002) Green revolution: A mutant gibberellins-synthesis gene in rice. Nature, 416: 701-702

Schaal BA, Gaskin JF, Caicedo AL (2003) Phylogeography, haplotype trees, and invasive plant species. Journal of Heredity, 94: 197–204

Schaal BA, Hayworth DA, Olsen KM, Rauscher JT, Smith WA (1998) Phylogeographic studies in plants: problems and prospects. Molecular Ecology, 7: 465–474

Second G (1982) Origin of the genic diversity of cultivated rice (*Oryza* spp.): Study of the polymorphism scored at 40 isozyme loci. The Japanese Journal of Genetics, 57: 25–57

Shannon C (1948) A mathematical theory of communication. Bell Systems Technological Journal, 27: 379–423

Shomura A, Izawa T, Ebana K, Ebitani T, Kanegae H, Konishi S (2008) Deletion in a gene associated with grain size increased yields during rice domestication. Nature Genetics, 40: 1023–1028

Sie M, Ghesquiere A. (1992) Isozyme polymorphism and classification of rice varieties from Burkina Faso in, Africa. Rice Genetics Newsletter, 9: 75-76

Singh N, Choudhury DR, Singh AK, Kumar S, Srinivasan K (2013) Comparison of SSR and SNP markers in estimation of genetic diversity and population structure of Indian rice varieties. PLoS ONE, 8: e84136

Sinha AK, Mishra PK (2013) Morphologybased multivariate analysis of phenotypic diversity of landrace of rice (Oryza sativa L.) of Bankura district of West Bengal Journal of Crop and Weed, 9:115-121

Song ZP, Lu B-R, Zhu YG, Chen JK (2003). Gene flow from cultivated rice to wild species *Oryza rufipogon* under experimental field conditions. New Phytologist, 157: 657-665

Song ZP, Zhu W, Rong J, Xu X, Chen JK, Lu BR (2006) Evidences of introgression from cultivated rice to *Oryza rufipogon* (Poaceae) populations based on SSR fingerprinting: implications for wild rice differentiation and conservation. Evolutionary Ecology, 20: 501-522

Staub JE (1994) Crossover: concepts in applications in genetics, evolution and breeding—an interactive computer-based laboratory manual. University OfWisconsin Press, Wisconsin

Sun CQ, Wang XK, Li ZC, Yoshimura A, Iwata N (2001) Comparison on the genetic diversity of common wild rice (*Oryza rufipogon* Griff.) and cultivated rice (*O. sativa* L.) using RFLP markers. Theoretical and Applied Genetics, 102: 157-162

Susanto U, Daradjat AA, Suprihatno B (2003) Advance in lowland rice breeding in Indonesia. Jurnal Litbang Pertanian, 22: 125-131 (Abstract in English)

Sweeney M, McCouch SR (2007) The complex history of the rice domestication. Annals of Botany, 100: 951–957

Tanaka K (1995) Malayan cultivated rice and its expansion. Agricultural Archaeology-Part I, 1: 73–78

Tanaka K (1998) Malayan cultivated rice and its expansion. Agricultural Archaeology-Part IV, 1: 344-350

Tanksley SD, McCouch SR (1997) Seed banks and molecular maps: Unlocking genetic potential from the wild. Science, 277:1063-1066

Thomson MJ, Polato NR, Prasetiyono J, Trijatmiko KR, Silitonga TS, McCouch SR (2009) Genetic diversity of isolated populations of Indonesian landrace of rice (*Oryza sativa* L.) collected in east Kalimantan on the island of Borneo. Rice, 2: 80–92

Thomson MJ, Septiningsih EM, Suwardjo F, Santoso TJ, Silitonga TS, McCouch SR (2007) Genetic diversity analysis of traditional and improved indonesian rice (*Oryza sativa* L.) germplasm using microsatellite markers. Theoretical and Applied Genetics 114: 559-568

Tripathi MP, Sthapit BR, Subedi LP, Sah SK, Gyawali S (2013) Agro-morphological variation in ‘‘Jhinuwa’’ rice landrace (*Oryza sativa* L.) of Nepal. Genetic Resources and Crop Evolution 60: 2261–2271

UNCTAD (2011) Historical evolution of the main paddy rice consuming countries (human food) in tones between 1961 and 2002, http://www.unctad.info/en/Infocomm/Agricultural_Products/Riz/Market/ (accessed March 2, 2015)

Vaughan DA (1994) The Wild Relatives of Rice. A Genetic Handbook. International Rice Research Institute, Manila

Vaughan DA, Lu BR, Tomooka N (2008) The evolving story of rice evolution. Plant Science, 174: 394–408

Vieira FG, Fumagalli M, Albrechtsen A, Nielsen R (2013) Estimating inbreeding coefficients from NGS data: Impact on genotype calling and allele frequency estimation. Genome Research, 23: 1852–1861

Watanabe S, Nakagawa A, Izumi S, Shimada H, Sakamoto A (2010) RNA interference-mediated suppression of xanthine dehydrogenase reveals the role of purine metabolism in drought tolerance in Arabidopsis. FEBS Letters, 584: 1181-1186

Weinberg, W., 1908 Über den Nachweis der Vererbung beim Menschen. Jahresh. Ver. Vaterl. Naturkd. Württemb. 64: 369–382 (English translations in Boyer 1963 and Jameson 1977)

Wright S (1951) The genetical structure of populations. Annual Eugenics, 15: 323-354

Wright S (1978) Evolution and the genetics of populations. Variability within and among natural populations. Vol 4. The University of Chicago Press, Chicago

Xu X, Liu X, Ge S, Jensen JD, Hu FY, Li X, Dong Y, Gutenkunst RN, Fang L, Huang L, Li JX, He WM, Zhang GJ, Zheng XM, Zhang FM, Li YR, Yu C, Kristiansen K, Zhang XQ, Wang J, Wright M, McCouch S, Nielsen R, Wang J, Wang W (2012)

Resequencing 50 accessions of cultivated and wild rice yields markers for identifying agronomically important genes. Nature Biotechnology, 7: 105–111

Xue W, Xing Y, Weng X, Zhao Y, Tang W, Wang L, (2008) Natural variation in *Ghd7* is an important regulator of heading date and yield potential in rice. Nature Genetics, 40: 761–767

Yang C-C, Kawahara Y, Mizuno H, Wu J, Matsumoto T, Itoh T (2012) Independent domestication of Asian rice followed by gene flow from *japonica* to *indica*. Molecular Biology and Evolution, 29: 1471–1479

Yang XC, Hwa CM 2008. Genetic modification of plant architecture and variety improvement in rice. Heredity. 101: 396-404

Yano M, Katayose Y, Ashikari M, Yamanouchi U, Monna L, Fuse T, Baba T, Yamamoto K, Umehara Y, Nagamura Y, Sasaki T (2000) *Hd1,* a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the *Arabidopsis* flowering time gene *CONSTANS*. Plant Cell, 12: 2473–2484

Yonemaru J-i, Ebana K, Yano M (2014) HapRice, an SNP haplotype database and a web tool for rice. Plant Cell Physiology, 55: e9 (1–12)

Yoshida T, Anas, Santi R, Ridwan S (2009) Genetic background of Indonesia rice germplasm and its relationship to agronomic characteristics and eating quality. The Japanese Journal of Crop Science, 78: 335-343

Yue B, Xue W, Xiong L, Yu X, Luo L, Cui K, Jin D, Xing Y, Zhang Q (2006) Genetic basis of drought resistance at reproductive stage in rice: separation of drought tolerance from drought avoidance. Genetics, 172: 1213–1228

Zhang P, Li J, Li X, Liu X, Zhao X, Lu Y (2011) population structure and genetic diversity in a rice core collection (*Oryza sativa* L.) Investigated with SSR Markers. PLoS ONE, 6: e27565

Zhu Q, Zheng X, Luo J, Gaut BS, Ge S (2007) Multilocus analysis of nucleotide variation of *Oryza sativa* and its wild relatives: severe bottleneck during domestication of rice. Molecular Biology and Evolution, 24: 875-888