Diversity Analysis of Rapeseed (*Brassica napus* L.) Genetic Resources in Japan

April 2017

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Diversity Analysis of Rapeseed (*Brassica napus* L.) Genetic Resources in Japan

A Dissertation Submitted to

The Graduate School of Life and Environmental Sciences,

the University of Tsukuba

in Partial Fulfillment of the Requirements

for the Degree of Doctor of Philosophy in Agricultural Science

(Doctoral Program in Biosphere Resource Science and Technology)

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Chapter 1: General Introduction

1.1 Introduction of rapeseed

Rapeseed (*Brassica napus* L.; genome AACC, 2n=38) also known as rape, oilseed rape (and, in the case of one particular group of cultivars, canola), is one of the most economically important oilseed crops over the world, which is thought to have multiple origins resulting from independent natural hybridization events between *B. rapa* (AA, 2n=18) and *B. oleracea* (CC, 2n=18) (U, 1935).

1.2 Origin and diffusion

The natural hybridization of *B. rapa* and *B. oleracea* was considered to occur at the Mediterranean region in the southern Europe (Röbbelen *et al.* 1989). The original wild rapeseeds were presumed to be grown on the steep cliffs along the coast of this region (Shiga 1971). From where it was introduced into Asia in the early 18th century (Röbbelen *et al.* 1989).

1.3 World Production

The cultivation of *Brassica* crops was believed to begin in the Middle Ages in Europe (Kimber and McGregor 1995), and some researcher conjectured that in Holland in the 17th century the rapeseed has been grown as a source of oil (Appelqvist and Ohlson 1972) but other thought it should be earlier than that time (Schröder-Lembke 1989).

Since the beginning of cultivation, with the improving of agronomic and processing technology, the utilization of both oil and meal of rapeseed is increasing gradually. As an important part of progress, the status of rapeseed oil has improved in recent decades. The new rapeseed cultivars with double low (low erucic acid and low glucosinolate)

traits were widely cultivated.

Nowadays, over 450 million tons of rapeseed seeds are harvested worldwide every year and accounts for about 20% of the world grain production (Carré and Pouzet 2014). In the year 2012, the EU leaded world′s rapeseed production with approximately 20 Mt, followed by Canada (15 Mt), China (12 Mt), and India (6 Mt) (ISTA Mielke Gmb 2012).

In recent years, genetic modified (GM) rapeseed have been grown widely and occupied at least 21% of the total rapeseed cultivation area (Nishizawa *et al.* 2010). Different type of GM rapeseed such as herbicide-tolerant (HT), insect-resistance (IR) and HT/IR have been constantly created and introduced into the farmland. In some developed countries such as Canada, 98% of the total rapeseed production was made up of GM rapeseed (Katsuta *et al.* 2015).

1.4Rapeseed cultivation and breeding in Japan

1.4.1 Cultivation history

The history of rapeseed in Japan was relatively clearer because of the late introduction to this country. Rapeseed was introduced into two areas of Japan during the Meiji period (19th century). The first one, a cultivar adapted to warm climates was introduced into Fukuoka Prefecture in the early Meiji period (Shiga 1971). This cultivar was called “Chosensyu” (Korean type), but some important traits of it were very consistent with European varieties such as late maturation, high plant height and large plant size. Plus, there is no record of the introduction via Korean, this cultivar was considered to be introduced from Europe (Shiga 1971). In 1886, three cold-resistant cultivars “Hamburg”, “Essex Rapeseeds”, “German Rape” were introduced from Germany to Hokkaido (Shiga 1971). These cultivars had the traits of very late maturation, fall sowing, strong cold-tolerance and tall but slim plants. Among them, “Hamburg” which most adapted to the local environment, was cultivated in some northern Japan areas such as Aomori, Iwate and Niigata Prefectures (Shiga 1971).

Until 1961, cultivation area and yield of rapeseed in Japan were increased only

except being affected by the Second World War. But the liberalization of soybean trade in 1961 and the liberalization of rapeseed trade after a few years caused the comprehensive declining of Japanese rapeseed cultivation (Shiga 1971, Honda 2009). In 2015, the cultivation area of rapeseed in Japan was 1620 ha, the total yield was 3100t (MAFF 2015). That was equivalent to only 0.1% of annual imports (2441480 t) (MOF 2016).

1.4.2 Breeding history

Since being introduced to Japan, the breeding of rapeseed has been carry out continuously. 1930 is an important time point in this process, before this year, the breeding work was mainly performed by agricultural research institute belongs to each prefecture government and the pure-line selection was the predominantly used method (Shiga 1971). Into the 1930s, the Japanese government started a formal breeding program to improve rapeseed which was expected to be used as a means of developing the rural economy. The initial purpose of the program was to breed out new cultivars which could maintain the good traits of Korean type and the maturation should be earlier. From the late 1950s, new cultivar with adverse environmental adaptability, lodging-resistance, disease-resistance, high yield and high quality became the breeding purpose (Shiga 1971, Yamamori 2006).

The breeding method that began in the 1930s were mainly crossbreeding, which including intraspecific hybridization of *B.napus* and the interspecific hybridization between *B. napus* and *B. rapa*. Heterosis breeding for F_1 lines was also used by the breeders (Shiga 1971). In recent years, the improvement of fatty acid composition has become the target of breeding, new cultivars such as “Nanashikibu”, “Kirariboshi” have the double-low quality same as canola (Yamamori 2006).

Until now, more than 200 rapeseed varieties were breed out in Japan.

1.4.3 Challenges in rapeseed breeding in Japan

Although large-scale cultivation has ceased, the Japanese breeders are still working on the improvement of rapeseed varieties. In 2014, NARO (National Agriculture and Food Research Organization) disclosed new rapeseed cultivar “nanaharuka”, which is suitable for growing in the warm southern region of Japan (NARO 2014). In 2015, another new cultivar “kirakiraginga” was disclosed, which is a double-low variety with higher yields (Honda *et al.* 2017). Besides these, other studies on vegetable rapeseed are ongoing too (Tsuge *et al.* 2015).

The aim of these studies is to provide new varieties and new cultivation methods for both actual and potential rapeseed requirements, which including the demand for vegetable rapeseed, demand for rapeseed meal for feed, demand for biofuels, green manure, landscape plant and the demand for high-quality varieties to meet future large-scale cultivation due to decline in cultivation inputs or the price increases of import rapeseed (Nonaka 2013, Fujii 2011, Honda *et al.* 2017).

1.5 Rapeseed genetic resources situation in Japan

1.5.1 Definition of genetic resources

According to the OECD definition, Genetic resources are genetic material of plants, animals or micro-organisms of value as a resource for future generations of humanity (OECD 1997). FAO also give a similar definition about plant genetic resources for food and agriculture: It means any genetic material of plant origin of actual or potential value for food and agriculture (FAO 2009).

In this study, I would like to use “Genebank resources” to refer to the rapeseed cultivars and landraces which were preserved in the genebank. And a phrase “Feral resources” would be used to refer to the rapeseed which are growing in the area with out of the human’s management.

1.5.2 Genebank resources

The gene bank belongs to the Genetic Resources Center, NARO (National Agriculture and Food Research Organization) is the largest one in Japan which collected more than 600 germplasms, including more than 300 Japanese germplasms and other overseas germplasms.

1.5.3 Feral resources

Although rapeseed is not a native species in Japan, many feral rapeseed populations were found around the country (Ministry of Agriculture, Forestry and Fisheries 2014). The observation and monitoring of some feral populations showed that there were no expansion or contraction of them (Katsuta *et al.* 2015). It is generally thought that most feral rapeseeds are derived from the escape of the domesticated plants (Nishizawa *et al.* 2010). Some studies also assumed that feral populations of rapeseed have formed after the 19th century (Kameda *et al.* 2010). But because rapeseed grows throughout Japan, the precise distribution records have not been created (Nishizawa *et al.* 2010).

In recent years, seeds spilled during transportation process of the large quantity of import rapeseed ware assumed to be a new origin of feral populations. But it needs to be pointed out that GM rapeseed occupied more than 80% of the imported rapeseeds, and most of the rapeseed plants in the port and roadside were proved to be non-GM ones (Saji *et al.* 2005).

Based on the above information, I can draw a conclusion that Japan's feral rapeseed is very likely to come from the escape of more ancient cultivars. Which may include cultivars that have been cultivated in Japan, and may also include varieties imported as raw material for oil extraction. These rapeseeds are affected by the natural environment after being introduced into Japan, resulting in some variations which adapt to the Japanese environment. If such variations are present, some of the feral rapeseeds will exhibit genetic characteristics different from NARO rapeseed accessions in the diversity analysis (Fig. 1.1). In this case, they may have the potential as breeding materials and become genetic resource that can be utilized. Here, I use "feral resources" this new name to refer to them, intended to emphasize their possibility.

1.6 Genetic diversity research of genetic resources

1.6.1 Importance of genetic diversity research

Definition of biological diversity could be written as the variation present in all species of plants and animals, their genetic material and the ecosystems in which they occur (Rao and Hodgkin 2002). The biological diversity can occur at three levels: genetic diversity (variation in genes and genotypes), species diversity (species richness) and ecosystem diversity (communities of species and their environment) (Rao and Hodgkin 2002). Diversity in plant genetic resources provides opportunity for plant breeders to develop new and improved cultivars with desirable characteristics (Govindaraj *et al.* 2015). But the cultivation, domestication and breeding will select elite individuals, this will lead to a decline in genetic diversity (Feuillet *et al.* 2008, Tester and Langridge 2010).

The availability of a gene bank with a high genetic diversity could provide a better range of parental selection for breeding, meanwhile, assessment of the genetic diversity of existing gene banks is also a means to improve the efficiency of utilization and construction of it (Govindaraj *et al.* 2015). It is also important to study the genetic diversity of feral resources. The remarkable diversity of regional landraces, local cultivars and related species offers a reservoir of genetic variation that has the potential to impact positively on crop improvement and sustainable agricultural production (Feuillet *et al.* 2008). Some agricultural traits such as male sterility, disease resistance, insect resistance were successfully introduced into cultivars from feral species (Duvick 1984, Feuillet *et al.* 2008, Yao *et al.* 1997).

1.6.2 Assessment methods of Genetic Diversity

Morphological, and biochemical measure can be used to analysis genetic diversity, but the assessment based on molecular markers has gained more importance due to the

speedy, quality of data generated and the widespread presence. Unlike other markers, molecular markers have an almost unlimited number and unaffected by environmental factors and/or the developmental stage of the plant (Govindaraj *et al.* 2015, Mondini *et al.* 2009).

Nowadays, new methods like Next Generation Sequencing (NGS) has become a useful way of genetic diversity assessment. Whole genome sequencing data could give comprehensive genetic information of the individuals (Pop and Salzberg 2008). However, although compared to traditional sequencing, the cost of NGS is much cheaper, it is still not very realistic in genetic diversity study of Japanese rapeseed resource.

1.6.3 Current Status of crop genetic diversity research in Japan

Japanese researchers have made a lot of achievements in the research of genetic diversity of several crop species partially included their wild relatives. Some major crops such as rice, maize, soybean, wheat, sorghum have been analyzed for genetic diversity in recent years (Kojima *et al.* 2005, Ebana *et al.* 2008, Kaga *et al.* 2012, Shehzad *et al* 2009). Several core collections, which can overcome the restriction of the utilization of large size of germplasm in breeding by using subsets of the entire collection of the species have been constructed too (Kojima *et al.* 2005, Ebana *et al.* 2008, Kaga *et al.* 2012, Shehzad *et al* 2009).

However, there is no comprehensive study on the genetic diversity of rapeseed in Japan, both of genebank resources and feral resources. And a core collection of the NARO Genebank which will be very useful for breeding work is still waiting to be constructed.

1.7 Objectives

The purposes of this thesis were to assess the genetic diversity of the rapeseed genetic resources in Japan, to explore their situation and potential for further exploitation for breeding efforts. The three main components of this study were

compiled in each chapter. In Chapter 2, the NARO genebank rapeseed resources were analyzed by SSR marker to obtain the information of its genetic diversity and genetic structure. Japanese germplasms from different regions were analyzed separately to explore the effects of breeding on germplasm resources. A core collection was constructed based on the polymorphic allele information to facilitate the future breeding work. In Chapter 3, the feral rapeseed resources collected from 13 locations of Japan were analyzed by SSR marker to reveal their genetic diversity and structure to reveal the possible formation way of them. Then, a comparison of the genebank resources and feral resources was preformed to understand whether there is similarity between them. Such comparisons will also help to determine whether the feral resources are valuable as breeding materials. Finally, in Chapter 4, the comprehensive discuss of the results of the above studies described in Chapter 2 and Chapter 3, and summarize the main conclusions of this study.

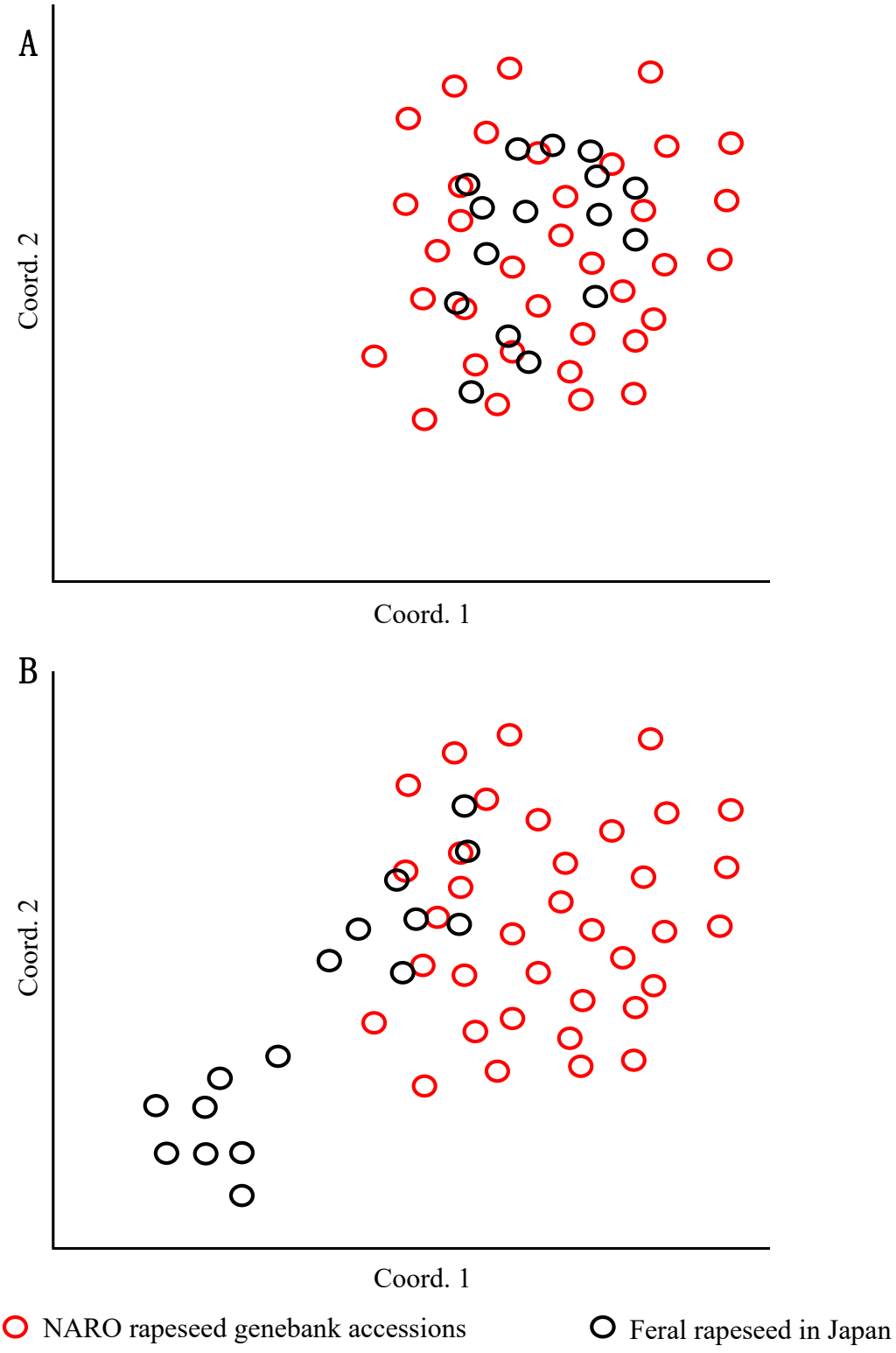

Fig. 1.1 The simulation PCoA of the relationship between NARO rapeseed genebank and feral rapeseed. A, assume that feral rapeseed comes entirely from the resources contained in the NARO rapeseed genebank; B, assume that feral rapeseed comes partily from the resources contained in the NARO rapeseed genebank. If the real situation is similar to B, then it can be considered feral rapeseed as genetic resources.

Chapter 2: Genetic diversity analysis and core collection construction in rapeseed genetic resources in Japan

2.1 Introduction

Genetic resources are key to progress in breeding, contributing to sustainable agriculture and associated industries. More than 600 accessions, including >300 Japanese landraces and cultivars, are maintained at the Genetic Resources Center of the National Agriculture and Food Research Organization (NARO Genebank). A collection of this size is expected to contain a wide range of genetic variation and offer opportunities for trait improvement. However, screening for target traits in the whole collection is often time-consuming, laborious, and costly. To efficiently use germplasm collections in breeding, knowledge of the genetic diversity and phylogenetic relationship among the germplasms is vital. In addition, establishing a representative subset of the whole collection, called a core collection, will be a feasible way for management, evaluation, and utilization of genetic resources.

A core collection should represent most of the diversity in the whole collection and should cover at least 70% of the alleles present in the whole collection (Brown 1989). Phenotypic traits have been used for constructing core collections, but they are easily affected by environmental conditions and are sometimes difficult to measure accurately. Recently, many core collections have been established by using DNA marker data, because DNA markers can be easily obtained and are highly stable and polymorphic (Taniguchi *et al.* 2014, Xu *et al.* 2016, Zhao *et al.* 2016). In rapeseed, several studies revealed genetic diversity of different collections using molecular markers such as RFLP (Song and Osborn 1992), RAPD (Ma *et al.* 2000), SRAP (Riaz *et al.* 2001), AFLP (Seyis *et al.* 2003), and SSR (Hasan *et al.* 2006). SSR markers are superior because they are able to detect multiple alleles per locus, are codominant at a single locus, and are relatively evenly distributed across genomes (Zalapa *et al.* 2012); they have been

widely used in recent diversity analyses of Brassica species (El-Esawi *et al.* 2016, Guo *et al.* 2016, Yao *et al.* 2012).

Over the last five decades, effort devoted in Japan to rapeseed breeding has resulted in considerable progress. Despite a large number of germplasms in NARO Genebank, only a few studies analyzed the small number of either Japanese or overseas accessions in this collection (Diers and Osborn 1994, Gyawali *et al.* 2013, Hasan *et al.* 2006, Ma *et al.* 2000). The number of rapeseed germplasms in the NARO Genebank has increased owing to both past studies and exploration of genetic resources.

The aims of this chapter are to examine the genetic diversity and population structure of NARO rapeseed collection using SSR markers, and to establish a core collection with good representation of the genetic diversity present in the whole collection.

2.2Materials and methods

2.2.1 Plant Material

The rapeseed collection in NARO Genebank hold 639 germplasms, the seeds are preserved in cool dry storage. I excluded accessions from our analysis if both parental lines were in the collection. The total number of accessions analyzed was 582 (Appendix 1), including 305 Japanese accessions (landraces, breeding lines, and cultivars) and 277 overseas accessions from all inhabited continents except Africa.

2.2.2 Genotyping

Seeds were germinated on moistened filter paper in 9-cm Petri dishes and then transplanted into a 72-cell tray filled with granular culture soil (Nippi-Engei-Baido-1gou soil, Nihon Hiryo Co., Ltd., Tokyo, Japan). Seedlings were grown in a climate chamber (25/22 °C 12 h/12 h) until the first true leaves had fully expanded. Genomic DNA was extracted from the first true leaf of each seedling by using the DNeasy Plant

Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol with some minor modifications.

A total of 504 Brassica SSR markers (Appendix 2) were prescreened against 8 representative accessions that were chosen on the basis of their origins and breeding histories: OOMINATANE (Japan), MICHINOKUNATANE (Japan), ASAHINATANE (Japan), CASCADE (USA), HAMBURG 1 (Germany), WESTAR (Canada), PROTA (Germany), RAPORA(Korea) (Wang *et al.* 2012, Cheng *et al.* 2009, Li *et al.* 2013, Li *et al.* 2011, Piquemal *et al.* 2005, Xu *et al.* 2010, Suwabe *et al.* 2002, Kim *et al.* 2009, Iniguez-Luy *et al.* 2009, Nagaoka *et al.* 2010, Lowe *et al.* 2002, AAFC Consortium 2016). SSR markers with clear reproducible polymorphic amplification products were then applied to all other accessions. PCR mixtures (10 μl) contained template DNA (10 ng), 1× KAPA 2G buffer A, 200 nM dNTP, 0.5 mM MgCl2, 0.1 U KAPA 2G Fast DNA polymerase (KAPA Biosystems Inc., Woburn, MA, USA), 2 pmol reverse primer, and 0.5 pmol forward primer. The forward primers were 5′-labeled with the fluorescent dyes 6-FAM, VIC, NED, or PET (Shimizu and Yano 2011). PCR was performed in a C1000 Thermal Cycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA) as follows: initial denaturation at 94 °C for 3 min; 30 cycles of 94 °C for 20 s, 54 °C for 30 s, and 62 °C for 30 s; 3 cycles of 94 °C for 20 s, 49 °C for 10 s, and 72 ° C for 5 s; and a final extension at 72 °C for 10 min. The size of the amplified fragments was estimated by using an automated DNA analyzer (model 3130xl) with a GeneScan-600LIZ size standard and GeneMapper v. 4.0 software (all Thermo Fisher Scientific, Inc., Waltham, MA, USA).

2.2.3 Genetic diversity and population structure analysis

The number of alleles, major allele frequency, number of rare alleles (frequency <5%), and polymorphism information content (PIC) were calculated for the whole collection and for each geographic group in PowerMarker v. 3.25 software (Liu and Muse 2005). Observed heterozygosity (Ho), expected heterozygosity (He), Shannon's information index (I), pairwise F statistics (FST), and Nei's genetic distance (Nei *et al.*

1983) were calculated in GenAlEx v. 6.502 software (Peakall and Smouse 2012). The components of variance between Japanese and overseas accessions, between different groups and among individuals within groups were estimated from the genetic distance matrix, as specified in the analysis of molecular variance (AMOVA) procedure using Arlequin 3.5.2.2 software (Excoffier and Lischer 2010). A nonparametric permutation procedure with 1000 permutations was used to test the significance of variance components associated with the different possible levels of genetic structure in this study.

Genotyping data for the SSR markers were analyzed by using the model-based STRUCTURE v. 2.3.4 software (Pritchard *et al.* 2000) to determine the most probable number of clusters (K value) and to assign rapeseed accessions to different clusters. The K value was determined by running an admixture and related frequency model with K = 1 to 20 (10 replications per K value); the burn-in period of each run and the Monte Carlo Markov Chain (MCMC) lengths were both set to 100,000. The website program STRUCTURE HARVESTER was used to estimate the optimal number of K value (Earl 2012). This program follows the ΔK method of Evanno *et al.* (2005). The same set of genotyping data was used to perform principal coordinates analysis (PCoA) in GenAlEx 6.502, and to construct the phylogenetic tree based on neighbor-joining (NJ) method implemented in MEGA7 software (http://www.megasoftware.net/).

2.2.4 Construction of core collection

Core collections were constructed using four different methods: two maximization (M) strategy methods with different algorithms, the Core Hunter method, and the random sampling method. Both M strategy methods select a core collection to maximize the number of alleles of the SSR markers. CoreFinder v. 1.1 (Cipriani *et al.* 2010) is based on the NP-complete set-covering problem and uses a Las Vegas–style randomized algorithm. PowerCore v. 1.0 (Kim *et al.* 2007) uses an advanced M strategy with a modified heuristic algorithm (A*) that can find the optimum path for sample selection. Core Hunter v. 2.0 (De Beukelaer *et al.* 2012) is a fast core-subset-selection program based on multiple genetic diversity measures and using a Mixed Replica

search algorithm. The software allows choosing sampling intensity and the genetic measures to be used as selection criteria. The Core Hunter software was run in R (http://www.r-project.org/); the modified Roger's distance (weight 0.7) and Shannon's information index (weight 0.3) were chosen to define a core collection comprising about 20% of the entry collection. In the random sampling method, before sampling, a neighbor joining (NJ) tree based on Nei's genetic distance is constructed in MEGA7 software. Sampling was performed according to Hu *et al.* (2000): initially, an accession is selected at random from each lower-order level subgroup, if there is just one accession in the subgroup, it is sampled; if there are two accessions, one is randomly selected. A new dendrogram was then generated, and the process was repeated until the number of selected accessions was reduced to 20% of the initial collection. To evaluate the representativeness of the different core collections, genetic diversity indices (allele number, allele retention ratio, number of effective alleles, Ho, He, I, and PIC) were calculated in PowerMarker v. 3.25 and GenAlEx 6.502 software. The differences between the whole collection and each core collection were tested by t-test.

2.2.5 Verify the core collection based on the phenotypic data

To validate the representativeness of the core collection based on phenotypic data is a common method. In this study, although no large-scale cultivation experiments were conducted to verify the representativeness of the core collection, the NARO rapeseed genebank's existing characterization data could still be used for a rough validation. In the NARO genebank database, rapeseed phenotype data recorded in different years and locations were preserved. These data do not contain all accessions, and a accession may be investigated several times. Therefore, it is not feasible to accurately evaluate the representativeness of the core collection base on these data. However, if the accessions contained in the core collection only cover a fraction of the total range of data, then the core collection could be considered to be unsuccessful. On the contrary, if the core collection can basically cover the range of the total data, it could be think that the core collection is basically successful. Therefore, I selected the characterization data of several important traits from the NARO rapeseed genebank to

analyze the representativeness of the core collection. These traits include: plant type, plant height, panicle length, seed color, cold tolerance, snow resistance, disease resistance, lodging resistance, oil content, erucic acid content, oleic acid content, linoleic acid content, linolenic acid content, llucosinolate content, 1000 grain weight.

2.3 Results

2.3.1 Genetic Diversity of the NARO rapeseed collection

Prescreening selected 30 of the 504 SSR markers. The selected markers amplified a total of 311 alleles in the 582 accessions, ranging from 3 (BoGMS0660 and BoEMS0049) to 39 (BrGMS0070) per marker, with a mean allelic richness of 10.37 (Table 2.1); 214 alleles (68.8%) with low frequency (<0.05) were regarded as rare alleles. In addition, the selected 30 SSR markers amplified only one or two alleles in each accession. Average genetic diversity indices were 0.56 for major allele frequency, 0.57 for He, and 0.52 for PIC. The average Ho value was 0.05 (range, 0.01–0.34), indicating that almost all accessions in the whole collection were highly homozygous.

The genetic diversity indices for geographical groups are summarized in Table 2.2. The average number of alleles per maker was 8.50 in Japanese germplasms and 9.37 in overseas germplasms. Among Japanese geographical groups, the average number of alleles per marker ranged from 2.90 (Chugoku-Shikoku area) to 5.43 (Chubu area), whereas overseas geographical groups had a wider variation, from 3.13 (Oceania) to 8.07 (Europe). The values of all other genetic diversity indices (Ho, He, I, PIC) were lower in Japanese than in overseas geographical groups. Hierarchical analysis of molecular variance revealed significant differences at all hierarchical levels (Table 2.3); the percentage of variation was highest among individuals within geographical groups (67.18%), followed by that between Japanese and overseas accessions (17.71%).

There was significant genetic differentiation between geographic groups in Japan, except between Kanto and Tohoku and between Kanto and Chubu. Pairwise F_{ST} ranged

from 0.017 (Kando and Chubu) to 0.192 (Hokkaido and Chugoku-Shikoku) (Table 2.4). The FST values between Hokkaido and western Japanese geographic groups (Kinki, Chugoku-Shikoku, and Kyushu) showed relatively high differentiation. Nei's genetic distance among Japanese geographic groups ranged from 0.044 (Kinki and Kyushu) to 0.173 (Hokkaido and Chugoku-Shikoku). Similar to pairwise F_{ST}, the genetic distances tended to be higher between the Hokkaido group and western Japanese geographic groups.

2.3.2 Population structure of the NARO rapeseed collection

The STRUCTURE analysis suggested K = 2 as the real value of K even though a secondary peak of ΔK exists at K = 4 (Fig. 2.1), indicating there are two genetic clusters in the NARO rapeseed collection (Fig. 2.2). With the membership probabilities (Q) threshold of 0.80, 227 accessions, mostly originating from overseas, were assigned to subgroup 1, and 276 accessions, mostly originating from Japan were assigned to subgroup 2 (Table 2.5). The remaining 79 accessions are assigned to admixed group, i.e., Q < 0.8. The result of PCoA was similar to that of STRUCTURE analysis (Fig. 2.3). The first and second factors of the PCoA explained approximately 19.1% and 7.3% of the variation in the genetic distance matrix, respectively. A bi-dimensional PCoA scatter plot indicated differentiation between Japanese and overseas accessions (Fig. 2.3). The neighbor-joining (NJ) tree further confirmed the differentiation between Japanese and overseas accessions (Fig. 2.4).

2.3.3 Construction of core collection

Four different core collections were developed by different methods. Sample size and genetic diversity indices of each core collection are shown in Table 2.6. The random sampling strategy and CoreHunter selected the largest number of accessions (116; 19.93% of the whole collection), whereas the CoreFinder method selected the smallest number (96; 16.49%). The core collections constructed by the two M strategy methods (PowerCore and CoreFinder) had the highest allele retention ratio (100%), indicating that they had all alleles observed in the whole collection, whereas approximately 30%

of alleles were lost in the two other core collections. Other indices (except Ho) were higher in the core collections constructed by the two M strategy methods than by the other two methods, although there were no significant differences among the core collections or between each core collection and the whole collection. Comparison of genetic diversity indices indicated that the core collections developed by PowerCore and CoreFinder were highly representative of the whole collection. Since the core collection by CoreFinder contains fewer accessions (96) than that by PowerCore (103), the former should be used as the core collection of the NARO rapeseed collection (Table 6). This core collection includes 39 Japanese accessions from seven geographic areas (Hokkaido, 2; Tohoku, 7; Kanto, 7; Chubu, 7; Kinki, 8; Chugoku-Shikoku, 1; Kyushu, 5; unknown, 2) and 57 overseas accessions (America, 6; Asia, 6; Europe, 41; Oceania, 2; unknown, 2) (Appendix 1). A bi-dimensional PCoA scatter plot also demonstrated that this core collection maintains genetic diversity, although it included fewer than 20% of accessions present in the whole collection (Fig. 2.5).

2.3.4 Verification of the core collection

Fig. 2.6-2.20 shows the representation of the core collection for the NARO rapeseed collection. Among them, for the data recorded in the form of grading (classification), I calculated the different levels (categories) as a percentage of the total number of samples, drawn into a histogram. For linear change data, it is performed by boxplot showing. From the figures, we can see that in the traits recorded in grading (classification), the core collection basically contains each level (category) (except for the extreme strong of lodging resistance. But at this level of this trait, NARO rapeseed genebank has only one accession) (Fig. 2.6, 2.9-2.13, 2.19). For linear change data, the core collection may cover most of the range of NARO rapeseed genebank (Fig. 2.7, 2.8, 2.14-2.18, 2.20).

Table 2.1. Summary statistics of the 30 SSR markers used for genotyping of 582 rapeseed accessions.

Marker name	Linkage group	Number of alleles	Number of rare alleles	Major allele frequency	Ho	He	PIC
BrGMS4028[a]	A1	9	5	0.46	0.34	0.71	0.67
BrGMS4031[a]	A1	5	3	0.86	0.04	0.24	0.22
BRAS084[b]	A1	22	17	0.30	0.10	0.83	0.81
BrGMS1411[a]	A2	7	4	0.58	0.05	0.59	0.55
BrGMS0667[a]	A2	15	8	0.51	0.06	0.71	0.69
BrGMS2498[a]	A3	4	2	0.52	0.04	0.52	0.40
sN2025[c]	A4	10	5	0.44	0.04	0.70	0.65
BrGMS2252[a]	A5	4	2	0.82	0.03	0.32	0.29
BrGMS0070[a]	A5	39	36	0.28	0.06	0.87	0.86
BnEMS0753[d]	A6	7	4	0.63	0.02	0.52	0.46
BrGMS3750[a]	A6	6	2	0.60	0.02	0.57	0.53
BrGMS3837[a]	A7	8	6	0.60	0.01	0.51	0.41
BnEMS0620[d]	A7	17	14	0.54	0.08	0.64	0.60
BrGMS0742[a]	A8	11	8	0.61	0.03	0.55	0.50
BnGMS0281[e]	A9	8	4	0.43	0.06	0.71	0.66
BrGMS3857[a]	A10	5	2	0.61	0.06	0.55	0.49
BrGMS3688[a]	A10	6	2	0.41	0.06	0.71	0.66
BrGMS0086[a]	A10	12	7	0.41	0.06	0.76	0.73
BnGMS271[e]	C1	9	6	0.48	0.02	0.59	0.50
BoGMS2016[f]	C2	15	9	0.30	0.03	0.84	0.82
BoEMS0016[g]	C2	9	6	0.50	0.05	0.65	0.60
BoGMS0660[f]	C2	3	1	0.61	0.01	0.48	0.37
BnGMS0289[e]	C3	21	18	0.44	0.03	0.62	0.55
BnGMS347[e]	C4	10	8	0.44	0.04	0.62	0.55
BoGMS0037[f]	C5	9	7	0.85	0.01	0.26	0.23
BoGMS1909[f]	C6	13	10	0.78	0.03	0.37	0.36
BnGMS0353[e]	C6	8	5	0.54	0.04	0.62	0.57
BoEMS0049[g]	C7	3	1	0.53	0.01	0.50	0.38
BnGMS0336[e]	C8	4	1	0.81	0.17	0.33	0.31
BoGMS0525[f]	C9	12	11	0.89	0.07	0.21	0.21
Average		10.37	7.13	0.56	0.05	0.57	0.52

Ho: observed heterozygosity, He: expected heterozygosity, PIC: polymorphism information content. [a]Detailed maker information is available in Xu *et al.* (2010), [b]Piquemal *et al.* (2005), [c]AAFC Consortium (2016), [d]Wang *et al.* (2012), [e]Cheng *et al.* (2009), [f]Li *et al.* (2011), [g]Li *et al.* (2013).

Table 2.2. Genetic diversity indexes for the NARO rapeseed collection and variations among geographic groups.

Geographical group	Number of accessions	Average number of alleles	Major allele frequency	Ho	He	I	PIC
Whole Collection	582	10.37	0.56	0.05	0.57	1.18	0.52
Japan	305	8.50	0.67	0.04	0.44	0.90	0.41
Hokkaido	9	3.30	0.58	0.03	0.52	0.91	0.47
Tohoku	69	5.27	0.65	0.06	0.46	0.88	0.44
Kanto	33	4.77	0.68	0.06	0.42	0.82	0.41
Chubu	40	5.43	0.65	0.04	0.46	0.90	0.44
Kinki	72	5.17	0.73	0.03	0.37	0.73	0.37
Chugoku-Shikoku	11	2.90	0.70	0.07	0.37	0.65	0.36
Kyushu	65	4.63	0.72	0.03	0.37	0.71	0.36
Unknown	6	–	–	–	–	–	–
Overseas	277	9.37	0.50	0.06	0.59	1.22	0.58
Europe	202	8.07	0.53	0.06	0.56	1.14	0.54
Asia	30	5.37	0.60	0.07	0.49	0.97	0.49
Oceania	8	3.13	0.61	0.05	0.48	0.84	0.45
America	27	4.40	0.63	0.05	0.47	0.88	0.45
Unknown	10	–	–	–	–	–	–

Ho: observed heterozygosity, He: expected heterozygosity, I: Shannon's information index, PIC: polymorphism information content.

Table 2.3. Result of the hierarchical analysis of molecular variance (AMOVA) for geographic groups in the NARO rapeseed collection.

Source of variation	d.f.	Parcentage of variation (%)	
Between Japanese and overseas accessions	1	17.71	***
Among geographical groups	9	7.01	***
Among individuals	555	67.18	***
Within individuals	566	8.10	**

***, **: significant at the 0.1% and 1% levels for 1,000 permutations, respectively.

Table 2.4. Estimates of pairwise F_{ST} (below diagonal) and Nei's genetic distance (above diagonal) among geographic groups in Japan.

	Hokkaido	Tohoku	Kanto	Chubu	Kinki	Chugoku -Shikoku	Kyushu
Hokkaido		0.105	0.125	0.110	0.148	0.173	0.143
Tohoku	0.059*		0.055	0.056	0.063	0.091	0.068
Kanto	0.102**	0.019		0.053	0.048	0.099	0.058
Chubu	0.122**	0.031**	0.017		0.056	0.091	0.049
Kinki	0.101**	0.039**	0.047**	0.058**		0.114	0.044
Chugoku-Shikoku	0.192**	0.084**	0.067*	0.076*	0.088**		0.075
Kyushu	0.138**	0.034**	0.063**	0.092**	0.055**	0.100**	

**: significant at the 1% level, *: significant at the 5% level.

Table 2.5. Geographic origin of rapeseed accessions assigned by STRUCTURE to two subgroups

Geographical group	Number of accessions	Subgroup 1[a]	Subgroup 2[a]	Admixed
Whole Collection	582	227	276	79
Japan	305	19	240	46
Hokkaido	9	4	3	2
Tohoku	69	5	46	18
Kanto	33	2	27	4
Chubu	40	4	27	9
Kinki	72	0	66	6
Chugoku-Shikoku	11	1	9	1
Kyushu	65	1	60	4
Unknow	6	2	2	2
Overseas	277	208	36	33
Europe	202	171	9	22
Asia	30	3	22	5
Oceania	8	5	0	3
America	27	25	1	1
Unknow	10	4	4	2

[a]Accessions are considered belonging to either of two subgroup when membership probabilities of ≥ 0.8.

Table 2.6. Comparison of genetic diversity indexes for four core collections constructed by different methods.

	Number of accessions	Number of alleles	Allele retantion ratio	Number of effective alleles	Ho	He	I	PIC
PowerCore[a]	103	10.37	100.0	3.77	0.05	0.64	1.41	0.59
CoreFinder[a]	96	10.37	100.0	3.57	0.06	0.62	1.39	0.58
CoreHunter	116	7.50	72.3	3.17	0.06	0.60	1.23	0.55
Random Strategy	116	7.20*	69.5	2.89	0.07	0.57	1.17	0.52

*: significant at the 5% level between core collection and whole collection.

Ho, observed heterozygosity; He, expected heterozygosity; I, Shannon's information index; PIC, polymorphism information content.

[a] Maximization strategy methods.

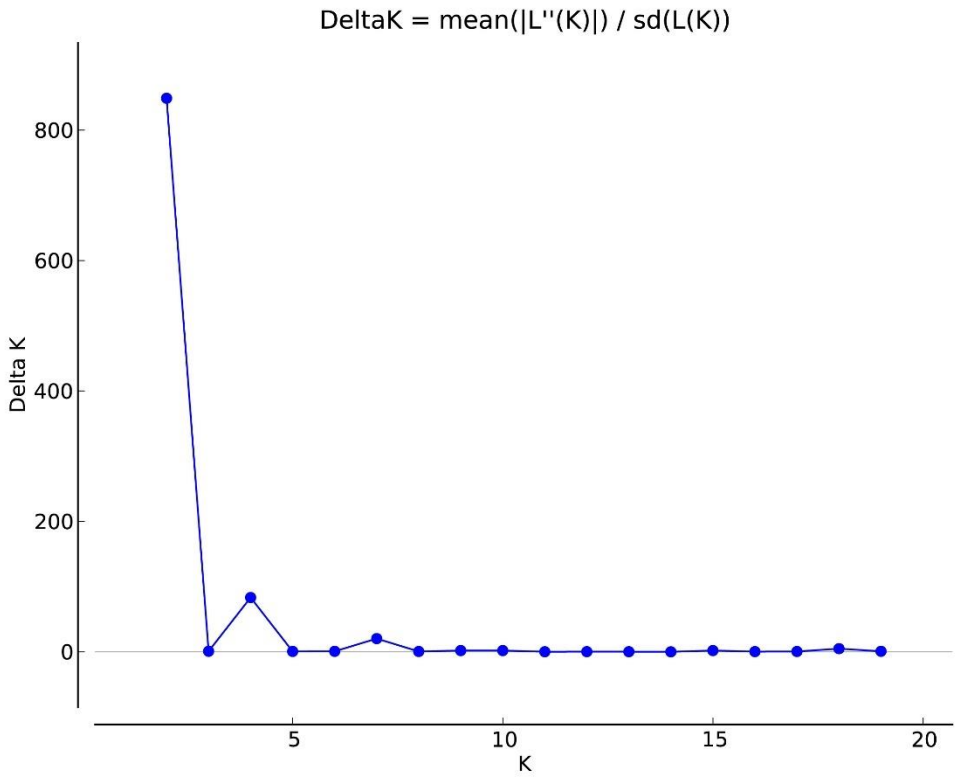

Fig. 2.1. ΔK, from the structure analysis of the 582 rapeseed accessions.

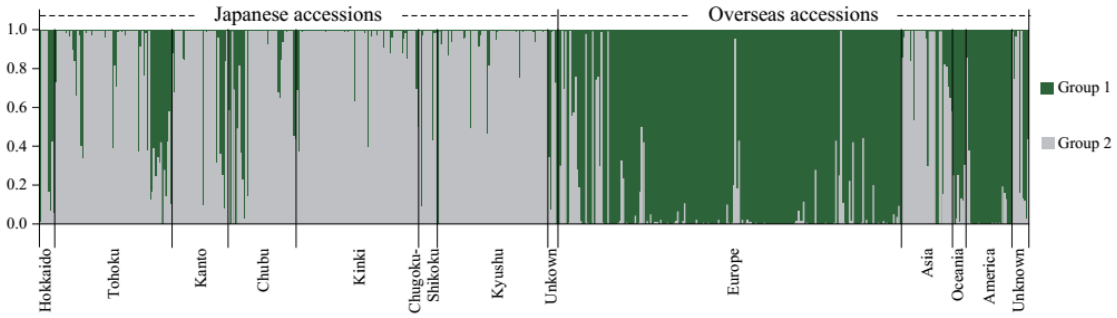

Fig. 2.2. Population structure of 582 rapeseed accessions based on 30 SSR markers. When K=2, the 582 germplasms were classified into two groups.

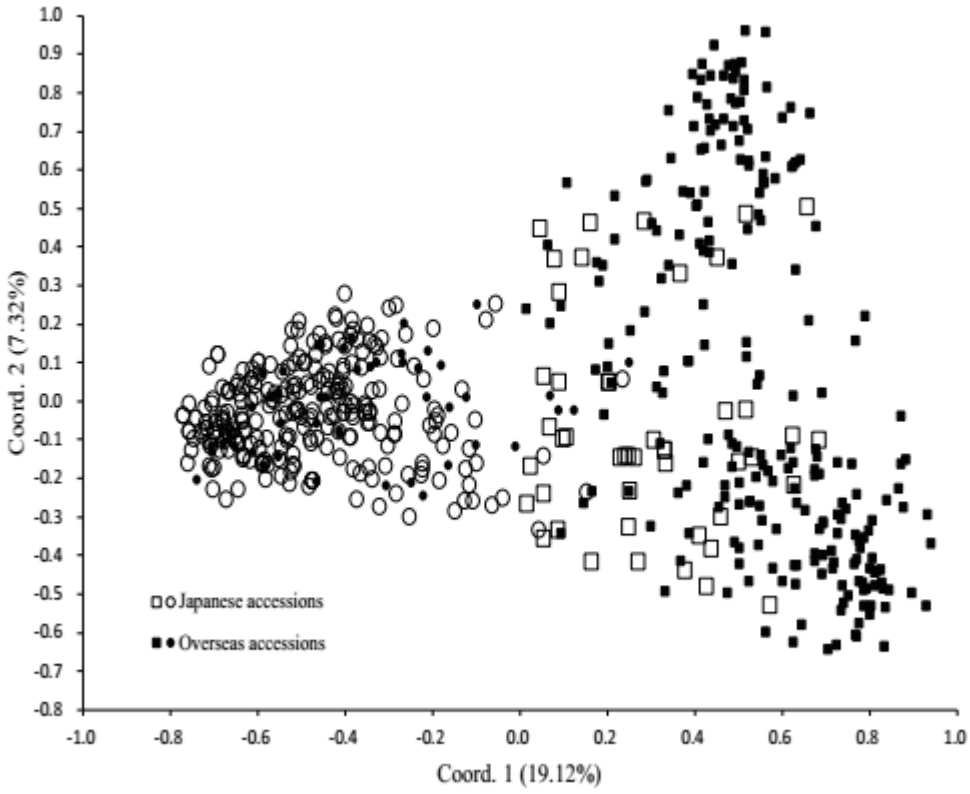

Fig. 2.3. Principal coordinates analysis (PCoA) of the 596 rapeseed accessions. Open symbols indicate Japanese accessions, and solid Symbols indicates overseas accessions. Square symbol indicates the rapeseed accessions assigned to group 1, and circle symbol indicates the accessions assigned to group 2 by the STRUCTURE analysis.

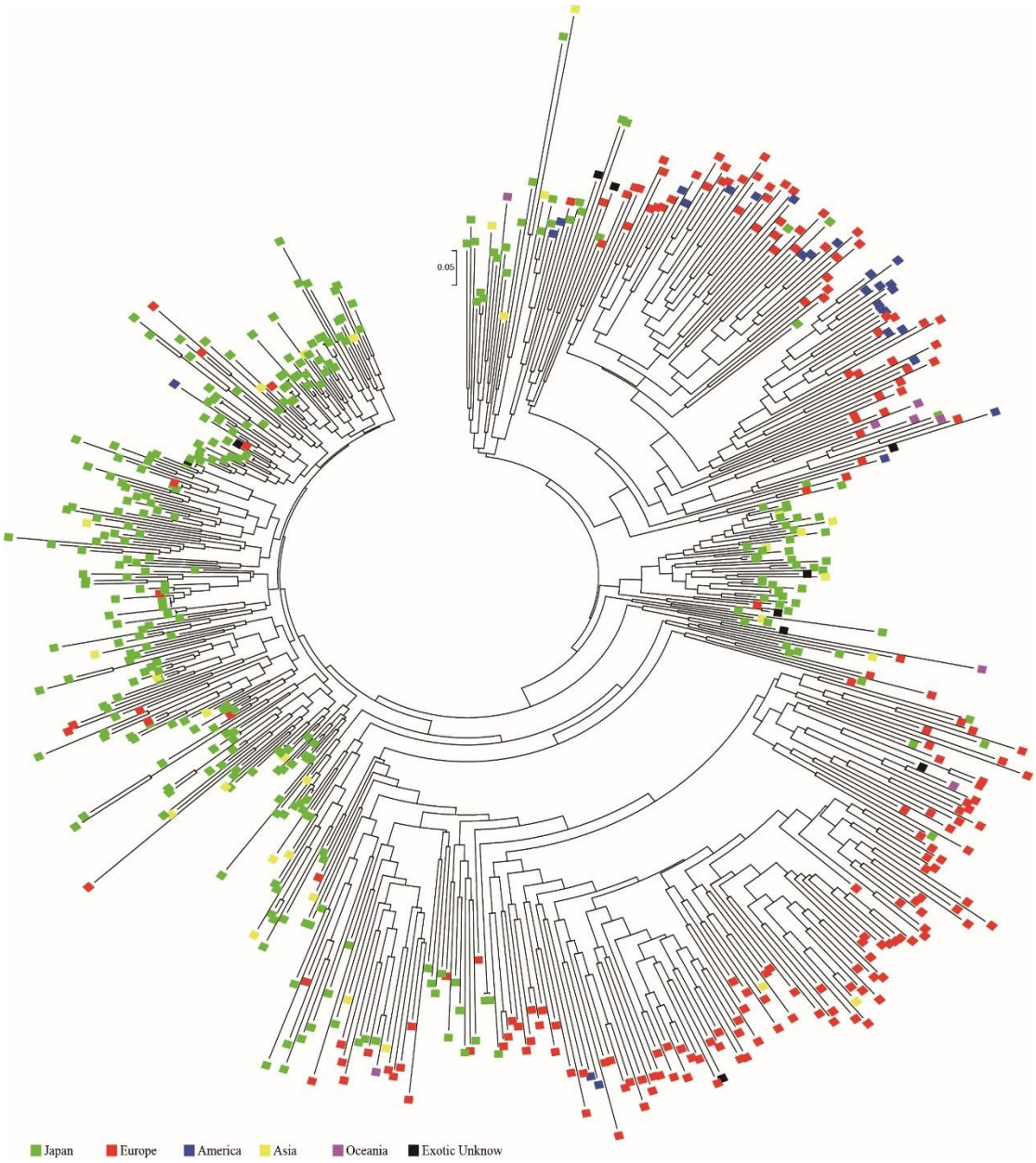

Fig. 2.4. The neighbor-joining (NJ) tree of the 582 rapeseed accessions constructed based on SSR analysis data.

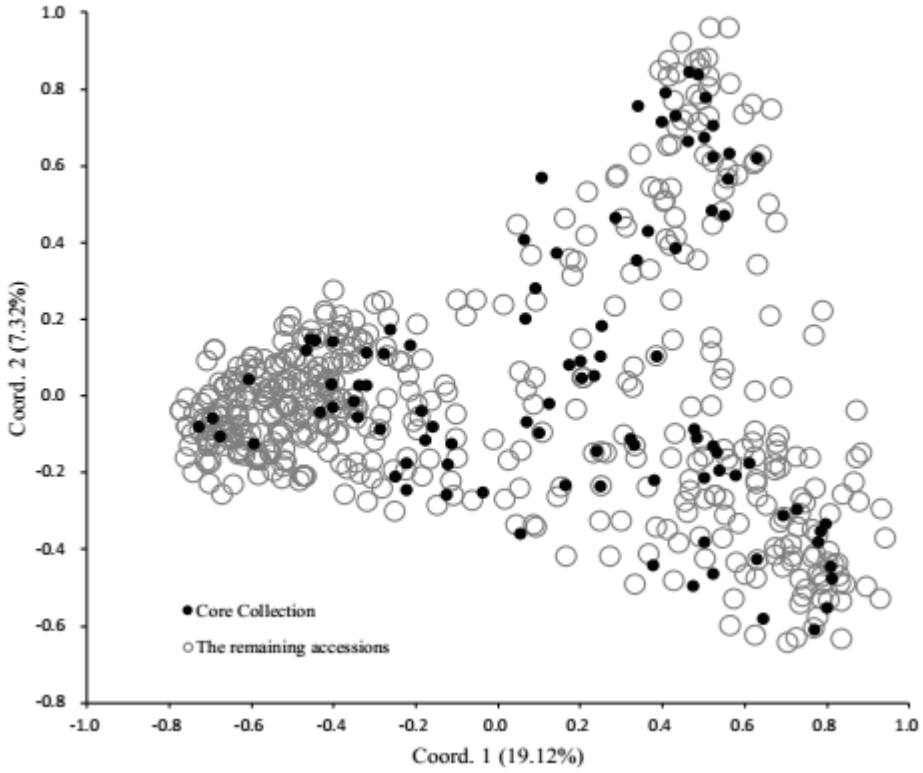

Fig. 2.5. Principal coordinate plot of the core collection constructed using the PowerCore and the whole collection. Solid and open symbols indicate the accessions included and not included in the core collection, respectively.

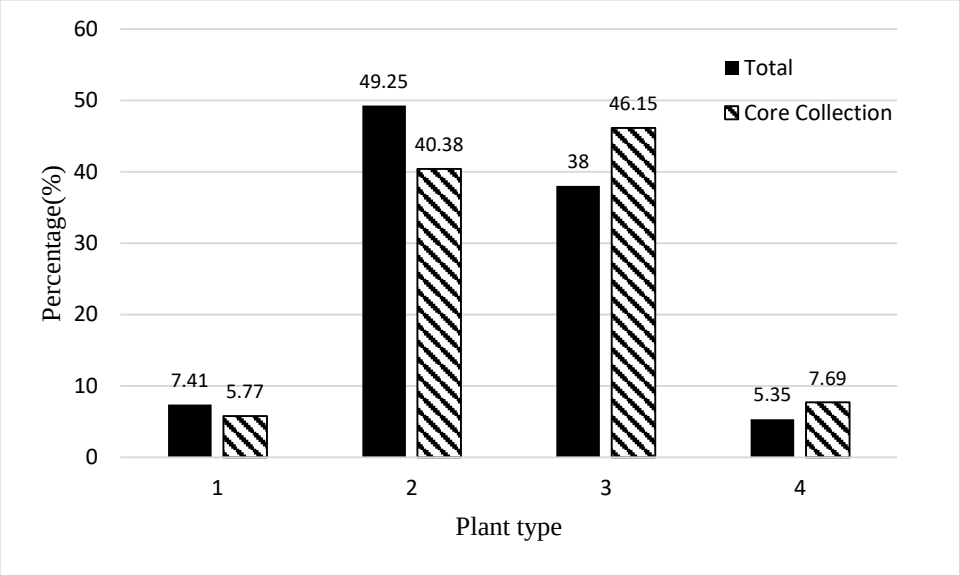

Fig. 2.6. The proportion of different plant types in NARO rapeseed genebank and core collection accessions.

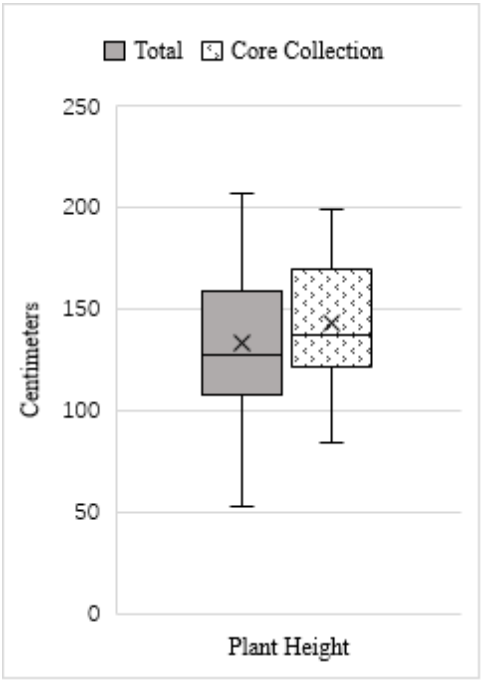

Fig. 2.7. Range of plant height in NARO rapeseed genebank and core collection accessions.

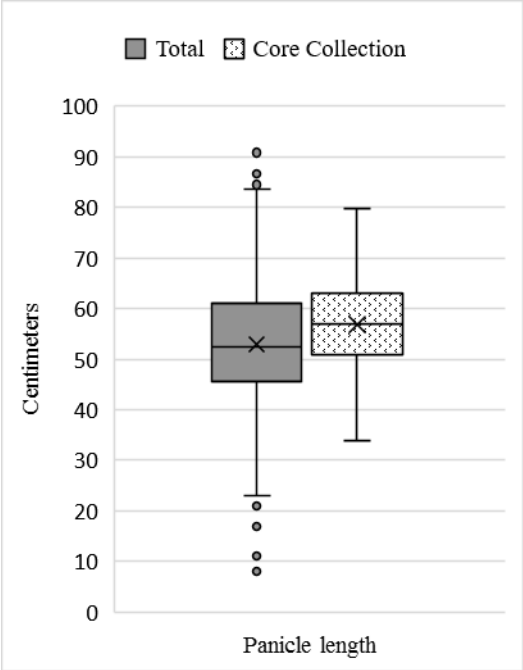

Fig. 2.8. Range of panicle length in NARO rapeseed genebank and core collection accessions.

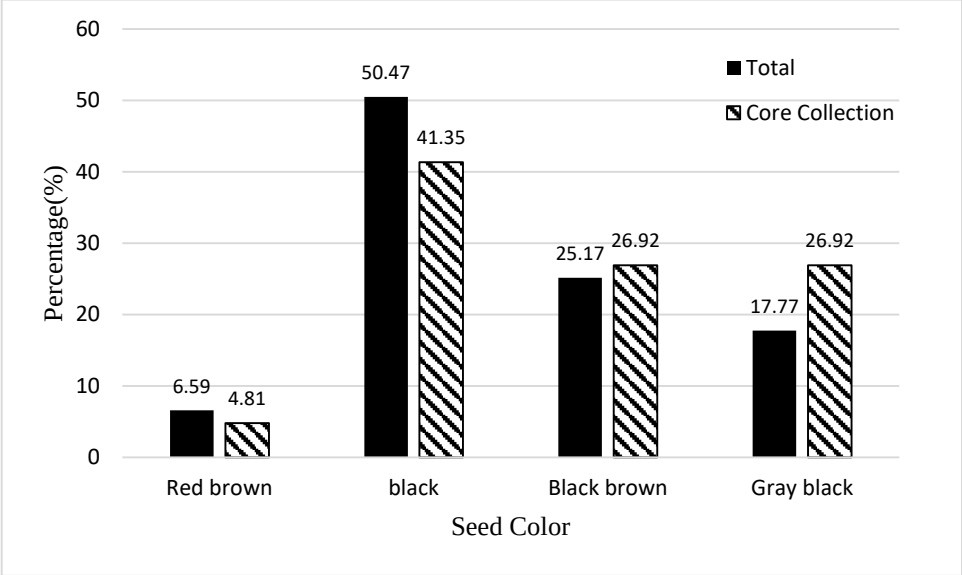

Fig. 2.9. The proportion of different seed colors in NARO rapeseed genebank and core collection accessions.

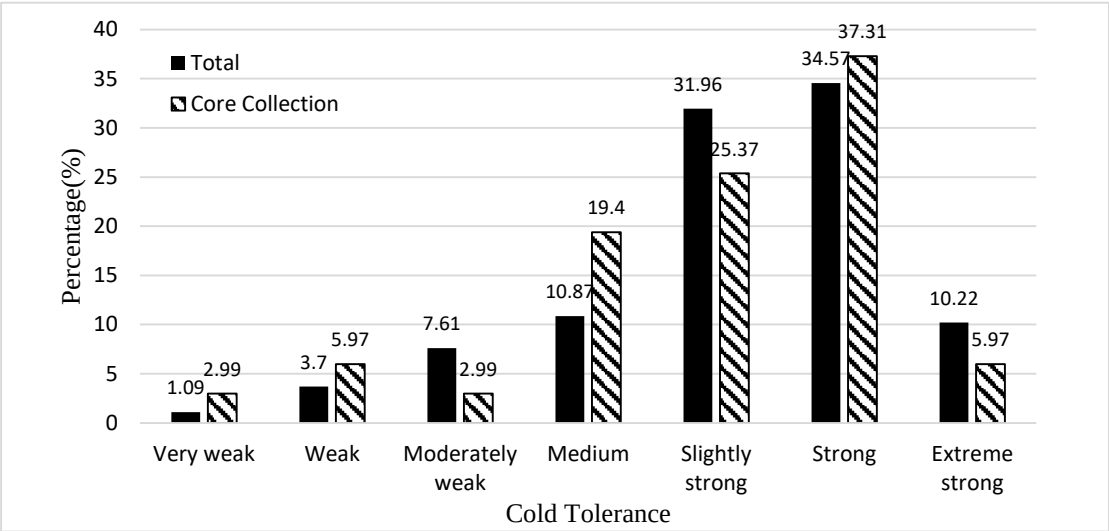

Fig. 2.10. The proportion of different cold tolerance abilities in NARO rapeseed genebank and core collection accessions.

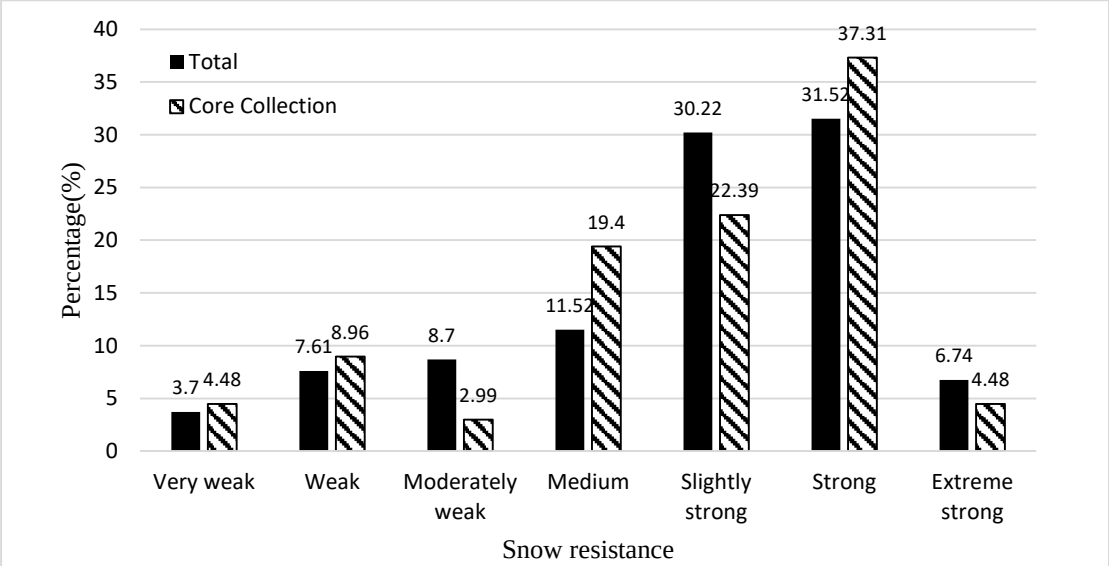

Fig. 2.11. The proportion of different snow resistance abilities in NARO rapeseed genebank and core collection accessions.

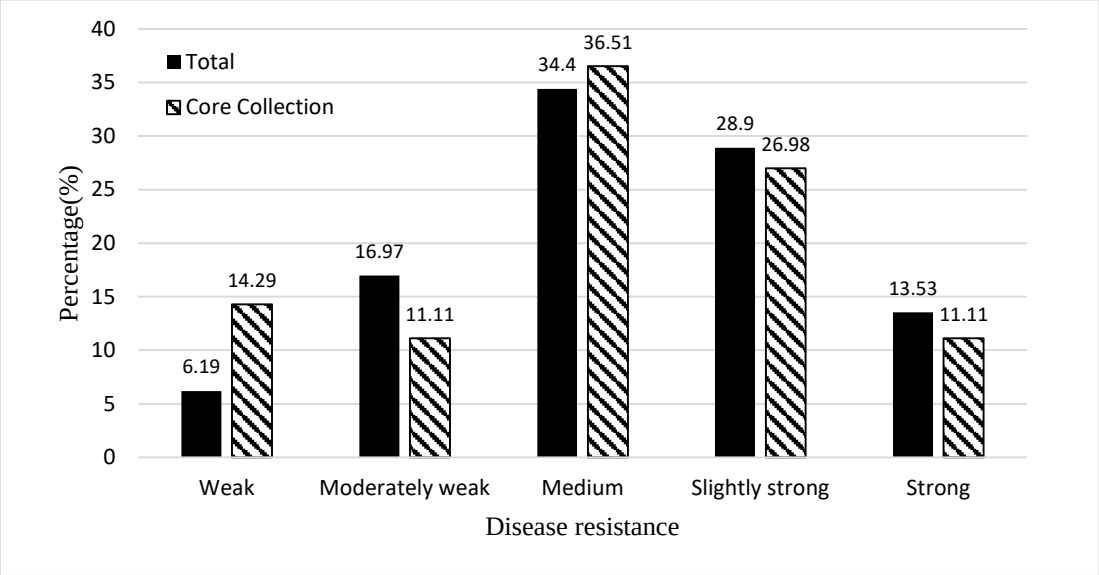

Fig. 2.12. The proportion of different disease resistance abilities in NARO rapeseed genebank and core collection accessions.

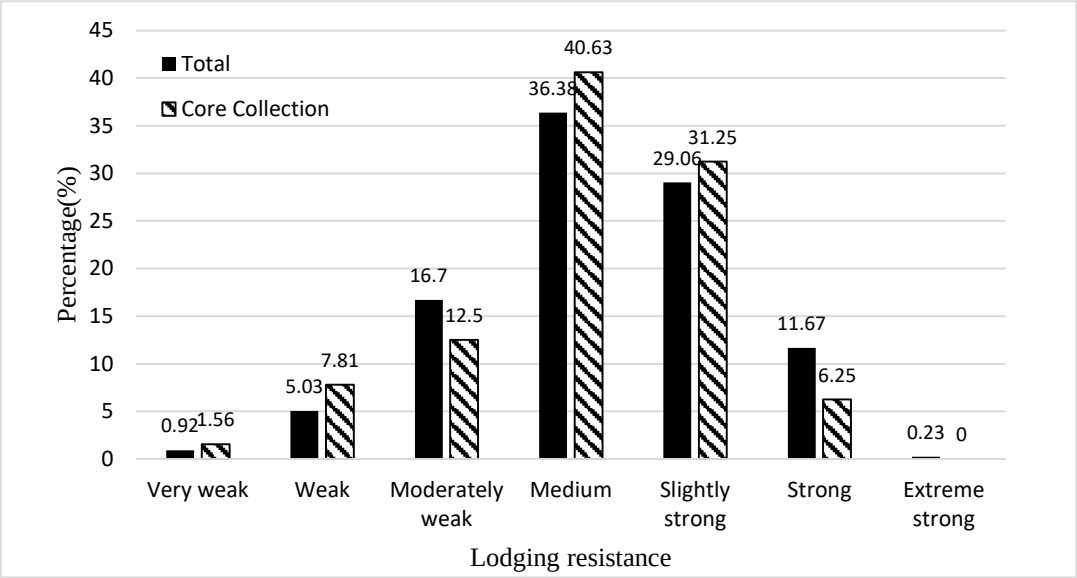

Fig. 2.13. The proportion of different lodging resistance abilities in NARO rapeseed genebank and core collection accessions.

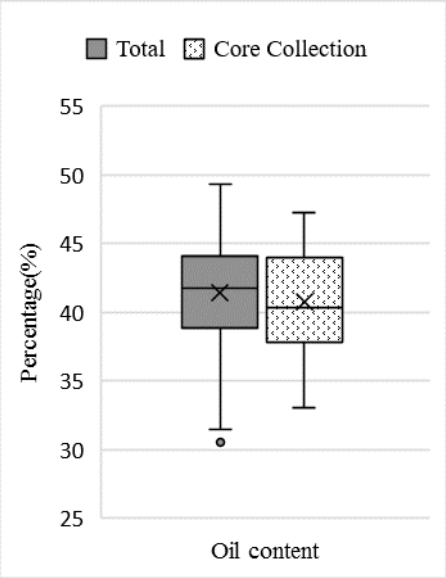

Fig. 2.14. Range of oil content in NARO rapeseed genebank and core collection accessions.

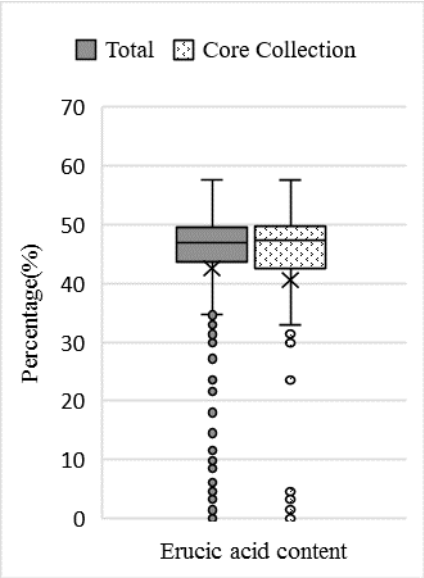

Fig. 2.15. Range of erucic acid content in NARO rapeseed genebank and core collection accessions.

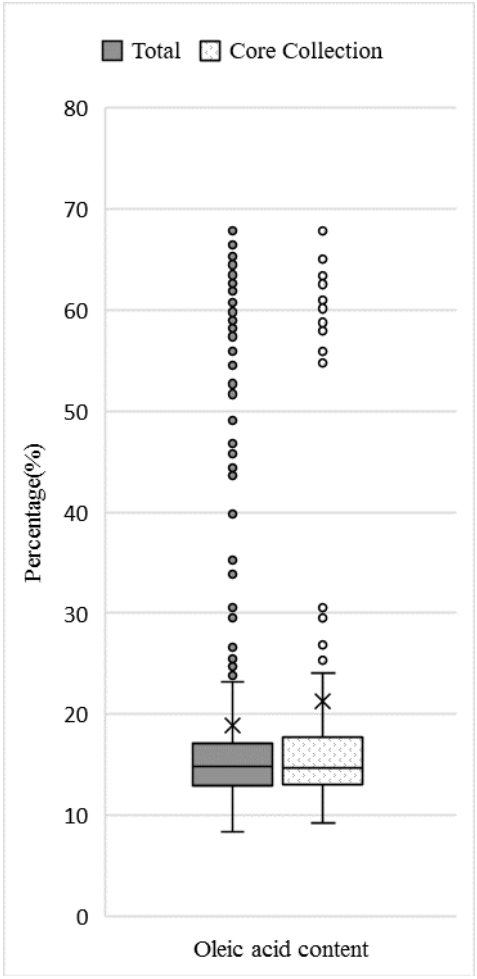

Fig. 2.16. Range of oleic acid content in NARO rapeseed genebank and core collection accessions.

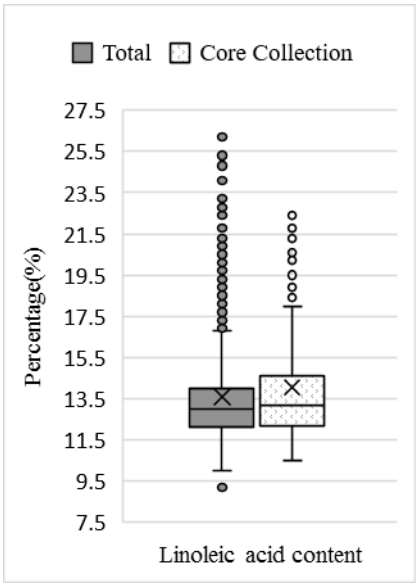

Fig. 2.17. Range of linoleic acid content in NARO rapeseed genebank and core collection accessions.

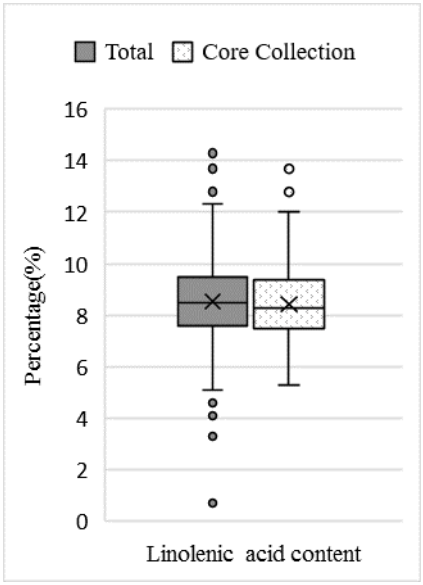

Fig. 2.18. Range of linolenic acid content in NARO rapeseed genebank and core collection accessions.

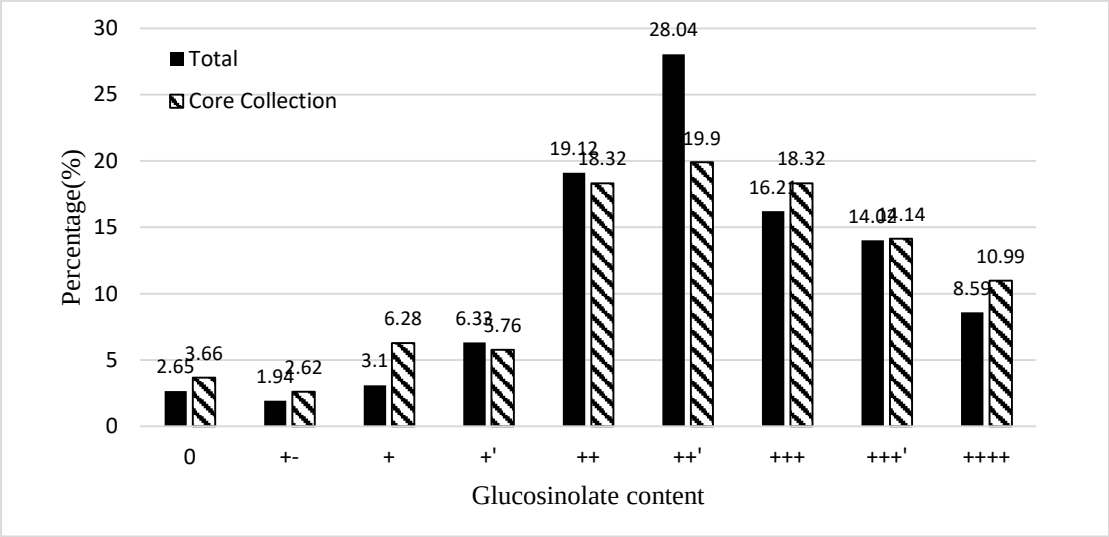

Fig. 2.19. The proportion of glucosinolate contents in NARO rapeseed genebank and core collection accessions.

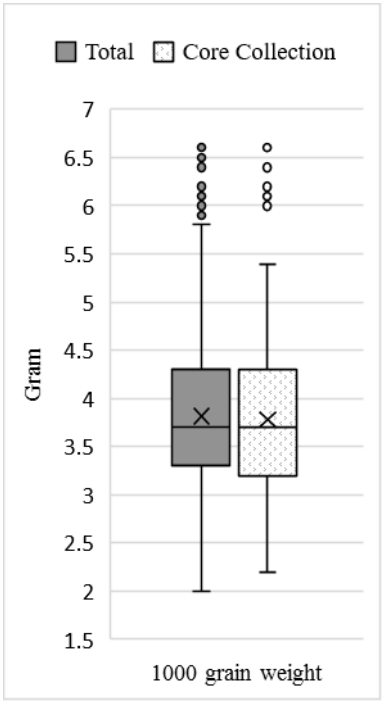

Fig. 2.20. Range of 1000 grain weight in NARO rapeseed genebank and core collection accessions.

2.4 Discussion

I evaluated the genetic diversity of the NARO rapeseed collection by using single-locus SSR markers. In allopolyploid plants, such as rapeseed, SSR primers usually amplify several alleles from multiple loci, which makes it difficult to assign these alleles to individual loci. Li *et al.* (2013) found a set of 230 single-locus rapeseed SSR markers. However, they used only 6 inbred lines and noted that not all of the markers might be true single-locus markers. In this study, I applied a total of 504 SSR markers, including the 230 markers of Li *et al.* (2013), to 582 rapeseed accessions, and found that 30 SSR markers (Table 2.1), distributed across all linkage groups, always amplified single alleles with high polymorphism. This finding indicates that these 30 SSR markers used in this study are useful for research on rapeseed genetic resources.

I identified 311 alleles in the whole collection, with an average of 10.37 alleles per locus and a PIC value of 0.52. Several studies calculated genetic diversity indices of diverse rapeseed collections on the basis of SSR markers. The average numbers of alleles per locus were 4.31 in 96 European rapeseed genotypes (Hasan *et al.* 2006), 3.4 in 192 inbred lines of various origins (Xiao et al 2012), and 7.3 in 169 worldwide rapeseed lines (Gyawali *et al.* 2013). The PIC value in the 192 lines analyzed by Xiao *et al.* (2012) was 0.37. The differences in allelic richness between the NARO collection and other rapeseed collections are due partly to the differences in the SSR markers and the number of accessions used in this study. Nevertheless, the higher values of the genetic diversity indices in this study indicate that the NARO rapeseed collection has moderately high genetic diversity. In contrast, the PIC value of the NARO rapeseed collection was lower than those reported in other *Brassica* species: 0.57 or 0.64 in *B. oleracea* (El-Esawi *et al.* 2016; Louarn *et al.* 2007) and 0.90 in *B. juncea* (Yao *et al.* 2012); this would reflect a narrower genetic base in rapeseed than in other *Brassica* species.

Rapeseed originated and was first domesticated in Europe, and then spread throughout the world. In Japan, rapeseed cultivation is considered to have spread after

the Meiji period (from 1868 to 1912) (Matsuo 1954); since then, many landraces have been developed throughout the country. Modern rapeseed breeding in Japan began in the 1930s; government-led large-scale breeding projects initially used a limited number of accessions (Matsuo 1954), which may have restricted the genetic diversity of Japanese accessions. As expected, I found a significant genetic differentiation between Japanese and overseas accessions (Fig. 2.3; Tables 2.3, 2.4), and comparison of the genetic indices indicated that genetic diversity was lower in Japanese accessions than in overseas accessions (Table 2.2). However, the values of genetic diversity indices were not very different between Japanese and overseas accessions. Over the last five decades, repeated introduction of overseas germplasms and subsequent breeding may have increased genetic diversity.

In general, differences in breeding materials and targets among different breeders and regions may lead to genetic differentiation. In Japan, I found small but significant differences among geographical groups (Table 2.4). Genetic differentiation tended to increase with increasing geographic distance. For example, I found the highest F_{ST} values and Nei's genetic distances between the Hokkaido group (northern Japan) and three groups in western Japan. The genetic differentiation might be caused not only by the diversity of germplasms used as breeding materials, but also by their adaptation to local environments. The genetic diversity indices PIC and I were higher in eastern than in western Japan. The genetic diversity of eastern Japanese accessions may have been further increased by recent active rapeseed breeding there.

The STRUCTURE analysis indicated the presence of two main genetic clusters in the NARO rapeseed collection and demonstrated differentiation between Japanese and overseas accessions: with a membership probability threshold of 0.80, most of the overseas accessions were assigned to the subgroup 1, and the Japanese accessions were assigned to the subgroup 2 (Table 2.5). Yet the presence of several accessions in each subgroup is not consistent with their geographical origins (Table 2.5), as similarly observed in previous studies (Chen *et al.* 2007, Hu *et al.* 2007, Li *et al.* 2012, Xiao *et al.* 2012). All of the Canadian cultivars, known by the trade name 'Canola' and bred in

the 1970s or later, were classified into the subgroup 1. Many accessions from Asian countries other than Japan belonged to the subgroup 2. The neighbor-joining (NJ) tree and principal coordinate analysis (PCoA) further confirmed the STRUCTURE results (Fig. 2.3, 2.4). These results indicate that Asian rapeseed may have genetically differentiated from that in other regions of the world, and that Japanese, Chinese, and Korean landraces and cultivars may be closely genetically related (Fig. 2.2). Some studies have revealed a wide diversity of Chinese and Australian rapeseed accessions (Chen *et al.* 2007, Guo *et al.* 2016, Wang *et al.* 2009). The NARO collection holds only 7 accessions from China and none from Australia. Obtaining accessions from these countries would further expand the diversity of the collection.

The most important factor in core collection construction is sample selection strategy, and many strategies based on stratified sampling and clustering methods are available (Zhang *et al.* 2011). Because different strategies result in different core collections (Thachuk *et al.* 2009, Wang et al 2007), I used four different methods. As the M strategy is based on maximizing the number of alleles, it can automatically generate a sampling ratio on the basis of the genetic diversity of the species; this strategy has been widely used in recent years (Belaj *et al.* 2012, Liu *et al.* 2015, Zhang *et al.* 2011). In this study, both PowerCore and CoreFinder, which are based on the M strategy, constructed core collections that retained 100% of alleles, and in this respect were superior to the other two methods used. The genetic diversity indices tended to be nearly same in both the PowerCore collection and the CoreFinder collection (Table 2.6). The number of accessions are fewer in the CoreFinder collection (Table 2.6), so I finally recommend it as the core collection of the NARO rapeseed collection. The core collection comprised 57 overseas accessions (20.6% of total) and 39 Japanese accessions (12.8%). The higher percentage of overseas accessions reflects their high allelic richness. However, the fact that approximately 40% of accessions in the core collection originated in Japan indicates their relatively high genetic diversity.

In conclusion, my study revealed the genetic structure of the NARO rapeseed collection and genetic relationships among accessions on the basis of single-locus SSR

markers. Some accessions were genetically identical or closely related to each other. This information is important for decreasing redundancy in the collection, thereby reducing the management cost and avoiding unnecessary distribution of such accessions to breeders. Further integration of the data from other collections maintained in different countries will make it possible to exploit and preserve the whole rapeseed gene pool and to retain the largest number of allelic variants for genes controlling the most important agronomic traits in the NARO collection. My candidate core collection can be used in further research such as genome-wide association studies to identify genomic regions controlling important agronomic traits.

I did not analyze the phenotypic data recorded in the NARO rapeseed collection because they have been obtained by different investigators and methods under various environmental conditions. However, based on these data, it can still be a qualitative analysis. So, I compared sixteen important traits in rapeseed production between NARO rapeseed collection and the core collection constructed by this study. Through the results we can see that the core collection based on genotypic data also has a considerable degree of representation of phenotype. This suggests that it is possible to establish a core collection by genotypic data. With the increase of the number of accession, the difficulty and cost of comprehensively assessing the polymorphism of accessions by cultivating test method is increasing. The results of this study tell us that the establishment of core collection only by genotypic data is a cost-effective and highly accurate method. However, just as mentioned before, phenotypic data used in this study was obtained at different times in different locations, therefore, it will be necessary to evaluate the phenotypes of all the accessions under the same environmental conditions, and integrate the data on genotypic and phenotypic diversity. This will make the core collection, which comprises accessions that are genetically diverse at both genotypic and phenotypic levels, available to breeders for enhancing the genetic potential of this crop.

Chapter 3: Genetic diversity analysis and origin presumption of feral rapeseed genetic resources in Japan

3.1 Introduction

At present, with climate change and the increasing demand for food all over the world, recovering lost genetic variability and making it available for plant breeding is becoming the consensus of many people. As described in Chapter 1, the feral resources have potential and practical significance for the improvement of crops due to the adaptability of different environment and the represent valuable storage of genetic diversity that has not been fully exploited (Feuillet *et al.* 2008, Pacheco-Olvera *et al.* 2012). In past decades, some new cultivars of the worldwide important crops such as cassava, wheat, millet, rice, maize, sunflower, lettuce, banana, potato, groundnut, tomato and chickpea were bred with many new traits such as pest/disease resistance, abiotic stress resistance, high yield/quality and male sterility or fertility restoration which were derived from their feral relatives (Reem and Toby 2007, Warschefsky *et al.* 2014).

To obtain outstanding cultivars, it is often need to be selected from a larger range of germplasm, on the other hand, it is difficult to discovery new genes and utilize them from a low-diversity germplasm pool (Tanksley and McCouch 1997, Reem and Toby 2007, Xiong *et al.* 2016). An accurate selection of materials based on the diversity information may help to achieve the breeding objectives and shorten the breeding period (Tanksley and McCouch 1997, McCouch 2004, Reem and Toby 2007, Xiong *et al.* 2016). Thus, understanding of the genetic diversity would be a crucial premise for breeding which using the feral resources.

Many global or regional genetic diversity studies of feral relatives of important crops have been performed. Among them, the researches of rice is a typical example. Feral rice species were widely used in modern rice breeding, so the researchers paid

early attention to it. No later than 1980s, studies based on morphological traits of feral rice have been performed exhaustively (Duncan 1994). With the rise of molecular marker technology in the 1990s, various markers such as RAPD, RFLP, ISSR, SSR were sonly been used to reveal the genetic diversity and population structure of feral rice resources all over the world (Buso *et al.* 1998, Qian *et al.* 2001, Sun *et al.* 2001, Lu *et al.* 2002, Zhou *et al.* 2003, Xie *et al.* 2010). Similar researches have also been down in other food crops such as wheat (Fahima *et al.* 1999, Ren *et al.* 2013), and oil crops such as soybean (Dong *et al.* 2001, Kuroda *et al.* 2006), peanuts (Huang *et al.* 2016) and olive (Belaj *et al.* 2010).

Feral rapeseed populations have been found in many countries. Researchers in different countries used different methods to reveale the genetic diversity status in different geographical areas, including Austria (Pascher *et al.* 2010), Germany (Elling *et al.* 2009), England (Bond *et al.* 2004), France (Bailleul *et al.* 2016). These studies confirmed the long-standing presence of feral populations, found that the feral rapeseed populations maintain an unignorable genetic diversity, and several of them even higher than the cultivars which were presumed to be the source of the feral plants. By comparing the field and feral rapeseed with molecular marker assessment, the source of the latter can also be speculated.

In Japan, the existence of feral rapeseed populations has been report many times in different geographic regions (Saji *et al.* 2005, Katsuta *et al.* 2015). Since rapeseed is a complete alien species, feral rapeseed should ideally be formed by escape of cultivars. However, there has no definite conclusion until now on which cultivars, and at what time they escaped. Moreover, due to the long-term survival of feral populations under natural conditions different from the original home, some new traits that enhance their ability to adapt to the environment may arise. Some traits such as secondary dormancy, adaptation of seed germination to annual cycles, and easier fruit dehiscence have been observed in other county (Pascher *et al.* 2010). By cultivation experiment, some important phenotypes of feral rapeseed from different geographic populations could be tested, and it would be helpful to know the degree of variation and adaptability to

different environments. Studies on the genetic diversity of genotypes and phenotypes of Japanese feral rapeseed can help to explore their origins and determine their value as breeding material.

Therefore, the purposes of this chapter are 1. Perform genetic diversity and population structure study of the typical feral rapeseed populations in Japan, to understand the composition and diversity of them; 2. In order to verify the genetic diversity status of feral populations, experiments of agronomic traits were conducted on individuals of each geographic population to observe their phenotypic diversity; and 3. Compare the feral samples with the genebank resources used in Chapter 2, to reveal the possible sources of feral populations.

3.2 Genetic diversity analysis of feral rapeseed resources in Japan

3.2.1 Plant materials

407 feral accessions of Japan *B. napus* were obtained from the National Institute for Environmental Studies (NIES) of Japan (143 accessions) and the Ministry of Agriculture, Forestry and Fisheries of Japan (268 accessions). The seeds of the former materials were collected in 2005 and the leaves of the latter materials were collected in 2015. The accessions were chosen based on their sampling site, which are covering a diverse geographical range of Japan (Fig 3.1).

3.2.2 Genotyping

Seeds obtained from the sampled individuals were germinated on moistened filter paper in 9-cm Petri dishes and then transplanted into a 72-cell tray filled with Nippi-Engei-Baido-1gou soil (Nihon Hiryo Co., Ltd., Tokyo, Japan). Seedlings were grown outside until the first true leaves had fully expanded. Genomic DNA was extracted from the first true leaf of each seedling and the collected leaves by using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol with

some minor modifications.

The same 30 SSR markers of Chapter 2 were used. PCR mixtures (10 μl) contained template DNA (10 ng), 1× KAPA 2G buffer A, 200 nM dNTP, 0.5 mM MgCl2, 0.1 U KAPA 2G Fast DNA polymerase (KAPA Biosystems Inc., Woburn, MA, USA), 2 pmol reverse primer, and 0.5 pmol forward primer. The forward primers were 5′-labeled with the fluorescent dyes 6-FAM, VIC, NED, or PET (Shimizu and Yano 2011). PCR was performed in a C1000 Thermal Cycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA) as follows: initial denaturation at 94 °C for 3 min; 30 cycles of 94 °C for 20 s, 54 °C for 30 s, and 62 °C for 30 s; 3 cycles of 94 °C for 20 s, 49 °C for 10 s, and 72 °C for 5 s; and a final extension at 72 °C for 10 min. The size of the amplified fragments was estimated by using an automated DNA analyzer (model 3130xl) with a GeneScan-600LIZ size standard and GeneMapper v. 4.0 software (all Thermo Fisher Scientific, Inc., Waltham, MA, USA).

3.2.3 Data analysis

The number of alleles, major allele frequency, polymorphism information content (PIC), F statistics (F_{is}, F_{ST}) and Nei's genetic distance (Nei *et al.* 1983) were calculated for the whole feral samples and for each geographic group in PowerMarker v. 3.25 software (Liu and Muse 2005). Observed heterozygosity (Ho), expected heterozygosity (He) and Shannon's information index (I) were calculated in GenAlEx v. 6.502 software (Peakall and Smouse 2012). Nei's genetic identity (Nei 1972) was calculated in POPGENE v. 1.32 (https://sites.ualberta.ca/~fyeh/popgene_download.html).

In addition to the analysis of each geographical populations, I also conducted another analysis of the populations that were sampled both in 2005 and 2015. This analysis contains 5 locations: Kashima, Yokohama, Shimizu, Yokkaichi and Fukuoka. The diversity indices of each populations for 2005 and 2015 were analyzed separately.

Hardy-Weinberg equilibrium test, AMOVA analysis and Mantel test between genetic distance and geographic distance was calculated in Arlequin v. 3.5.2.2 (Excoffier and Lischer 2010). The geographic distance was measured using Google

Earth based on the sampling location information. According to the allele frequency of each locus, Bottleneck v. 1.2.02 (Cornuet and Luikart, 1996; Piry et al, 1999) was used to detect the bottleneck effect of geographical population. Bottleneck software uses the following principles: Within an ideal population, the alleles number of selectively neutral loci decreased more rapidly than the gene diversity (or observed heterozygosity), so, for a population that has recently experienced bottleneck effect, there may be an excess of heterozygosity (Maruyama and Fuerst 1985). For the reliability of the results, three mutation models provided by the software were used in the detection of population bottleneck: Infinite allele model (IAM), Stepwise mutation model (SMM), Two-phased mutation model (TPM). The significance of heterozygosity excess was determined using the two-tailed test of Wilcoxon sign-rank test (Cornuet and Luikart 1996).

Genotyping data for the SSR markers were analyzed by using the model-based STRUCTURE v. 2.3.4 software (Pritchard *et al.* 2000) to determine the most probable number of clusters (K value) and to assign rapeseed accessions to different clusters. The K value was determined by running an admixture and related frequency model with K = 1 to 15 (10 replications per K value); the burn-in period of each run and the Monte Carlo Markov Chain (MCMC) lengths were both set to 100,000. The website program STRUCTURE HARVESTER was used to estimate the optimal number of K value (Earl 2012). This program follows the ΔK method of Evanno *et al.* (2005). The same set of genotyping data was used to perform principal coordinates analysis (PCoA) in GenAlEx 6.502 to verify the population structure determined by STRUCTURE. The Nei's genetic distance between each individual and geographic population was used to construct neighbor joining (NJ) trees using MEGA7 software (http://www.megasoftware.net/).

3.2.4 Results

3.2.4.1 Genetic diversity of feral rapeseed resources

The 30 markers amplified a total of 208 alleles, ranging from 2 (BoEMS0049) to 36 (BrGMS0070) per marker, with a mean allelic richness of 10.37 (Table 3.1). Average genetic diversity indices were 0.50 for major allele frequency, 0.48 for He, and 0.59 for PIC. The average Ho value was 0.15 (range, 0.04- 0.28), indicating that almost all individuals of the feral populations were highly homozygous. The average inbreeding coefficient F_{is}, which is the deviation of the actual frequency of the genotype from the theoretical expected frequency in the population, is 0.70, indicating a lack of heterozygous individuals in all populations and implying that all populations may deviate from Hardy-Weinberg equilibrium. The average differentiation coefficient F_{ST}, which indicating the degree of genetic differentiation between populations, is 0.25, indicating that 25% of the genetic variation existed among the populations, while 75% were present in each population.

The genetic diversity indices for geographical populations are summarized in Table 3.2. The average number of alleles per maker ranging from 1.80 (Kitakyushu) to 6.37 (Nagoya) (Table 3.2). Variation range of He, I, PIC were 0.19–0.63, 0.33–1.30, 0.16–0.59. The Kitakyushu population has the lowest values of these indices, and Shimizu population has the highest He, I and PIC value. The Ho value of 13 populations range from 0.06 (Yokohama and Kitakyushu) to 0.26 (Shibushi). Observed heterozygosity of all populations were lower than expected heterozygosity, and the inbreeding coefficient F_{is} in each population was significantly greater than zero (0.24–0.88), which implying that each population may deviate from Hardy-Weinberg equilibrium. Hardy-Weinberg equilibrium test was performed to obtain direct evidence: The result showed that all of the geographic populations of feral rapeseed strongly deviate from the Hardy-Weinberg equilibrium (Table 3.3).

Analysis of diversity indices from the five locations which were sampled twice in ten years did not show a consistent trend. Based on the results of the analyzed diversity indices, the Kashima, Shimizu and Yokkaichi populations showed a decline in genetic diversity, while the remaining two populations, Yokohama and Fukuoka, showed an increase in genetic diversity (Table 3.2).

Based on IAM, TPM and SMM three different assumptions, bottleneck effect of 13 geographic populations was shown in Table 3.4. Under the IAM model, the Hachinohe, Nagoya and Fukuoka populations showed significant heterozygosity excess ($P < 0.05$), while the Shimizu, Kobe, Sakai and Shibushi populations reached a very significant level ($P < 0.01$). Under the TPM model, just Sakai population showed significant heterozygosity excess ($P < 0.05$). Under the SMM model, Kashima populations reached a significant level ($P < 0.05$) while the Tomakomai, Hachinohe, Chiba and Nagoya population showed very significant heterozygosity excess ($P < 0.01$).

The analysis based on F statistic shows that the genetic differentiation coefficient (F_{ST}) ranges from 0.028 (Fukuoka and Yokkaichi) to 0.495 (Kitakyushu and Tomakomai) (Table 3.5). Among the 13 populations, Kitakyushu population showed a relative higher differentiation with other populations (all of the $F_{ST} > 0.2$). The similar tendency can also be seen in Nei's genetic distances results: Kitakyushu population showed relatively high distance away from other populations (average 0.60) (Table 3.6). Nei's unbiased measures of genetic identity ranged from 0.391 to 0.966, the Kitakyushu population showed the lowest genetic identity with other populations (Table 3.6). Mantel test showed that there was only an insignificant weak positive correlation ($r=0.097$, $p= 0.296$) between geographical distance and genetic distance (Fig 3.2).

The hierarchical analysis of geographic populations gives a clearer picture of the major sources of genetic variation (Table 3.7). As the AMOVA analysis shows, the genetic variation among geographic populations was only 15.39%, and the genetic variation within individuals was larger (24.4%). However, the genetic variation among individuals within geographic populations was 60.21%. All of these differences reached very significant level.

3.2.4.2 Population structure of the feral rapeseed resources

The optimal K value was 4, indicating the presence of 4 main groups (Fig. 3.3). Using 0.8 as the likelihood to cluster for each accession in the four populations, a total

of 334 accessions (82.1%) were grouped to one of the four groups. Table 3.8 showed the percentage of accessions in each group, Chiba, Yokkaichi, Sakai and Kitakyusyu populations showing a higher proportion (>0.8) of membership in one group, while the accessions of other populations were assigned to different groups or have a high percentage of admixture. The Hachinohe and Shibushi population have the highest percentage of admixture (>0.7). The grouping of STRUCTURE is not related to the geographic distance between populations and is consistent with the results of the Mantel test. The result of PCoA was similar to that of STRUCTURE analysis (Fig. 3.5). The first two factors of the PCoA explained approximately 23.83% of the variation in the genetic distance matrix. A bi-dimensional PCoA scatter plot indicated differentiation between 13 feral rapeseed populations (Fig. 3.5). It is noteworthy that, about 20 accessions of Kashima population, and each of one accession of Tomakomai and Shimizu population were clearly different from other accessions, moreover, the accessions belongs to G2 group were also distinct from other accessions. And the accessions from Hachinohe and Kitakyushu populations distributed closer while other populations were mixed together.

The results of cluster analysis were similar to those of PCoA (Fig 3.6), but unlike PCoA analysis, the trend of separation of some populations and other populations can be observed more clearly. Some individuals from Shibushi, Hachinohe, Yokohama Kitakyushu, Nagoya and Kashima populations were clustered together, showing that they have a close genetic background. However, in addition to the Kitakyushu population, other populations were clustered in more than one cluster. In Nagoya population, for example, its accessions were mainly clustered in two clusters, in addition to some samples distributed in other clusters.

When cluster at the population level, we can see that Kitakyushu population is first separated from other populations, followed by Shimizu population, and the genetic distance between the remaining populations is relatively small (Fig 3.7).

Table 3.1. Summary statistics of the 30 SSR markers used for genotyping of 407 wild rapeseed accessions.

Marker name	Linkage group	Number of alleles	Major allele frequency	Ho	He	PIC	Fis	F_{ST}
BrGMS4028[a]	A1	5	0.37	0.08	0.47	0.59	0.82	0.29
BrGMS4031[a]	A1	6	0.34	0.24	0.62	0.76	0.62	0.20
BRAS084[b]	A1	36	0.15	0.16	0.81	0.92	0.81	0.13
BrGMS1411[a]	A2	8	0.54	0.18	0.50	0.61	0.64	0.20
BrGMS0667[a]	A2	13	0.74	0.11	0.25	0.31	0.55	0.28
BrGMS2498[a]	A3	4	0.63	0.09	0.39	0.41	0.76	0.12
sN2025[c]	A4	13	0.50	0.09	0.49	0.68	0.82	0.30
BrGMS2252[a]	A5	4	0.74	0.06	0.29	0.39	0.81	0.45
BrGMS0070[a]	A5	36	0.26	0.20	0.71	0.86	0.72	0.20
BnEMS0753[d]	A6	9	0.54	0.24	0.52	0.61	0.54	0.19
BrGMS3750[a]	A6	6	0.50	0.22	0.53	0.58	0.60	0.18
BrGMS3837[a]	A7	13	0.36	0.16	0.60	0.72	0.74	0.22
BnEMS0620[d]	A7	13	0.66	0.17	0.45	0.44	0.62	0.19
BrGMS0742[a]	A8	15	0.38	0.20	0.53	0.72	0.62	0.31
BnGMS0281[e]	A9	8	0.47	0.18	0.48	0.55	0.62	0.27
BrGMS3857[a]	A10	6	0.54	0.17	0.46	0.54	0.63	0.25
BrGMS3688[a]	A10	7	0.30	0.28	0.63	0.71	0.56	0.19
BrGMS0086[a]	A10	13	0.36	0.16	0.58	0.76	0.73	0.29
BnGMS271[e]	C1	8	0.47	0.06	0.47	0.62	0.88	0.32
BoGMS2016[f]	C2	14	0.34	0.10	0.59	0.75	0.83	0.26
BoEMS0016[g]	C2	9	0.57	0.09	0.42	0.50	0.79	0.28
BoGMS0660[f]	C2	4	0.70	0.04	0.28	0.38	0.84	0.41
BnGMS0289[e]	C3	12	0.48	0.22	0.50	0.68	0.57	0.29
BnGMS347[e]	C4	5	0.48	0.09	0.47	0.58	0.82	0.24
BoGMS0037[f]	C5	8	0.73	0.15	0.35	0.43	0.58	0.17
BoGMS1909[f]	C6	15	0.26	0.21	0.69	0.83	0.69	0.20
BnGMS0353[e]	C6	6	0.68	0.11	0.33	0.41	0.68	0.26
BoEMS0049[g]	C7	2	0.61	0.05	0.33	0.36	0.85	0.26
BnGMS0336[e]	C8	7	0.46	0.18	0.50	0.58	0.64	0.26
BoGMS0525[f]	C9	6	0.82	0.11	0.26	0.29	0.57	0.26
Average		10.37	0.50	0.15	0.48	0.59	0.70	0.25

Ho: observed heterozygosity, He: expected heterozygosity, PIC: polymorphism information content, Fis: inbreeding coefficient, F_{ST}: differentiation coefficient.

[a]Detailed maker information is available in Xu *et al.* (2010), [b]Piquemal *et al.* (2005), [c]AAFC Consortium (2016), [d]Wang *et al.* (2012), [e]Cheng *et al.* (2009), [f]Li *et al.* (2011), [g]Li *et al.* (2013).

Table 3.2. Genetic diversity indexes for the wild rapeseed geographic populations.

Geographical population	Sample size	Number of alleles	Major allele frequency	Ho	He	I	PIC	Fis
Tomakomai	6	2.87	0.65	0.21	0.46	0.79	0.40	0.56
Hachinohe	24	3.80	0.63	0.14	0.48	0.86	0.42	0.68
Kashima	89	5.73	0.62	0.09	0.50	0.95	0.44	0.81
Kashima 2005	26	3.60	0.67	0.06	0.44	0.81	0.39	0.84
Kashima 2015	63	4.63	0.73	0.10	0.40	0.75	0.35	0.73
Chiba	13	3.60	0.66	0.10	0.46	0.86	0.42	0.76
Yokohama	34	4.73	0.54	0.06	0.57	1.07	0.51	0.88
Yokohama 2005	11	3.10	0.66	0.07	0.43	0.78	0.39	0.81
Yokohama 2015	23	3.80	0.66	0.06	0.47	0.86	0.42	0.88
Shimizu	18	5.40	0.47	0.23	0.63	1.30	0.59	0.67
Shimizu 2005	11	4.13	0.53	0.16	0.56	1.08	0.51	0.73
Shimizu 2015	7	3.07	0.62	0.32	0.43	0.79	0.42	0.24
Nagoya	78	6.37	0.54	0.17	0.59	1.21	0.54	0.73
Yokkaichi	59	4.60	0.61	0.12	0.51	0.95	0.45	0.75
Yokkaichi 2005	36	3.73	0.62	0.08	0.49	0.89	0.43	0.83
Yokkaichi 2015	23	3.13	0.71	0.16	0.40	0.71	0.35	0.53
Kobe	10	2.53	0.69	0.17	0.36	0.64	0.35	0.61
Sakai	6	2.50	0.71	0.16	0.39	0.65	0.34	0.53
Kitakyushu	9	1.80	0.85	0.06	0.19	0.33	0.16	0.71
Fukuoka	49	5.40	0.58	0.13	0.55	1.08	0.50	0.75
Fukuoka 2005	31	4.17	0.62	0.10	0.50	0.93	0.45	0.78
Fukuoka 2015	18	4.07	0.60	0.17	0.53	1.00	0.48	0.67
Shibushi	12	4.10	0.52	0.26	0.60	1.12	0.55	0.55

Ho: observed heterozygosity, He: expected heterozygosity, I: Shannon's Information Index, PIC: polymorphism information content, Fis: inbreeding coefficient.

Table 3.3. Hardy-Weinberg equilibrium test of wild rapeseed geographic populations.

Marker name	Geographic population													
	Tomakomai	Hachinohe	Kashima	Chiba	Yokohama	Shimizu	Nagoya	Yokkaichi	Kobe	Sakai	Kitakyushu	Fukuoka	Shibushi	Total
BrGMS4028	*	***	***	***	***	**	***	***	mo	ns	mo	***	**	***
BrGMS4031	ns	***	***	***	***	**	***	***	ns	ns	**	***	***	***
BRAS084	ns	***	***	***	***	***	***	***	*	**	***	***	***	***
BrGMS1411	ns	**	***	**	***	**	***	***	ns	ns	ns	***	**	***
BrGMS0667	ns	**	ns	ns	***	***	***	***	**	mo	mo	***	*	***
BrGMS2498	ns	**	***	***	***	**	***	***	**	*	mo	***	ns	***
sN2025	**	***	***	***	***	***	***	***	ns	*	mo	***	***	***
BrGMS2252	mo	**	***	***	***	***	***	***	*	mo	mo	***	ns	***
BrGMS0070	ns	***	***	***	***	***	***	***	***	*	**	***	**	***
BnEMS0753	ns	***	***	**	***	***	***	***	ns	ns	**	***	*	***
BrGMS3750	ns	***	***	***	***	**	***	***	*	ns	**	***	*	***
BrGMS3837	ns	***	***	***	***	***	***	***	ns	**	**	***	**	***
BnEMS0620	ns	**	***	***	***	ns	***	***	*	ns	ns	***	***	***
BrGMS0742	ns	ns	***	ns	***	***	***	***	ns	ns	mo	***	*	***
BnGMS0281	*	***	***	*	***	**	***	***	ns	*	mo	***	**	***
BrGMS3857	ns	***	***	**	***	**	***	***	ns	ns	mo	***	ns	***
BrGMS3688	ns	***	***	***	***	*	***	***	**	ns	ns	***	*	***
BrGMS0086	ns	***	***	***	***	***	***	***	mo	ns	mo	***	ns	***
BnGMS271	*	***	***	**	***	*	***	***	mo	ns	**	***	*	***
BoGMS2016	*	***	***	***	***	**	***	***	mo	ns	ns	***	***	***
BoEMS0016	*	***	***	***	***	***	***	***	**	ns	mo	***	**	***
BoGMS0660	*	***	***	*	***	mo	***	***	mo	ns	mo	***	**	***
BnGMS0289	ns	***	***	***	***	*	***	***	mo	ns	mo	***	**	***
BnGMS347	*	***	***	***	***	**	***	***	mo	*	**	***	ns	***
BoGMS0037	ns	***	***	mo	***	***	***	***	mo	ns	mo	***	*	***
BoGMS1909	**	***	***	***	***	***	***	***	***	ns	*	***	***	***
BnGMS0353	*	*	***	ns	***	**	***	***	mo	ns	*	***	ns	***
BoEMS0049	ns	***	***	*	***	ns	***	***	mo	mo	mo	***	ns	***
BnGMS0336	mo	***	***	***	***	***	***	***	ns	ns	ns	***	ns	***
BoGMS0525	ns	ns	***	mo	***	**	***	ns	*	mo	mo	***	ns	***

ns: no significance, mo: locus is monomorphic, ***: significant at the 0.1% level, **: significant at the 1% level, *: significant at the 5% level.

Table 3.4. Population bottleneck analysis in wild rapeseed geographical populations.

Geographical population	Loci with heterozygosity excess			P-value		
	IAM	TPM	SMM	IAM	TPM	SMM
Tomakomai	18	14	14	0.359	0.274	0.004**
Hachinohe	19	16	12	0.010*	0.191	0.002**
Kashima	20	12	5	0.172	0.338	0.046*
Chiba	18	15	9	0.116	0.050	0.000**
Yokohama	25	20	17	0.195	0.426	0.520
Shimizu	25	22	21	0.003**	0.126	0.480
Nagoya	23	17	7	0.012*	0.543	0.000**
Yokkaichi	22	21	12	0.266	0.552	0.408
Kobe	17	14	12	0.000**	0.068	0.222
Sakai	15	14	14	0.001**	0.036*	0.094
Kitakyushu	10	8	8	0.155	0.440	0.292
Fukuoka	21	14	9	0.017*	0.083	0.052
Shibushi	26	23	19	0.001**	0.197	0.502

**: significant at the 1% level, *: significant at the 5% level.

Table 3.5. Estimates of pairwise F_{ST} (below diagonal) among wild rapeseed geographic populations.

	Tomakomai	Hachinohe	Kashima	Chiba	Yokohama	Shimizu	Nagoya	Yokkaichi	Kobe	Sakai	Kitakyushu	Fukuoka	Shibushi
Tomakomai													
Hachinohe	0.184**												
Kashima	0.142*	0.195**											
Chiba	0.173**	0.310**	0.199**										
Yokohama	0.125*	0.139**	0.097**	0.165**									
Shimizu	0.134**	0.203**	0.172**	0.155**	0.111**								
Nagoya	0.129**	0.161**	0.165**	0.185**	0.075**	0.078**							
Yokkaichi	0.092*	0.213**	0.200**	0.098**	0.165**	0.152**	0.184**						
Kobe	0.195**	0.292**	0.254**	0.236**	0.203**	0.108**	0.179**	0.214**					
Sakai	0.166*	0.352**	0.200**	0.084	0.188**	0.150**	0.169**	0.101*	0.272**				
Kitakyushu	0.495**	0.457**	0.352**	0.446**	0.342**	0.262**	0.318**	0.381**	0.434**	0.478**			
Fukuoka	0.071	0.187**	0.160**	0.060*	0.119**	0.101**	0.131**	0.028**	0.168**	0.072	0.325**		
Shibushi	0.091*	0.152**	0.144**	0.200**	0.071*	0.080**	0.100**	0.135**	0.151**	0.183**	0.351**	0.105**	

F_{ST}: differentiation coefficient.

**: significant at the 1% level, *: significant at the 5% level.

Table 3.6. Estimates of Nei's unbiased measures of genetic identity (above diagonal) and Nei's genetic distance (below diagonal) among wild rapeseed geographic populations.

	Tomakomai	Hachinohe	Kashima	Chiba	Yokohama	Shimizu	Nagoya	Yokkaichi	Kobe	Sakai	Kitakyushu	Fukuoka	Shibushi
Tomakomai		0.665	0.818	0.790	0.746	0.709	0.727	0.872	0.560	0.756	0.391	0.834	0.767
Hachinohe	0.409		0.723	0.620	0.758	0.587	0.684	0.649	0.530	0.571	0.474	0.660	0.786
Kashima	0.201	0.324		0.826	0.782	0.684	0.747	0.772	0.513	0.804	0.490	0.794	0.785
Chiba	0.236	0.479	0.192		0.758	0.745	0.782	0.887	0.553	0.929	0.549	0.924	0.751
Yokohama	0.293	0.277	0.246	0.277		0.738	0.807	0.770	0.630	0.742	0.551	0.795	0.858
Shimizu	0.344	0.532	0.38	0.294	0.304		0.837	0.749	0.623	0.724	0.627	0.800	0.750
Nagoya	0.319	0.380	0.291	0.247	0.215	0.178		0.771	0.577	0.771	0.583	0.848	0.810
Yokkaichi	0.137	0.433	0.258	0.119	0.262	0.289	0.260		0.612	0.861	0.499	0.966	0.805
Kobe	0.579	0.635	0.668	0.592	0.462	0.473	0.550	0.492		0.533	0.392	0.622	0.650
Sakai	0.279	0.560	0.218	0.074	0.299	0.323	0.260	0.150	0.629		0.563	0.895	0.735
Kitakyushu	0.939	0.746	0.714	0.600	0.596	0.467	0.541	0.696	0.937	0.574		0.567	0.546
Fukuoka	0.182	0.415	0.230	0.080	0.230	0.223	0.165	0.035	0.475	0.111	0.567		0.813
Shibushi	0.266	0.241	0.242	0.286	0.154	0.288	0.210	0.217	0.432	0.308	0.606	0.207	

**: significant at the 1% level, *: significant at the 5% level.

Table 3.7. Reuslt of the hierarchical analysis of molecular variance (AMOVA) for wild rapeseed geographic populations.

Source of variation	d.f.	Parcentage of variation (%)	
Among geographica populations	12	15.39	***
Among individuals	394	60.21	***
Within individuals	407	24.4	***

***: significant at the 0.1% levels for 1,000 permutations.

Table 3.8. Number and percentage of feral rapeseed accessions in STRUCTURE groups among the 13 populations.

Population	Accessions No.	No. of accessions in each group					Percentage of accessions in each group				
		G1	G2	G3	G4	Admixture	G1	G2	G3	G4	Admixture
Tomakomai	6	0	0	1	4	1	0	0	16.67	66.67	16.67
Hachinohe	24	0	1	5	1	17	0	4.17	20.83	4.17	70.83
Kashima	89	0	10	41	24	14	0	11.24	46.07	26.97	15.73
Chiba	13	0	1	0	12	0	0	7.69	0	92.31	0
Yokohama	34	14	2	1	10	7	41.18	5.88	2.94	29.41	20.59
Shimizu	18	2	11	0	4	1	11.11	61.11	0	22.22	5.56
Nagoya	78	25	34	1	9	9	32.05	43.59	1.28	11.54	11.54
Yokkaichi	59	4	0	0	51	4	6.78	0	0	86.44	6.78
Kobe	10	0	8	0	2	0	0	80	0	20	0
Sakai	6	0	0	0	6	0	0	0	0	100	0
Kitakyushu	9	9	0	0	0	0	100	0	0	0	0
Fukuoka	49	2	5	0	32	10	4.08	10.2	0	65.31	20.41
Shibushi	12	1	0	0	1	10	8.33	0	0	8.33	83.33

Fig. 3.1. Map of the sample collection regions for Japan feral rapeseed.

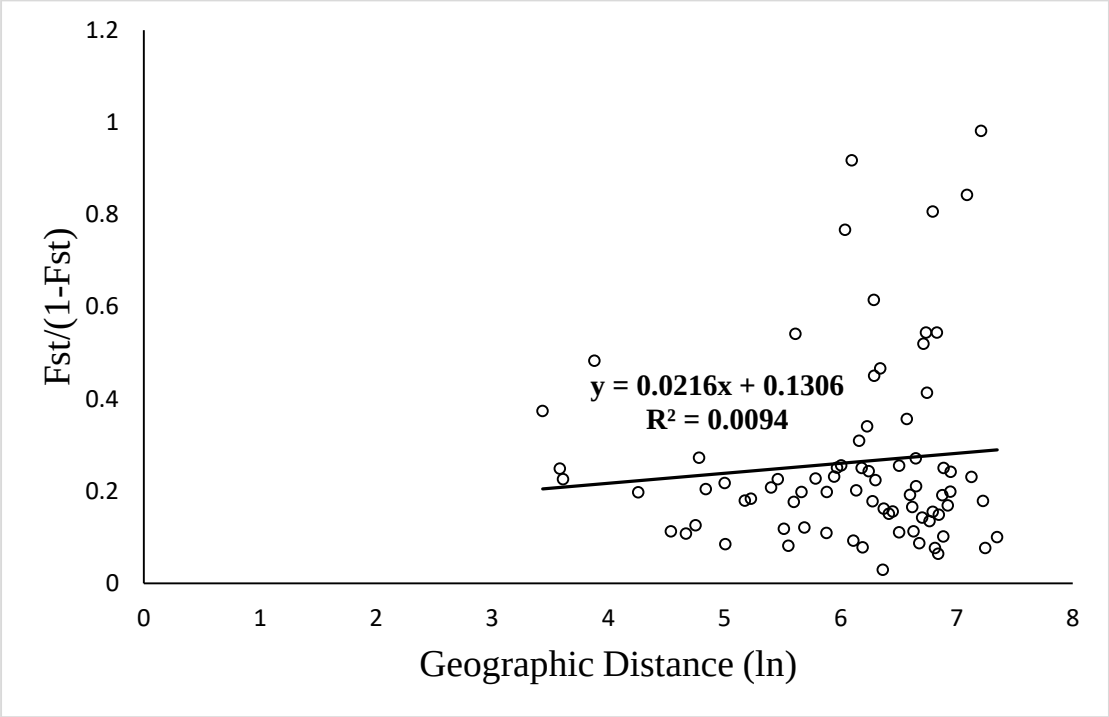

Fig. 3.2. Mantel test result of the correlation between geographic distance and genetic distance of Japan feral rapeseed populations.

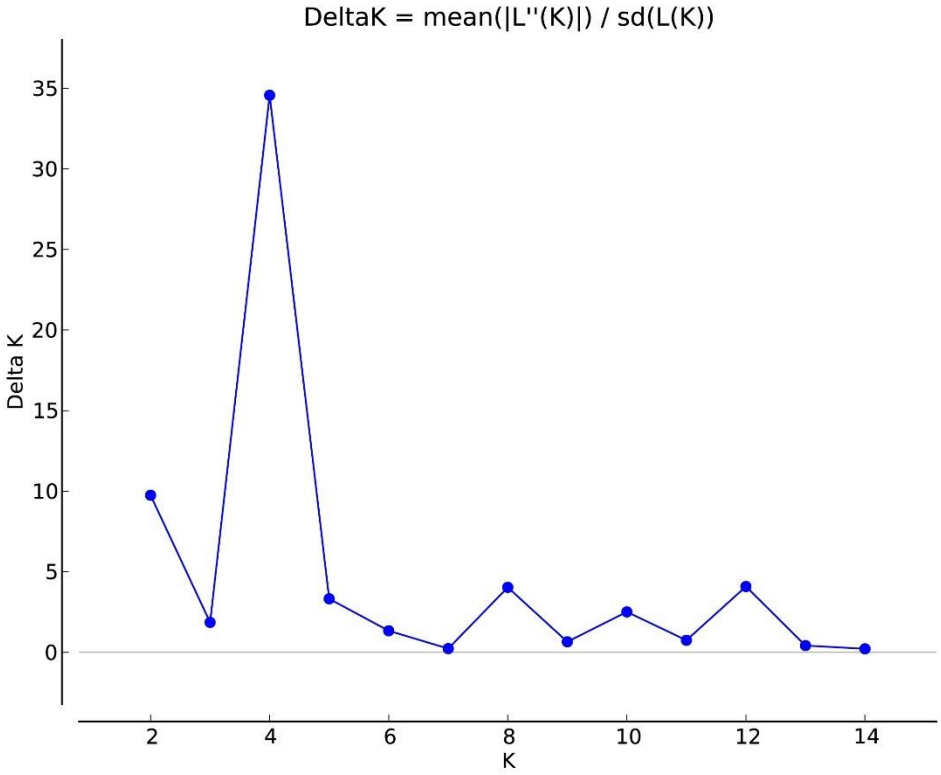

Fig. 3.3 ΔK, from the structure analysis of the 407 feral rapeseed accessions.

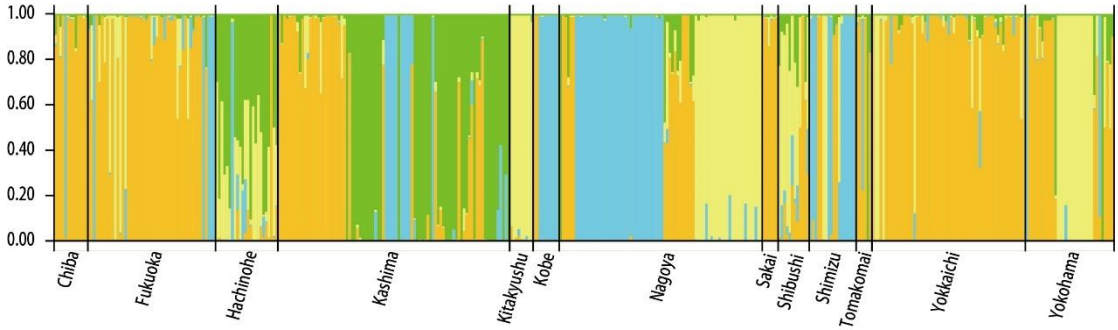

Fig. 3.4. Population structure of 407 feral rapeseed accessions based on 30 SSR markers. When K=4, the 407 germplasms were classified into four subgroups.

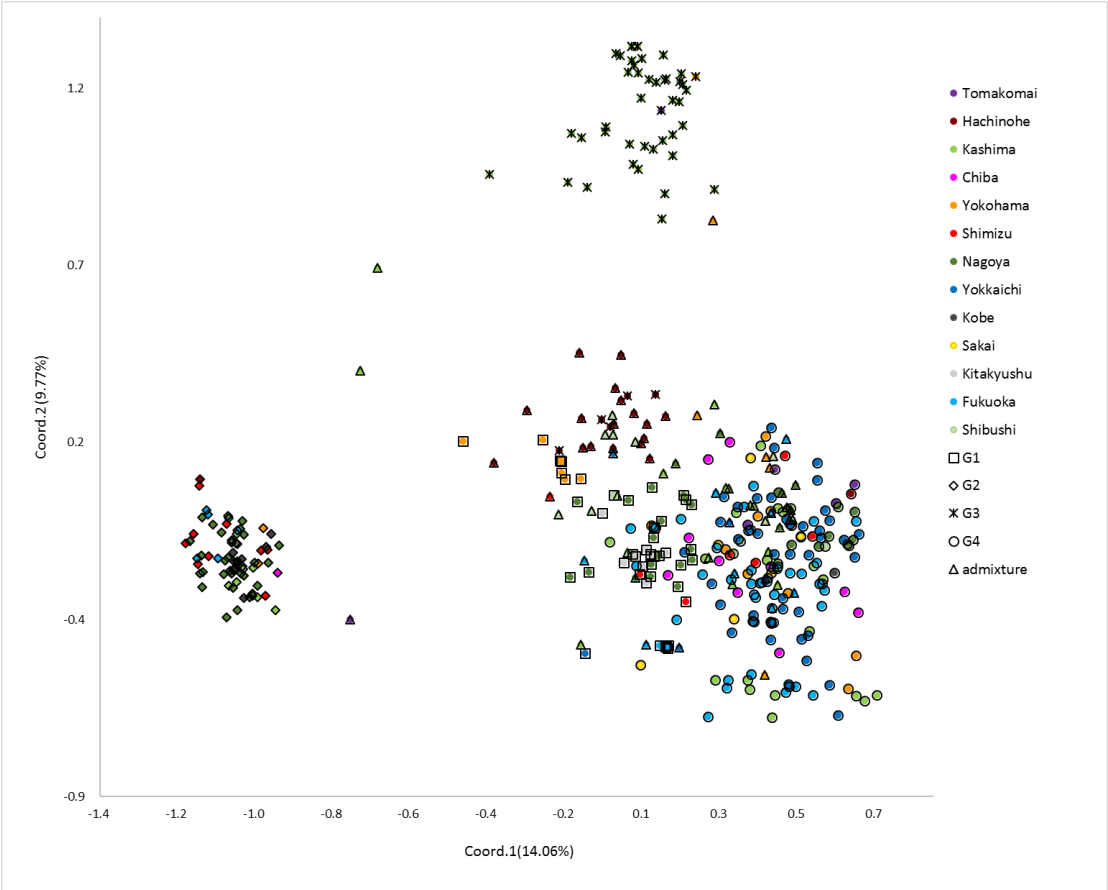

Fig. 3.5. Principal coordinates analysis (PCoA) of the 407 feral rapeseed accessions. Each colored spot is representative of one geographic population and each different shape represents one group matching the STRUCTURE group.

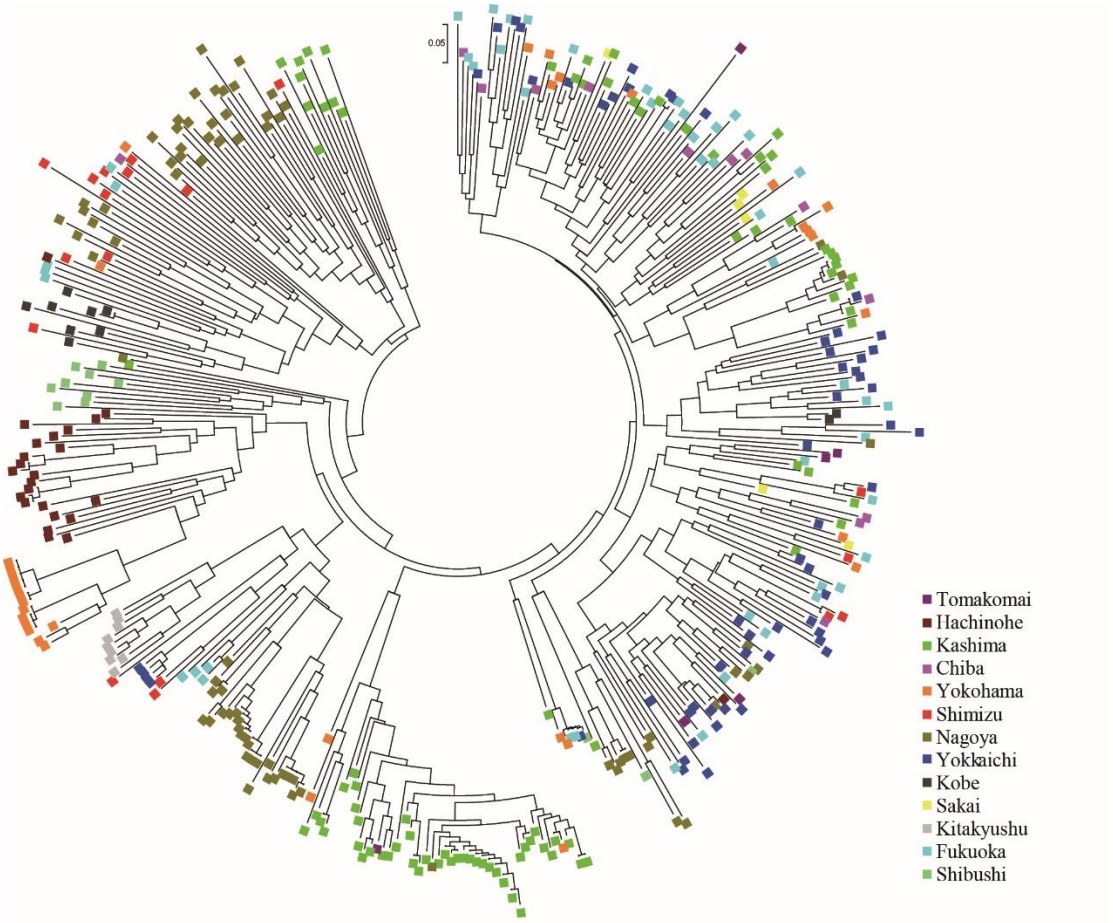

Fig. 3.6. NJ dendrogram of 407 feral rapeseed accessions based on the Nei's genetic distance (1983). Germplasms from different populations were identified by different colors.

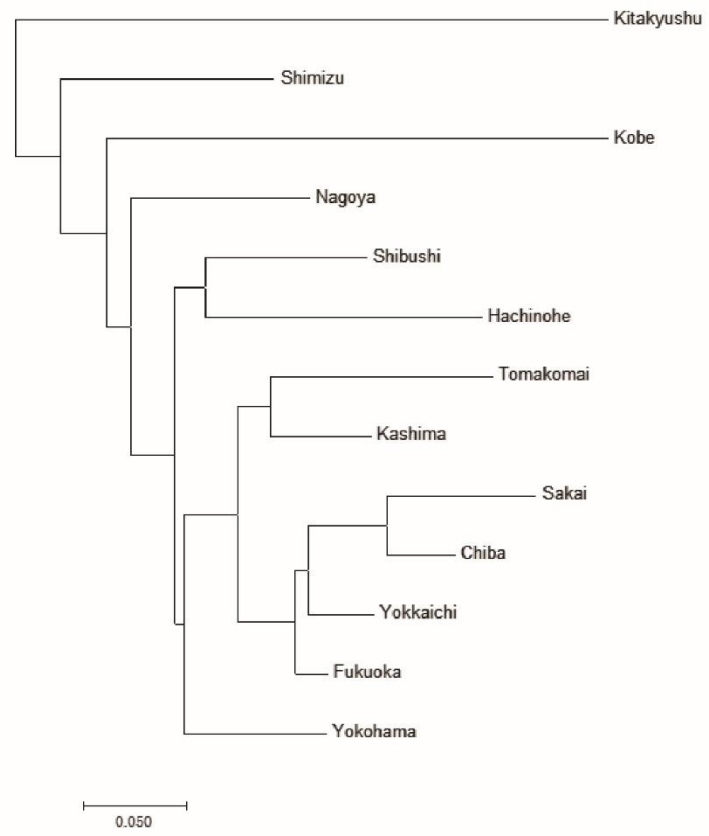

Fig. 3.7. NJ dendrogram of 13 feral rapeseed populations based on the Nei's genetic distance (1983).

3.3 Determination of the agronomic traits of environmental adaptability of the feral rapeseed resources.

3.3.1 Plant materials and methods

3.3.1.1 Plant materials

22 accessions of the 143 NIES accessions from the 8 geographic regions were chose for this experiment, three cultivars were chosen as comparison. 3 Kashima accessions, 4 Chiba accessions, 2 Sakai accessions, 5 Yokkaichi and Fukuoka accessions and 1 Yokohama, 1 Shimizu and 1 Kitakyushu accession were selected. The cultivars were Westar, Asakanonatane and Prota, form Japan and oversea countries. Seeds of these accessions were obtained by self-cross of the individuals of the diversity study in Chapter 3.2.

3.3.1.2 Experimental site and cultivation method

The experiment was conducted in the experimental field of the Agriculture and Forestry Research Center, University of Tsukuba (Ibaraki, Japan), which is located on about 28m above sea level (36°07′01″ latitude and 140°05′41″ longitude). The climate type belongs to warm moist climate. In July, August and September 2014, 12 seeds of each accession were planted into a 72-cell tray filled with Nippi-Engei-Baido-1gou soil (Nihon Hiryo Co., Ltd., Tokyo, Japan), and repeated three times. 3 or 4 weeks later, 15 seedlings of each accession were randomly selected and transplanted into field. The seedlings were planted in two rows, with an interval of 40 cm between them. Twenty-five seedlings (each containing 22 feral accessions and 3 cultivars) were one block, and fifteen blocks were planted each month. When transplanting, 50g of slow-release fertilizer Ekorongu413 type100 (JCAM AGRI. Co., Ltd., Tokyo, Japan) were applied per plant.

3.3.1.3 Data collection

The number of germinated individuals was counted after two weeks of sowing and the average germination rate of each accession was calculated. The first flowering date

of each individuals was recorded during the cultivation process and the number of days until flowering was calculated on the basis of sowing date. The average days until flowering were calculated when all individuals of an accession were flowering.

3.3.1.4 Evaluation of secondary dormancy

The secondary dormancy of the seeds was induced by water stress, which produced by Polyethylene Glycol (PEG) solution (Gruber *et al.* 2008). The water stress should be -15000kPa (-15bar) at 20℃. The concentration of the PEG solution was calculated according to Michel and Kaufmann (1973). In the induction of secondary dormancy stage, place 20 seeds of one accession in Φ9cm Petri dishes with a double layer of filter paper, and add 8ml of PEG solution, and repeated three times. This stage would last for 14d in dark condition at 20℃. After that wash and transfer the seeds to new Petri dishes and add 6ml deionized water under a green safety light. Then maintain the seeds in darkness of another 14d at 20℃. The seed without secondary dormancy will germinate during this period. All germinated seeds will be counted and removed at the 2nd, 4th, 7th and 14th day. Dormancy breaking treatment to identify viable but dormant seeds with alternative light and temperature conditions: light/darkness; 30/5℃, 12/12h, it will last 7d. Seeds that remained undisturbed after 7d were considered dead seeds and would be excluded from the statistical analysis.

3.3.2 Results

3.3.2.1 Field germination rate

The results of the field germination rate are summarized in Fig. 3.8. In the case of feral accessions, the germination rates of the 2014/07 and 2014/08 showed a great difference between individuals at different geographic populations and between individuals of the same population, ranged from 38.9% to 100%. While the difference between cultivars is small, ranged from 79.2% to 100%. In addition, the germination rate of different accession was increased gradually with the decrease of temperature in the different months, and finally leads to a reduction in the disparity between different accessions.

3.3.2.2 Number of days until flowering

The results for number of days until flowering are summarized in Fig. 3.9. The time required from sowing to blooming is quite different in different feral accessions, and similar to field germination results, different accessions of the same geographical population showed a big difference. As for the results of different sowing dates, this difference ranged from 97 days to 249 days in 2014/07. The range of difference in 2014/08 was smaller, which ranged from 136 days to 241 days. The difference in 2014/09 is small, ranged from 165 days to 203 days. On the average, the difference between feral accessions and cultivar accessions is not significant.

3.3.2.3 Secondary dormancy

The secondary dormancy rate between different accessions has a large variation range (Fig. 3.10). Among the feral accessions, 7 accessions had a secondary dormancy rate of less than 10%, but there were 6 accessions above 50%, of which the highest was higher than 80%. Similar to the field germination rate and number of days until flowering, the difference in accessions of the same geographic population was also very large. The cultivars also had some secondary dormancy, their average was 43.7%, slightly higher than the feral population of 31.0%.

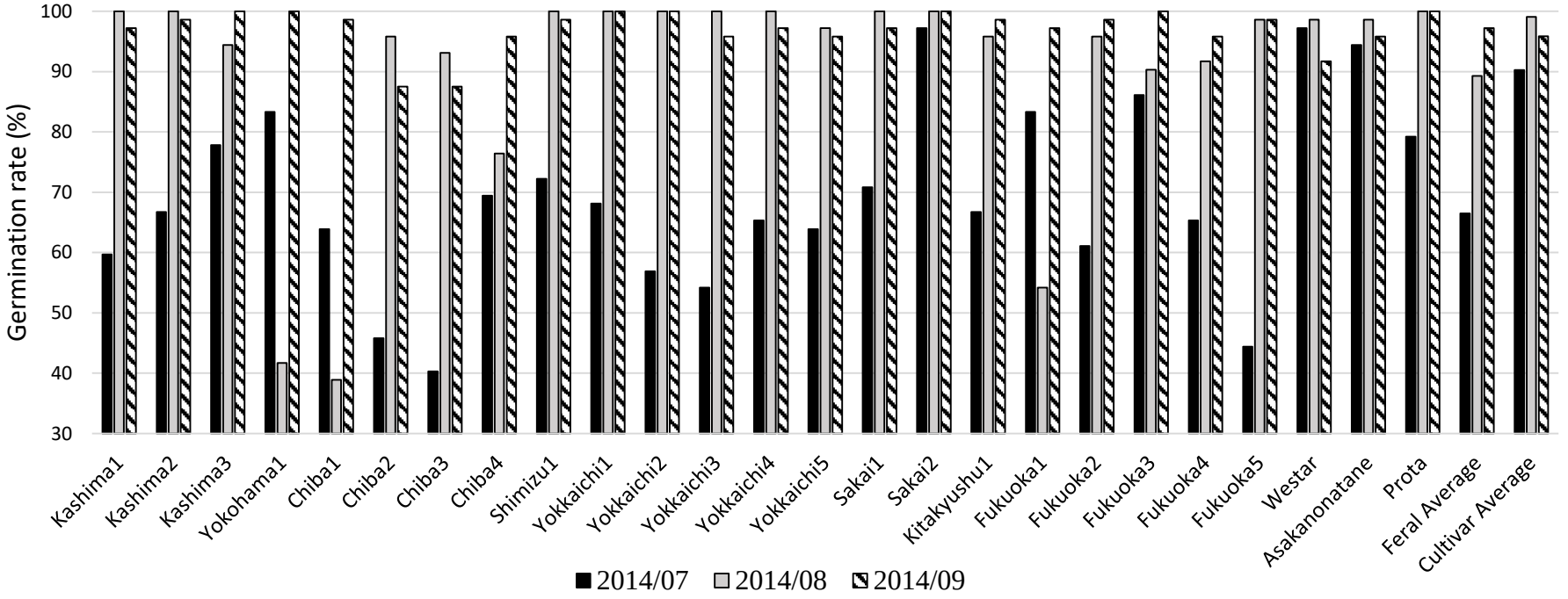

Fig. 3.8. Field germination rate of 26 accessions sowed in 2014/07,08,09.

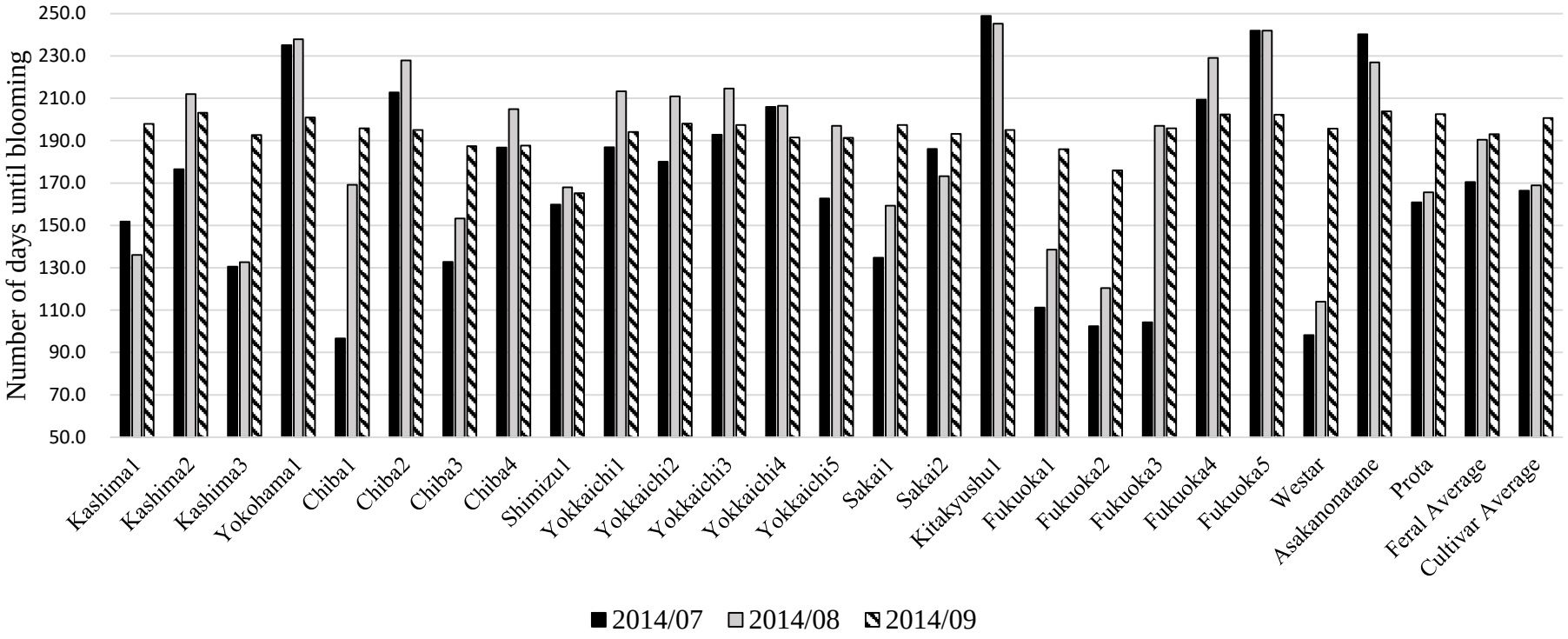

Fig. 3.9. Number of days until flowering of the 26 accessions sowed in 2014/07,08,09.

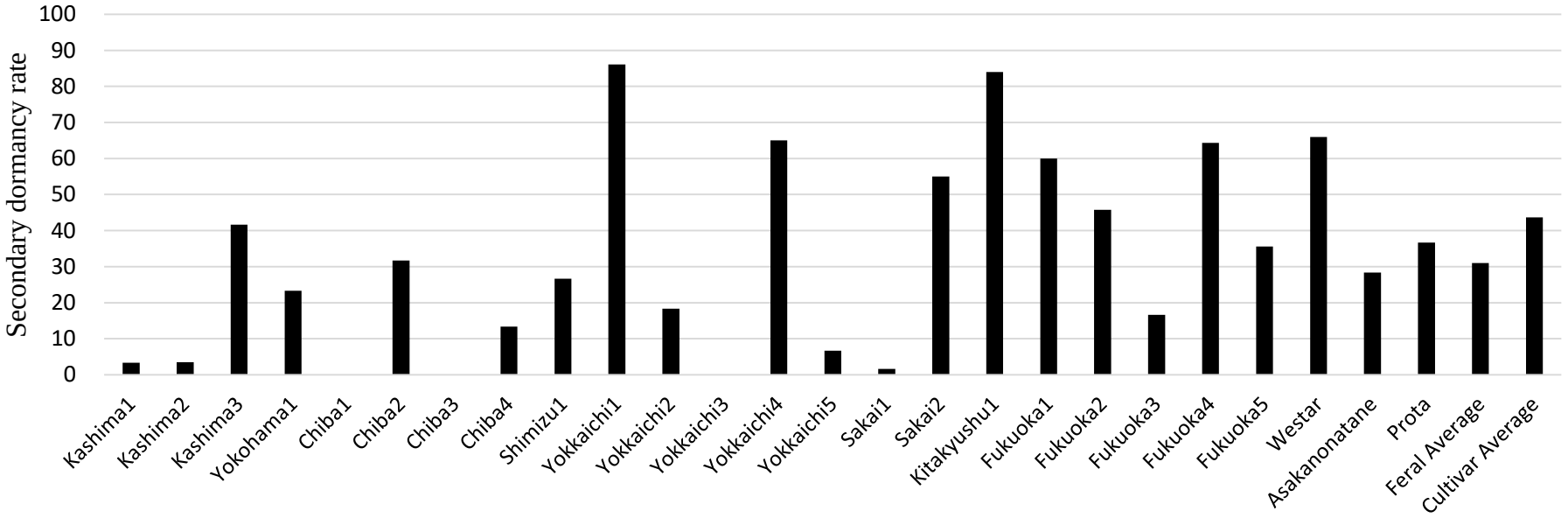

Fig. 3.10. Secondary dormancy rate of 26 accessions.

3.4 Comparison of genebank resources and feral resources

3.4.1 Data used in this study

Genotyping data of 582 NARO rapeseed genebank accessions and 407 feral rapeseed accessions in Chapter 2 and Chapter 3.2 were used.

3.4.2 Data analysis

In PCR amplification, I found that some specific alleles only appeared in accessions of feral populations but not in NARO rapeseed genebank accessions. The number of these alleles was counted. Principal coordinates analysis (PCoA) was performed in GenAlEx 6.502 to show the relationships between genebank and feral accessions. Cluster method was also used to analyze the relationship between them. A dendrogram applying neighbor joining (NJ) method based on a matrix of Nei's genetic distances was obtained using MEGA7 software. In order to more clearly show the relationship between feral rapeseed and Japan/Overseas accessions, another NJ tree was constructed based on grouping these three kind of accessions.

3.4.3 Results

Number of specific alleles in feral populations and its proportion to allele number in NARO genebank was summarized in Table 3.9. In 30 SSR markers, a total of 57 feral population specific alleles were found. There were minimal of 0, maximum of 15 (BRAS084) in each marker. They account for 18.32% of the total allele number of the NARO rapeseed genebank.

It can be seen from the PCoA result that the coverage of the feral population is smaller than that of the genebank population and the distribution area is also different (Fig 3.11). Most of the feral accessions were distributed in the vicinity of the genebank accessions, indicating that they have close genetic relationship. Among them, the majority of accessions are located more closely with the oversea accessions. However, part of the feral accessions was distributed in the absence of the genebank accessions,

indicating that they were genetically distinct from the genebank accessions. It is worth noting that some of the accessions were located at a relatively distant distance, revealing that they are genetically different from other samples. Their nearest cultivated variety is a variety from overseas (Germany), MIRANDER.

Similar trends can be seen from the NJ tree (Fig 3.12). Feral accessions and cultivars were mainly characterized by two ways: one is clustered together with feral accessions or cultivars separately; the other is that they were clustered together in a mixed way. Individuals of the feral population were roughly distributed over four subclusters at a distance, which was consistent with the results of their STRUCTURE analysis. In addition, there is no feral accessions that were clustered in subsluster where cultivar Westar is located. The cluster analysis of the feral accessions and the genebank accessions from different sources shows that Japan and overseas accessions are closer, and feral accessions are slightly farther away from them (Fig 3.13). In Chapter 2, I found the Japan and overseas accessions could be divided into two groups in STRUCTURE and PCoA analysis. After adding the feral rapeseeds, we can find that the genetic difference within the NARO rapeseed genebank is less than the genetic difference between it and feral rapeseed.

Table 3.9. Specific alleles number in feral populations and relative percentage.

Marker name	Allele number in NARO genebank （A）	Allele number in feral populations	Unique alleles number in feral populations (B)	B/A (%)
BrGMS4028	9	5	0	0.00
BrGMS4031	5	6	1	20.00
BRAS084	22	36	15	68.18
BrGMS1411	7	8	3	42.86
BrGMS0667	15	13	0	0.00
BrGMS2498	4	4	0	0.00
sN2025	10	13	5	50.00
BrGMS2252	4	4	0	0.00
BrGMS0070	39	36	3	7.69
BnEMS0753	7	9	2	28.57
BrGMS3750	6	6	0	0.00
BrGMS3837	8	13	5	62.50
BnEMS0620	17	13	2	11.76
BrGMS0742	11	15	4	36.36
BnGMS0281	8	8	0	0.00
BrGMS3857	5	6	1	20.00
BrGMS3688	6	7	2	33.33
BrGMS0086	12	13	1	8.33
BnGMS271	9	8	0	0.00
BoGMS2016	15	14	3	20.00
BoEMS0016	9	9	1	11.11
BoGMS0660	3	4	1	33.33
BnGMS0289	21	12	0	0.00
BnGMS347	10	5	0	0.00
BoGMS0037	9	8	2	22.22
BoGMS1909	13	15	2	15.38
BnGMS0353	8	6	1	12.50
BoEMS0049	3	2	0	0.00
BnGMS0336	4	7	3	75.00
BoGMS0525	12	6	0	0.00
Average	10.37	10.37	1.9	18.32

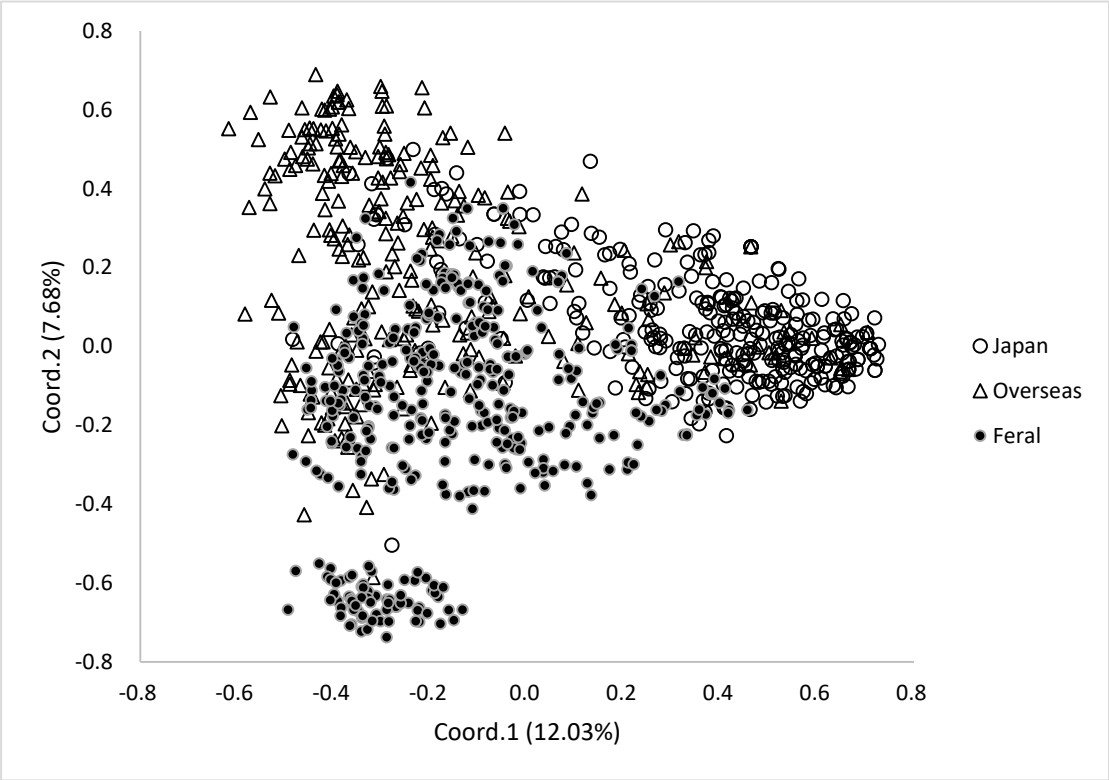

Fig. 3.11. Principal coordinates analysis (PCoA) of the 407 feral and 582 NARO genebank rapeseed accessions.

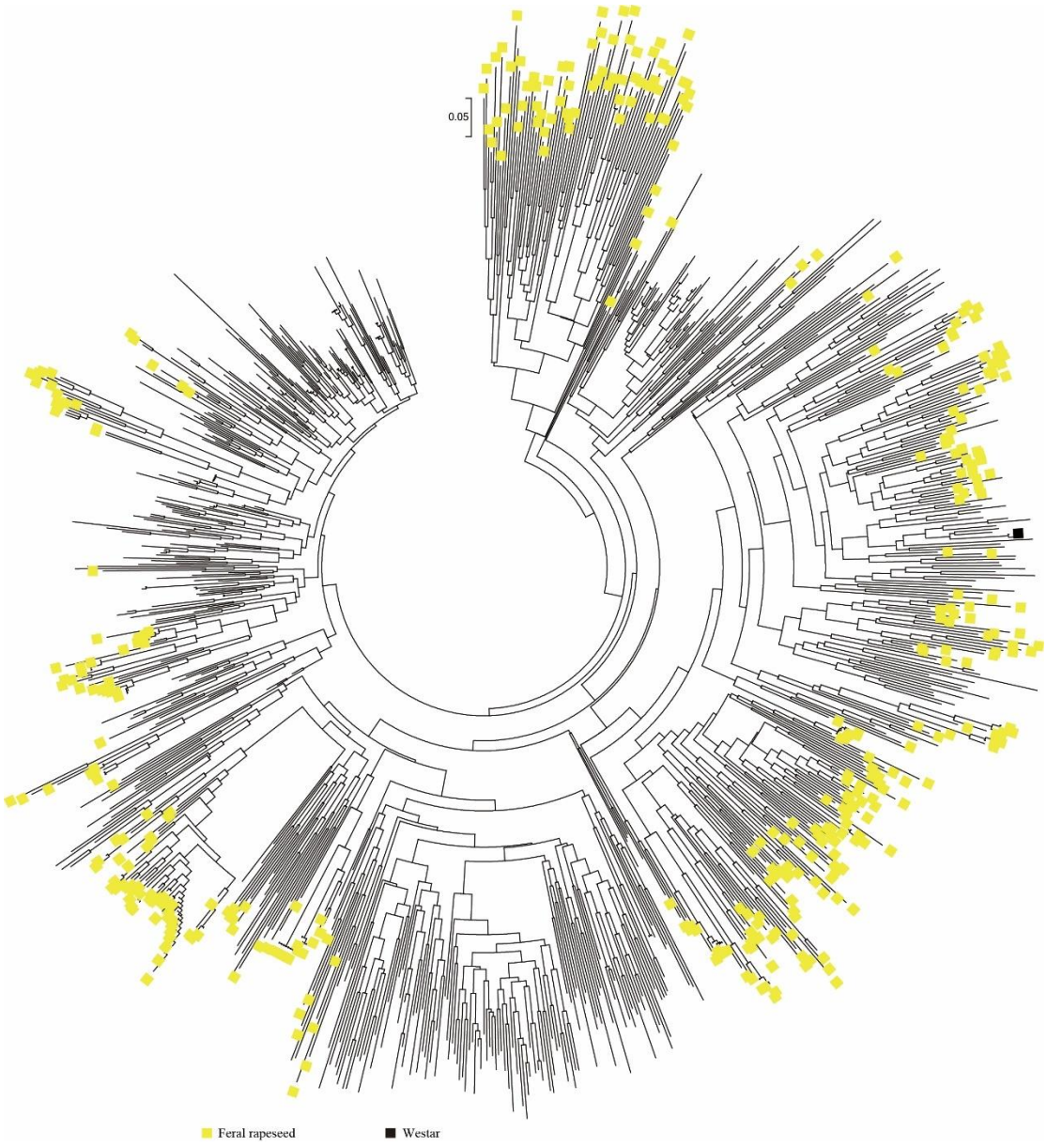

Fig. 3.12. NJ dendrogram of the 407 feral and 582 NARO genebank rapeseed accessions based on the Nei's genetic distance (1983). Feral accessions were identified by yellow mark, the cultivar "westar" was marked by black.

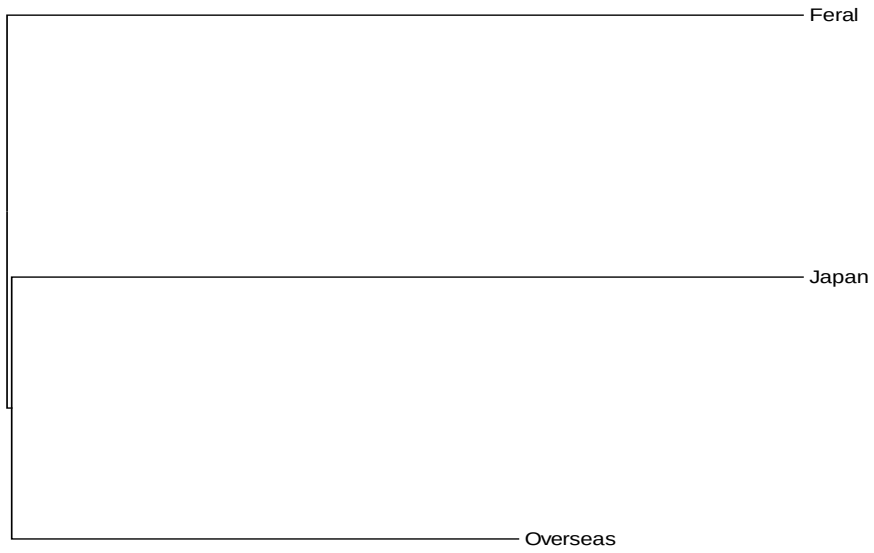

Fig. 3.13. NJ dendrogram of the grouped feral and Japan/Overseas genebank rapeseed accessions based on the Nei's genetic distance (1983).

3.5 Discussion

Genetic diversity and population structure of feral rapeseed populations that persisted outside of Japan's farmland was studied for the first time. By genotyping analysis with SSR markers, I revealed that the Japan feral rapeseed populations have a relatively high genetic diversity (Table 3.1). The relatively high number of alleles (average 10.37, same with NARO genebank collection), expected heterozygosity (He) (average 0.48) and PIC values (average 0.59) indicated that the SSR markers used in the Chapter 2 could be applied to the diversity analysis of feral rapeseed. In addition to Japan, feral rapeseed populations were found in many other countries (Liu *et al.* 2010), but there is not much research on their genetic diversity. By contrast with existing studies, the mean expected heterozygosity (He) of feral populations in Japan was found to be higher than that of Austria feral rapeseed populations (0.48 > 0.32) (Pascher *et al.* 2010). This suggests that compared with Austria, which is close to the origin place of rapeseed, Japan may have feral populations with higher genetic diversity due to the introduction of different rapeseed varieties from multiple areas of the world.

The inbreeding coefficient between individuals (F_{is}) is a numerical value indicating the degree of inbreeding, which ranges from -1 to 1. When the value of F_{is} is an extremely significant positive value, it means that inbreeding occurs mainly in the population; if it is extremely significant negative value, it means there is outcrossing in the population (Weir and Cockerham 1984). The mean F_{is} value of the feral populations is 0.70, indicating that the feral populations were mainly reproduce by selfing. Other evidence that could support this view is the low observed heterozygosity (Ho = 0.15), which is the number of heterozygotes for one locus divided by the total number of observed individuals. Rapeseed is often cross-pollinated plant, which produces seeds mainly by selfing (Katsuta *et al.* 2015), this study shows that rapeseed populations grown under natural conditions are consistent with this theory.

The definition of Hardy-Weinberg equilibrium is: in an infinite random-mating

population, if migration happened but mutation and selection are absent, the population gene frequency and genotype frequency would remain unchanged for generations, that is in an equilibrium condition. In this study, all of the loci are deviated from Hardy-Weinberg equilibrium, and in addition to the Kitakyushu and Tomakomai population, most loci of other populations also deviate from the Hardy-Weinberg equilibrium. This may be due to the selfing reproduce in the population, which lead to not meet the prerequisite of Hardy-Weinberg equilibrium, which is random mating. Similar situation was also found in rice and its wild relatives (Agrama and Eizenga 2008, Melaku *et al.* 2013), and as with my inference, the high degree of selfing is considered to be the cause of this situation.

In this study, three mutation models (IAM, TPM, and SMM) were used to detect the bottleneck effects of each geographic population. Among them, TPM is intermediate model, between IAM and SMM models. In the treatment of microsatellite data, TPM consists mostly of one-step mutations, of which a small number (5% to 10%) consists of multiple mutations (Luikart *et al.* 1998). Compared with SMM and IAM, the TPM model is better in most of the microsatellite data (Yang *et al.* 2012). If only use the TPM model to detect the bottleneck effect, in this study, only the Sakai population has affected by significant bottleneck effects. From this point of view, the bottleneck effect of the feral rapeseed populations is not very strong, which may be a reason for their high genetic diversity, because the richness of the genetic diversity of a species is affected by the population bottleneck effect (Rao and Hodgkin 2002).

Temporal trend of genetic diversity was studied in several species both in cultivars, landraces and wild species (Malysheva-Otto 2007, Singh *et al.* 2016, Chabane *et al.* 2007, Wongtamee *et al.* 2017). In the five sites compared in this study, the trend of genetic diversity change is not uniform. It should be noted that the number of samples of two-time sampling is not consistent, which will have an adverse effect on the final result. When we focus on Kashima population, it can be found that although the number of samples in 2015 was more than double that of 2005, the genetic diversity index

declined slightly. In contrast, the number of samples for the Fukuoka population in 2015 was about 0.6 times that of 2005, but the genetic diversity index was slightly higher. Through the above comparison, we can think that in different locations, feral rapeseed population genetic diversity change with the passage of time, and may be caused by different reasons. In order to further clarify the mechanism of this change, long-term monitoring and analysis are needed.

Population genetic differentiation coefficient (F_{ST}) is an important indicator of genetic differentiation among populations. In general, the F_{ST} value among populations above 0.15 are considered to have significant genetic differentiation (Frankham *et al.* 2010). The differentiation of feral populations could be seen in Table 3.5, which shows that most of the differentiation between populations reached significant and very significant levels. Most of the populations except Kitakyushu population have a F_{ST} value around 0.2, indicating that the differentiation among these populations was moderately strong. Similar results can be seen from the results of the STRUCTURE, PCoA and NJ dendrogram of the 407 feral accessions (Fig. 3.4, 3.5, 3.6). These results show that individuals within these different populations were not significantly different from those of other populations, which indicate that most of the feral populations may be derived from different cultivars. This also explains why there is no significant correlation between genetic distance and geographical distance among different populations revealed by mantel test. The result of AMOVA hierarchical analysis showed that genetic variation mainly existed among individuals and not between populations. This also indicates from the side that feral populations are made up of individuals that are different from each other.

Phenotypic data are intuitive for assessing diversity, many studies assess the genetic diversity of *Brassica* crops using phenotypic data. In different agronomic traits, such as seed yield, pod number, thousand-seed weight, and root morphology, leaf ionomic, and so on, rapeseed showed high polymorphism (Chen *et al.* 2014, Thomas *et al.* 2016). Since genotypic polymorphism may not be able to reflect phenotypic

polymorphism, direct study of phenotypic polymorphism of feral rapeseed accessions can directly reflect the variation range of feral rapeseed. On the other hand, some authors demonstrated that the ability to respond to natural selection is a major factor in the success of introduced species (Bossdorf *et al.* 2005, Suarez and Tsutsui 2008). It has been found that an introduced *B.rapa* exhibits a rapid adaptive evolution in flowering time (Franks *et al.* 2007). So, the environmental adaptability-related traits: field germination rate, number of days until flowering and secondary dormancy were chosen in this study, to see whether the feral rapeseed has a wide range of variation. The results showed that the field germination rate and the number of days until flowering varied greatly in different feral rapeseed accessions. The ability of secondary dormancy is also similar. It can be seen that the feral rapeseeds have a relatively high degree of polymorphism, and differences between accessions of the same feral rapeseed population is large, which is consistent with the results of SSR genotyping. Furthermore, the dormancy rate of accessions was above 80% in the secondary dormancy, indicating that some feral rapeseed accessions have already showed wildness characteristics, suggesting that they may have been genetically altered since escaped.

Based on the above discussion, we can come to a point, that is the source of the existing feral rapeseed population in Japan, due to its high overall diversity and diversity within the populations, should be the escape of different varieties which were cultivated or imported as oilseed in the history. These feral rapeseeds have a large range of variation both in the phenotypic and molecular levels, indicating the potential as breeding materials. In addition, due to their high homozygosity, they are good materials for either selective breeding or cross breeding materials.

Chapter 4: General Discussion

Work on arrangement of genetic resources is organized throughout the history of exploitation of them. Researchers have collected wild relatives of crops, landraces and cultivars, and established a large number of genebanks. A good genebank should have a wide variety of diversity, and a core collection of it which could facilitate selection of breeding material. At the same time, the diversity study of feral populations will also help to discover new breeding materials. This kind of works have been down in many crops in many countries, but so far no studies have focused on Japanese rapeseed, although it still needs to be further improved to meet the different needs. This study is the first study on the diversity of genetic resources of Japanese rapeseed, including both genebank and feral resources, which would fill the gaps in this field and the achievements of this study will be an important background information for further breeding works.

In Chapter 2, my study revealed the genetic structure of the NARO rapeseed collection and genetic relationships among accessions on the basis of single-locus SSR markers. Some accessions were genetically identical or closely related to each other. This information is important for decreasing redundancy in the collection, thereby reducing the management cost and avoiding unnecessary distribution of such accessions to breeders. Further integration of the data from other collections maintained in different countries will make it possible to exploit and preserve the whole rapeseed gene pool and to retain the largest number of allelic variants for genes controlling the most important agronomic traits in the NARO collection.

The candidate core collection constructed in the study can be used in further research such as genome-wide association studies to identify genomic regions controlling important agronomic traits. The phenotypic data recorded in the NARO rapeseed collection was not used in this study because they have been obtained by different investigators and methods under various environmental conditions. However,

I still use these data for a rough assessment. The results showed that the core collection established in this study has good represention, and the diversity was preserved in the case of reducing the number of germplasms. However, I have also found that some of the important traits in rapeseed production, such as seed production ability, number of effective pods per plant, number of seed per pod, were not recorded in the data base of NARO rapeseed genebank (Or only evaluated a few varieties). Therefore, in this study, I did not compare core collection and whole collection with these traits. Because the period and place of the investigation are different, and coupled with the lack of the data of some important traits, so I think on the basis of this study, it will be necessary to evaluate the phenotypes of all the accessions under the same environmental conditions, and integrate the data on genotypic and phenotypic diversity. This will make the core collection, which comprises accessions that are genetically diverse at both genotypic and phenotypic levels, available to breeders for enhancing the genetic potential of this crop. In addition, in view of the possibility to be used as a leaf vegetable in the future, I think the knowledge of other traits of rapeseed is also necessary. So far, because the rapeseed has been used mainly as oil crop, so there is not much research which focus on the performance of its vegetative production. Therefore, in the future, when assessing the phenotype of the core collection, the evaluation criteria of other *Brassica* vagetables (Especially which have close relationship with rapeseed, such as Komatsuna, etc.) could be referred and the establishment of suitable evaluation criteria for rapeseed could be set up.

Collect crop varieties with broad genetic background is one of the important work to build genebank. In the discussion of Chapter 2, I have mentioned that it could be considered that the introduction of rapeseed varieties from the emerging rapeseed product countries. Similar work has been carried out between China, Australia and India. A project funded by these three countries evaluated the genetic diversity, phylogenetic relationships, and heterosis of rapeseed varieties in the three countries, and it was sought to improve the production ability of their own rapeseed by introducing each other's rapeseed varieties with different characteristics (Oilseed *Brassica* improvement

in China, India and Australia. Project ID: CIM/1999/072). The report of the project pointed out that in the countries such as China and Australia, although the rapeseed production and breeding began with the introduction of European rapeseed varieties, but in the long history (China started in the 1930s, Australia started in the 1970s) of breeding, a lot of excellent varieties with a wide range of diversity were breed based on introducing varieties from other countries and crossing with native *Brassica* species. However, in NARO rapeseed genebank, there are very few varieties of these countries. I suggest that in the future, we could not only to expand the NARO rapeseed collection with rapeseed varieties of these countries, but also promote associated research with them to improve the quality of Japanese rapeseed varieties.

In Chapter 3, SSR markers were used to study the genetic diversity of 13 major feral rapeseed populations in Japan, and some individuals were selected from each population for phenotypic analysis to verify the existence and variability of the diversity. Since there is no natural distribution in history, current Japanese feral rapeseed has an obvious source, that is the escaped individuals from the cultivated varieties which once been introduced to Japan. It may also include the varieties that have only been introduced into Japan as oilseed but have not been cultivated in Japan. Besides, genetically modified rapeseed also belongs to one of them. Feral rapeseed has been tested for many years and a number of genetically modified rapeseed individuals have been identified, the source of which can be determined (Saji *et al.* 2005). However, in feral rapeseed populations, most of the individuals are non-transgenic and their origin is not clear (Saji *et al.* 2005).

In Chapter 3, the analysis of genetic diversity and population structure of feral rapeseed populations shows that they may come from different varieties and escaped in different periods. And the individuals of the same geographic population are genetically different, indicating that multiple cultivars were involved in the escape process at the same site. These results are further verified in the results of cultivation experiments. But for rapeseed, some other traits are important indicator of evaluation of germplasm

resources, including seed yield, oil content, erucic acid and glucosinolate content, disease and stress resistance and so on (Abbadi and Leckband 2011). In the present study, I just wanted to show the diversity of feral rapeseed resources and therefore did not assess these traits. In future studies, a comprehensive study of these important traits will help to better understand the diversity of feral rapeseed resources and identify valuable accessions. This work will be very important, because the SSR markers used in this study did not consider the linkage of traits with them, so what kind of interesting traits the feral rapeseed may have is still unknown. In addition, in the feral rapeseed distribution area, it has been found that many local residents pick up rapeseed flower stalks for eating. This shows that the current feral rapeseed has been accepted by the public to a certain extent. It is possibile to screen some elementary leafy rapeseed lines based on the precise test evaluation of them.

From the perspective of suitability as breeding material considerations, higher genetic diversity and high homozygosity of feral rapeseed resources have shown that they are having such potential. However, only the above results are insufficient to draw firm conclusions. Because they are descendants of the cultivars, if they are genetically identical to the cultivars, they will be negligible utility value. If there are individuals belong to feral rapeseed populations which are genetically different from the cultivars by natural selection or is not conserved by the NARO rapeseed genebank, then their utility value will be greatly increased. In order to answer this question, we need to compare the results of Chapter2 and Chapter 3 to explore the relationship between the two kind germplasm resources. It is worth mentioning that, in Chapter 3, the same SSR markers of Chapter 2 were used. This is not the most efficient way of studying genetic diversity, because the SSR markers which showed polymorphism in NARO rapeseed genebank do not necessarily have polymorphism in feral rapeseed. But it is necessary to do in order to make direct comparisons.

PCoA and cluster analysis based on total NARO rapeseed genebank and feral rapeseed were summarized in Fig. 3.11 and Fig. 3.12. Lots of the feral accessions are

distributing next to the NARO rapeseed genebank accessions, indicating that they have a close relationship. On the other hand, it can be seen that there are some feral accessions distributed on subclusters which without cultivars and landraces. This suggests that these accessions are genetically distant from accessions present in the NARO rapeseed genebank. This difference may come from the germplasm not included in the genebank, and also may come from genetic variation of early escaped individuals in the long-term field survival. Previous studies on feral rapeseed in England, Germany and Austria also reached similar conclusions (Bond *et al.* 2004, Elling *et al.* 2009, Pascher *et al.* 2010). It is noteworthy that in their study, the number of cultivars involved in the comparison was 13, 5, and 19, respectively, whereas the number in this study was 582. In contrast to large numbers of cultivars, feral rapeseed still has some accessions diverge from them, suggesting that they are likely to be strongly influenced by selection pressure. The presence of specific alleles also provides support for this conjecture. The NJ tree based on grouping the feral, Japan and Overseas accessions shows a slightly distant kinship between feral and Japan/Overseas accessions, which tells us the uniqueness of the feral rapeseed accessions from the other side.

This is an important finding. In the previous thinking, the Japanese feral rapeseed was considered to have low diversity, and should very similar with NARO rapeseed genebank accessions, because it is not a native crop in Japan and is distributing nearby the rapeseed import ports. But this study gives a completely different result. The long-time existing of these feral populations suggests that they have adapted to the Japanese environment, and moreover, they are genetically distant from the existing germplasm, so that in the future breeding work, the feral accessions which have a far genetic distance to the genebank germplasms can be incorporated into the parental candidate.

Although there is no cultivation of GM rapeseed in Japan, but some GM rapeseed has been found to be distributed in the feral rapeseed distribution area (Katsbuta et al. 2015). These GM rapeseeds are the same specie with non-GM feral rapeseed, so they can be crossed to produce fertile offspring. Therefore, in the GM rapeseed population,

in addition to the ones directly came from the import GM rapeseed, there may be other ones came from the hybridisation with local non-GM feral rapeseeds. Finding out the genetic background of GM rapeseed could help assess the risk of environmental impact of GM rapeseed. The genetic diversity data of feral rapeseed and single-locus SSR markers obtained in this study could be applied to such assessments.

In summary, the results of this study show that rapeseed has a considerable genetic diversity in the whole NARO rapeseed genebank and in different geographical feral rapeseed populations for the first time. They could provide a good germplasm base for the future breeding works. A core collection of the former was constructed to facilitate the selection of breeding parents, and after the necessary phenotypic characterization studies, feral rapeseed can also be added to the genebank to enrich its germplasm diversity. The single-locus SSR markers found by this study could also be used in future studies of rapeseed such as the genetic diversity study of Chinese and Austrain rapeseed varieties, or the GM rapeseeds in Japan.

Summary

Brassica napus L., or commonly known as rapeseed, oilseed rape (and, in the case of one particular group of cultivars, canola), is one of the most economically important oilseed crops over the world. Over 450 million tons of rapeseed seeds are harvested worldwide every year and accounts for about 20% of the world grain production. Japan is not the original home of rapeseed, the existing rapeseed in Japan was introduced into two areas during the Meiji period (19th century). Rapeseed showed far more than Japanese traditional oil crop "Aburana" (*B. rapa*) production capacity and was promoted quickly. Japanese government started a formal breeding program in 1930s to improve rapeseed which was expected to be used as a means of developing the rural economy. Until now, more than 200 rapeseed varieties were breed out in Japan. In order to better promote breeding work, NARO (National Agriculture and Food Research Organization) collected a large number of Japanese rapeseed varieties and many foreign varieties. In addition, there are stable feral rapeseed populations in various locations of Japan, which constitute another important component of Japanese rapeseed resources. Their source is unknown, and lack of genetic background information, so it cannot determine whether they have utility value.

Knowledge of the genetic diversity and phylogenetic relationship among the germplasms is vital to efficiently use germplasm collections in breeding. In addition, establishing a representative subset of the whole collection, called a core collection, will be a feasible way for management, evaluation, and utilization of genetic resources. But until now, there is no genetic diversity study of Japanese rapeseed (genebank and feral resource). Therefore, the objective of this study is to assess the genetic diversity of the rapeseed genetic resources in Japan, to explore their situation and potential for further exploitation for breeding efforts via: 1) analysis the genetic diversity and genetic structure of NARO genebank rapeseed resources by SSR markers, and construct a core collection based on the polymorphic allele information to facilitate the future breeding

work; 2) analysis the feral rapeseed resources collected from 13 locations of Japan by SSR marker to reveal their genetic diversity and structure to reveal the possible formation way of them, and preform a comparison of the genebank resources and feral resources to understand whether there is similarity between them.

In the study of NARO rapeseed genebank, 582 accessions were genotyped with 30 SSR markers covering all 19 rapeseed chromosomes. These markers amplified 311 alleles (10.37 alleles per marker; range, 3–39). Analysis of molecular variance indicated significant genetic differentiation between Japanese and overseas accessions, which is similar with the result of STRUCTURE analysis. The genetic diversity of Japanese accessions was lower than that of overseas accessions. Small but significant differences were found among geographical groups, and genetic differentiation tended to increase with geographical distance. The core collection constructed comprises 96 accessions of diverse origin. It represents the whole collection well and thus it may be useful for rapeseed genetic research and breeding programs. The core collection improves the efficiency of management, evaluation, and utilization of genetic resources.

The genetic diversity study of the feral rapeseed sampled in 13 locations in Japan was performed with the same 30 SSR markers. These markers amplified 311 alleles (10.37 alleles per marker; range, 2–36). Genetic diversity indices showed that these feral populations have relatively high genetic diversity. Mantel test showed that there was no correlation between genetic distance and geographic distance between populations. Similar results can be seen from the STRUCTURE, PCoA and cluster analysis. These analyzes also showed that the genetic structure of a population is usually complex, indicated the multiple source of the feral rapeseed. Low heterozygosity and the Hardy-Weinberg equilibrium analysis showed that these populations reproduce mainly by selfing. The cultivation experiment results revealed that feral rapeseed had different phenotypes. These finding suggest that Japanese feral rapeseed has the potential to be used as breeding material.

A comprehensive comparison is carried out by combing the SSR genotype data.

Some unique alleles of the feral population were found to occupy 18.32% of the total number of alleles. Based on the PCoA and cluster analysis, some feral rapeseeds that was genetically far away from NARO rapeseed genebank cultivars was found. They may come from early cultivars that have not been collected and/or cultivars that have changed in the course of adapting to the natural environment of Japan. These results further confirmed the potential value of feral rapeseed.

I have successfully accessed the genetic diversity of rapeseed in Japan, mainly focus on NARO rapeseed genebank and representative feral accessions. A core collection of the NARO rapeseed genebank also be constructed. Based on these work, the basic panorama of genetic diversity of Japan rapeseed resources has just been drawn. This valuable information could provide a great help for future rapeseed breeding.

Acknowledgments

First, I would like to express my sincere gratitude to Professor Dr. Ryo Ohsawa, my supervisor, who provided an invaluable opportunity to study in Plant Breeding Laboratory of the University of Tsukuba, and who provided continues support and advising with patience and kindness. My thanks are also extended to Assistant Professor Dr. Yosuke Yoshioka of Plant Breeding Laboratory of the University of Tsukuba for his careful, rigorous guidance and encouragement during my PhD program. I also deeply appreciate the guidance of Assistant Professor Dr. Takashi Hara of Plant Breeding Laboratory of the University of Tsukuba who helped me a lot in the molecular experiment. And I also would like to express my thanks to Associate Professor Dr. Ayako Shimono of the Plant Ecology Laboratory of TOHO University for the extraordinary efforts of the sampling work.

I am also want to express my gratitude to Professor Dr. Hiroshi Ezura of the Laboratory of Olericulture & Floriculture of the University of Tsukuba, and Professor Dr. Chikako Kiribuchi-Otobe of the Division of Wheat and Barley Research Institute of Crop Science, NARO for reading and providing me with valuable comments on my thesis.

Agriculture and Forestry Research Center of University of Tsukuba provided the field for cultivation experiments. The center staff provided me with help in farmland finishing, I am extremely grateful for their help. The same thanks also to NARO's staff, who provided genebank samples for this study. I also thank for the help from lab staff Noriko Nishi and other lab members during my study in Tsukuba, they helped me a lot not only about the experiment but also the understanding of Japanese culture. My thanks also to those contributed in any way to this work but are not mentioned here due to the space limitation.

Finally, I would like to thank my parents and my wife, they broke through the secular prejudices, decided to support me studying in Japan. They are the spiritual support of me.

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Appendix

Appendix 1. The list of 582 accessions used in this study.

Sample ID in this study	品種・系統名	Variety name	JP No.	Origin 1	Origin 2	Cultivar/Landrace	Corecollection
NR001	芯切菜	SHIN KIRINA	25942	Japan	Kantō	Landrace	Included
NR002	芯切菜	SHIN KIRINA	25951	Japan	Kantō	Landrace	Included
NR003	高系 10-1 号	KOUKEI 10-1	26139	Japan	Chūbu	Cultivar	
NR004	高系 11 号	KOUKEI 11	26140	Japan	Chūbu	Cultivar	Included
NR005	つみ菜	TSUMINA	26141	Japan	Japan Unknow	Landrace	
NR006	樺太	KARAFUTO	26142	Overseas	Europe		
NR007	岩内種	IWANAISHU	26143	Japan	Hokkaido	Landrace	
NR008	ハンブルグ 1 号	HAMBURG 1	26144	Overseas	Europe		Included
NR009		MR 1	26145	Japan	Hokkaido	Landrace	
NR010	タイセツナタネ	TAISETSUNATANE	26146	Japan	Hokkaido	Cultivar	
NR011	なたね農林 2 号	NATANE NOURIN 2	26147	Japan	Tōhoku	Cultivar	
NR012	なたね農林 5 号	NATANE NOURIN 5	26148	Japan	Tōhoku	Cultivar	
NR013	なたね農林 10 号	NATANE NOURIN 10	26149	Japan	Tōhoku	Cultivar	
NR014	東北 3 号	TOUHOKU 3	26150	Japan	Tōhoku	Cultivar	
NR015	東北 4 号	TOUHOKU 4	26151	Japan	Tōhoku	Cultivar	
NR016	東北 5 号	TOUHOKU 5	26152	Japan	Tōhoku	Cultivar	
NR017	東北 7 号	TOUHOKU 7	26153	Japan	Tōhoku	Cultivar	
NR018	東北 8 号	TOUHOKU 8	26154	Japan	Tōhoku	Cultivar	
NR019	東北 9 号	TOUHOKU 9	26155	Japan	Tōhoku	Cultivar	
NR020	東北 10 号	TOUHOKU 10	26156	Japan	Tōhoku	Cultivar	
NR021	東北 12 号	TOUHOKU 12	26157	Japan	Tōhoku	Cultivar	Included
NR022	東北 14 号	TOUHOKU 14	26158	Japan	Tōhoku	Cultivar	
NR023	東北 16 号	TOUHOKU 16	26159	Japan	Tōhoku	Cultivar	
NR024	東北 19 号	TOUHOKU 19	26160	Japan	Tōhoku	Cultivar	
NR025	東北 22 号	TOUHOKU 22	26161	Japan	Tōhoku	Cultivar	
NR026	東北 23 号	TOUHOKU 23	26162	Japan	Tōhoku	Cultivar	
NR027	東北 24 号	TOUHOKU 24	26163	Japan	Tōhoku	Cultivar	Included
NR028	東北 28 号	TOUHOKU 28	26165	Japan	Tōhoku	Cultivar	
NR029	東北 36 号	TOUHOKU 36	26169	Japan	Tōhoku	Cultivar	
NR030	東北 42 号	TOUHOKU 42	26173	Japan	Tōhoku	Cultivar	
NR031	東北 46 号	TOUHOKU 46	26177	Japan	Tōhoku	Cultivar	
NR032	東北 48 号	TOUHOKU 48	26178	Japan	Tōhoku	Cultivar	

Apendix1　continue

Sample ID in this study	品種・系統名	Variety name	JP No.	Origin 1	Origin 2	Cultivar/Landrace	Corecollection
NR033	東北 49 号	TOUHOKU 49	26179	Japan	Tōhoku	Cultivar	
NR034	東北 50 号	TOUHOKU 50	26180	Japan	Tōhoku	Cultivar	
NR035	東北 56 号	TOUHOKU 56	26181	Japan	Tōhoku	Cultivar	
NR036	東北 58 号	TOUHOKU 58	26183	Japan	Tōhoku	Cultivar	
NR037	東北 61 号	TOUHOKU 61	26184	Japan	Tōhoku	Cultivar	
NR038	ミホナタネ	MIHONATANE	26185	Japan	Tōhoku	Cultivar	
NR039	東北 6 号	TOUHOKU 6	26186	Japan	Tōhoku	Cultivar	
NR040	東北 11 号	TOUHOKU 11	26187	Japan	Tōhoku	Cultivar	
NR041	東北 15 号	TOUHOKU 15	26188	Japan	Tōhoku	Cultivar	
NR042	東北 18 号	TOUHOKU 18	26189	Japan	Tōhoku	Cultivar	
NR043	東北 20 号	TOUHOKU 20	26190	Japan	Tōhoku	Cultivar	
NR044	東北 30 号	TOUHOKU 30	26193	Japan	Tōhoku	Cultivar	
NR045	札幌	SAPPORO	26194	Japan	Tōhoku	Landrace	
NR046	北海道種	HOKKAIDOUSHU	26195	Japan	Hokkaido	Landrace	
NR047	遠州黒種	ENSHUU KURODANE	26196	Japan	Tōhoku	Landrace	Included
NR048	東山種	TOUSANSHU	26197	Japan	Chūbu	Landrace	
NR049	宮城晩生	MIYAGI BANSEI	26198	Japan	Tōhoku	Landrace	
NR050	蟹	KANI	26199	Japan	Tōhoku	Landrace	
NR051	蕪菜	KABURANA	26200	Japan	Tōhoku	Landrace	
NR052	ムツナタネ	MUTSUNATANE	26203	Japan	Tōhoku	Cultivar	Included
NR053	東北 64 号	TOUHOKU 64	26204	Japan	Tōhoku	Cultivar	
NR054	バンダイナタネ	BANDAINATANE	26205	Japan	Kinki	Cultivar	Included
NR055	なたね農林 4 号	NATANE NOURIN 4	26206	Japan	Chūbu	Cultivar	
NR056	なたね農林 7 号	NATANE NOURIN 7	26207	Japan	Chūbu	Cultivar	
NR057	なたね農林 8 号	NATANE NOURIN 8	26208	Japan	Chūbu	Cultivar	
NR058	北陸 7 号	HOKURIKU 7	26209	Japan	Chūbu	Cultivar	
NR059	北陸 13 号	HOKURIKU 13	26210	Japan	Chūbu	Cultivar	
NR060	北陸 19 号	HOKURIKU 19	26211	Japan	Chūbu	Cultivar	Included
NR061	北陸 23 号	HOKURIKU 23	26212	Japan	Chūbu	Cultivar	
NR062	スエヒロナタネ	SUEHIRONATANE	26214	Japan	Kinki	Cultivar	Included
NR063		HANBAAKU(HAMBURG)	26215	Overseas	Europe		
NR064	なたね農林 13 号	NATANE NOURIN 13	26216	Japan	Chūbu	Cultivar	
NR065	北陸 25 号	HOKURIKU 25	26217	Japan	Chūbu	Cultivar	
NR066	Landrace 朝鮮	ZAIRAI CHOUSEN	26218	Overseas	Asia		
NR067	鴻分 2 号	KOUBUN 2	26219	Japan	Kantō	Cultivar	
NR068	鴻分 3 号	KOUBUN 3	26220	Japan	Kantō	Cultivar	
NR069	鴻分 7 号	KOUBUN 7	26221	Japan	Kantō	Cultivar	
NR070	鴻分 17 号	KOUBUN 17	26222	Japan	Kantō	Cultivar	
NR071	遠州	ENSHUU	26223	Japan	Kantō	Landrace	

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Sample ID in this study	品種・系統名	Variety name	JP No.	Origin 1	Origin 2	Cultivar/Landrace	Corecollection
NR072	晩生菜	BANSEINA	26224	Japan	Kantō	Landrace	
NR073	四石晩	YONKOKU OKU	26225	Japan	Kantō	Landrace	
NR074	ドワフエセックス	DWARF ESSEX	26226	Overseas	Exotic Unknow		
NR075	Cultivar ナプス	IKUSEI NAPUS	26227	Japan	Kantō	Landrace	
NR076	遠州 3 号	ENSHUU 3	26228	Japan	Kantō	Landrace	
NR077	吾妻種	AZUMASHU	26229	Japan	Japan Unknow	Landrace	
NR078	苔	UNDAI	26230	Japan	Kantō	Landrace	
NR079	晩生菜種	OKUTE NATANE	26231	Japan	Kantō	Landrace	
NR080	晩菜	OKUNA	26232	Japan	Chūbu	Landrace	
NR081	野中 1 号	NONAKA 1	26233	Japan	Kantō	Landrace	
NR082	中野種（矮性）	NAKANOSHU(DWARF TYPE)	26234	Japan	Kantō	Landrace	
NR083	水原種	MIZUHARASHU	26235	Japan	Kantō	Landrace	
NR084	吾妻（淡黄色）	AZUMA(LIGHT YELLOW)	26236	Japan	Kantō	Landrace	
NR085	吾妻（黄色）	AZUMA(YELLOW)	26237	Japan	Kantō	Landrace	
NR086	吾妻（肉色）	AZUMA(SALMON PINK)	26238	Japan	Kantō	Landrace	Included
NR087		INTEGRIFORIA	26239	Overseas	Exotic Unknow		
NR088		WAX-LESS MUTANT	26240	Japan	Kyushu	Cultivar	
NR089	普通朝鮮	FUTSUU CHOUSEN	26241	Overseas	Asia		
NR090		B.NAPUS,RAPIFERA	26242	Overseas	Exotic Unknow		
NR091	なたね農林 1 号	NATANE NOURIN 1	26243	Japan	Kyushu	Cultivar	
NR092	なたね農林 3 号	NATANE NOURIN 3	26244	Japan	Kyushu	Cultivar	
NR093	なたね農林 6 号	NATANE NOURIN 6	26245	Japan	Kinki	Cultivar	
NR094	なたね農林 9 号	NATANE NOURIN 9	26246	Japan	Kinki	Cultivar	
NR095	なたね農林 11 号	NATANE NOURIN 11	26247	Japan	Kyushu	Cultivar	
NR096	なたね農林 12 号	NATANE NOURIN 12	26248	Japan	Kinki	Cultivar	
NR097	なたね農林 14 号	NATANE NOURIN 14	26249	Japan	Kyushu	Cultivar	
NR098	なたね農林 15 号	NATANE NOURIN 15	26250	Japan	Kyushu	Cultivar	
NR099	なたね農林 16 号	NATANE NOURIN 16	26251	Japan	Tōhoku	Cultivar	
NR100	なたね農林 18 号	NATANE NOURIN 18	26253	Japan	Tōhoku	Cultivar	
NR101	ムラサキナタネ	MURASAKINATANE	26254	Japan	Chūbu	Cultivar	
NR102	ミチノクナタネ	MICHINOKUNATANE	26255	Japan	Tōhoku	Cultivar	
NR103	イスズナタネ	ISUZUNATANE	26257	Japan	Kinki	Cultivar	
NR104	チサヤナタネ	CHISAYANATANE	26258	Japan	Tōhoku	Cultivar	
NR105	アブラマサリ	ABURAMASARI	26259	Japan	Kyushu	Cultivar	Included
NR106	ホウマンナタネ	HOUMANNATANE	26260	Japan	Kyushu	Cultivar	
NR107	ミユキナタネ	MIYUKINATANE	26261	Japan	Tōhoku	Cultivar	
NR108	アサヒナタネ	ASAHINATANE	26263	Japan	Tōhoku	Cultivar	
NR109	コンゴウナタネ	KONGOUNATANE	26266	Japan	Kinki	Cultivar	
NR110	ハヤナタネ	HAYANATANE	26267	Japan	Kyushu	Cultivar	

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Sample ID in this study	品種・系統名	Variety name	JP No.	Origin 1	Origin 2	Cultivar/Landrace	Corecollection
NR111	オオミナタネ	OOMINATANE	26268	Japan	Kinki	Cultivar	
NR112	ゲンカイナタネ	GENKAINATANE	26269	Japan	Kyushu	Cultivar	
NR113	近畿 9 号	KINKI 9	26270	Japan	Kinki	Cultivar	
NR114	近畿 17 号	KINKI 17	26271	Japan	Kinki	Cultivar	
NR115	近畿 19 号	KINKI 19	26273	Japan	Kinki	Cultivar	
NR116	近畿 20 号	KINKI 20	26274	Japan	Kinki	Cultivar	
NR117	近畿 29 号	KINKI 29	26278	Japan	Kinki	Cultivar	
NR118	近畿 30 号	KINKI 30	26279	Japan	Kinki	Cultivar	
NR119	近畿 31 号	KINKI 31	26280	Japan	Kinki	Cultivar	
NR120	近畿 32 号	KINKI 32	26281	Japan	Kinki	Cultivar	
NR121	北陸 3 号	HOKURIKU 3	26282	Japan	Chūbu	Cultivar	
NR122	北陸 4 号	HOKURIKU 4	26283	Japan	Chūbu	Cultivar	
NR123	北陸 9 号	HOKURIKU 9	26284	Japan	Chūbu	Cultivar	
NR124	北陸 12 号	HOKURIKU 12	26285	Japan	Chūbu	Cultivar	
NR125	北陸 14 号	HOKURIKU 14	26286	Japan	Chūbu	Cultivar	
NR126	北陸 15 号	HOKURIKU 15	26287	Japan	Chūbu	Cultivar	
NR127	北陸 16 号	HOKURIKU 16	26288	Japan	Chūbu	Cultivar	Included
NR128	北陸 17 号	HOKURIKU 17	26289	Japan	Chūbu	Cultivar	
NR129	北陸 18 号	HOKURIKU 18	26290	Japan	Chūbu	Cultivar	
NR130	北陸 20 号	HOKURIKU 20	26291	Japan	Chūbu	Cultivar	
NR131	東海 1 号	TOUKAI 1	26295	Japan	Kinki	Cultivar	
NR132	東海 3 号	TOUKAI 3	26296	Japan	Kinki	Cultivar	
NR133	東海 4 号	TOUKAI 4	26297	Japan	Kinki	Cultivar	
NR134	東海 9 号	TOUKAI 9	26300	Japan	Kinki	Cultivar	
NR135	東海 11 号	TOUKAI 11	26302	Japan	Kinki	Cultivar	
NR136	東海 12 号	TOUKAI 12	26303	Japan	Kinki	Cultivar	
NR137	東海 14 号	TOUKAI 14	26304	Japan	Kinki	Cultivar	
NR138	東海 16 号	TOUKAI 16	26306	Japan	Kinki	Cultivar	
NR139	東分 3 号（W）	TOUBUN 3(W)	26311	Japan	Kinki	Cultivar	
NR140	東分 3 号（R）	TOUBUN 3(R)	26312	Japan	Kinki	Cultivar	
NR141	東分 4 号	TOUBUN 4	26313	Japan	Kinki	Cultivar	
NR142	東分 5 号	TOUBUN 5	26314	Japan	Kinki	Cultivar	
NR143	東分 6 号	TOUBUN 6	26315	Japan	Kinki	Cultivar	
NR144	東分 7 号	TOUBUN 7	26316	Japan	Kinki	Cultivar	
NR145		GANMAA M(40)2-3-1-1	26320	Japan	Kinki	Cultivar	Included
NR146		GANMAA M(80)4-1-2-1	26321	Japan	Kinki	Cultivar	
NR147		4X 8-1-1	26322	Japan	Kinki	Cultivar	
NR148		4X 20-1-1	26323	Japan	Kinki	Cultivar	
NR149	合成 5 号	GOUSEI 5	26324	Japan	Kinki	Cultivar	

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Sample ID in this study	品種・系統名	Variety name	JP No.	Origin 1	Origin 2	Cultivar/Landrace	Corecollection
NR150	早生朝鮮	WASE CHOUSEN	26325	Overseas	Asia		
NR151	早生朝鮮 2 号	WASE CHOUSEN 2	26326	Overseas	Asia		
NR152	中生朝鮮	NAKATE CHOUSEN	26327	Overseas	Asia		
NR153	早生村松	WASE MURAMATSU	26328	Japan	Kantō	Landrace	
NR154	四日市黒種	YOKKAICHI KURODANE	26329	Japan	Kinki	Landrace	
NR155	三重黒種	MIE KURODANE	26330	Japan	Kinki	Landrace	
NR156	伊勢黒種	ISE KURODANE	26331	Japan	Kinki	Landrace	
NR157	伊勢黒種 1 号	ISE KURODANE 1	26332	Japan	Kinki	Landrace	
NR158	伊勢黒	ISEGURO	26333	Japan	Kinki	Landrace	
NR159	水原種	SUIGENSHU	26334	Overseas	Asia		Included
NR160	吾妻	AZUMA	26335	Japan	Japan Unknow	Landrace	
NR161	京都吾妻	KYOUTO AZUMA	26336	Japan	Kinki	Landrace	
NR162	小型吾妻	KOGATA AZUMA	26337	Japan	Kinki	Landrace	
NR163	吾妻種	AZUMASHU	26338	Japan	Kinki	Landrace	
NR164	吾妻種	AZUMASHU	26339	Japan	Kinki	Landrace	Included
NR165	吾妻種	AZUMASHU	26340	Japan	Kinki	Landrace	
NR166	吾妻中生	AZUMA NAKATE	26341	Japan	Chūgoku-Shikoku	Landrace	
NR167	早生不二種	WASE FUJISHU	26342	Japan	Chūbu	Landrace	
NR168	不二種	FUJISHU	26343	Japan	Chūbu	Landrace	Included
NR169	不二	FUJI	26344	Japan	Chūbu	Landrace	
NR170	早生不二	WASE FUJI	26345	Japan	Chūbu	Landrace	
NR171	Landrace 朝鮮種	ZAIRAI CHOUSENSHU	26346	Overseas	Asia		
NR172	朝鮮種	CHOUSENSHU	26347	Overseas	Asia		
NR173	大朝鮮	DAICHOUSEN	26348	Overseas	Asia		
NR174	大朝鮮 33 号	DAICHOUSEN 33	26349	Overseas	Asia		Included
NR175	糟屋種	KASUYASHU	26350	Japan	Kyushu	Landrace	
NR176	唐種	TOUDANE	26351	Japan	Kyushu	Landrace	
NR177	村山種	MURAYAMASHU	26353	Japan	Kyushu	Landrace	Included
NR178	筑紫種	CHIKUSHISHU	26354	Japan	Kyushu	Landrace	
NR179	筑紫	CHIKUSHI	26355	Japan	Kyushu	Landrace	
NR180	晩生	OKUTE	26356	Japan	Japan Unknow	Landrace	Included
NR181	晩生菜	OKUTENA	26357	Japan	Chūbu	Landrace	
NR182	青森 1 号	AOMORI 1	26358	Japan	Tōhoku	Landrace	
NR183	宮城晩生	MIYAGI OKUTE	26359	Japan	Tōhoku	Landrace	
NR184	六ッ美早生	MUTSUMI WASE	26360	Japan	Chūbu	Landrace	
NR185	六ッ美晩生	MUTSUMI OKUTE	26361	Japan	Chūbu	Landrace	Included
NR186	六ッ美中生	MUTSUMI NAKATE	26362	Japan	Chūbu	Landrace	
NR187	中生六ッ美	NAKATE MUTSUMI	26363	Japan	Chūbu	Landrace	
NR188	六ッ美	MUTSUMI	26364	Japan	Chūbu	Landrace	

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Sample ID in this study	品種・系統名	Variety name	JP No.	Origin 1	Origin 2	Cultivar/Landrace	Corecollection
NR189	糸島種	ITOSHIMASHU	26365	Japan	Kantō	Landrace	Included
NR190	岩内	IWANAI	26366	Japan	Hokkaido	Landrace	
NR191	ハンブルグ	HAMBURG	26367	Overseas	Europe		
NR192	露 5 号	RO(USSR)5	26368	Overseas	Europe		
NR193	露 6 号	RO(USSR)6	26369	Overseas	Europe		
NR194	墺 3 号	OUAUSTRIA)3	26370	Overseas	Europe		
NR195	波 3 号	PO(POLAND)3	26371	Overseas	Europe		
NR196	独 1 号	DOKU 1(GERMANY 1)	26372	Overseas	Europe		
NR197	羅 1 号	RU 1(ROUMANIA 1)	26373	Overseas	Europe		Included
NR198	仏 1 号	FUTSU 1(FRANCE 1)	26374	Overseas	Europe		
NR199	仏 2 号	FUTSU 2(FRANCE 2)	26375	Overseas	Europe		
NR200	仏 3 号	FUTSU 3(FRANCE 3)	26376	Overseas	Europe		
NR201	仏 5 号	FUTSU 5(FRANCE 5)	26377	Overseas	Europe		
NR202	仏 7 号	FUTSU 7(FRANCE 7)	26379	Overseas	Europe		Included
NR203	仏 9 号	FUTSU 9(FRANCE 9)	26380	Overseas	Europe		
NR204	仏 10 号	FUTSU 10(FRANCE 10)	26381	Overseas	Europe		
NR205	仏 11 号	FUTSU 11(FRANCE 11)	26382	Overseas	Europe		
NR206		COLZA	26383	Overseas	Europe		Included
NR207		SVALOFSORIG	26384	Overseas	Exotic Unknow		
NR208		B.NAPUS,RAPIFERA	26385	Overseas	Exotic Unknow		Included
NR209		B.NAPUS,OLEIFERA	26386	Overseas	Europe		
NR210		NABO	26387	Overseas	America		
NR211	長州黒種	CHOUSHUU KURODANE	26388	Japan	Chūgoku-Shikoku	Landrace	
NR212	四石晩	YONKOKUBAN	26389	Japan	Japan Unknow	Landrace	Included
NR213	早生黒 8 号	WASEGURO 8	26390	Japan	Kinki	Landrace	
NR214	伊勢黒種 125 号	ISE KURODANE 125	26391	Japan	Kinki	Landrace	Included
NR215	晩菜	BANSAI	26392	Japan	Chūbu	Landrace	
NR216	吾妻種	AZUMASHU	26393	Japan	Kinki	Landrace	
NR217	吾妻種	AZUMASHU	26394	Japan	Kinki	Landrace	
NR218	吾妻 1 号	AZUMA 1	26395	Japan	Kinki	Landrace	
NR219	新不二	SHIN FUJI	26396	Japan	Kinki	Landrace	
NR220	中生不二	NAKATE FUJI	26397	Japan	Kantō	Landrace	
NR221	糟屋選種	KASUYA SENSHU	26398	Japan	Chūgoku-Shikoku	Landrace	
NR222	糟屋	KASUYA	26399	Japan	Chūgoku-Shikoku	Landrace	
NR223	小朝鮮	KOCHOUSEN	26400	Overseas	Asia		
NR224	大朝鮮（福）	DAICHOUSEN(FUKU)	26401	Overseas	Asia		
NR225	大朝鮮 1 号	DAICHOUSEN 1	26402	Overseas	Asia		
NR226	大朝鮮 2 号	DAICHOUSEN 2	26403	Overseas	Asia		Included
NR227	雑種	ZASSHU	26404	Japan	Tōhoku	Landrace	Included
NR228	青柳 1 号	AOYAGI 1	26405	Japan	Kyushu	Cultivar	

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Sample ID in this study	品種・系統名	Variety name	JP No.	Origin 1	Origin 2	Cultivar/Landrace	Corecollection
NR229	九大新 2 号	KYUUDAI SHIN 2	26406	Japan	Kyushu	Landrace	
NR230	福岡朝鮮 1 号	FUKUOKA CHOUSEN 1	26407	Japan	Kyushu	Landrace	
NR231	波 4 号	PO(POLAND)4	26408	Overseas	Europe		
NR232	波 5 号	PO(POLAND)5	26409	Overseas	Europe		
NR233	佛 4 号	FUTSU 4(FRANCE 4)	26410	Overseas	Europe		
NR234	佛 8 号	FUTSU 8(FRANCE 8)	26411	Overseas	Europe		
NR235	仏 12 号	FUTSU 12(FRANCE 12)	26412	Overseas	Europe		
NR236	西洋種	SEIYOUSHU	26413	Overseas	Exotic Unknow		
NR237	波 1 号	PO(POLAND)1	26414	Overseas	Europe		
NR238	中野種（矮性）	NAKANOSHU(DWARF)	26415	Japan	Kantō	Landrace	
NR239	水原種（帯化性）	SUIGENSHU(FASCIATION)	26416	Overseas	Asia		Included
NR240	吾妻種	AZUMASHU	26417	Japan	Kinki	Landrace	Included
NR241	一色 2 号	ISSHIKI 2	26418	Japan	Chūbu	Landrace	Included
NR242	吉田種	YOSHIDASHU	26419	Japan	Chūbu	Landrace	
NR243		C.O.	26420	Japan	Kantō	Landrace	Included
NR244	鴻種系 1409	KOUSHUKEI 1409	26421	Japan	Kantō	Landrace	
NR245	近畿早生	KINKI WASE	26422	Japan	Kinki	Landrace	
NR246	福本一	FUKUMOTO 1	26423	Japan	Japan Unknow	Landrace	
NR247		SPRING RAPE REGINA	26424	Overseas	Europe		
NR248		SPRING RAPE GOLDEN ARGENTINE	26425	Overseas	Europe		
NR249		WINTER RAPE MATADOR	26426	Overseas	Europe		Included
NR250	東海 23 号	TOUKAI 23	26427	Japan	Kinki	Cultivar	
NR251	東海 27 号	TOUKAI 27	26428	Japan	Kinki	Cultivar	
NR252	水原	SUWEON	26429	Overseas	Asia		
NR253	吾妻種	AZUMASHU	26430	Japan	Kinki	Landrace	
NR254	近畿 26 号	KINKI 26	26431	Japan	Kinki	Cultivar	Included
NR255	近畿 27 号	KINKI 27	26432	Japan	Kinki	Landrace	
NR256	吾妻種	AZUMASHU	26433	Japan	Kinki	Landrace	
NR257	吾妻種	AZUMASHU	26434	Japan	Kinki	Landrace	
NR258	東海 21 号	TOUKAI 21	26436	Japan	Kinki	Cultivar	
NR259	東海 24 号	TOUKAI 24	26437	Japan	Kinki	Cultivar	
NR260	唐種	KARADANE	26438	Japan	Kyushu	Landrace	
NR261	九州 4 号	KYUUSHUU 4	26439	Japan	Kyushu	Cultivar	
NR262	九州 6 号	KYUUSHUU 6	26440	Japan	Kyushu	Cultivar	
NR263	九州 7 号	KYUUSHUU 7	26441	Japan	Kyushu	Cultivar	
NR264	九州 8 号	KYUUSHUU 8	26442	Japan	Kyushu	Cultivar	
NR265	九州 9 号	KYUUSHUU 9	26443	Japan	Kyushu	Cultivar	
NR266	九州 11 号	KYUUSHUU 11	26444	Japan	Kyushu	Cultivar	
NR267	九州 17 号	KYUUSHUU 17	26448	Japan	Kyushu	Cultivar	

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Sample ID in this study	品種・系統名	Variety name	JP No.	Origin 1	Origin 2	Cultivar/Landrace	Corecollection
NR268	九州 18 号	KYUUSHUU 18	26449	Japan	Kyushu	Cultivar	Included
NR269	九州 19 号	KYUUSHUU 19	26450	Japan	Kyushu	Cultivar	
NR270	九州 22 号	KYUUSHUU 22	26451	Japan	Kyushu	Cultivar	
NR271	九州 24 号	KYUUSHUU 24	26452	Japan	Kyushu	Cultivar	
NR272	九州 25 号	KYUUSHUU 25	26453	Japan	Kyushu	Cultivar	Included
NR273	九州 26 号	KYUUSHUU 26	26454	Japan	Kyushu	Cultivar	
NR274	九州 27 号	KYUUSHUU 27	26455	Japan	Kyushu	Cultivar	
NR275	九州 29 号	KYUUSHUU 29	26456	Japan	Kyushu	Cultivar	
NR276	九州 32 号	KYUUSHUU 32	26457	Japan	Kyushu	Cultivar	Included
NR277	九州 33 号	KYUUSHUU 33	26458	Japan	Kyushu	Cultivar	
NR278	九州 34 号	KYUUSHUU 34	26459	Japan	Kyushu	Cultivar	
NR279	九州 36 号	KYUUSHUU 36	26461	Japan	Kyushu	Cultivar	
NR280	九州 39 号	KYUUSHUU 39	26463	Japan	Kyushu	Cultivar	
NR281	九州 40 号	KYUUSHUU 40	26464	Japan	Kyushu	Cultivar	
NR282	九州 41 号	KYUUSHUU 41	26465	Japan	Kyushu	Cultivar	
NR283	九州 42 号	KYUUSHUU 42	26466	Japan	Kyushu	Cultivar	
NR284	九州 47 号	KYUUSHUU 47	26468	Japan	Kyushu	Cultivar	
NR285	九州 49 号	KYUUSHUU 49	26470	Japan	Kyushu	Cultivar	
NR286	九州 50 号	KYUUSHUU 50	26471	Japan	Kyushu	Cultivar	
NR287	九州 51 号	KYUUSHUU 51	26472	Japan	Kyushu	Cultivar	
NR288	九州 52 号	KYUUSHUU 52	26473	Japan	Kyushu	Cultivar	
NR289	九州 54 号	KYUUSHUU 54	26475	Japan	Kyushu	Cultivar	
NR290	九州 56 号	KYUUSHUU 56	26477	Japan	Kyushu	Cultivar	
NR291	九州 57 号	KYUUSHUU 57	26478	Japan	Kyushu	Cultivar	
NR292	東北 51 号	TOUHOKU 51	26481	Japan	Tōhoku	Cultivar	
NR293	東北 52 号	TOUHOKU 52	26482	Japan	Tōhoku	Cultivar	
NR294	東北 53 号	TOUHOKU 53	26483	Japan	Tōhoku	Cultivar	
NR295	東北 54 号	TOUHOKU 54	26484	Japan	Tōhoku	Cultivar	
NR296	東海 25 号	TOUKAI 25	26485	Japan	Kinki	Cultivar	
NR297	東海 26 号	TOUKAI 26	26486	Japan	Kinki	Cultivar	
NR298	南九州 5 号	MINAMIKYUUSHUU 5	26491	Japan	Kyushu	Cultivar	
NR299	南九州 7 号	MINAMIKYUUSHUU 7	26493	Japan	Kyushu	Cultivar	
NR300	南九州 8 号	MINAMIKYUUSHUU 8	26494	Japan	Kyushu	Cultivar	
NR301	吾妻種	AZUMASHU	26495	Japan	Kinki	Landrace	Included
NR302	吾妻種	AZUMASHU	26496	Japan	Kinki	Landrace	
NR303	吾妻種	AZUMASHU	26497	Japan	Kinki	Landrace	
NR304	吾妻種	AZUMASHU	26498	Japan	Kinki	Landrace	
NR305	吾妻種（28）	AZUMASHU(28)	26499	Japan	Kinki	Landrace	
NR306	吾妻種（31）	AZUMASHU(31)	26500	Japan	Kinki	Landrace	

Apendix1　continue

Sample ID in this study	品種・系統名	Variety name	JP No.	Origin 1	Origin 2	Cultivar/Landrace	Corecollection
NR307	大朝鮮（中野）	DAICHOUSEN(NAKANO)	26501	Overseas	Asia		
NR308	大朝鮮（光安）	DAICHOUSEN(MIZUYASU)	26502	Overseas	Asia		
NR309	大朝鮮（村山）	DAICHOUSEN(MURAYAMA)	26503	Overseas	Asia		
NR310	粕屋種 21 号	KASUYASHU 21	26504	Japan	Kyushu	Landrace	
NR311	唐種（50）	TOUDANE(50)	26505	Japan	Chūgoku-Shikoku	Landrace	
NR312	唐種（51）	TOUDANE(51)	26506	Japan	Chūgoku-Shikoku	Landrace	
NR313	唐種（52）	TOUDANE(52)	26507	Japan	Chūgoku-Shikoku	Landrace	Included
NR314	魚成 Landrace	UONARU ZAIRAI	26508	Japan	Chūgoku-Shikoku	Landrace	
NR315	近永 Landrace	CHIKANAGA ZAIRAI	26509	Japan	Chūgoku-Shikoku	Landrace	
NR316	Landrace 種（成妙）	ZAIRAISHU(NARITAE)	26510	Japan	Chūgoku-Shikoku	Landrace	
NR317	清満 Landrace	SEIMAN ZAIRAI	26511	Japan	Chūgoku-Shikoku	Landrace	
NR318	大川種	OOKAWASHU	26512	Japan	Kyushu	Landrace	
NR319	中野種（矮性）	NAKANOSHU(DWARF)	26514	Japan	Kantō	Landrace	
NR320		SVALOFS ORIG	26517	Overseas	Europe		
NR321	北海 2 号	HOKKAI 2	26518	Japan	Hokkaido	Landrace	
NR322		SV.REGINA II SUMMER RAPE	26519	Overseas	Europe		Included
NR323		N.Z.CALDER SWEDE	26521	Overseas	Oceania		Included
NR324		BROAD LEAF ESSEX RAPE	26522	Overseas	Oceania		
NR325		APHIS RESISTANT RAPE	26523	Overseas	Oceania		
NR326		N.Z.WYE SWEDE(CLUB ROOT RES)	26524	Overseas	Oceania		Included
NR327		HOSTRAPS VESTAL	26525	Overseas	Europe		
NR328		HOSTRAPS PLUS	26526	Overseas	Europe		
NR329		WINTER RAPE GIESSEN	26528	Overseas	Europe		Included
NR330		EXP.SYNTHETEC VAR C(HIGH OIL)	26529	Overseas	America		
NR331		GOLDEN	26531	Overseas	America		
NR332		RAPE UNIT 13 PLANT 5	26532	Overseas	Europe		
NR333		RAPE UNIT 13 PLANT 8	26533	Overseas	Europe		Included
NR334		RAPE UNIT 15 PLANT 1	26534	Overseas	Europe		
NR335		RAPE UNIT 15 PLANT 8	26535	Overseas	Europe		Included
NR336		SCHERWITZ WINTER RAPE	26536	Overseas	Europe		
NR337		WINTER RAPE WEIBULLS MARGO	26537	Overseas	Europe		
NR338		WINTER RAPE WEIBULLS 506	26538	Overseas	Europe		
NR339		JANETZKI WINTER RAPE	26539	Overseas	Europe		
NR340	南九州 9 号	MINAMIKYUUSHUU 9	26541	Japan	Kyushu	Cultivar	
NR341	南九州 15 号	MINAMIKYUUSHUU 15	26547	Japan	Kyushu	Cultivar	
NR342	チクゼンナタネ	CHIKUZENNATANE	26556	Japan	Kyushu	Cultivar	
NR343	ダイリュウナタネ	DAIRYUUNATANE	26557	Japan	Kyushu	Cultivar	
NR344	南九州 24 号	MINAMIKYUUSHUU 24	26558	Japan	Kyushu	Cultivar	

Apendix1　continue

Sample ID in this study	品種・系統名	Variety name	JP No.	Origin 1	Origin 2	Cultivar/Landrace	Corecollection
NR345	九州 61 号	KYUUSHUU 61	26561	Japan	Kyushu	Cultivar	
NR346	福系 1141-1	FUKUKEI 1141-1	26565	Japan	Kyushu	Landrace	
NR347	福系 1141-2	FUKUKEI 1141-2	26566	Japan	Kyushu	Landrace	
NR348	福系 1143-2-3	FUKUKEI 1143-2-3	26567	Japan	Kyushu	Landrace	
NR349	福系 1145-1-7	FUKUKEI 1145-1-7	26568	Japan	Kyushu	Landrace	
NR350		CUBS ROOT	26569	Overseas	Asia		
NR351		ORLE	26570	Overseas	Asia		
NR352		RAPORA	26571	Overseas	Asia		
NR353		YUDAL	26572	Overseas	Asia		
NR354	華 318	KA 318	26573	Overseas	Asia		
NR355	華 322	KA 322	26574	Overseas	Asia		
NR356	華 325	KA 325	26575	Overseas	Asia		
NR357	華 336	KA 336	26576	Overseas	Asia		
NR358		MARIS HAPLONA	26577	Overseas	Europe		Included
NR359		NILLA=1022	26578	Overseas	Europe		
NR360		NILLA(GLOSSY LEAF,RECESSIVE)	26579	Overseas	Europe		
NR361		ARTIFICIAL B.NAPUS I WINTER	26580	Overseas	Europe		
NR362		ARTIFICIAL B.NAPUS II WINTER	26581	Overseas	Europe		Included
NR363		WINTER RAPE REGAL 267/5	26582	Overseas	Europe		
NR364		WINTER RAPE MATADOR 267/3	26583	Overseas	Europe		
NR365		SVALOFS VICTORIA	26584	Overseas	Europe		
NR366		SVALOFS FENIX BANGHOLM	26585	Overseas	Europe		Included
NR367		HOST RAPE REGAL	26586	Overseas	Europe		
NR368		SVALOFS REGINA II	26587	Overseas	Europe		
NR369		REGAL	26588	Overseas	Europe		
NR370		GYLLE	26589	Overseas	Europe		
NR371		RIGO	26590	Overseas	Europe		
NR372		NORDE	26591	Overseas	Europe		Included
NR373		LINUS	26592	Overseas	Europe		Included
NR374		R JANUS	26593	Overseas	Europe		
NR375		R CRESUS	26595	Overseas	Europe		
NR376		SVALOFS GULLE	26596	Overseas	Europe		
NR377		GERMANY	26597	Overseas	Europe		
NR378		RAPOL	26598	Overseas	Europe		Included
NR379		LEMBKES	26599	Overseas	Europe		Included
NR380		FONTO	26600	Overseas	Europe		
NR381		GEBR.DIPPES	26601	Overseas	Europe		
NR382		JANETZKIS WEIHENSTEPHANER	26602	Overseas	Europe		
NR383		LIHO=PETRA	26603	Overseas	Europe		Included
NR384		SPATS ZOLLERNGOLD	26604	Overseas	Europe		Included
NR385		KOMET	26605	Overseas	Europe		
NR386		KRAPPHAUSER	26606	Overseas	Europe		

Apendix1　continue

Sample ID in this study	品種・系統名	Variety name	JP No.	Origin 1	Origin 2	Cultivar/Landrace	Corecollection
NR387		LEMBKES MALCHOWER	26607	Overseas	Europe		
NR388		QUEDLINBURGER PLATZFESTER	26608	Overseas	Europe		
NR389		OLQUELL	26609	Overseas	Europe		
NR390		MALI	26610	Overseas	Europe		Included
NR391		JANETZKIS SOMMERRAPS	26611	Overseas	Europe		
NR392		ERLE	26612	Overseas	Europe		
NR393		LIFURA	26613	Overseas	Europe		Included
NR394		LINOLA	26614	Overseas	Europe		
NR395		MATADOR	26615	Overseas	Europe		
NR396		OMI	26616	Overseas	Europe		
NR397		PETRANOVA=LIHONOVA	26617	Overseas	Europe		
NR398		ERGLU	26618	Overseas	Europe		
NR399		ARMANDER	26619	Overseas	Europe		
NR400		EXPANDER	26620	Overseas	Europe		
NR401		PANTER	26622	Overseas	Europe		
NR402		SALAMANDER	26623	Overseas	Europe		
NR403		SYNRA	26624	Overseas	Europe		
NR404		DIAMANT	26625	Overseas	Europe		
NR405		LENORA	26626	Overseas	Europe		
NR406		LESIRA	26627	Overseas	Europe		
NR407		POLNOSLASKI	26628	Overseas	Europe		Included
NR408		BRONOWSKI	26633	Overseas	Europe		
NR409		MLOCHOWSKI	26634	Overseas	Europe		Included
NR410		WIELKOPOSKI	26636	Overseas	Europe		
NR411		SZNESZOWICKI	26638	Overseas	Europe		
NR412		WARCZAWSKI	26639	Overseas	Europe		
NR413		BOROWSKI	26640	Overseas	Europe		
NR414		B.NAPUS OLEIFERA	26642	Overseas	Europe		
NR415		CESKA	26643	Overseas	Europe		
NR416		NUAPSKA	26644	Overseas	Europe		
NR417		TREBICSKA	26645	Overseas	Europe		
NR418		JARNI CESKA	26646	Overseas	Europe		
NR419		FERTODI	26647	Overseas	Europe		Included
NR420	羅 1 号	RUMANIA 1(RU 1)	26648	Overseas	Europe		
NR421		COLZA 18 MAROC	26649	Overseas	Europe		
NR422		CZ-YY MAROC	26650	Overseas	Europe		
NR423		TONUS	26651	Overseas	Europe		
NR424		CRESUS	26654	Overseas	Europe		
NR425		MAJOR	26655	Overseas	Europe		
NR426		TOWER	26656	Overseas	America		Included
NR427		ORO	26657	Overseas	America		
NR428		TARGET	26658	Overseas	America		
NR429		NUGGET	26659	Overseas	America		
NR430		TANKA	26660	Overseas	America		

Apendix1　continue

Sample ID in this study	品種・系統名	Variety name	JP No.	Origin 1	Origin 2	Cultivar/Landrace	Corecollection
NR431		MIDAS	26661	Overseas	America		
NR432		ZEPHYR	26663	Overseas	America		
NR433		TARGET	26664	Overseas	America		
NR434		CLUB ROOT RESISTANT RAPE	26665	Overseas	Oceania		
NR435	晩生つみな CO	BANSEI TSUMINA CO	26666	Japan	Kantō	Landrace	
NR436	川流れ	KAWA NAGARE	26667	Japan	Chūbu	Landrace	
NR437	宮内菜	MIYAUCHI NA	26668	Japan	Kantō	Landrace	
NR438		CO 4	26669	Japan	Kantō	Landrace	
NR439		BRIDGER	36289	Overseas	America		
NR440		CASCADE	36290	Overseas	America		
NR441		SHINKIRINA(KAKINA)	37340	Japan	Kantō	Landrace	
NR442	かぶれ菜	KABULENA	37341	Japan	Kantō	Landrace	Included
NR443		C O NA	37397	Japan	Kantō	Landrace	Included
NR444		JUNO	40676	Overseas	Europe		
NR445		SV 0212	40677	Overseas	Europe		
NR446		TOPAS	40678	Overseas	Europe		
NR447		GLOBAL	40679	Overseas	Europe		
NR448		EMIL	40680	Overseas	Europe		
NR449		HANNA	40681	Overseas	Europe		
NR450		OLIVIA	40682	Overseas	Europe		
NR451		OMEGA	40683	Overseas	Europe		
NR452		JUVEL	40684	Overseas	Europe		
NR453		KARAT	40685	Overseas	Europe		Included
NR454		NIKLAS	40686	Overseas	Europe		
NR455		GULLIVER	40687	Overseas	Europe		Included
NR456		DIPLOM	40688	Overseas	Europe		
NR457		LERGO	40689	Overseas	Europe		Included
NR458		ELVIRA	40690	Overseas	Europe		
NR459		BELINDA	40691	Overseas	Europe		
NR460		KORINA	40692	Overseas	Europe		
NR461		GUNDULA	40693	Overseas	Europe		
NR462		RIDANA	40694	Overseas	Europe		Included
NR463		TAMARA	40695	Overseas	Europe		
NR464		ELENA(00)	40696	Overseas	Europe		Included
NR465		SANTANA(00)	40697	Overseas	Europe		
NR466		ARABELLA(00)	40698	Overseas	Europe		
NR467		KURANDER	40699	Overseas	Europe		
NR468		MIRANDER	40700	Overseas	Europe		
NR469		PRIMOR	40701	Overseas	Europe		
NR470		ANJA	40702	Overseas	Europe		Included
NR471		MAJA	40703	Overseas	Europe		
NR472		EDITA	40704	Overseas	Europe		Included
NR473		NURA	40705	Overseas	Europe		Included
NR474		AKELA	40706	Overseas	Europe		

Apendix1　continue

Sample ID in this study	品種・系統名	Variety name	JP No.	Origin 1	Origin 2	Cultivar/Landrace	Corecollection
NR475		BARCOLI	40707	Overseas	Europe		
NR476		BISHOP	40708	Overseas	Europe		Included
NR477		CANARD	40709	Overseas	Europe		
NR478		EMERALD	40710	Overseas	Europe		
NR479		VIVA	40711	Overseas	Europe		
NR480		WINFRED	40712	Overseas	Europe		
NR481		DORAL	40713	Overseas	Europe		
NR482		GLORIA	40714	Overseas	Europe		
NR483		BINERA	40715	Overseas	Europe		
NR484		RUBIN	40716	Overseas	Europe		
NR485		CERES	40717	Overseas	Europe		
NR486		COBRA	40718	Overseas	Europe		
NR487		GARANT	40719	Overseas	Europe		
NR488		QUINTA	40720	Overseas	Europe		
NR489		ORBIS	40721	Overseas	Europe		
NR490		GERMANDER	40722	Overseas	Europe		
NR491		LIRAMA	40723	Overseas	Europe		
NR492		LINE	40724	Overseas	Europe		
NR493		OPTIMA	40725	Overseas	Europe		
NR494		RALLY	40726	Overseas	Europe		
NR495		LORAS	40727	Overseas	Europe		
NR496		LISANDRA	40728	Overseas	Europe		
NR497		BRUTOR	40729	Overseas	Europe		
NR498		DARMOR	40730	Overseas	Europe		
NR499		ALTEX	40731	Overseas	America		
NR500		ANDOR	40732	Overseas	America		
NR501		REGENT	40733	Overseas	America		
NR502		WESTAR	40734	Overseas	America		
NR503		ATR RESISTANT TOWER	40735	Overseas	America		Included
NR504	ネムロルタバカ	NEMURO RUTABAKA	41101	Japan	Hokkaido	Cultivar	Included
NR505	マジェスティク１号	MAGESTIC 1	41102	Overseas	Europe		
NR506	グリーントップ	GREEN TOP	41103	Overseas	Exotic Unknow		
NR507		N-501	46068	Japan	Kantō	Landrace	
NR508		C-463	46069	Overseas	Europe		
NR509		N-261	46070	Overseas	Europe		
NR510		N-346	46071	Overseas	America		
NR511		N-349	46072	Overseas	America		
NR512		N-404	46073	Overseas	America		Included
NR513		N-264	46074	Overseas	Oceania		
NR514		N-345	46075	Overseas	Oceania		
NR515		N-405	46076	Overseas	Oceania		
NR516	トワダナタネ	TOWADANATANE	67870	Japan	Tōhoku	Cultivar	
NR517	東北 68 号	TOUHOKU 68	67871	Japan	Tōhoku	Cultivar	

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Sample ID in this study	品種・系統名	Variety name	JP No.	Origin 1	Origin 2	Cultivar/Landrace	Corecollection
NR518	東北 72 号	TOUHOKU 72	67872	Japan	Tōhoku	Cultivar	
NR519	東北 73 号	TOUHOKU 73	67873	Japan	Tōhoku	Cultivar	
NR520	青系 258	AOKEI 258	67874	Japan	Tōhoku	Cultivar	
NR521	青三系-1	AOSANKEI-1	67875	Japan	Tōhoku	Cultivar	Included
NR522	郡脂 5 号	GUNSHI 5	67876	Japan	Tōhoku	Cultivar	Included
NR523	北系 56-6-6-4	HOKUKEI 56-6-6-4	67877	Japan	Hokkaido	Cultivar	Included
NR524	Windal	WINDAL	67878	Overseas	Europe		
NR525		61･3A-14-2	67879	Overseas	Europe		
NR526		70･12･714-1	67880	Overseas	Europe		
NR527		70･34･114-3	67881	Overseas	Europe		Included
NR528	Cresor	CRESOR	67882	Overseas	Europe		
NR529		B･12･122･122･1-1	67883	Overseas	Europe		
NR530		B 23-1	67884	Overseas	Europe		Included
NR531	Ramses	RAMSES	67885	Overseas	Europe		
NR532	R39:Jetneuf-1	R39:JETNEUF-1	67886	Overseas	Europe		
NR533		RAFAL	67887	Overseas	Europe		
NR534	Blako	BLAKO	67888	Overseas	Europe		
NR535	Erra-1	ERRA-1	67889	Overseas	Europe		
NR536	Erra-2	ERRA-2	67890	Overseas	Europe		
NR537	Vertis	VERTIS	67891	Overseas	Europe		Included
NR538	北海 3 号	HOKKAI 3	67892	Japan	Hokkaido	Cultivar	
NR539	郡脂 5 号-1	GUNSHI 5-1	67893	Japan	Tōhoku	Cultivar	
NR540	郡脂 5 号-2	GUNSHI 5-2	67894	Japan	Tōhoku	Cultivar	
NR541	郡脂 5 号-3	GUNSHI 5-3	67895	Japan	Tōhoku	Cultivar	
NR542		Z･E･N	67896	Overseas	America		Included
NR543	青油 2 号	QUINGYOU 2	67897	Overseas	Asia		Included
NR544	青油 5 号	QUINGYOU 5	67898	Overseas	Asia		Included
NR545	青油 6 号	QUINGYOU 6	67899	Overseas	Asia		
NR546		D 26/79	67900	Overseas	Europe		
NR547		BK 27/78	67901	Overseas	Europe		
NR548	Prota	PROTA	67902	Overseas	Europe		
NR549	Da 5/81	DA 5/81	67903	Overseas	Europe		
NR550	Sedo 2	SEDO II	67904	Overseas	Europe		
NR551	東北 78 号	TOUHOKU 78	67905	Japan	Tōhoku	Cultivar	
NR552	東北 79 号	TOUHOKU 79	67906	Japan	Tōhoku	Cultivar	
NR553	東北 74 号	TOUHOKU 74	67907	Japan	Tōhoku	Cultivar	
NR554	東北 75 号	TOUHOKU 75	67908	Japan	Tōhoku	Cultivar	
NR555	カミキタナタネ	KAMIKITANATANE	67909	Japan	Tōhoku	Cultivar	
NR556		LAULENTIAN	76713	Overseas	America		Included

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Sample ID in this study	品種・系統名	Variety name	JP No.	Origin 1	Origin 2	Cultivar/Landrace	Corecollection
NR557		WILHELMSBURGER	76714	Overseas	America		Included
NR558		ECD 06	76715	Overseas	Europe		
NR559		ECD 07	76716	Overseas	Europe		Included
NR560		ECD 08	76717	Overseas	Europe		
NR561		ECD 09	76718	Overseas	Europe		
NR562		ECD 10	76719	Overseas	Europe		
NR563		BGR 468	80935	Overseas	America		
NR564		KRASNOSELSKAYA	128131	Overseas	Exotic Unknow		Included
NR565		BOUNTY	146914	Overseas	America		
NR566		CELEBRA	146915	Overseas	Europe		Included
NR567		DELTA	146916	Overseas	Europe		
NR568		GOLDA	146917	Overseas	Europe		Included
NR569		STALLION	146920	Overseas	Europe		Included
NR570		TRIBUTE	146921	Overseas	America		
NR571		VANGUARD	146922	Overseas	Europe		Included
NR572		AZTEC	146923	Overseas	Europe		
NR573		FALCON	146924	Overseas	Europe		
NR574		INCA	146925	Overseas	Europe		
NR575		ONYX	146926	Overseas	Exotic Unknow		
NR576		SABRINA	146927	Overseas	Europe		
NR577		MONETA	146931	Overseas	Europe		Included
NR578		HELIOS	147431	Overseas	Exotic Unknow		
NR579	高系 10 号-2	KOUKEI 10-2	207962	Japan	Chūbu	Cultivar	Included
NR580	高系 10-2 号	KOUKEI 10-2	207963	Japan	Chūbu	Cultivar	
NR581	長島 Landrace1	NAGASHIMA ZAIRAI 1	220360	Japan	Kinki	Landrace	
NR582	長島 Landrace2	NAGASHIMA ZAIRAI 2	220361	Japan	Kinki	Landrace	

Appendix 2. Primer information of the 504 SSR markers used in this study.

Marker name	Primer sequence (5'-3')		Citation
	Forward	Reverse	
BnEMS0004	CTCGTCTCTCCAACTTCTGT	TCAACATCAACATCCACATC	Wang *et al*. (2012)
BnEMS0006	CATCTCCTCTGTCTTTATCCTT	GAATCGTGTTCGTGAGTGA	Wang *et al*. (2012)
BnEMS0008	TTCTCTCCATCTCTCTTCCA	ATGAATCGTGTGCGTGAGT	Wang *et al*. (2012)
BnEMS0020	CTCTATCCGAAGTGAATGATG	GTCGTCAACCCACACAAA	Wang *et al*. (2012)
BnEMS0031	GGACCAGAACCAGAGAGATT	TAATAACGATGAACCCTACGA	Wang *et al*. (2012)
BnEMS0048	TCACTCTACAACCCTGTTTCA	TCTTTCTCTTCTTCATCCTCC	Wang *et al*. (2012)
BnEMS0056	ACGACATCCTTCTTCTTCTTC	CAGTTTATCATCAGACAATCCA	Wang *et al*. (2012)
BnEMS0112	AAAGGAACCACATTTCTCTCT	CCCATCTCTCTCTCTCTCTTC	Wang *et al*. (2012)
BnEMS0139	TTGTCGGAGATAATGAGAGAA	GAGGAGTTGGCTTGTGAG	Wang *et al*. (2012)
BnEMS0169	ATCGCTTCATCAGCAAGA	AGAGACACGACAGAACACAAG	Wang *et al*. (2012)
BnEMS0214	TTGCACGATCTTACTCTCTTC	TTTCTAGTCTTTATTGCGCC	Wang *et al*. (2012)
BnEMS0273	CCGCATACAAGATTGTGTTA	TCGGAATTGAAAGGTACAGT	Wang *et al*. (2012)
BnEMS0287	TCATTTCTACGGAGGAGTTG	GGAGGAGAAGAATCTAGGGA	Wang *et al*. (2012)
BnEMS0294	ATCCTCCGTTAGAGATCACA	TGGCAAACAAATGAATGATA	Wang *et al*. (2012)
BnEMS0340	ATGGAAATGAAGAAAGACGA	CATAGAGAGGATGGCTTGAG	Wang *et al*. (2012)
BnEMS0395	AACCACAACCATAGACGAAC	TTCATTTCTCCCTTTGTCC	Wang *et al*. (2012)
BnEMS0446	GAAGAAGAGAGGCTAGGGAG	TCTGGTGAAGACAGATGTGA	Wang *et al*. (2012)
BnEMS0472	ATTATAACCGACAACAACCG	TTTAGCAGCCAAGATAGGAG	Wang *et al*. (2012)
BnEMS0481	CTTGAGATCGTTCCTCTACG	TCTAACCTCTGCTGCTCTTC	Wang *et al*. (2012)
BnEMS0525	GCATTCCTATTTGAGCAATC	GATCAAGCCAATAGATTGTA	Wang *et al*. (2012)
BnEMS0536	ATTCTTGGCTTCTCAATCAA	TTATTAGTTTCAGTGGCGGT	Wang *et al*. (2012)
BnEMS0537	CTCAGACAAAGCGACCAC	CGTTAAGGAAAGGTCTGTTG	Wang *et al*. (2012)
BnEMS0612	CGGAAAGTTTGGTAATTGAG	GAGGAGGATGATGAATCAGA	Wang *et al*. (2012)
BnEMS0619	GTTTAGTTTCGCCTTGTGTC	CACAAACTGATTCATCAACG	Wang *et al*. (2012)
BnEMS0620	TCCTCTCAATTTCTTCAGGA	AAGATTCATGCCACTTCAAC	Wang *et al*. (2012)
BnEMS0694	AGATTTCTTCCCATCTCCAT	AAGCAGAAACCCACTAGACA	Wang *et al*. (2012)
BnEMS0718	GTCTCGAAATCTCAATACCG	GTAGATCTCGGAACCAACAG	Wang *et al*. (2012)
BnEMS0727	GAGGGATCTCATGTTCACTG	AAAGAAGACGAAATCTAGTTGAA	Wang *et al*. (2012)
BnEMS0753	CAATCAATCTCTTCAAAGCC	CCTTACCTTCCTCACTTCCT	Wang *et al*. (2012)
BnEMS0872	TTGAAGAAGAGAAGATGGGA	CTCTCACTGGAGAATTGAGC	Wang *et al*. (2012)
BnEMS0885	TCAGGGTCACTTTCTCAATC	TTTCTGGAAAGAAGGATGAA	Wang *et al*. (2012)
BnEMS0891	CGAGTAGACGGGATTTACAG	AAGTTAGGGTCGAAGAGGAC	Wang *et al*. (2012)
BnEMS1001	CCTCCTCCTCTTCTCAGTCT	ATCTGTGGATTTGAATTTGG	Wang *et al*. (2012)
BnEMS1009	AACCCTAGCTTCCTCAATTC	AAGGAGGAGGAGAGGTTTAG	Wang *et al*. (2012)
BnEMS1010	AAGACAAAGTCGGTGAAGAA	GCTACGAATCTCATCCTCTG	Wang *et al*. (2012)
BnEMS1061	TTTACATGGAAAGAGGCAGT	ACCTATGATGAGCATGGAAC	Wang *et al*. (2012)
BnEMS1094	TTTAGTTTCATCAAACACTCTCT	CCACTATGTTAGCTTCGTCC	Wang *et al*. (2012)
BnEMS1119	ACTGGAGTTTCAATTGGATG	CATCATCTTCAGCACTAGCA	Wang *et al*. (2012)
BnEMS1132	GTCGAAACCCAATCTGTTTA	TACAGGATATCGCTTCGTCT	Wang *et al*. (2012)
BnEMS1136	AAGGTTATAATCTGCGACGA	ATATTTGTAACCGCAAATGG	Wang *et al*. (2012)
BnEMS1150	ATCTAGAGGCGATTCTCTGA	GAGATGGATCCGAGTTTGTA	Wang *et al*. (2012)
BnEMS1215	GCCAGATCAACACTCCCTTCT	ACAACAGAAGAACACGGAGGA	Wang *et al*. (2012)
BnGMS0002	TTCACATGTTTGTCTCAACG	TACCATTCCTAAGGTGCCTA	Cheng *et al*. (2009)
BnGMS0014	ATCCGAAGAGAGGATAGAGG	GTATGGACGACGAATCATCT	Cheng *et al*. (2009)
BnGMS0103	GTGAGTGGAAACGTTCTAGC	AAGACTGTTACGGCTCGTC	Cheng *et al*. (2009)
BnGMS0271	ATGTTTCTCCATTTGTCCTG	GTTGTGTGTTCTGGGAGAAT	Cheng *et al*. (2009)
BnGMS0281	TATAACACGGTGTGTGCTGT	CCAACAAATTTAATGCATGTT	Cheng *et al*. (2009)
BnGMS0289	CATTACAAACTCAGCGTCAA	CAGGACACTCGGTTATCAAA	Cheng *et al*. (2009)
BnGMS0293	ACGTTTCACTTGAGGATCTG	CTATACGATGTCTGGTCGGT	Cheng *et al*. (2009)
BnGMS0336	ACCGAATAACAAGTCGAACA	TTGAAACACACCCATTTACA	Cheng *et al*. (2009)
BnGMS0353	AACCAAAGAATACATCCGAA	TTGCAGAAGTTGAGACAATG	Cheng *et al*. (2009)
BnGMS0373	CAGATAAATTTGGGTAGCTCA	TATGGATCTGGAAACTCGAA	Cheng *et al*. (2009)
BnGMS0386	TTGGCTCATCAATGACAATA	ACAATGTGGTAAACACGAAA	Cheng *et al*. (2009)
BnGMS0509	TGAATGTGCTTCTTCGTATG	TTCAAGCTCGTTCACTCTCT	Cheng *et al*. (2009)
BnGMS0574	ATCTATACGCACACTTCCGT	ATGGCTTTGTCTAATCGAGA	Cheng *et al*. (2009)
BnGMS0615	GGCACGGATCTTAACCTAGT	TCAAGGGCTTTCATATCTTG	Cheng *et al*. (2009)
BnGMS0634	CAATTTGGGACTGAAGAAAT	CGCATTCTTCAAACAAACTC	Cheng *et al*. (2009)
BnGMS0635	CAAAGCAAATCAAACAATCA	CAATTCACGTATGATCCACA	Cheng *et al*. (2009)
BnGMS0646	TTTACGAGAGTCGGAAGAT	CATTCACATCCACTCTGATG	Cheng *et al*. (2009)
BnGMS0662	CGATCGAATTGCACTGTACT	ATGCACAGAGCTGAAGAAAT	Cheng *et al*. (2009)
BnGMS0713	CCTCCGTGTATTCTTTTCCTG	CAGGTCCATATCATACAACTTCC	Cheng *et al*. (2009)
BnGMS0749	CGGTCTAGTTACCCAGATCACC	AATCCCCTTGCCACTTATCAT	Cheng *et al*. (2009)
BnGMS0771	GTGATTTGGGATCAAAGACGA	GACGTCGCGAAACTTCTAATG	Cheng *et al*. (2009)
BnGMS0808	CTCGTGAGAAAAACTGTGATGC	CATGTCCCTGGAGCTCTTGT	Cheng *et al*. (2009)
BnGMS0945	AGTGTCAGCCCCTCAGAGAAC	CACATCTCTTGGAAATGCAAAC	Cheng *et al*. (2009)
BnGMS0966	ACAAAGTCGATTGATCGAGGA	CTCCATCGGTGGTGTCTTAAA	Cheng *et al*. (2009)
BnGMS0968	TACATGGCAAAGAACCTCGTC	AAGTCTCAGGAAGCACGTCAA	Cheng *et al*. (2009)
BnGMS1004	ATGTATCTCAGCAGCCCACAA	CAATCACAACAGACGTACATGC	Cheng *et al*. (2009)
BnGMS124	GGAGATTCAAGCTCAAGATG	GAGCTTTCTCAGCTTTACCA	Cheng *et al*. (2009)
BnGMS14	ATCCGAAGAGAGGATAGAGG	GTATGGACGACGAATCATCT	Cheng *et al*. (2009)
BnGMS2	TTCACATGTTTGTCTCAACG	TACCATTCCTAAGGTGCCTA	Cheng *et al*. (2009)
BnGMS205	AAGAGAGACAGCGTGTTGTT	GACTCTAGGAGAGTAGACGGC	Cheng *et al*. (2009)

Appendix 2 continue

Marker name	Primer sequence (5'-3')		Citation
	Forward	Reverse	
BnGMS239	GCGACATGGTAATTGAAGTT	TTTAATGGGTCCATGATAGC	Cheng *et al*. (2009)
BnGMS271	ATGTTTCTCCATTTGTCCTG	GTTGTGTGTTCTGGGAGAAT	Cheng *et al*. (2009)
BnGMS291	TGGGCAGTTATAGGATATGAA	ATGTCAATAACGTACTCGGG	Cheng *et al*. (2009)
BnGMS3	AAAGAGCCCACATGAAAGTA	TGAACTAGGCACCAAGAACT	Cheng *et al*. (2009)
BnGMS314	TTGCTACAATTTACTTCGTTTG	GTAACCCGTATAAGTGGCTG	Cheng *et al*. (2009)
BnGMS315	TTACGGTATATTCTCGCCAT	GTCTACCAGAACACCCTCAA	Cheng *et al*. (2009)
BnGMS317	TATTGGGTCAAGAACTTGCT	CAATGAATCCCTGTTCAAGT	Cheng *et al*. (2009)
BnGMS338	ATTATATGGCAAGCGATGTT	TCTCACAGCAGAGAACCTTT	Cheng *et al*. (2009)
BnGMS347	TCACACAAATCTCCTCCTCT	AGGTATCAGCCAATGACTTC	Cheng *et al*. (2009)
BnGMS349	CCAGGAGGGAGTAAACTCTT	GGTCAATAAACAATGGTGTTG	Cheng *et al*. (2009)
BnGMS353	AACCAAAGAATACATCCGAA	TTGCAGAAGTTGAGACAATG	Cheng *et al*. (2009)
BnGMS373	CAGATAAATTTGGGTAGCTCA	TATGGATCTGGAAACTCGAA	Cheng *et al*. (2009)
BnGMS386	TTGGCTCATCAATGACAATA	ACAATGTGGTAAACACGAAA	Cheng *et al*. (2009)
BnGMS422	TGGAGAAGAAGGCTATACACA	TCACACCAGGGTAAAGGTAG	Cheng *et al*. (2009)
BnGMS452	TAGATGGTCCTTGACCCATA	AACATGTCTTTGATGAAGCC	Cheng *et al*. (2009)
BnGMS454	TGTATCTGGTTCATGGTTGA	ATGGAAATGGAACGTGATAG	Cheng *et al*. (2009)
BnGMS488	CGTCGCAGTCTGTAATTGT	TGTTTAACTTCAAATATGGCTTT	Cheng *et al*. (2009)
BnGMS492	AAATTATAACCAATGTCTGAAA	AGATTTAAGTGCAATCGGAA	Cheng *et al*. (2009)
BnGMS513	ATGCCAGCATGAAGAGTTAG	GCTTTGTGGTCGTATTTCTC	Cheng *et al*. (2009)
BnGMS598	TGGTAGGTCCATTCTCTGTC	GAAGATGAAGAGGAAGGGTT	Cheng *et al*. (2009)
BnGMS602	GGAAATTTGCTTCTTACCAA	TTGTGTTGTGTTTGTAACCC	Cheng *et al*. (2009)
BnGMS603	TGTATGGTCTGATGGTCTC	CGTTCCTGATGATCTCAAAT	Cheng *et al*. (2009)
BnGMS616	CGCTATGGATAATGTGTTCA	ATTAACAGCGAGGATAGCAA	Cheng *et al*. (2009)
BnGMS631	CCATTATTCATGTTTACCACC	CCTCGCTCCTAGTAGGTACA	Cheng *et al*. (2009)
BnGMS632	CCATTATAAGAGTGTCCATGAGT	AACGGGTTGTTGACATTAAG	Cheng *et al*. (2009)
BnGMS634	CAATTTGGGACTGAAGAAAT	CGCATTCTTCAAACAAACTC	Cheng *et al*. (2009)
BnGMS646	TTTACGAGAGTCGGAAGAT	CATTCACATCCACTCTGATG	Cheng *et al*. (2009)
BnGMS659	GCCAAATTCATTAGTAACCG	CCAAATTTGATTAAGTAGTGCT	Cheng *et al*. (2009)
BnGMS662	CGATCGAATTGCACTGTACT	ATGCACAGAGCTGAAGAAAT	Cheng *et al*. (2009)
BoEMS0004	AGAGCGCAGTCCACACAC	ATGGATCAGAAACAGTGGAG	Li *et al*. (2013)
BoEMS0016	AATCTTCGCCGCTGTTTA	TTAACCTTTATGCTAACGCC	Li *et al*. (2013)
BoEMS0049	GAATCTCGTAAATTTGATGG	CGACTAATTCTTGGAGTTGG	Li *et al*. (2013)
BoGMS0026	CGAGAACAAGAGACAAGTGAG	TGATTAGACCCTGATTGAAAG	Li *et al*. (2011)
BoGMS0030	GCTAATCCAGACCCAAACA	AGAAACACCATTCAACTCAAA	Li *et al*. (2011)
BoGMS0037	ACTGCTCTCTCCCTTCATCT	TATCTTCCGCCTCTTCTGT	Li *et al*. (2011)
BoGMS0081	AGTCCTAATGGTGCTCTTTGT	CTGTTGAGGTGTTGTCCTTT	Li *et al*. (2011)
BoGMS0112	ACTGGTCCGAATAGGTGAG	CGATAAATGGTTCAATGTCC	Li *et al*. (2011)
BoGMS0116	TGTCACCAGTCTCTCTCTCTC	TCAGTGCTTCCTCAAACATAA	Li *et al*. (2011)
BoGMS0168	GTCTTGTATGAAGCCCAGTAG	AGAGGAAGTGTCGGGAAG	Li *et al*. (2011)
BoGMS0254	CTACGGCTTCGTCTCGTC	TACTTCCCAACCATTCTGTAA	Li *et al*. (2011)
BoGMS0281	ATCCTTCTGCCTTCTCTG	TGACATCCATCCACACATT	Li *et al*. (2011)
BoGMS0282	CCCTTGTAGAGAGAGAGAGGA	AAACGAAATAAGATGACGAGA	Li *et al*. (2011)
BoGMS0299	GCAGGAATCAAACAGAAACT	TCTCTCTCTCTCTCTCTCTCGT	Li *et al*. (2011)
BoGMS0342	ATGAATAACCAAGCGACAAG	TAAGCCAGAAGGCACTGTT	Li *et al*. (2011)
BoGMS0358	GGCTCGCTACACTATGCT	TTCACCAAGAAAGAGTTCATC	Li *et al*. (2011)
BoGMS0364	CCTGTCTTTGCTCATCTATTT	GCGTTAGGTTGTTGTAGGATT	Li *et al*. (2011)
BoGMS0394	CCCTTACTTTGTTCAGGTTTC	CATCTCTACCCACCACACA	Li *et al*. (2011)
BoGMS0420	AAATGTTATGTGTGGGTCTATT	AGAGAGAGAGAGAATGGTGAGA	Li *et al*. (2011)
BoGMS0454	GCACTGAGAGAGAAAGAGTCA	TGTTGTATGGCAAGTATGTGT	Li *et al*. (2011)
BoGMS0456	TAACAAACGGAAAGACGA	GTGAAACCACAGAGGATAGAA	Li *et al*. (2011)
BoGMS0486	AAGGAGGAACCAAATGCC	TGATAATGCCACTGATAGGAC	Li *et al*. (2011)
BoGMS0501	ATGATGAGTTTGCTCGTTAGG	AAATCCTTCCTCCTTTCAC	Li *et al*. (2011)
BoGMS0505	CTGTGGTGAGTGCTATTGG	TGTTGCTCGTCATTTCTATCT	Li *et al*. (2011)
BoGMS0507	TACGATTTCTGTTCTATTCTATC	ATCCCTGCGGTTATCAAA	Li *et al*. (2011)
BoGMS0513	CTGACCATCTATCAAACCAAA	ACCGTCTTTACCATTCATC	Li *et al*. (2011)
BoGMS0525	AGTCCCATCAAGTCCAAATAC	GTCGTCTTCAGCCATCAG	Li *et al*. (2011)
BoGMS0537	AAGACAACATCCCTGAAGAAC	GAGAGTCGCTGAACTAAAGAA	Li *et al*. (2011)
BoGMS0545	CCTCTGTTTCTTTGCTCTTTG	GATTCATTGTGTGTGTGATGT	Li *et al*. (2011)
BoGMS0573	TTTGAGGTATTGTAGCAGATT	AGCATTTGTAGTTGAGGACAG	Li *et al*. (2011)
BoGMS0576	ACCTGGAGTTGAGACGGG	CAGTGTTGAGTGTTCTTCTATT	Li *et al*. (2011)
BoGMS0582	CCTGAGTCTTGGAGCCTT	TCGTTATTAGATTTGAGTATTTG	Li *et al*. (2011)
BoGMS0584	GAAACCCACCTCGTATTTAG	AAACCAAACCTTCTCTCTTCA	Li *et al*. (2011)
BoGMS0594	TTGTTCGGTTTATTTGGTTT	GGTCCATTTACTACTTATTCCT	Li *et al*. (2011)
BoGMS0607	GTTCCTTTCCTTGGTTCATAC	CCGCCATCTTCATCCTTT	Li *et al*. (2011)
BoGMS0619	TCAAGACCCGCTCCAAAG	ATGTTCGTTGCTCCAAATAG	Li *et al*. (2011)
BoGMS0624	AAGACGAAGTCAAGTCAAGGT	CGTATCATCCAGAGTATCCAG	Li *et al*. (2011)
BoGMS0660	CCTTGTTCTTTGTAGGAAATG	GGTGCTTGTGCTTTGTTT	Li *et al*. (2011)
BoGMS0678	CTCTTTCTTGCTTCTTCCTTC	TCTTCTCTTCCTTCCTCCTT	Li *et al*. (2011)
BoGMS0687	GACAACACAACAGACGCA	GCATTTCCCATTACTTCCA	Li *et al*. (2011)
BoGMS0705	GTCACCCTATCTCTCTCCATT	TAGCCACCAAGTCCTGTTC	Li *et al*. (2011)
BoGMS0738	TTGAGGAAGGAACACGAA	GTGGGAGAGTGAGGGTAGTAA	Li *et al*. (2011)
BoGMS0741	CTCAAACTCCGTCGCTCT	TCCTCCTCACTACTTTCTTCA	Li *et al*. (2011)
BoGMS0811	GCACTGTCAAATCACTCAAA	ACTTCTCCCAATCTCTGTCTC	Li *et al*. (2011)
BoGMS0819	AGGGAGATGGACACATTTAG	GAGAGAGGGCAAAGAAGATAG	Li *et al*. (2011)

Appendix 2 continue

Marker name	Primer sequence (5'-3')		Citation
	Forward	Reverse	
BoGMS0836	CATAAACACACCGAACAAGAC	ACGCAATGACACACATACAC	Li *et al*. (2011)
BoGMS0845	CCTTTGTCTTCTTCACTCTCC	ACCAGGCTCTTTCTTTCTCT	Li *et al*. (2011)
BoGMS0867	GACTCAGACCACAAACACTCA	GCAAAGAGACTTACTTACCCA	Li *et al*. (2011)
BoGMS0868	AAATCCCAACGAGATAGGTAG	AGAAAGAAAGGAAGAAAGTGG	Li *et al*. (2011)
BoGMS0927	ACCAGAGAAGGCATACATAGA	CGAAGGAGTTTGTGAGGATAA	Li *et al*. (2011)
BoGMS0929	TCAGACCCAAAGCCAGTT	TTGTGGAAGATGAAACCATT	Li *et al*. (2011)
BoGMS0934	GGTCAGAGTAAGGGATGGA	TGTAAAGATGTCGGCTAATGT	Li *et al*. (2011)
BoGMS1017	TTTGTTGTTGTTGTTGTGTTC	TATCTCTCGCTCGTCTCA	Li *et al*. (2011)
BoGMS1028	ACAAGTTCTCTCTCACCGAA	TTCACAACCCTCTAACATCTC	Li *et al*. (2011)
BoGMS1037	CCGTAACTTTCTCTCTTCCTC	GTCAGCAGCACGATTCAG	Li *et al*. (2011)
BoGMS1055	CGGATAGGAGAGGTTTCAG	TGACTTTGCTTCATCTTCCA	Li *et al*. (2011)
BoGMS1065	GGGTTGATTGGGAAGTGT	CTTAGCACCATTTGTTTGTATT	Li *et al*. (2011)
BoGMS1118	AGACATCAAAGGCAAGACAG	TCAACAGAACTACTCTCCTCAA	Li *et al*. (2011)
BoGMS1145	CTTCTCTCTTCGCATCATAAC	GTCCCTCTCCTCTCTCTCTCT	Li *et al*. (2011)
BoGMS1218	GTAAACCGAAATCAGAATGG	GACGAAGATGGAAGGGTAA	Li *et al*. (2011)
BoGMS1235	ATTCATCATCTCTCTCGGAA	TAAGGCTCATCTCAACTCTCA	Li *et al*. (2011)
BoGMS1259	ACAAGGAGAAGGAGAAGACAC	TGAGGAAGATGAAGTTGAAGA	Li *et al*. (2011)
BoGMS1264	GATTGTGGAGGTGGGTTT	TCAAGAAGAAAGAGAAAGAGAGA	Li *et al*. (2011)
BoGMS1283	TTGTCATCATCCTCTTCACTC	TGCTATCCACTCTTCTTCTCA	Li *et al*. (2011)
BoGMS1287	GTGAAGCAAGGCATAAATAAA	GTAGAAACAGGTCCAACCAA	Li *et al*. (2011)
BoGMS1330	AGGAGAAGAAGGAAGATACCA	AGAAAGGAAAGAAAGACCAGA	Li *et al*. (2011)
BoGMS1360	GAGACCAGAGAAGGAGGAAC	CACTCACTATCACACACACTCA	Li *et al*. (2011)
BoGMS1412	GGTTTAGTGTTGGTTCGGT	GCGAGAGAAAGTTGAGGTT	Li *et al*. (2011)
BoGMS1419	AAAGGTCGTATTTCTCTCTCTC	GATTTGATTCTGGTGTGAAGT	Li *et al*. (2011)
BoGMS1432	AAGTCGGCACAAGGTGTT	AGGGATTTAGAGTTTCGTGTT	Li *et al*. (2011)
BoGMS1472	ACTCGCACACACATACATACC	GAATAGGGAGAGAGATTTGAG	Li *et al*. (2011)
BoGMS1486	AAATGTGTTCTTGGTGATG	AGGAGGGTAAGTTGGTGATT	Li *et al*. (2011)
BoGMS1495	TAACACTGAAACACATTGGCT	GTGAGAAAGATGACGAAGATG	Li *et al*. (2011)
BoGMS1565	GATGACTTGGCGTTGATT	CTGAGAGAGAAGAAGGAGAAGA	Li *et al*. (2011)
BoGMS1567	TTCAGTAGGGAGGGATAGGT	GGTTATGATGCTCGTCGG	Li *et al*. (2011)
BoGMS1615	TGCCTGAGAAGAACATGAAGAC	ACTCACTCACCCATGGTTTCTT	Li *et al*. (2011)
BoGMS1652	TGCGGTCTTATAGGAGATGTGA	GGGACAATTATCATGTGGACCT	Li *et al*. (2011)
BoGMS1697	AGAGACAAACCAGCCTTGGAC	GCGATTTCAGAGAGGTAGAGGA	Li *et al*. (2011)
BoGMS1740	CTTCCGTGGATGATGTCTTGT	AACAATGGTCGTGAAAGGGTAT	Li *et al*. (2011)
BoGMS1746	GCTCGGCTAACACCCTATTCTA	AGCAAGACAAGTGAGTTGGTTC	Li *et al*. (2011)
BoGMS1747	TGTTATCGGAACCCTCTTGAAT	ATATAATTGACCCGTGCGTAGC	Li *et al*. (2011)
BoGMS1764	TTGGAGCATCACGAAACTACAC	TGCAACGTTATCACGCTTCTAT	Li *et al*. (2011)
BoGMS1795	AAGAACTGACCCGAATAGATCG	GAGATTGGAGAGATGGATTTGTG	Li *et al*. (2011)
BoGMS1813	TTGTCTTCTTCCGTTCCAATCT	TACAGACGAGTTGTGGATCGTC	Li *et al*. (2011)
BoGMS1897	AAGGAGGACGATCAGCTAAACC	CCCTCTCTCTCTCCTCTTAGGG	Li *et al*. (2011)
BoGMS1909	AGGTGCAATAAGCTGTCTCTCC	AAACTCGAGCTTATCACGCTGT	Li *et al*. (2011)
BoGMS1937	TCTCTAAACCGCAGGAAATCAT	GAATGTTTGATGGAACCAAAGG	Li *et al*. (2011)
BoGMS2016	GTATCCGAACTCAAATCCGAAC	CTGTGAGAGAAGCAAACCCTTT	Li *et al*. (2011)
BoGMS2026	CCTTTCGTTCGTGATTACACAA	CCAGTAACCACGTTTAAATCCTC	Li *et al*. (2011)
BoGMS2030	AACACTAGCCAAGGGTTTGTAA	AAGCAAATGAACCATCCACTCT	Li *et al*. (2011)
BoGMS2064	ATTTCGACCCACCGAAAGTATT	CATCGGACGGGTCAGATATT	Li *et al*. (2011)
BoGMS2095	CCATGCTGGTAGGCAATAAGTA	TGGACCATGGGACCATAGTAGT	Li *et al*. (2011)
BoGMS2228	CGTCGTCACTAGCTTCTCTCCT	GTTCCTTCCTTGGATTCTTCAA	Li *et al*. (2011)
BoGMS2256	AGGGAGAGAGGAAACAAACTGA	ATAAGAACCTCCGCTCTCTGCT	Li *et al*. (2011)
BoGMS2319	ACTGGTGCGGAATGAATAGAAG	CTTTGGTGATGCCACGATAGTA	Li *et al*. (2011)
BoGMS2351	TTGGCGCTAGTCAGATCTTCTC	CCTAATGATTCCACCCTCATTC	Li *et al*. (2011)
BoGMS2360	CGATTCAGTCTCGCATTATCAA	AGAATCTCCGATACCATTCGTC	Li *et al*. (2011)
BoGMS2387	ATGTCCCTGTGAAATGTGTGTT	TTTCTCGGACAACCTTTGTGTA	Li *et al*. (2011)
BoGMS2431	CAACCACCGACTCAAATACAGA	GCACCTGCAATCTCCATACATA	Li *et al*. (2011)
BoGMS2468	CATTCGGGTTATTCAACTTTGG	CGAACGGATCGGATCTTATTT	Li *et al*. (2011)
BoGMS2477	TCTGTACAGTGCCGTGTCAAAT	TGACTCTTACATCAATCACGGTCT	Li *et al*. (2011)
BoGMS2499	TATACGATTGCTCTCCCTCCTC	TCGTACAGGTGAATTACGCAAG	Li *et al*. (2011)
BoGMS2504	TTTGGCCTTATCCAAATCTCTC	AGGCGACAAGTTGAATCTAAGG	Li *et al*. (2011)
BoGMS2590	GAGCAACAGTAATCAGGGTAACAA	ATTGTTCGGAAACTTCCATCTC	Li *et al*. (2011)
BoGMS2759	GATATTCGTCATTGCCCAGTGT	AGGATGCTTATGAATGGCTAGG	Li *et al*. (2011)
BoGMS2791	ACCGATTTCTACCCGACTTAAA	TGACATACCAACTCCCATCAAA	Li *et al*. (2011)
BoGMS2965	AGAGACAGCTTAAACCCTTCCA	TCTTAGTGGAGTGACTTCGGAAC	Li *et al*. (2011)
BoGMS2992	TGTTGTTTCCTCATACGTTTCG	TCACCCACACAAGTCAAATACTG	Li *et al*. (2011)
BoGMS3000	CGTGAGAGAGAGAGGGAGAGAG	CAAACTCCTAGCCCAACTCCTA	Li *et al*. (2011)
BoGMS3009	AAAGTTCGCTGCTTTACTCGAC	TAAGCTGATAGCTTCCGGTTTC	Li *et al*. (2011)
BoGMS3139	CCAAATATTCAGGTTCCCATGT	GTTCGTAGGAGAGAGGCATCAT	Li *et al*. (2011)
BoGMS3309	AAGGTTGCCTTCTTCCTCACTT	ACAACAGATGACTGGCAAACAC	Li *et al*. (2011)
BoGMS3409	AGTTCGTTGAGCTCTTTGGAAG	TTCAATCCTTCTTCTGCTGGA	Li *et al*. (2011)
BoGMS3432	CTTGATCACCAACAGAATCCTC	GCTATCTGCTCCGAGTCCTAAA	Li *et al*. (2011)
BoGMS3538	TTCACCGTCCTCTTCAGTATCA	AGGAGAATCGTCAGAATCAAGC	Li *et al*. (2011)
BoGMS3581	TTCCTCATCAGGATCAACATCA	GGTTCCATCATCAATGGTTTCT	Li *et al*. (2011)
BoGMS3608	TTAGAGCGATAGGGCTTAACGA	ACACAAGCTCAACCGAGAAGAT	Li *et al*. (2011)
BoGMS3654	AGATGGGAGACTCGATGTTGAT	TTGTTGTGATGCTCTGTCACTG	Li *et al*. (2011)
BRAS019	CTCAAGACAAACGACCAGTAA	GAGAAGAAATCGCCAAGA	Piquemal *et al*. (2005)

Appendix 2 continue

Marker name	Primer sequence (5'-3')		Citation
	Forward	Reverse	
BRAS084	ATTGGGTTCTGACCTTTTCTC	TTTTCCTTCATCGCTACCAC	Piquemal *et al*. (2005)
BrEMS0005	AGGAGTTCAAAGAGGAAACC	TCCTCATCTTCACCATCTTC	Li *et al*. (2013)
BrEMS0015	GATCACCACTGAAACTGAA	GCTTCTTGTAGCAGAGGAAA	Li *et al*. (2013)
BrEMS0028	TCTCTCAAGGGATTCTCATC	AATCAAGATCCGAGCCAT	Li *et al*. (2013)
BrGMS0060	GTTCTTATACTCGCGTTGGAGC	TTTAGCTTCTTCTCGAAATCGC	Xu *et al*. (2010)
BrGMS0070	TACAATGAAGATGTGATCCCGA	CGTGCGTGAGCTTATCAATACA	Xu *et al*. (2010)
BrGMS0086	ATATCCCAACACCGCACTAAAC	TTCGTCTTCATCTACGCTCAAA	Xu *et al*. (2010)
BrGMS0108	TGACTCCAAACATCACCACAA	TGAAGACCAAGCGAACTTGATA	Xu *et al*. (2010)
BrGMS0140	ACTGACTCCAAACATCACCACA	TGAAGACCAAGCGAACTTGATA	Xu *et al*. (2010)
BrGMS0283	TACCATAGAGCCAAGCGGTATT	CCCTTCTTCTTCTTCCTTCCTC	Xu *et al*. (2010)
BrGMS0366	TTTGCCGAGTAACCTTTGTCAT	AAAGTTGGGCTTTTAGCTTTCC	Xu *et al*. (2010)
BrGMS0454	ACCCTAGCCCCATAATAACCTT	TATCCACTTTGAGCGTTAAGCA	Xu *et al*. (2010)
BrGMS0485	CTTAACCTCGCAATCATAACCTG	GTGAAACGGTAAGGAGTTTGCT	Xu *et al*. (2010)
BrGMS0579	GCTTTCTTCAGATCCTCCTTGA	CCTTTCACTTGATCGTTCTTCC	Xu *et al*. (2010)
BrGMS0667	TCTTCTTCTTCCTCATCCCCTT	GGATTCTCACTTCGGAGACCTA	Xu *et al*. (2010)
BrGMS0734	ATCTTCACTCGAACTCTCGCTC	AACGCCATTGCTAATCTACACA	Xu *et al*. (2010)
BrGMS0742	ACACGTTGAAGCAAGTCACCTA	CAAAGTCCTTCCAAAACCAAAC	Xu *et al*. (2010)
BrGMS0753	CTCTTGTCGGATTTTGAGTTGA	TTGTAGGATGGTGACTGGTGAG	Xu *et al*. (2010)
BrGMS0758	AAGTTCTAGTCCGCGAGTTTT	TCAACATTCACAACCTTACCAG	Xu *et al*. (2010)
BrGMS0899	AGATACAATCGTTGGGCTCTGT	ATTGCTCACAGTTTCGTCCATA	Xu *et al*. (2010)
BrGMS1218	GCGACCAAACACGTAAGAGAG	ATCATCGAAAGCCCCATAAGT	Xu *et al*. (2010)
BrGMS1411	GCTCCGAATCTCAAATCTAACG	AATCCGCCACTTAATCTCCAAT	Xu *et al*. (2010)
BrGMS1436	CATAATCAACGAGCATGAGCTT	GTGTTTGGTATTTGGGCTTCTC	Xu *et al*. (2010)
BrGMS1450	TGCTATCAACGAGACAATCCTG	AGACGATGACGATGAGAATGTG	Xu *et al*. (2010)
BrGMS1474	ATTTGTTTGTGGCCTTCTGTTC	GATTTATTTGTTGAGTTGCGGG	Xu *et al*. (2010)
BrGMS1490	ATCAAATCATCCTCCTCTCCAC	ATTTCACCATCCAATTCCCA	Xu *et al*. (2010)
BrGMS1562	TTTCAGTCGGTGAGACGATATG	TTCTTGGAATCATAGCTGGACA	Xu *et al*. (2010)
BrGMS1569	TTGCTTCATTTGCTTGATGG	CCTACGTTGCAGTTTCCAATAAC	Xu *et al*. (2010)
BrGMS1713	AGGATTAGGCTGTTGAATGCTT	AGAGAGAATCGTCATGGAGGAG	Xu *et al*. (2010)
BrGMS1778	TCACCACCTTCTTCAGACTCAC	GAGACAGAGATTTCGGAGGAGA	Xu *et al*. (2010)
BrGMS1786	CGCCAATAGAGTTGAGCAATAAC	CCATCGGTTCAGTTCAGCATA	Xu *et al*. (2010)
BrGMS1804	CACAGTTGGGTATATTTGTAAGTGC	ACGATCCCCGTAGTAAAAGATT	Xu *et al*. (2010)
BrGMS1894	AAAGGGGACGTAGAACAAGTGA	GTCACACAACTAGGACACAGCC	Xu *et al*. (2010)
BrGMS2025	CATTGCATCTGACTCTCACCAT	CAAGCAGCCTAAGAAGGAGTGT	Xu *et al*. (2010)
BrGMS2130	GGCCACACATCACATTACCAT	TGGAGGAGGAAGCTAGAGAAGA	Xu *et al*. (2010)
BrGMS2193	AGTTTAAACCTGCGGATGATGT	GGCTGTTTGCTGAATAGGAGTT	Xu *et al*. (2010)
BrGMS2244	CCCAAAGCGTATAGCATAGTCC	TGTGCGCGTAGTCTTTAGTTTC	Xu *et al*. (2010)
BrGMS2252	GCACATGATCACAAAGATCTCA	ATTACCTTCGGTCTCGGTTCAT	Xu *et al*. (2010)
BrGMS2375	TGGCAAGAGAGTCACATTTGTT	GATTGTGCTGTGTCTCTGTGGT	Xu *et al*. (2010)
BrGMS2431	GGTTTGGGCTTCTAGGAAACAT	GATAGACCAGAGCAGACCAGAAA	Xu *et al*. (2010)
BrGMS2498	AGGAAACTTTCACCGAGTGCTA	AATTGTGTTCGTGTTGAGCAGT	Xu *et al*. (2010)
BrGMS2505	TATCCGTTAAGACAATGCAACG	CATAGTAGGCGTTAGGGTCCTG	Xu *et al*. (2010)
BrGMS2628	TCCTTGGTGGTAAATCCTCAAA	GGCGAAATTCAGTTGACATCAT	Xu *et al*. (2010)
BrGMS2767	GGGCTCCACTTTGTACTACACTC	CTGGATCCAATCCAAATCCAAT	Xu *et al*. (2010)
BrGMS2771	AGTTCGTTCTCACGTTCTCTCC	TAAGCAATAACGGGAGCTTCTC	Xu *et al*. (2010)
BrGMS2849	CCTCTCATGAACCTGTAGTGTGA	CTCCCACTTTCCTGTCTCCTTA	Xu *et al*. (2010)
BrGMS2864	GGGTCCTTGGTACCATATTTGC	TATTCTCGGTCTGTTCCACCTT	Xu *et al*. (2010)
BrGMS2901	TTTATTACGACTGCCACCAAGA	CAGATGCAGGAATAGTGACGTT	Xu *et al*. (2010)
BrGMS2969	TGTGGTCCAACAACTTAAGCAC	TTCTTCTCGCACTCATCTTCAA	Xu *et al*. (2010)
BrGMS2989	GTTCACCAGGCTCAATTCTGTT	AGCTCCTGAAATTTAGCAAAGC	Xu *et al*. (2010)
BrGMS2998	AAGTGTGCAGCAGAGGAAAGA	AAAGACCCGGAGTGAACACTTA	Xu *et al*. (2010)
BrGMS3032	CGTTGCTTGCTCCTATACAACA	AGATTCTTCTGCTCGAGTCCAC	Xu *et al*. (2010)
BrGMS3041	TCACTCACGACTTCATCCTACG	ACCAGAAGGTTACAGAGGTGCT	Xu *et al*. (2010)
BrGMS3080	TGTTCAGTACTCTGGCATGAGG	TAGATTATCACGCTGCAGGCTA	Xu *et al*. (2010)
BrGMS3093	GAGTTTGATGGCTCTCGTCTTT	CCATACCAGAACCTTGTCCACT	Xu *et al*. (2010)
BrGMS3114	TCTGATCTAAACCAAGCCCAGT	AGGGCATGATAGTTGCTTCATT	Xu *et al*. (2010)
BrGMS3124	TCCCAAGGTTCTTCTCCAATAA	ATGAAACGAATTGGGTCCTAGT	Xu *et al*. (2010)
BrGMS3125	CACACACATTCGACAACCAAAT	CCTAGCTACTTGAACGATGCAC	Xu *et al*. (2010)
BrGMS3126	TGTTGCTACTGAGAAAGCATCTTC	GGAGAAAGTAAGAAGCTTTGAGG	Xu *et al*. (2010)
BrGMS3582	TTAACAAGACGGGCCTTACG	TGCTAATCTCATAAGCCGATCA	Xu *et al*. (2010)
BrGMS3607	GCATGCATCCACTACTCCATTA	CAGGCATGAATACACACCCTTT	Xu *et al*. (2010)
BrGMS3653	AGCTGTGAGAACAGTGACATGG	GAATGGAACCAAGTGACATCAA	Xu *et al*. (2010)
BrGMS3688	GTTGAATTGCCTCCAGTGAGTT	TTTAGACGCAATGGTAAGAGCA	Xu *et al*. (2010)
BrGMS3750	GTTGGGCAAAGGTATGATGTAA	TCCATAAATCGCCAGCTATCAT	Xu *et al*. (2010)
BrGMS3755	TTATCAAGGGATTCGGTTTGAC	GATTGCAGCTTTATCATCCACA	Xu *et al*. (2010)
BrGMS3784	CTTTCTCTCCGCAGACATCAAT	TCTTGATCAAACCAACATACGG	Xu *et al*. (2010)
BrGMS3787	GCTAACGTTGCTCGTCACACTA	CGAATCAAAGGCACCTAAAGAT	Xu *et al*. (2010)
BrGMS3807	AACCTTGAGGACCCATATCCTT	GGAGACATGGACATGATGACTG	Xu *et al*. (2010)
BrGMS3837	GCAGAACCGGATAGAATGTGTA	CCAATTTCATGCCTAAGTGGTA	Xu *et al*. (2010)
BrGMS3857	GATAAGAGACAAGCCATTGTGC	GAGCTCGTCAGATTCTTGTTGA	Xu *et al*. (2010)
BrGMS3925	AGCAAGATCCCAAATATGCAG	GCACAACATTAGCCAGGTCATA	Xu *et al*. (2010)
BrGMS4027	ATCCGCATTGTCTCCATTAGAT	CCTGCTTACAGGAGAAGAGGAA	Xu *et al*. (2010)
BrGMS4028	TGTAAACAACATCGCCTAGTGG	TTTCCGGCTCAAAGTTTCTC	Xu *et al*. (2010)
BrGMS4031	CTCGAGTATTGTGTGGCGAGTA	TGCAGGATTAGTCAGAATCCAA	Xu *et al*. (2010)

Appendix 2 continue

Marker name	Primer sequence (5'-3')		Citation
	Forward	Reverse	
BrGMS4057	AGCACAGGAGTGAGACCAACAT	GCTAATGTGTCATCAGCTTTCC	Xu *et al*. (2010)
BrGMS4086	CCTTAAACTGATGCTGAATACACG	GTCACAGACTGAGAGCGAGAGA	Xu *et al*. (2010)
BrGMS4110	CAAGAATCCGACCCAAGTTTA	TGACTATGGATGATCGATTTGG	Xu *et al*. (2010)
BrGMS4149	CCCAACCTAACTGATACAAATGAG	TAGCATGGGCAATCAGGAAATA	Xu *et al*. (2010)
BrGMS4151	GAACGACGGAAATGTGAGTGTA	CATTATCCCTCGTCGAACCTT	Xu *et al*. (2010)
BrGMS4194	GCATCATCAATTGGCATATGAG	ATCTCTCGAATCGGTGTTCTTC	Xu *et al*. (2010)
BrGMS4196	CTTCTTTGGCATGTTGTACGAT	ATTGGCATCAGGGTTTCACTAA	Xu *et al*. (2010)
BrGMS4252	GCCGACCCTAGTAGACCATAAG	ATTCAGATGACCATGGGTTTG	Xu *et al*. (2010)
BrGMS4289	ACAACGACGATTAGCTGTTTCA	TTTCAAAGGAAGAGGAGCAAAG	Xu *et al*. (2010)
BrGMS4320	TTGGGTAACAGATAACCCATGC	CAACTCCACCCTCTTACAGTCC	Xu *et al*. (2010)
BrGMS4450	CTCAGGTTCTGTTCAACCAACA	CAACAGGAAATGCGATGTGTAT	Xu *et al*. (2010)
BRMS-001	GGTGGCTCTAATTCCTCTGA	ATCTTTCTCTCACCAACCCC	Suwabe *et al.* (2005)
BRMS-007	AAATTGTTTCTCTTCCCCAT	GTGTTAGGGAGCTGGAGAAT	Suwabe *et al.* (2005)
BRMS-008	AGGACACCAGGCACCATATA	CATTGTTGTCTTGGGAGAGC	Suwabe *et al.* (2005)
BRMS-008	AGGACACCAGGCACCATATA	CATTGTTGTCTTGGGAGAGC	Suwabe *et al.* (2005)
BRMS-014	CCGTAAGGAATATTGAGGCA	TTCCCAATTCTCAAACGGTA	Suwabe *et al.* (2005)
BRMS-016	CTAGGGTTATGGTTTGAATCAGACA	TCGTATAGCTCAAATCAAATGTTCA	Suwabe *et al.* (2005)
BRMS-017	GGAAAGGGAAGCTTCATATC	CTGGAAAGCATACACTTTGG	Suwabe *et al.* (2005)
BRMS-018	TCCCACGCCTTCTAGCCTTC	ACCGGAGCTTTTCTGTTGCC	Suwabe *et al.* (2005)
BRMS-018	TCCCACGCCTTCTAGCCTTC	ACCGGAGCTTTTCTGTTGCC	Suwabe *et al.* (2005)
BRMS-019	CCCAAACGCTTTTGACACAT	GGCACAATCCACTCAGCTTT	Suwabe *et al.* (2005)
BRMS-026	CCTATCCTCGGACTAATCAG	CTTGATGAGTTTCACATTGC	Suwabe *et al.* (2005)
BRMS-031	TGCCACCAATGACAATGACACTATC	GATGCACTGGGACCACTTACATTTT	Suwabe *et al.* (2005)
BRMS-036	GGTCCATTCCTTTTTGCATCTG	CATGGCAAGGGGTAACAAACAT	Suwabe *et al.* (2005)
BRMS042	TCGGAATTGGATAAGAATTCAA	GGATCAGTTATCTGCACCACAA	Suwabe *et al.* (2005)
BRMS-042	GGATCAGTTATCTGCACCACAA	TCGGAATTGGATAAGAATTCAA	Suwabe *et al.* (2005)
BRMS-043	GCGATGTTTTTTCTTCAGTGTC	TTAATCCCTACCCACAATTTCC	Suwabe *et al.* (2005)
BRMS050	AACTTTGCTTCCACTGATTTTT	TTGCTTAACGCTAAATCCATAT	Suwabe *et al.* (2005)
BRMS-050	AACTTTGCTTCCACTGATTTTT	TTGCTTAACGCTAAATCCATAT	Suwabe *et al.* (2005)
BRMS-051	GGCCAAGCCACTACTGCTCAGA	GCGGAGAGTGAGGGAGTTATGG	Suwabe *et al.* (2005)
BRMS-056	GATCAAGGCTACGGAGAGAGAG	CGTGACGCTAGAGTAATCGAGT	Suwabe *et al.* (2005)
BRMS-058	GCAGACAAGAAATTCTCGCCATGTC	GACATTGGCGAAAGTCTTGAACTGG	Suwabe *et al.* (2005)
BRMS-061	CTAGGGTTATGGTTTGAATCAGACA	TCGTATAGCTCAAATCAAATGTTCA	Suwabe *et al.* (2005)
BRMS-062	CTGCTGTGGAGATCGTTCATTGTAT	CCTTTTCTTCTGGGTTTCCTGACTT	Suwabe *et al.* (2005)
BRMS-070	AGGCCGAGGGTTAGACAAAAGAGAT	CGTTGGGAGGCTTCTTTAGTCACTT	Suwabe *et al.* (2005)
BRMS-071	CAAAGCGAGAAAGTGCAGTTGAGAG	TCCACGAAACTACTGCAGATTGAAA	Suwabe *et al.* (2005)
BRMS-075	GTTTCACATATTTTCTCTGTTTATT	ACCTTAAATGTTAAGTAAGCTAAAC	Suwabe *et al.* (2005)
BRMS-079	AATTTCTACTGGGGCGGGTGCTATC	TTAATGGTGTGATGGCGTGACGATG	Suwabe *et al.* (2005)
BRMS-085	ACTCCACACTCTCACTTCCTCTATT	TTACGCTTGTTTCTGTTTTTGAATA	Suwabe *et al.* (2005)
BRMS-090	AACGAACAACAACCAGCAAGGAGAG	GATCTGAAAACCCTAGCCGTCAATG	Suwabe *et al.* (2005)
BRMS-094	TTACCACTATGCGCCTTGTTACAGC	TGAACTGGGGAAAAGAAAGCAAAGA	Suwabe *et al.* (2005)
BRMS-096	AGTCGAGATCTCGTTCGTGTCTCCC	TGAAGAAGGATTGAAGCTGTTGTTG	Suwabe *et al.* (2005)
BRMS-097	AAGAGCAAGAGCCAAGACAGCCTAC	TGGTGATGGAGAAGTACATGGACGT	Suwabe *et al.* (2005)
BRMS-098	TGCTTGAGACGCTGCCACTTTGTTC	CATTCCTCCCCACCACCTTCACATC	Suwabe *et al.* (2005)
BRMS-100	CTCTTGAGAATCAGAGAGAGATTAC	GATCTTCATTATATTCATCTCTCTC	Suwabe *et al.* (2005)
BRMS-105	GAATCTCTGCAAGAAACAAATGTTA	ATCAAGTAACCTCCTTTTCTCTCTC	Suwabe *et al.* (2005)
BRMS-108	ATTCAAAGACAAAGGAATGCCTGAG	GATCCTGTCGAATGGCATTAATAAA	Suwabe *et al.* (2005)
BRMS-120	GTCAGCATACACGGCAAAACTCGCA	GGTACGGCAGTTCTCGGTCGGTTAA	Suwabe *et al.* (2005)
BRMS-124	GAGACGAGTGTTTTGTTGGCAGTTG	CGACGAGAACCAACACATAACAACC	Suwabe *et al.* (2005)
BRMS-125	GTTCTCAAAGGGAAACCGAAAAACA	GAGTTGGCCAGAGATTTACATGCGT	Suwabe *et al.* (2005)
BRMS-128	CCTGCATTTGGAAAACAAAACATTA	TTCATCACTTCCTCCTCAAATACCG	Suwabe *et al.* (2005)
BRMS-129b	TGAGGTTAGACATGGCGCTGCTTGC	TTTGATCATTGTGGTCGCGAGTTCG	Suwabe *et al.* (2005)
BRMS-151	ACAGGAACCTGTAGAACCAGTAGG	CTTCACCAATGGCTTCTCTCAT	Suwabe *et al.* (2005)
BRMS-153	GCAATAAAACCCTCCAACTTTCTAT	AAAACACAGGAGGAAAATTAAAAGG	Suwabe *et al.* (2005)
BRMS-158	AAAAGACAAAACCATCCAAACACTA	AAGCCTTTTTGAACTTCTCTGTGAT	Suwabe *et al.* (2005)
BRMS-166	AAGTCACTACTTCCATTGAGGAACC	GATGATTGGTGGTTTGGGTTT	Suwabe *et al.* (2005)
BRMS-173	GAGGATGCAGTTGCTGTTGTT	CTTCTTCGATGGATTCAAGAGAAC	Suwabe *et al.* (2005)
BRMS-175	GTGATACTGAAAGGGAGAGAGTGAG	AATCCTCATGAGCAAATCAACTAAC	Suwabe *et al.* (2005)
BRMS-176	ATGTCTACAGATGTGGAACCTATGG	AGGAAGACTGATTAACTCGTTTTGA	Suwabe *et al.* (2005)
BRMS-184	AGAACAATCTAACCAAAAAGACTCG	AAACAAAATAGGTCCGAAGAACTTT	Suwabe *et al.* (2005)
BRMS-185	ATCAAACCAGCAGTTCTATACCAAT	TCTCTTTCTGACCCGAAGAAGAC	Suwabe *et al.* (2005)
BRMS-195	AATACTTTCTGAAGTTGTCCGCTAA	AACCTACGCAAGATGCTTCTACTT	Suwabe *et al.* (2005)
BRMS-198	CGAGAGCAGTTAGGAAGCTTATAGA	AGAGATACTCTGTCCTCCACCTCTT	Suwabe *et al.* (2005)
BRMS-201	GTAAATAACAGTTCTGCCTCTGCTC	CTGCTGAATTAATTGCTGCTTCT	Suwabe *et al.* (2005)
BRMS-206	ATTAGACTCTTTCAAAAACGCAAAG	GATGACAACAACTCTTCCTCTGTTA	Suwabe *et al.* (2005)
BRMS-218	CCAAATATGTTTTCGTAAAGAAGGA	CTCCATTACTAAACAGTTTCCCATC	Suwabe *et al.* (2005)
BRMS-221	AAAGTCCTTGACGTTTGAGGAAGAA	CAGGTTCTTATGAAGGACCATGCAT	Suwabe *et al.* (2005)
BRMS-227	ACCATCTCGCTATTTATTTATGAAG	GACGATTTGATAGAGGAAAGGAAT	Suwabe *et al.* (2005)
BRMS-231	ATTCATAGCAAACCACGTGTGTAGT	TTCTGGACTTCAATTAGAGATGGTC	Suwabe *et al.* (2005)
BRMS-232	AAAACAATACGACTGATTGAACCAT	CAAATCATAGTCGAAACTAGCTAAAA	Suwabe *et al.* (2005)
BRMS-235	GGATCACAATCGTGTCCTAGTAATC	AGCATATCCATCAAGAGCTGGT	Suwabe *et al.* (2005)
BRMS-240	CAAGAGTATTTGTGTGGGTTGACTC	AAATAACGAACGGAGAGAGAGAGAG	Suwabe *et al.* (2005)
BRMS-245	CTCCTGTCCTCAACTATGTTTCCTT	TTGATTAGAAATAGAAGAAGAGAGA	Suwabe *et al.* (2005)
BRMS-247	TCTCTTTTGAGGTTTTCACTTTCAG	GCTGATTAAACTCTTACCACCAGAG	Suwabe *et al.* (2005)

Appendix 2 continue

Marker name	Primer sequence (5'-3')		Citation
	Forward	Reverse	
BRMS-252	ACTGGACTTATGTCTGAACAAGGAC	CTGGCCAACATCAACATATAAACTA	Suwabe *et al.* (2005)
BRMS-260	AGAGATCACCAACAGAAAGTTGAAT	TTCTCGTAATTGAAACTTGGGTTTA	Suwabe *et al.* (2005)
BRMS-261	CACCTGTCATGTCTTCTTCTGG	TTGTCTTTGTTTTCTTCTCATTCG	Suwabe *et al.* (2005)
BRMS-276	GACCGTTTTGCATTTTAAGAGCATT	TCACCACCAGTATCTTCAACAATCA	Suwabe *et al.* (2005)
BRMS-298	CCACTGTTTTATGACTCCAGTGCTT	TGACCTGGTGAAGTAGTTGTCTCGT	Suwabe *et al.* (2005)
BRMS-303	ACTCAACAACCGAACAAGAAAAACA	CGGTAGAGAACAGAGGAAGCCTAAG	Suwabe *et al.* (2005)
BRMS-312	CGCAGCAATTAAAAGCTTCACAGTA	ATTTCAACATGTCAGACGCTCTTTC	Suwabe *et al.* (2005)
BRMS-314	CATCATTTGTCTGTATCCGTCACTG	AATTGCAGGCTATGGTAACTGTCTG	Suwabe *et al.* (2005)
BRMS-317	GCAAAACAGATGAAGAAGATGGATG	GAGCTTATTGGGAGGCTTAACTCTG	Suwabe *et al.* (2005)
BRMS-322	CAGCAAACTAATAAAACCTACAACTCA	ACAGGAAAGAGAGGAAGGTTCAGTG	Suwabe *et al.* (2005)
BRMS-324	AACTTAACCGAAACCGAGATAGGTG	AATCTCGAAATTCATCGACTTCCTC	Suwabe *et al.* (2005)
BRMS-327	TCGTATTTTGTAGGCTCTGTTACGG	GCTTCAGCTTATCAGTCTCCTCTCC	Suwabe *et al.* (2005)
BRMS-330	TAATTTCCGTTTCGCCTCCACTTAT	TTGCTTTAATCAGGATGCTCTTGTC	Suwabe *et al.* (2005)
BRMS-333	ACTCTTCAGTTAGAAAAGCTAAATCT	CTCTTTCTGTGTGCTTACCGTTTTC	Suwabe *et al.* (2005)
BRMS-336	GGAGTGATGAGCGAGTGAGTATGTT	GTTGTGATGGTAGCAGTGGCAGT	Suwabe *et al.* (2005)
BRMS-339	ATTGATTCCGTACAATGACGAGTCT	TTCTGGAAAACGACAAATGACAAGT	Suwabe *et al.* (2005)
BRMS-342	CAACCAAAACGGGCCAATATAGTTA	TGTTTTCAAATAATCTCCCGTCTAA	Suwabe *et al.* (2005)
cnu_m250a	CAGATTTTCGAAAGGTGGTTGG	CCATCACCCGAAAATCCAAA	Kim *et al.* (2009)
cnu_m316a	TCAAGCATGTCCTTAAAACTCTGA	GCGTTCACGTTTCCCATATC	Kim *et al.* (2009)
FITO017	TTTTTGTATCCTCCATCATTTTTG	TGATATGTTTGACAATTTCCCC	Iniguez-Luy *et al.* (2009)
FITO018	TGCCAAGCTTTGAGTAGCAA	TGCCTTCTTCCATGCTTTCT	Iniguez-Luy *et al.* (2009)
FITO019	GTGAGACACCATCCCTCTT	TAGGAACGCTTGTAAACCTG	Iniguez-Luy *et al.* (2009)
FITO026	TATGGCAGAGTATGTGGGA	TACAAAGGTGTTTACCCGAA	Iniguez-Luy *et al.* (2009)
FITO034	CTGGTTTTCTCCTTCATCAG	AGCTTTTAGCTTTTCTCTCTCTC	Iniguez-Luy *et al.* (2009)
FITO036	GGATTGCCTGAGTTTATTCTT	TCTGGAGTAGATGCTTTGGT	Iniguez-Luy *et al.* (2009)
FITO040	GATTGTTTGTTTCTAACTGTGG	GATTGTTTGTTTCTAACTGTGG	Iniguez-Luy *et al.* (2009)
FITO043	AAAGTCGTGGGAAGTATCGT	AGGTGTAAGGATGGTGGTAGT	Iniguez-Luy *et al.* (2009)
FITO045	ATGGCTGTAGAAACACATTGA	CTGACAACACGAGCATCTTAC	Iniguez-Luy *et al.* (2009)
FITO050	AGCACAACTACCTGAACCTCT	TTTATCCTCGGTCTTCTCTCT	Iniguez-Luy *et al.* (2009)
FITO066	AGCCCATTTACCTGCTGA	GAAAGACGATGCTTAGGGT	Iniguez-Luy *et al.* (2009)
FITO067	TTTCCCATAAACTTCTCATTT	GCTTCATCGCTAATACTGCT	Iniguez-Luy *et al.* (2009)
FITO081	AACTAACTCGGGAAACAACC	GAATGTCCGTCAGAATACC	Iniguez-Luy *et al.* (2009)
FITO083	CCCTTTGTTTCTTCTTTATTCA	TCAACTATTCTATCACATCTCCA	Iniguez-Luy *et al.* (2009)
FITO088	CCATCACTCATCTCACTCTTT	ATAAATCTCGTTGTCGGAAGT	Iniguez-Luy *et al.* (2009)
FITO094	TTTATTTCTTTGGACTTGGG	CTACCGCATCATACATTCATT	Iniguez-Luy *et al.* (2009)
FITO095	AGATTTCATCCACAGCCTC	TTTGATTCTTGCGTTCTCTC	Iniguez-Luy *et al.* (2009)
FITO098	ATGGGAAGAGAGTGAGGG	TCTAAACCTAAACTACACCTGCT	Iniguez-Luy *et al.* (2009)
FITO100	GATGAGAGAAGGAAACCCTAA	ACAGCAGGAGAAGAGAGAGAA	Iniguez-Luy *et al.* (2009)
FITO102	TAACTCATCAAACCAATGCGA	CGTCCAACACAAACACTTACT	Iniguez-Luy *et al.* (2009)
FITO110	TAACCAACAACCTCCAATGA	CAATCCGAGAACAAAGATAAA	Iniguez-Luy *et al.* (2009)
FITO120	AAATACAAACCTCTACTCCCTG	GCTTGCTCACAGTCTTCTTC	Iniguez-Luy *et al.* (2009)
FITO122	CTATGTGATTAGTTGGTTGGG	AGCAGCGGAAGAGGAGTT	Iniguez-Luy *et al.* (2009)
FITO131	CTACAACGGGCAGGGTGA	AAATCCCATCTACTGGTTTGA	Iniguez-Luy *et al.* (2009)
FITO132	TTGGGATGTAGGAACTGA	TCACACTAAAGGTCAATCAAA	Iniguez-Luy *et al.* (2009)
FITO139	CCTCCATTACCACCACAA	CGTAGACAAACAACACCTGA	Iniguez-Luy *et al.* (2009)
FITO146	ATTACTGAACGGACGAAA	GGAGCAGATGGAAGTTGTTAG	Iniguez-Luy *et al.* (2009)
FITO156	TATGTGTGTGTGTGTGTGTGT	ATAACCTGACAACGAAGATTG	Iniguez-Luy *et al.* (2009)
FITO161	AGCCAGGGTTACACAGAGA	CCCAGAAGAGGGAGTCAG	Iniguez-Luy *et al.* (2009)
FITO163	AGGTGGATTTCTGAGTTTGAG	GTGCTGTTGAATGTAGTCCTG	Iniguez-Luy *et al.* (2009)
FITO165	ATCTACCTATTGAGGGTTTGG	CCAGGGCTACACAGAGAA	Iniguez-Luy *et al.* (2009)
FITO190	AGTCACACATTCCCAGCC	GTAATCCCAGCATCCAGAA	Iniguez-Luy *et al.* (2009)
FITO203	AAGTCGTTAGGCGAATCTG	ATTGAAGAGGAAGAAGGAGAA	Iniguez-Luy *et al.* (2009)
FITO204	TCTGATGGAGAAGAAGAAGAC	ATTGAAGAGGAAGAAGGAGAA	Iniguez-Luy *et al.* (2009)
FITO227	GTAACAGCAGAAGCAGAAGCA	CAGGTTCACGATACACAAGA	Iniguez-Luy *et al.* (2009)
FITO237	ATGAACAGCAACAACAACAA	TGTCTTCTCAAGTCCAGCA	Iniguez-Luy *et al.* (2009)
FITO239	TAAGAGATTTGAAGTTGGGA	GATAGTGTTGAAGCAGAGAAC	Iniguez-Luy *et al.* (2009)
FITO250	TTTATTCGGTTTATTCGGTTT	AGTATTTCCTTGATGTTTCCA	Iniguez-Luy *et al.* (2009)
FITO259	ACCAAATCCTGTTCACTTCTT	ATCACCCTCTCCCTCTCTATT	Iniguez-Luy *et al.* (2009)
FITO262	TCATAATAGCACAAGTCATAGTC	GAATCAAACCCAACACCTC	Iniguez-Luy *et al.* (2009)
FITO272	CTGGGTGACAGAGCAAGA	AGGCTGGTCCTGAACTCC	Iniguez-Luy *et al.* (2009)
FITO279	AGAGACCTGCCACACAGA	GCACTTATTACAGACCCAAAC	Iniguez-Luy *et al.* (2009)
FITO281	CACACACAAACACACACAGTT	GAAGAGAGTGAGGACGAAGAG	Iniguez-Luy *et al.* (2009)
FITO282	CAGAGGTGGAGTGAGAAAGA	GATGAAGAATGGAACCCTAAA	Iniguez-Luy *et al.* (2009)
FITO287	TGATTAGAAGGACAGCACACT	CCTCACACCACTCGGTTC	Iniguez-Luy *et al.* (2009)
FITO289	ACTGTCATTTCTCTCTCTCCTC	GTGCCGACTCTCTTCTCAC	Iniguez-Luy *et al.* (2009)
FITO294	ATCACTTTCCTCCAAATCACT	TACGACCTCAATCAATCAATC	Iniguez-Luy *et al.* (2009)
FITO306	AGATTGTCCGTTCTTGTATTT	CCCTGTTTCTTGTAGTGTTATG	Iniguez-Luy *et al.* (2009)
FITO316	ACGGATAAAGGAGGAGAAGA	CTGGTTAGTTAGCGTTGAGAA	Iniguez-Luy *et al.* (2009)
FITO353	ATCGCTGTTCGGTGGGTT	GTGCCAATAAAGTGTAAGGGA	Iniguez-Luy *et al.* (2009)
FITO355	GAACAAACCAACAGATTGAAC	GTGACGATGAGGAAGTGAA	Iniguez-Luy *et al.* (2009)
FITO373	TCCCGAAACTCAAATACATAA	CCCTAAGTCCATAACTACTACAA	Iniguez-Luy *et al.* (2009)
FITO375	TAGACGAACCTTGACAGCC	CGTGGAAGAAGAGATGATG	Iniguez-Luy *et al.* (2009)
FITO377	CATCACGCTTCACACTTATTT	TATTTGCCTTTCCATCATT	Iniguez-Luy *et al.* (2009)
FITO389	CATCTCCTAAACCTTCATCCT	GTCCTTGTGACTTATGCGTT	Iniguez-Luy *et al.* (2009)

Appendix 2 continue

Marker name	Primer sequence (5'-3')		Citation
	Forward	Reverse	
FITO394	GCTGACCTCCAACACTAAACT	TGTGAAGCAAGCCTAAATG	Iniguez-Luy *et al.* (2009)
FITO398	CAGAAACTTGGAAAGAAACAA	TACTAAACGGAAACGAAACTG	Iniguez-Luy *et al.* (2009)
FITO400	AGCCACGAAGAGAGAGACTT	TAAGAGGAGAAAGAAGAGCGT	Iniguez-Luy *et al.* (2009)
FITO424	AAACTGAGAATCAAGGGTT	ATGTCAAGAACAAATCAACGA	Iniguez-Luy *et al.* (2009)
FITO426	TGTCATAGTCAGTCAAAGTAGG	AAAGTGGAGAGAGGCAAATAC	Iniguez-Luy *et al.* (2009)
FITO429	ATTAGGGAGGAAGCGGTG	AGACAGAGAGACGAGGAGAGA	Iniguez-Luy *et al.* (2009)
FITO434	CTTTCTGTCTCTCCCTCATTC	CGCTGTTATCCGACTCCA	Iniguez-Luy *et al.* (2009)
FITO472	CGTCTTTGACCTTTCTATCTG	TGCCCTTGTTTCTTTCTTT	Iniguez-Luy *et al.* (2009)
FITO476	GAAACCATCCTTGAACACTC	CACACGCACTATCTCTCCTT	Iniguez-Luy *et al.* (2009)
FITO477	GAGAGAGGGAGTCTGTTTGTT	TTTACGCAGTGTTATGTTGG	Iniguez-Luy *et al.* (2009)
FITO482	CTTGCTCACCTCACACCC	TAACTGCGAACAGAAACGA	Iniguez-Luy *et al.* (2009)
FITO485	GGAATCAAACCGAACATAAA	CAAGAAGAGAGGCTGAATAGA	Iniguez-Luy *et al.* (2009)
FITO486	TATTTGGTTGGGCAGATTAC	ACTTTATTGGAACTATCAGGGA	Iniguez-Luy *et al.* (2009)
FITO488	TTTCTAAGGCGTATCAAAGTG	ATTTCTCCTCAACCAACAGAC	Iniguez-Luy *et al.* (2009)
FITO497	TGGTGTTTGGTATTTCTTTGT	ATTTATTCATTCCTACGGG	Iniguez-Luy *et al.* (2009)
FITO503	TTTCTATGATTTGCGGGA	GAGGATGAACACCACACAAC	Iniguez-Luy *et al.* (2009)
FITO505	CTGGATGGTCTTCTGTGTTT	AGGATTTCTTTAGTGGGTTGT	Iniguez-Luy *et al.* (2009)
FITO514	TCTCAAGGTTTGTGTCTCTGT	ACGGTTGCGTTCTATGTAAGT	Iniguez-Luy *et al.* (2009)
FITO520	AGCCCTTCTTCTTCTTCTTCT	GCTCTTCTTCCAATGCCC	Iniguez-Luy *et al.* (2009)
FITO565	GGTCTCTCCAAAGTAATGGTC	TCAATCCTCTCTCAAATCAAG	Iniguez-Luy *et al.* (2009)
KBrB005M19R	TGGTCCGGACTCCTAAGTTAATGC	CAAATCCCAATGTACAACGAACGA	Hatakeyama *et al.* (2010)
KBrB016E19R	ATGTTGAGCTGCGAATACGTTCCT	GAGCAATCTTCACCCTCTTCGTGT	Hatakeyama *et al.* (2010)
KBrB019C14F	GTGGAATGGAGTGTTGACAAATGG	TGTTTGTTGGTTCTTGCTCAAAGC	Hatakeyama *et al.* (2010)
KBrB022M07F	TTAAGCTTTGCATGTGCCTCTCTG	ATATGGGGTGATATTGGGGTGTGT	Hatakeyama *et al.* (2010)
KBrB024M19R	GCATCCTTGTTCCTGATTCCAAAC	TTAGATCCACCGGCAAAAATCACT	Hatakeyama *et al.* (2010)
KBrB027H17F	GGCAGATTCATCAAGATCCAAAAC	GCTCTTCAAGGCAAGGAAGATCAG	Hatakeyama *et al.* (2010)
KBrB065O24R	GTAGGGGCAGAGATATGGGGTAGG	ATGTCGTGTTTGATGCGGATATTG	Hatakeyama *et al.* (2010)
KBrB070L18R	TTTGTTGGTCGAGCTGTTTCAGAG	CACAGGGAGCCGTAGAAGAAAAGA	Hatakeyama *et al.* (2010)
KBrB078P21R	GAGAAGTACAATTTCGCAGGCGTT	CAAACCAGTCAGCAAACCTGAAAA	Hatakeyama *et al.* (2010)
KBrB092H15F	GGGCTATGTAAACATCTGGCATGG	CCGCTGGATTTCTATTCATAGTCG	Hatakeyama *et al.* (2010)
KBrH006K06R	GAGAGAGAGACGACACACACTCGG	TAATCGCAATCCCCAAAAGAGAGA	Hatakeyama *et al.* (2010)
KBrH015H02R	TGAAGTAACCACGTGACATTGATGA	TCCAGATTCATATCCACATTACGCA	Hatakeyama *et al.* (2010)
KBrH057L10F	TCAAACATTTGTCAACGCACAC	GTAGCCTTCGGTCAATGCAAAGAA	Hatakeyama *et al.* (2010)
KBrH071B03R	GGACCGGCACGTATATTACCTGAA	ATCATCGAGATCCGAGAAACGAAC	Hatakeyama *et al.* (2010)
KBrH079A14F	TGCTTTCCTACCTTTTTCCCCTTC	GAGTGCGACTTGGAATTTCTCCAT	Hatakeyama *et al.* (2010)
KBrH094B16F	CCTTCTTCTCGATCTCACTCTCGG	TTCGAACTCCTCCTTGAACGACTC	Hatakeyama *et al.* (2010)
KBrH102J11R	GGGCTTTGATCCATTCCCTTAGTG	CCCATCTCAGCAAACACAGTTACG	Hatakeyama *et al.* (2010)
KBrH103G17F	ACAGACAAAAGCTTCATTGCCACA	GAGACAAAGGCTGATTCCTCCAAA	Hatakeyama *et al.* (2010)
KBrH104H02R	ATGTGAGCTCGGGATGAGAGTAGG	TCTCGCTCCTAAACCCTTTCTTCC	Hatakeyama *et al.* (2010)
KBrH107C06R	CCATCAAGTGTTTACAAACGAGCA	TGCAGGTGTTTTCCTTACCTCTCC	Hatakeyama *et al.* (2010)
KBrH107O12F	TCAATGCTGGGAAGAAACTCTGTG	AGATCTCTCCTCTCGCACCTCAAA	Hatakeyama *et al.* (2010)
KBrH108F14R	AAGGTTCCAGCTCTGCATTTCAAG	AAGGCTTCAGTAATCCCTTCCCAG	Hatakeyama *et al.* (2010)
KBrH110H05R	TTCCACTCCCACTTCGTCTTCTTC	CTCAGATCTGCAATCTAACAGATGAAC	Hatakeyama *et al.* (2010)
KBrH110I17R	CGCTAAGCTAGAGCTTGCACTGAA	GCGGAACAAAGAGAAATGGAGAAA	Hatakeyama *et al.* (2010)
KBrH114A18F	AACCATTTGAGCTGAGATTTTGCC	TGCAGTTTTCATCACAACCTTCCA	Hatakeyama *et al.* (2010)
KBrH120N01R	AAGCTGCCTCAAAACACAAACCTT	AGAAACTGTATCAGTCTCCGGCCA	Hatakeyama *et al.* (2010)
KBrH123B08R	TTTCTTGAAAGATCCGGACGATGT	TGTGGCACAAATGGAGTCAAATCT	Hatakeyama *et al.* (2010)
Na10-B01	CAAGTGTCTGCTAGGTGGGG	TCGATCGAAGAAACCAGACC	Lowe *et al.* (2002)
Na12-A07	TCAAAGCCATAAAGCAGGTG	CATCTTCAACACGCATACCG	Lowe *et al.* (2002)
Na12-B05	CAAATATCCGTCATCGGAGC	CCTGCGGGATATTGAAGACC	Lowe *et al.* (2002)
Na12-C06	AACGGATGAAGAACACATTGC	TAGGGCCTGTTATTCGATGG	Lowe *et al.* (2002)
Ni4-F08	GAGAAAGAAGCAAACACAAAGC	TCTCTTTCTTCTCGTTGCCG	Lowe *et al.* (2002)
nia_m043a	CCATTCGAGGTGGTCGTAAA	AGAAAACGGACCTCGATTCA	Li *et al.* (2013)
OI10-B08	AAGCTGTTCGATGAAATGCC	ACTTGTTTGCATCCATTGCC	Lowe *et al.* (2002)
OI10-C01	ATGACTGCTTAAACAGCGCC	CTTCTCCAACAAAAGCTCGG	Lowe *et al.* (2002)
OI10-D08	TCCGAACACTCTAAGTTAGCTCC	GAGCTGTATGTCTCCCGTGC	Lowe *et al.* (2002)
Ra2-G08	ATGTCCGGATAACCGAATCC	GAAGCTTTTCAATTTTTAAGTTCTCTC	Lowe *et al.* (2002)
sN2025	AAGGAGCAAACACAAGCGAT	GCACAAGCACTCTACGGTGA	AAFC Consortium (2016)
sR11644	GCAAACTGGTAAACCCTGGA	GGGTAGACTGGTCCCGAGAT	AAFC Consortium (2016)
sR6293	CCAAACGCTTTTCTTTCTGC	CCAATGACGCTCCAAGATTT	AAFC Consortium (2016)