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Rice Germplasm in Korea and Association Mapping

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1. Introduction

Rice is a traditional staple food crop in Korea and many other countries. Although the center of rice origin is still unclear, it is believed to be introduced from China to the Korean Peninsula in the early Bronze Age via one of two possible routes—across the West Sea or along the northeastern seashore from China according to Hammer (2005) and Vavilov (1935). Rice germplasm has evolved through several millennia of cultivation and selection by our farming ancestors. An important consequence of the domestication of both plants and animals is a reduction of genetic variability (Hancock, 2004). Maintaining biodiversity is an important worldwide problem and different countries have various policies intended to preserve biodiversity. Because conservation of biodiversity and ecosystems is closely linked to the quality of human life, the preservation and improvement of ecosystems are problematic for agriculture.

Genetic diversity in a crop species is essential for sustained high productivity. Breeding efforts have been devoted to improving grain quality, yield potential, resistance to diseases and insect pests, and environmental stress tolerance. Progress in plant breeding requires a continuous supply of genes or gene-complexes. In this respect, the researcher is often handicapped by the limited availability of germplasm resources. The assembly of large varietal collections, systematic screening for desired traits and subsequent incorporation of the relevant genes into existing cultivars is imperative to meet these needs. The use of landrace varieties has increased in recent years. Wild rice accessions have contributed greatly to rice breeding as a source of resistance genes (*e.g.*, *Xa21*, *BPH14*, *BPH15*) (Ronald *et al.*, 1992; Song *et al.*, 1995; Yang *et al.*, 2004; Du *et al.*, 2009; Hu *et al.*, 2012). Much of the diversity in the rice gene pool is contained in gene banks around the world. Molecular biology has contributed significantly to an increased

understanding of many aspects of plant biology by generating technologies and methods of analysis that provide new approaches or supplement classical methods of analysis. Plant genetic resource scientists and other researchers are increasingly aware of the potential benefits of applying new technologies to germplasm conservation and research.

The integration of genetic data with molecular biotechnology will help breeders produce new rice varieties with the desired traits and make the conservation of rice genetic resources more efficient. Because of newly developed methods for association mapping of genes or QTLs related to desired traits, many genome-wide association analyses have been conducted in rice and resulted in valuable genome-wide association maps to describe the genetic architecture of complex traits. However, further efforts are needed to obtain more genomic information to fill in the gaps of our knowledge and meet the needs and challenges of rice breeding. This chapter will focus on the status of rice germplasm preservation activities, research programs, and outcomes of association mapping in rice in Korea.

2. Research on rice germplasm in Korea

The Ministry of Foreign Affairs and Trade (2009) had outlined eight major environmental issues as current threats to Korea; global warming, desertification, wildlife extinction, rain forest reduction, acid rain, depletion of the ozone layer, marine pollution, and air pollution. The rate of climate change is faster in Korea than the global average, leading to a rapid reduction in national biodiversity. One hundred and ninety families comprising 4000 species of vascular plants and ferns occur in Korea (Lee and Yim, 1978). Approximately 3700 different kinds of flowering plants are estimated to occur naturally (Chung, 1957; Lee, 1980). Four hundred and seven different endemic taxa in six genera are distributed throughout Korea (Lee, 1982). However, some plant species are on the verge of extinction because of pollution and a wide range of developmental activities during the last 20 years in Korea (Ministry of Environment, 1994), highlighting the importance of conservation efforts (Ahn *et al.*, 1994). Conservation programs usually involve activities such as collection, characterization, evaluation, regeneration, documentation, and storage of each germplasm accession.

The National Biodiversity Strategy was implemented in 1997 to integrate and consolidate plans formulated by various ministries and government institutes, including Comprehensive Biological Resources Conservation Plans. The Rural Development Administration (RDA) Gene Bank is one of the institutions responsible for these plans. Rice research programs covering agronomic practices, physiology, post-harvest technology, grain quality evaluation, rice breeding and genetics, and biotechnology, are led by the National Institute of Crop Science (NICS) under the RDA. Other institutions affiliated with NICS carry out rice research programs to target specific problems in various regions of Korea. From 1980 to 1990, rice sheath blight (*Acrocyldirum oryzae*) was the most destructive disease affecting production from damaging approximately 555,000 hectares of rice paddy fields in Korea. Furthermore, rice pests including brown plant hopper, white-backed plant hopper, and small brown plant hopper attacked 586,000 hectares of rice nationwide during the same period (NASTI, 1996). A continuous

cultivation of only five or six cultivars countrywide should be responsible to the extensive losses from the pests.

Rice season normally begins in mid-April and ends in mid-October in Korea. The lowest temperature in both April and October is 13°C. Because of the unprecedented yield loss due to cold damage in 1980 (damage to 80% of total rice hectareage and a yield reduction of 3.9 tons per hectare), cultivation of high-yielding “Tongil-type” rice cultivars declined rapidly, and only high-yielding japonica cultivars have been grown since 1990. In 2010, 20 mid- to late-maturing japonica cultivars were grown on 891,493 hectares, accounting for 92.9% of the total rice production area (Kang and Kim, 2012). Large decrease of temperature also occurred in 1971 and 2003, causing damage to 17% and 20% of total rice hectareage, respectively. Preharvest sprouting may become a serious problem for rice production, as well as for other crops, because of the trend in recent years for frequent and unusually heavy rain at harvest time. Breeders are making efforts to address this problem.

Rice breeders see the development of genetically improved cultivars using modern breeding techniques as an efficient way to reduce the losses in rice production caused by biotic and abiotic stresses. Sequencing the rice genome for genotyping and developing marker-assisted selection (MAS) system have fast-tracked research efforts. In the past, most national programs gave a lower priority to collecting wild relatives of rice than to collecting rice cultivars. Wild rice resources are agronomically unattractive, relatively expensive to conserve, and difficult to use. However, wild rice germplasms are known to contain a broad array of useful genes (Hodgkin, 1991). The benefits for the landrace germplasms to be used in breeding new cultivars in response to climate and environment changes in Korea are resistances to diseases in order to maintain superior qualities suited to consumers’ preferences. Plant germplasm resource activities in Korea are performed by The Basic Conservation Programme for Nature and Environment (1994–2003) under the Ministry of Environment (NASTI, 1996).

The RDA Gene Bank conserves 24,673 rice accessions, including Korean landraces and wild types. Many gene banks are having financial difficulty to maintain germplasm collections due to a rapid increase of accession number. These problems may restrict a full exploitation, evaluation, and utilization of these accessions, thus managing such collections presents major challenges (Holden, 1984). The concept of a core collection for resolving these problems has received increased attention over the last few years. Germplasm sampling methods include sequential, stratified, biased (for example, by ecology or country), and random sampling. An understanding of factors underlying the traits being sought will help reduce the time required for identification of useful genes. For very rare traits, such as some associated with resistance to virus infection, searching among wild *Oryza* species and *O. glaberrima* may be most appropriate. Efficient methods for evaluation of germplasm to identify genes for crop improvement will promote the use of conserved germplasm. Frankel and Brown (1984) proposed the concept of a core set of lines to resolve such problems. A desire core set should include the maximum genetic diversity in a crop species including its wild relatives with minimum repetition and provide a manageable set of accessions to gene bank managers, plant breeders, and research scientists. Such a core collection would become the focus of the search for desirable new characteristics, detailed evaluation, and development of new techniques. An initial set of 4406 rice accessions was selected based on ecological types and accession passport information,

including their countries of origin. Using simple sequence repeat (SSR) genotype information, a final core set comprising the 166 conserved accessions currently used by the RDA was generated by a heuristic approach using the PowerCore software developed by Kim *et al.* (2007). Based on this resulted core set, some association mapping studies have been conducted and further researches are still being undertaken.

3. Association mapping in rice

Association mapping analyzes loci in diverse populations and associates them with both one another and with phenotypes. It is a powerful genetic mapping tool for crops and provides high-resolution, broad allele coverage, and cost-effective gene tagging for the evaluation of plant germplasm resources. Genetic mapping of QTL can be performed in two main ways (Ross-Ibarra *et al.*, 2007): (1) Linkage-mapping as well as “gene tagging” using experimental populations (also referred to as “biparental” mapping populations) and (2) LD-mapping or “association mapping” using diverse lines from the natural populations or germplasm collections (Abdurakhmonov and Abdulkarimov, 2008). LD mapping is based on identification of associations between phenotype and allele frequencies. The advantage of LD mapping for the breeder is that mapping and commercial variety development can be conducted simultaneously (Langridge and Chalmers, 2005). For phenotypes or traits that are governed by multiple genes or QTLs, diverse alleles or advantageous allele combinations should be mined by association mapping followed by gene-tagging efforts using biparental crosses.

The localization of alleles relies on creating a statistical association between markers and QTL alleles and on the efficacy of markers. For markers to be effective, they must be closely linked to the target locus and be able to detect polymorphisms in material likely to be used in a breeding program. Improvements in marker screening techniques have facilitated the tracking of genes (Subudhi *et al.*, 2006). Isoenzyme and other protein-based marker systems had in wide long been used before DNA markers became popular (Langridge and Chalmers, 2005). Since then, a variety of DNA-based molecular markers have been developed, including restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs) (Williams *et al.*, 1990), amplified fragment length polymorphisms (AFLPs) (Vos *et al.*, 1995), SSRs (Litt and Luty, 1989), single-strand conformational polymorphisms (SSCPs), cleaved amplified polymorphic sequence (CAPS) markers (Konieczyn and Asubel, 1993), sequence tagged sites (STSs) (Olson *et al.* 1989), sequence-characterized amplified region (SCAR) markers (Martin *et al.*, 1991), and single nucleotide polymorphisms (SNPs) (Brookes, 1999). A total of 2740 SSRs were experimentally confirmed for rice in 2002 or approximately one SSR for every 157 kb (McCouch, 2002). The highly polymorphic nature of many microsatellites is of particular value (Banni *et al.*, 2012, Yoon *et al.*, 2012, Moe and Park, 2012, Zhao *et al.*, 2012a; Khaing *et al.*, 2013) for analysis of closely related genotypes or within narrowly adapted gene pools. Thus, the availability of a high-density SSR map is a valuable public resource for interpretation of the functional significance of the rapidly emerging rice genome sequence information.

The next generation of genetic markers is based on SNPs, which provide an attractive tool for QTL mapping studies and marker-assisted selection in plant breeding programs (Mohler and

Schwarz, 2005). SNP discovery is performed primarily *in silico* or using new sequencing approaches (Henry and Edwards, 2009). Large-scale SNP analysis is now possible in plants using a range of platforms. The increasing ease of sequencing and automated genotyping has made association mapping in plants a more attractive option by altering the conventional plant genome mapping method, which involves linkage analysis in a segregating population. This trend is likely to continue as the sequencing of genomes increases. Recently, genome-wide association studies (GWAS) with SNP variants have been conducted using new sequencing platforms (Table 1).

3.1. International rice association-mapping activity

Genome mapping of rice was first attempted using linkage analysis of appearance or phenotype (Nagao and Takahashi, 1963). Nowadays, improvement of the linkage map has been achieved using isozymes (Nakagahra, 1977) and RFLPs and SSRs (McCouch *et al.*, 1988; Saito *et al.*, 1991; McCouch *et al.*, 1991 and Yu *et al.*, 1991, Tanksley *et al.*, 1991, Causse *et al.*, 1994; Kurata *et al.*, 1994; Harushima *et al.*, 1998; McCouch *et al.*, 2002). Relatively few association-mapping studies in rice have been performed. Some rice association-mapping studies using various populations and molecular markers are summarized in Table 1 in which most are conducted using SSR markers.

Whole-genome resequencing is a promising strategy to identify the relationship between sequence variation and normal or mutant phenotypes. High-throughput genome resequencing - if accurate - has the advantage of allowing researchers to identify the specific nucleotides associated with a given phenotype, and allowing the effective detection and analysis of genetic variations important for molecular breeding. An important application of NGS is the resequencing of targeted regions for the identification of mutant alleles, and we believe that mapping by sequencing will become a centerpiece in efforts to discover the genes responsible for QTLs. Generally speaking, the availability of a wide range of low- and high-multiplex single nucleotide polymorphism (SNP) assay methods (sequencing accuracy and depth of coverage relies on the experimental design) makes SNPs an ideal marker option for QTL mapping, association analysis, MAS, and the construction of high-density genetic maps for fine mapping and cloning of agronomically important genes (McCouch *et al.*, 2010).

SNP discovery by resequencing whole-genome or subgenome of sample materials is often among the first use of a reference genome sequence. For inbreeding species such as rice, lines to be resequenced are normally purified through 1 or 2 generations of inbreeding (via single seed descent). After a DNA sample is resequenced using NGS technology, SNPs can be identified by comparing the sequenced genome with a reference genome like Nipponbare for japonica rice. For example, using information on the features of the B73, Gore *et al.* (2009) targeted the gene fraction of the maize genome for resequencing in the founder inbred lines of the nested association mapping population. Two data sets comprising 3.3 million SNPs were used to produce a first haplotype map ("HapMap") and to analyze the distribution of recombination and diversity along the maize chromosomes.

A suitable example is the construction of a comprehensive HapMap for rice that was used for the genome-wide associate study of 14 agronomic traits, such as heading date and tillering (Huang *et al.*, 2010). The researchers made use of low-coverage (1-fold per rice line) sequence

data across lines, for a combined coverage of ~508-fold, and detected 3.6 million SNPs which can explain ~36% of the phenotypic variance for 14 agronomic traits. This work provided a new approach to low-fold sequence coverage, which can be used to detect not only SNPs but also more complex polymorphisms, and partially overcome the need for deeper sequence coverage (Clark, 2010). Further study was performed with the similar strategy for 950 world-wide rice varieties by Huang et al. (2012) and thirty-two novel loci associated with flowering time and ten grain-related traits were identified. Additionally, 40 cultivated accessions selected from the major groups of rice and 10 from their wild progenitors (*O. rufipogon* and *O. nivara*) were resequenced to >15X raw data coverage (Xu et al., 2012). After mapping the sequence read back to an IRGSP reference genome, the authors investigated the genome-wide variation pattern in a comparative analysis. The data revealed examples of structural variation in genomes and included 6.5 million high-quality SNPs after excluding sites with missing data in any accession. Using these population and SNP data, the authors also identified thousands of new rice genes and tracked down those with a significantly lower diversity in cultivated, but not wild rice. These variants represent a valuable resource for those interested in improving rice cultivars.

Preferences in terms of the processing, cooking, and eating qualities of rice differ globally. Plant breeders are attempting to fulfill consumer demand for rice varieties with specific qualities. The major components of rice grain quality include appearance, milling, cooking, eating, and nutritional aspects. The chemical composition of rice grain is important because of its relationship with eating quality of rice. Amylose content is one of the most important traits that determine cooking quality, which is controlled by a major locus *waxy* (*Wx*) on chromosome 6 (Wang et al., 1992; He et al., 1999; Tan et al., 1999). Genes associated with amylose content, such as starch synthase IIa (*SSIIa*) and *Wx*, are of particular interest. Sano et al. (1986) identified two alleles of the *Wx* locus using RFLP markers that correspond to the indica and japonica subspecies. Most grain quality mapping studies have used the *O. sativa* germplasm (He et al., 1999; Tan et al., 1999, 2000, 2001; Zhou et al., 2003). Borba et al. (2010) conducted association mapping study on yield traits and grain quality traits including amylose content, and the significant association detected between amylose content and RM190 was in agreement with previous QTL analyses. Zhao et al. (2011) identified 44,100 SNP variants across 413 diverse rice accessions collected from 82 countries and observed GWAS for six categories of traits covering morphology related traits; yield-related traits; seed and grain morphology related traits; stress-related phenotypes; cooking, eating and nutritional-quality-related traits; and plant development, represented by flowering time. This study demonstrated that different traits have different genetic architectures.

Olsen and Purugganan (2002) elucidated the origin and evolution of glutinous rice based on the haplotype of the *Wx* gene. By using dCAPS markers, waxy mutations and waxy rice cultivation were shown to have occurred predominantly in the japonica line during the evolution of domestic rice cultivation (Yamanaka et al., 2004). Genetic polymorphisms of starch-synthesis genes have been demonstrated to be associated with starch physicochemical properties using molecular markers such as SSRs, SNPs, and STSs. These markers can be extremely useful in marker-assisted breeding (Bao et al., 2002; Bao et al., 2006a). *SSIIa* was identified as the major gene responsible for determination of gelatinization temperature (GT). Among four SNPs in the *SSIIa* gene, some that cause amino acid substitutions have been

identified. The GC/TT SNP is strongly associated with GT (Bao *et al.*, 2006b; Nakamura *et al.*, 2005; Umemoto and Aoki, 2005; Waters *et al.*, 2006).

Rice nutritional quality is another important factor for consumer acceptance. In developing countries where rice is the main food, its nutrient content makes a significant contribution to the intake of some essential nutrients. Interest in natural antioxidants in rice is growing due to their role in preventing oxidative stress-related diseases (Aguilar-Garcia *et al.*, 2007; Willcox *et al.*, 2004; Zhang *et al.*, 2013). Rice contains antioxidant compounds such as γ -oryzanols, tocopherols, and polyphenols, which are associated with a reduced risk of developing chronic diseases such as cardiovascular disease, type 2 diabetes, and some cancers (Liu, 2007; Tan *et al.*, 2001; Toyokuni *et al.*, 2002). Pigments and flavonoids in colored rice are positively correlated with the antioxidant capacity (Xia *et al.*, 2003; Yawadiao *et al.*, 2007). Association mapping of pigments and flavonoid contents was carried out in brown rice using SSR markers. Significant correlations between phytochemical content and marker loci were found and markers associated with multi-phenotypic traits such as grain color, phenolic content and antioxidant capacity were identified (Shao *et al.*, 2011).

The amino acid composition of rice grain is an important characteristic related to nutrient quality. Environmental conditions, potassium, and nitrogen dramatically influence the amino acid and protein contents of rice (Wu *et al.*, 2004). Few reports of mapping of QTLs for the contents of protein and amino acids in rice grain have been published. Twelve main effect QTLs (MQTLs) were identified for 10 components of amino acid content in milled rice. Most of the main effect QTLs for amino acid content tended to co-localize within the genome (Lu *et al.*, 2009).

Although many QTL analyses and genetic mapping studies of grain quality have been conducted, association-mapping studies of biotic and abiotic traits in rice are few in number. The genes or QTLs related to these traits are complex. Genetic mapping, including association mapping and linkage mapping, are useful methods of identifying alleles for these traits. As shown in Table 1, most association-mapping studies focused on morphological and agronomic characteristics. Four studies were related to grain and eating quality and only one addressed disease resistance and aluminum tolerance. Biotic and abiotic stress-tolerance traits remain to be explored by association mapping.

Reference	Number of accessions and population type	Number and types of markers used	Traits
Virk <i>et al.</i> , 1996	200 rice accessions	7 RAPD	Ten morphological traits; culm number, culm length, culm diameter, grain length, grain width, leaf length, leaf width, days to 50% flowering, panicle length and seedling height
Zhang <i>et al.</i> , 2005	218 inbred lines, worldwide germplasms	60 SSR, 114 RFLPs	Multiple agronomic traits such as plant height, heading date, flag leaf length and width, tiller number, stem diameter, panicle length, grain length and width, grain length/width ratio, grain thickness, 1000-grain weight

Reference	Number of accessions and population type	Number and types of markers used	Traits
Iwata <i>et al.</i> , 2007	332 rice accessions	179 RFLPs	Size and shape of milled rice grains
Agrama <i>et al.</i> , 2007	183 rice accessions	123 SSRs	Grain length and width, grain length/width ratio, 100 grain weight, grain thickness
Yan <i>et al.</i> , 2009	90 accessions	108 SSRs + 1 indel	Single, dual and total stigma exertions and spikelet characteristics
Wen <i>et al.</i> , 2009	170 rice accessions	126 SSRs, 6 indels	Heading date, plant height, panicle length
Borba <i>et al.</i> , 2010	242 inbred lines, worldwide germplasms	86 SSRs	Yield, amylose content, head-milled rice
Huang <i>et al.</i> , 2010	517 landraces including japonica and indica	~3.6 million SNPs	Fourteen agronomic traits
Iwata <i>et al.</i> , 2010	332 rice accessions	179 RFLPs	Grain shape variation
Jin <i>et al.</i> , 2010	416 rice accessions	100 SSRs	Grain color
Ordóñez Jr. <i>et al.</i> , 2010	192 elite rice breeding lines and tropical japonica germplasm base	97 SSRs	Grain quality and flowering time
Zhao <i>et al.</i> , 2010	395 diverse <i>O. sativa</i> accessions	1,536 SNPs	Amylose content
Famoso <i>et al.</i> , 2011	373 diverse <i>O. sativa</i> accessions	36,901 SNPs	Al tolerance
Hu <i>et al.</i> , 2011	303 <i>O. sativa</i> accessions	24 SSRs	Awness
Zhao <i>et al.</i> , 2011	413 diverse accessions of <i>O. sativa</i>	44,100 SNPs	Thirty-four traits of agronomic characteristics, cooking and eating quality, disease resistance
Bryant <i>et al.</i> , 2011	174 accessions	156 SSRs	Silica concentration in rice hulls
Li <i>et al.</i> , 2011	217 accessions	154 SSRs and 1 indel	Yield and yield components among 14 traits
Lou <i>et al.</i> , 2011	48 accessions	218 markers (SSRs + indels)	Grain metabolites
Shao <i>et al.</i> , 2011	416 rice accessions including 361 white rice, 50 red rice, and 6 black rice	100 SSRs	Color parameters of brown rice grain, phenolic content, flavonoid content and antioxidant activity
Zhang <i>et al.</i> , 2011	A core collection consisting of 150 rice varieties	274 SSRs	Six morphological traits: glume hair, phenol reaction, length of 1st-2nd rachis internode, glume color at heading, leaf hair, and grain length/width
Zhou <i>et al.</i> , 2012	128 japonica rice varieties	152 SSRs	Eleven quantitative traits of agronomic and economic importance

Reference	Number of accessions and population type	Number and types of markers used	Traits
Huang <i>et al.</i> , 2012	950 worldwide rice cultivars	1,345,417 SNPs	Flowering time and grain yield traits
Jia <i>et al.</i> , 2012	217 entries	154 SSR markers and 1 indel marker	Sheath blight resistance
Li <i>et al.</i> , 2012	203 accessions	154 SSRs and 1 indel	Harvest index and related components among 14 traits
Clark <i>et al.</i> , 2013	233 rice (<i>Oryza sativa</i>) accessions	36,901 SNPs	Root growth and development

Table 1. International association-mapping studies of various traits using various markers in rice.

3.2. Association mapping of rice in Korea

To identify useful alleles from a representative core set of rice lines for transferring into elite lines, an allele-mining set of 166 accessions (Zhao *et al.*, 2010) was successfully developed from 4046 rice accessions which were selected from 10368 accessions in the Korea RDA Gene Bank by 39 phenotype traits (Chung and Park, 2009), through a modified heuristic algorithm approach based on 15 SSR markers using the PowerCore software (Kim *et al.*, 2007). Chung *et al.* also employed the PowerCore software of Kim *et al.* to develop the first preliminary core set by phenotypes. The gene diversity and population structure (Q) were analyzed using PowerMarker 3.25 (Liu and Muse, 2005) and Structure 2.2 (Evanno *et al.*, 2005) based on 170 SSR markers. Analysis of these data identified the major substructure groups when the number of populations was set at four (Fig. 1).

Association mapping was conducted on this core set of lines over the past 2 years (as shown in Table 2). Zhao *et al.* (2012b) analyzed 130 accessions from the core set using 170 SSR markers for association analysis of physicochemical traits related to eating quality. Linkage disequilibrium (LD) patterns and distributions are of fundamental importance for genome-wide mapping associations. The mean r^2 value for all intrachromosomal loci pairs was 0.0940. LD between linked markers decreased with distance. Marker–trait associations were investigated using the unified mixed-model approach considering both Q and kinship (K). In total, 101 marker–trait associations ($P < 0.05$) were identified using 52 SSR markers covering 12 chromosomes (Fig. 2.). Although direct comparisons of the chromosomal locations of marker–trait associations with previously reported QTLs are difficult because different materials and mapping molecular markers were used, most marker–trait associations were located in regions containing QTLs associated with a given trait. Indeed, some were located in similar or proximal regions related to starch synthesis. The new markers related to eating quality will facilitate the understanding of QTLs and marker-assisted selection (Zhao *et al.*, 2012b).

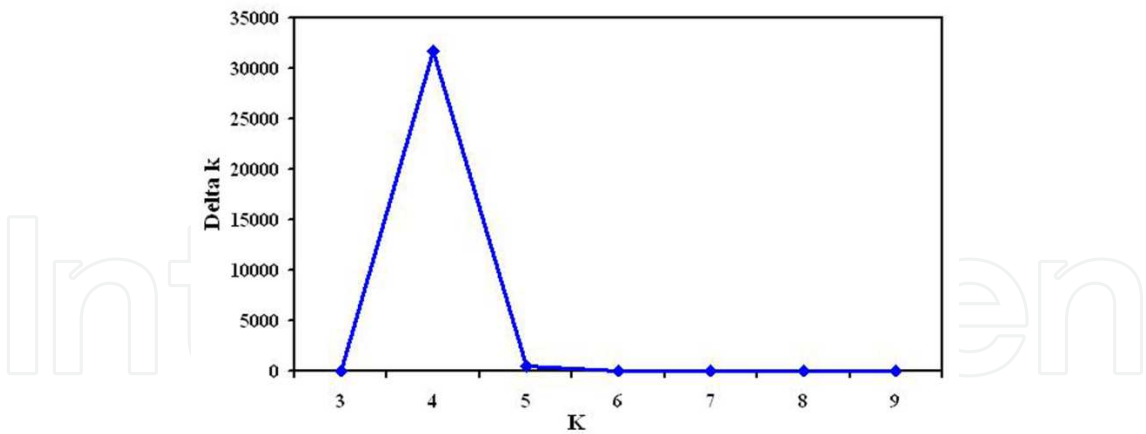


Figure 1. Values of ΔK , with its modal value used to detect the true K of four groups ($K = 4$). For each K value, five independent runs (blue diamonds) were considered and averaged over the replicates (Zhao *et al.*, 2012b).

Reference	Numbers of lines and population type	Number and types of markers used	Traits
Zhao <i>et al.</i> , 2009	84 accessions from land race core set	25 SSRs	16 amino acids
Zhao <i>et al.</i> , 2012	130 accessions from core set	170 SSRs	Eating quality
Lu <i>et al.</i> , 2012a	104 accessions from core set	86 SNPs and indels	Amylose content, RVA
Lu <i>et al.</i> , 2012b	107 accessions from core set	83 SNPs, indels, and SSRs	Amylose content, RVA

Table 2. Rice association-mapping studies for various traits and marker types in Korea.

Association analysis of candidate genes has been used to trace the origin of agronomically important traits. Lu *et al.* (2012a) used the rice core lines for association-mapping to investigate the relationship between sequence variations from parts of 10 SSRGs and the amylose content (AC) and rapid viscosity analysis (RVA) profiles. Eighty-six sequence variations were found in 10 sequenced amplicons including 79 SNPs, six insertion-deletions (indels), and one polymorphic SSR. Among them, 61 variations were exon-based, of which 41 should lead to amino acid changes. The association mapping results showed a sum of four significant associations between three phenotypic indices and three sequence variations. An ADP - glucose pyrophosphorylase small subunit 1 (*OsAGPS1*) SNP (A to G) was significantly associated with increased AC ($P < 0.001$, $r^2 = 15.6\%$) while a 12-bp deletion of an *AGPase* large subunit 4 (*OsAGPL4*) (Table 3) was significantly related to decreased breakdown viscosity ($P < 0.001$, $r^2 = 16.6\%$) in both general linear model (GLM) and mixed linear model (MLM) (Lu *et al.*, 2012a). One SNP with a g/c transversion at the 63rd nucleotide downstream of the *OsBEIIb* gene termination codon on rice chromosome 2 was significantly associated with multiple trait

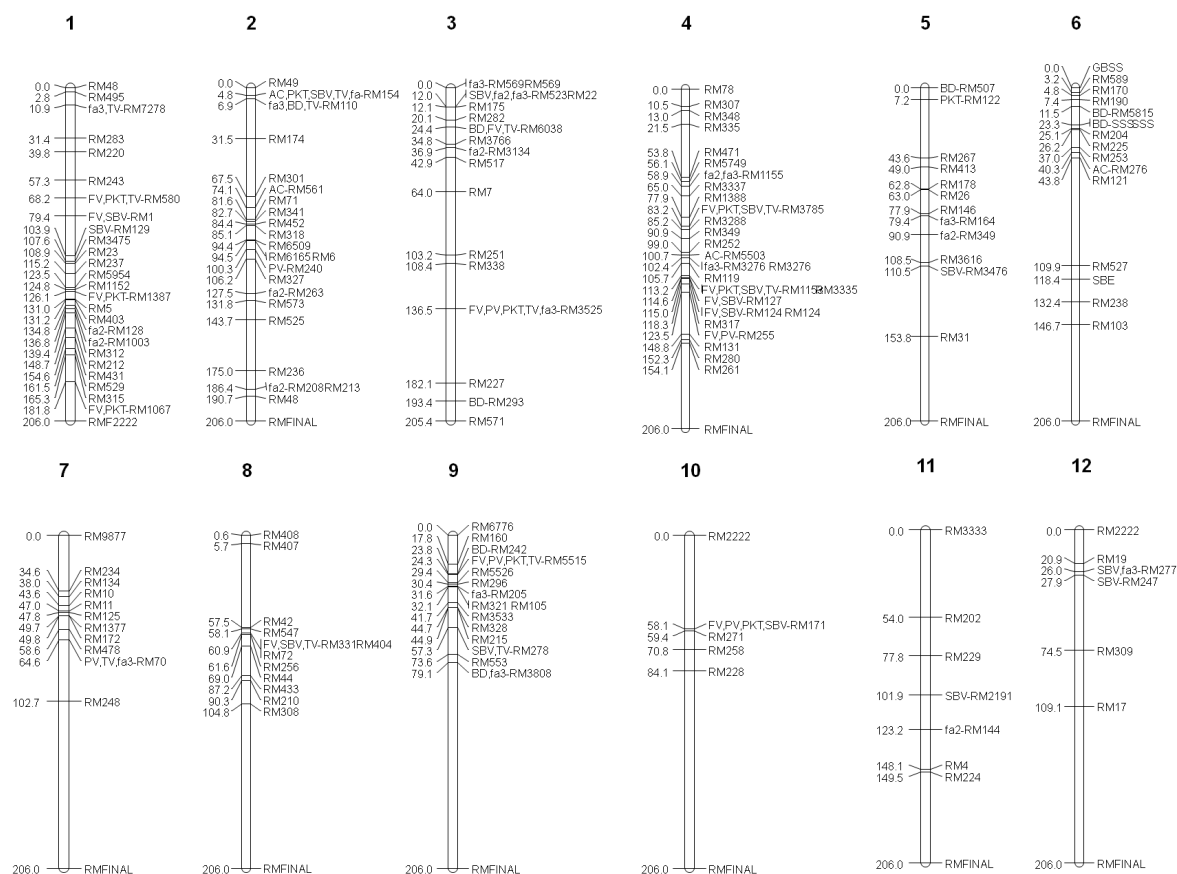


Figure 2. The positions of markers used and marker–trait associations on 12 chromosomes except unmapped markers. Genetic distances are indicated as cM on the left of each map and the corresponding trait-marker names are indicated on the right. AC, amylase content; PKV, peak viscosity; TV, trough viscosity; BD, breakdown viscosity; FV, final viscosity; SBV, setback viscosity; PKT, peak time; fa, degree of polymerization (DP) ≤ 12 ; fb₂, $24 < \text{DP} \leq 36$; fb₃, $\text{DP} < 36$ (Zhao *et al.*, 2012b).

indices in both the GLM and MLM, including the final viscosity ($P < 0.001$, $r^2 = 23.87\%$), in both 2009 and 2010, and AC ($P < 0.01$, $r^2 = 11.25\%$) and trough viscosity ($P < 0.01$, $r^2 = 20.43$) in 2010 (Table 4). This study provided a new perspective on the use of allele mining in breeding strategies based on marker-assisted selection (Lu *et al.*, 2012b).

Gene	Location	Nucleotide variations	AAC	Trait	P_GLM	P_MLM	Rsq_Marker
<i>OsAGPL4</i>	1st exon 106–117th	del(TCCGCCGCCGCC)	del(SAAA)	BDV	9.99E–04	2.64E–05	0.1656
<i>OsAGPL4</i>	1st intron 36th	del(cgcgtgcg)	–	BDV	9.99E–04	2.64E–05	0.1656
<i>OsAGPSI</i>	3rd exon 133rd	AAT→GAT	N→D	AC	9.99E–04	2.03E–04	0.1555
				FV	6.00E–03	9.05E–04	0.1236

AAC, amino acid changes; P_GLM, adjusted P -values with 1000 permutations; P_MLM, P -values significant in the FDR test; amino acid code: S, serine; A, alanine; N, asparagine; D, aspartic acid; BDV, breakdown viscosity; AC, amylase content; FV, final viscosity (Lu and Park, 2012a).

Table 3. Association between sequence variations and phenotype

Gene	Location	SNP	Trait	Year	P_GLM	P_MLM	Qvalue	R ²
<i>OsBEIIb</i>	3end63 rd	g/c	AC	2010	2.10E-03	2.19E-04	9.64E-03	0.1125
			PV	2010	3.10E-03	5.82E-04	2.56E-02	0.1590
			TV	2009	2.00E-04	7.41E-04	1.12E-02	0.1893
				2010	1.00E-04	9.76E-05	3.14E-03	0.2043
			FV	2009	1.00E-04	4.77E-05	1.31E-03	0.2267
				2010	1.00E-04	3.85E-06	1.69E-04	0.2387
<i>OsBE</i>	-224 th	c/t	TV	2010	3.60E-03	7.10E-03	3.80E-02	0.1784
<i>OsSSSIIb</i>	-1042 nd	t/g	TV	2010	ns	5.90E-03	3.80E-02	0.0986
	-984 th	g/t	TV	2010	ns	6.90E-03	3.80E-02	0.0885
	-957 th	c/t	TV	2010	ns	6.90E-03	3.80E-02	0.0885
	-782 nd	a/g	TV	2010	ns	6.90E-03	3.80E-02	0.0885

P_GLM: adjusted *P*-values with 10,000 permutations in GLM; P_MLM: nominal *P*-values in MLM; Q value: adjusted nominal *P*-value in MLM by false discovery rate; AC: amylose content; PV: peak viscosity; TV: trough viscosity; FV: final viscosity (Lu and Park, 2012b).

Table 4. Associations between sequence variations and eating quality indicators.

Zhao *et al.* (2009) evaluated the contents of 16 amino acids in brown rice by genotyping using 25 SSR markers. A total of 42 marker-trait associations for amino acid content covering three chromosomes ($P < 0.05$) were identified by the MLM model (Fig. 4), which accounted for more than 40% of the total variation (Zhao *et al.*, 2009). In our research group, association mapping of rice traits related to cold-stress tolerance during germination, preharvest sprouting resistance, salt tolerance, blast disease resistance, and grain physicochemical properties are undertaken using SSRs and SNP variants on advanced resequencing platforms.

In conclusion, association mapping is a promising approach to overcoming the limitations of conventional linkage mapping in plant breeding. Recent research has demonstrated the significant potential of LD-based association mapping of physicochemical traits and other important agronomic traits in rice accessions using SSR/SNP markers. This type of mapping could be a useful alternative to linkage mapping for the detection of marker-trait associations, and lead to implementation of marker-assisted selection in rice breeding programs.

4. Future directions

4.1. Genomics and GWAS in germplasm research

With the development of next- and third-generation sequencing technologies, the whole genomes of individual rice accessions can now be sequenced with less than \$ 1000 (US dollar).

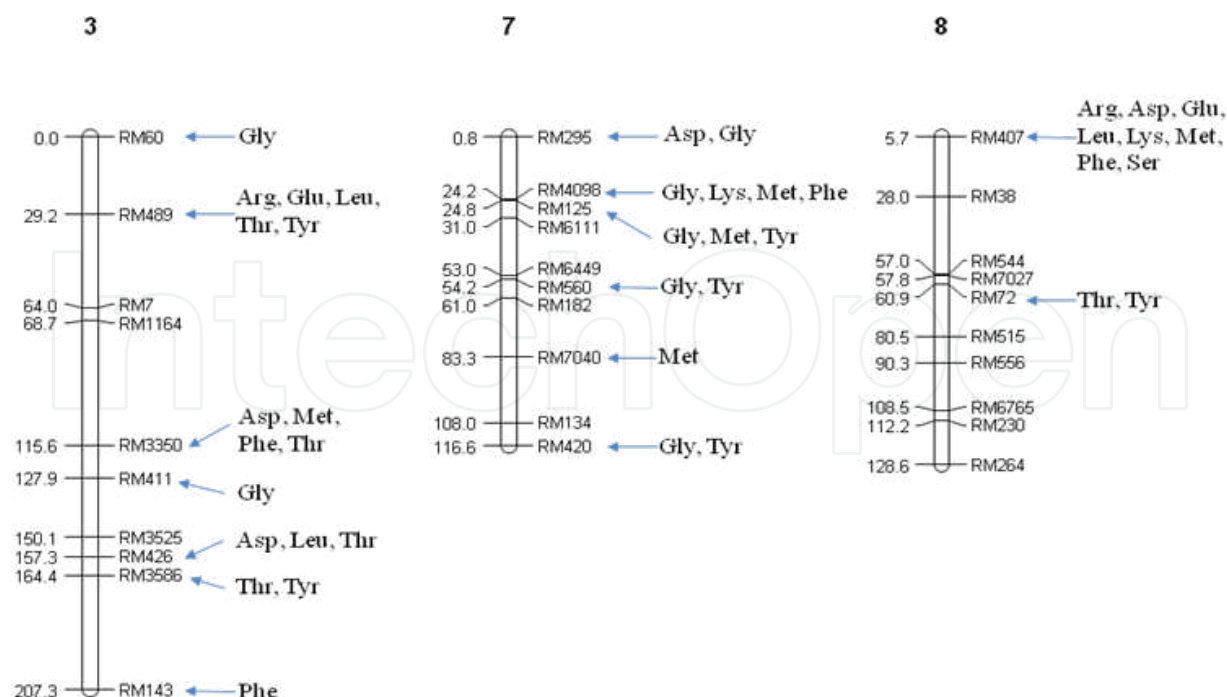


Figure 3. Three regions of putative marker-trait associations on three chromosomes (3, 7, and 8) for amino acid content in brown rice. Genetic distances are indicated in cM on the left of each map and the corresponding marker names are indicated on the right. Ala, alanine; Arg, arginine; Asp, aspartic acid; Glu, glutamine; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Tyr, tyrosine; Val, valine (Zhao *et al.*, 2009).

Also, new efficient genotyping technologies, such as RADs (Restriction Associated DNAs) (Baird *et al.*, 2008) and GBS (Genotyping-by-Sequencing) allow the generation of genotyping data for up to 40,000 genes at low cost in few days.

Natural alleles and alleles obtained from artificially mutagenized populations provide an important resource for crop breeding. By using all available alleles and detailed phenotypic data from core sets of rice lines, new genes and useful traits can be identified. Molecular tags for useful traits developed using GWASs based on genotypic and phenotypic information can be used to track target traits during segregation of populations in rice breeding (Figure 4).

To identify new alleles from a representative core set of rice lines and transfer them into elite lines, we finally selected 166 from ~25,000 accessions in the RDA Gene Bank. We completed whole-genome resequencing of 84 core accessions with 7x coverage in 2012. We plan to resequence the whole genomes of the remaining 82 core accessions in addition to 84 bred varieties from a validation set in 2013. We are currently undertaking the phenotyping of the core accessions for agronomic traits, and chemical composition for the GWAS analysis with the resequence information. We are also planning to improve the software algorithm for the association analysis to increase the ability to identify alleles from the core set of lines using whole genomic SNP or indel genotype data and phenotypic information. More precise characterization of rice traits that confer resistance to stress from climate change is required to

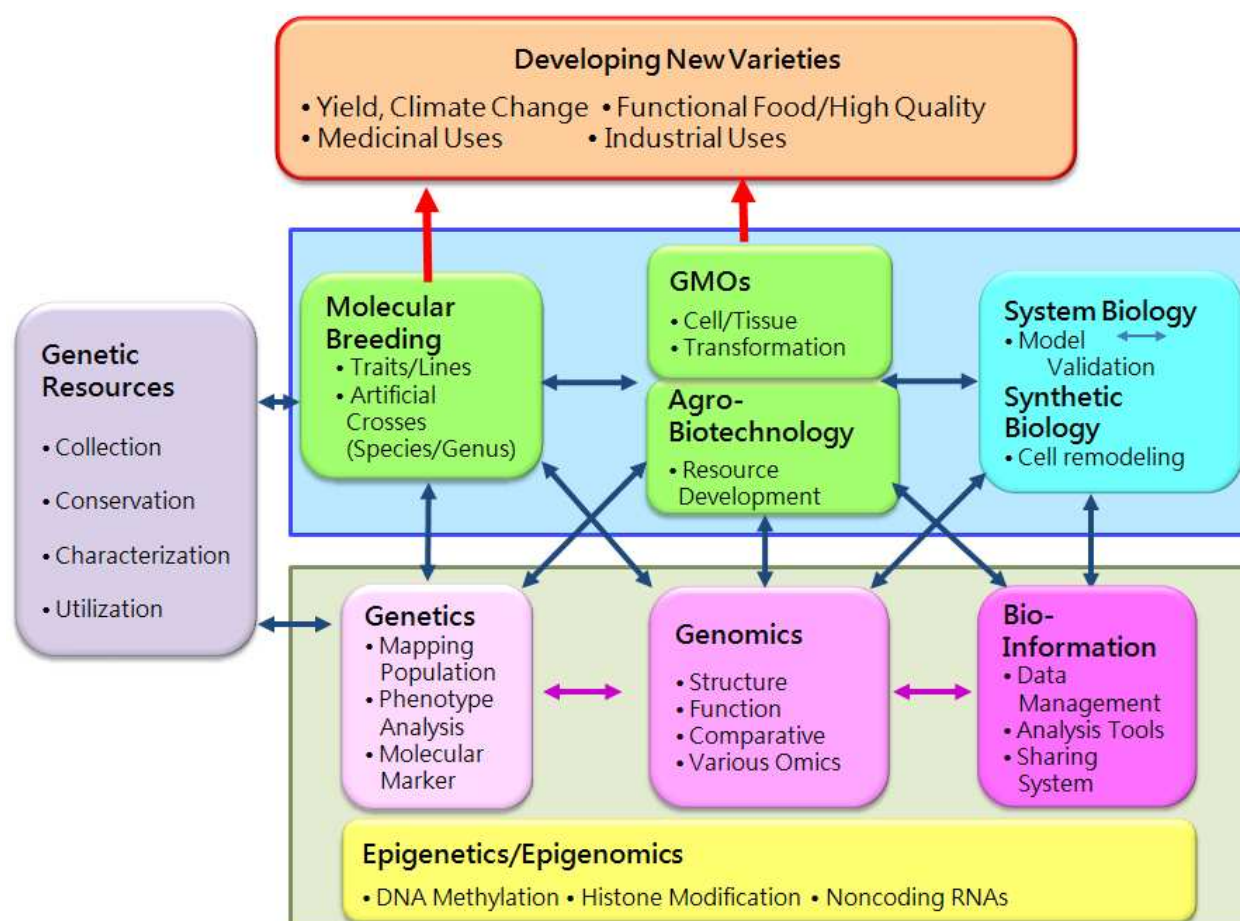


Figure 4. A schematic illustration of inter-disciplinary relationships between genomic research and other fields in the breeding of crop species.

screen useful alleles using GWASs. Using whole-genome genotype information, we are able to develop large numbers of molecular tags across 12 different rice linkage groups based on their contributions to specific phenotypes.

4.2. Strategy for identification of major and minor QTLs for molecular breeding

The core accessions are highly diverse with many traits useful for rice breeding. Upon selection of an accession with a desirable trait, bi-parental mapping populations will be developed using two japonica varieties (Shindongjinbyeon and Junambyeon) and one indica variety (Hanareumbyeon). Major QTLs will be surveyed with an F_8 RIL-segregating population using whole-genome resequencing of 96 samples for first mapping, and then, we can resequence this target region using the expanded 3000 to 5000 samples for fine mapping till the targeted gene can be cloned. We expect that all major QTLs will contribute more than 10% to target traits. To identify minor QTLs that contribute less than 10% to a target trait, BC_4F_1 population will be first developed, and then, selfing will be done till BC_4F_8 . The recurrent parent will be an elite line for the purposes of QTL mapping and for transferring target traits into the elite lines. Mapping of minor QTLs will be performed using a BC_4F_8 segregating population (as shown in Fig. 5).

Natural variation results from the expression of different alleles during evolution. As a result of the contributions to farmers over the past ~8000 years, many important traits have been accumulated in the natural germplasm collections currently maintained in seed banks. Whole genome resequencing allows efficient identification of unused alleles from conserved germplasm. We are at present developing a platform for allele mining in rice breeding systems using GWAS approaches and diverse germplasm accessions with the support of the Next-Generation BioGreen 21 Program (No.PJ009099) from Rural Development Administration, Republic of Korea. We believe our effort will facilitate the molecular breeding of rice.

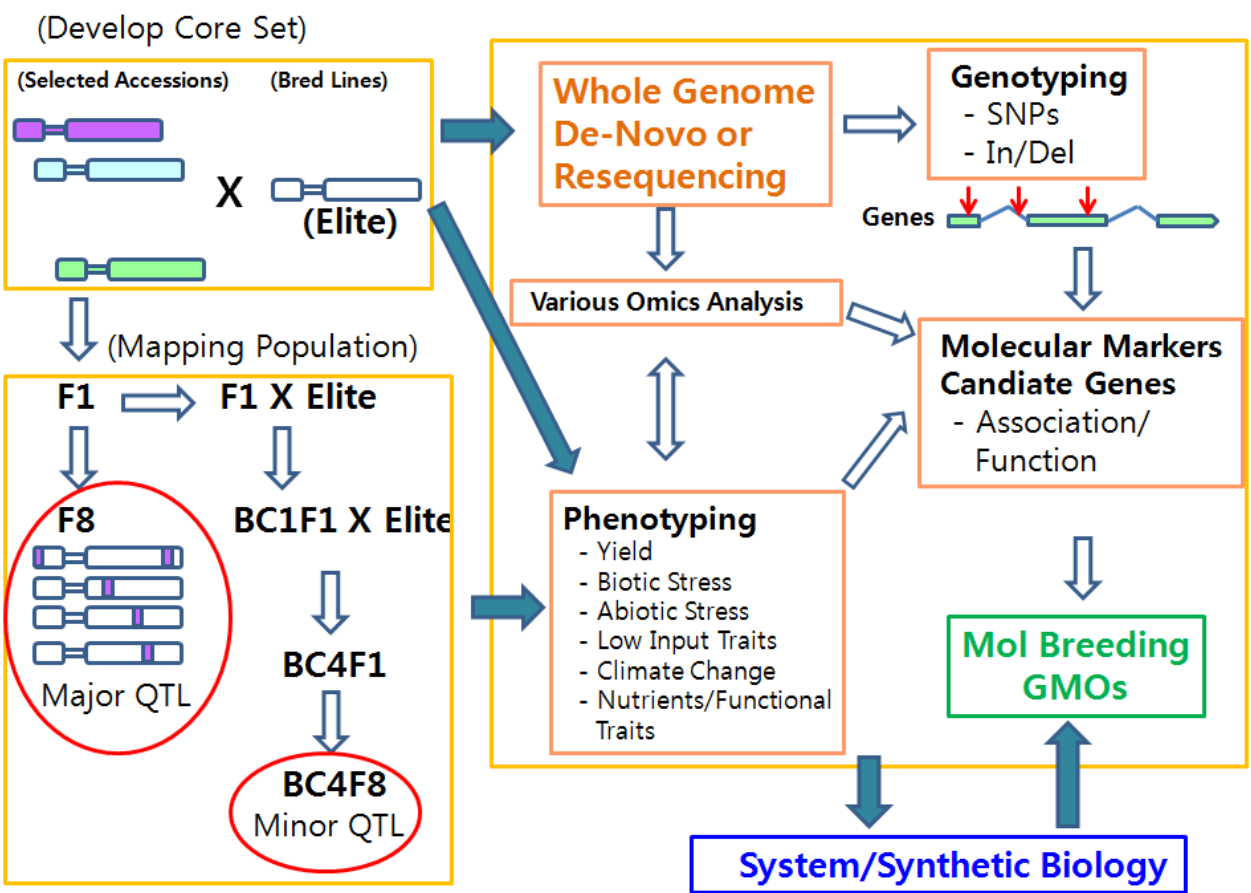


Figure 5. Strategies for identification of major and minor QTLs in rice from selected accessions carrying useful traits through GWAS. The major QTLs will be localized and tagged by molecular markers in the F₈ generation. Minor QTLs will be localized using a BC₄F₈ population.

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