SUMMARY OF Ph.D DISSERTATION

Studies of Cold Resistance through Identification and Evaluation of Genetic Loci for Rice Breeding and Cultivation Technology Development in East African Highlands

In sub-Saharan Africa, rice production is very low compared to the consumption, and therefore boosting rice production has become a major issue of concern in order to meet the rapidly growing demand for rice. Promotion of New Rice for Africa (NERICA) for production in highland elevation regions has been initiated in order to increase rice production. Low temperature stress is however a major factor limiting rice production in temperate regions and at high altitude areas in the tropics by reducing spikelet fertility at the booting stage, which is the most sensitive stage to this stress. In this dissertation, three studies were carried out with the main objective of developing cold tolerant plant breeding materials and cultivars for production in highland regions of East Africa. The studies had three specific objectives: (1) to evaluate cold tolerance of eight upland NERICA varieties and their recurrent parents (*O. sativa*) at the reproductive stage in comparison with Japanese standard rice varieties. (chapter 2), (2) to identify quantitative trait loci (QTLs) associated with rice CT at booting stage on the basis of spikelet fertility and other agronomic traits in F_2 population derived from Hananomai and WAB56-104 (chapter 3), and (3) to investigate the potential of a new cold stress adaptation strategy (cold escape) by evaluating the functional role of a mutation allele in flowering time control of rice grown under alternate wetting and drying (AWD) water saving technique of rice cultivation (chapter 4).

In chapter 2, CT of eight NERICA was evaluated at the reproductive stage on the basis of filled grain ratio (FGR) and other agronomic characteristics after cold water irrigation controlled at 19 °C, in comparison with Japanese standard rice varieties used for CT evaluation at Aichi

Prefecture Mountainous Agricultural Research Institute. The FGR was greatly affected by cold water irrigation. NERICA 1, 2, and 7 had higher FGR (51.9–57.9 %) while NERICA 6, 15, and 16 had lower FGR (6.2–14.5 %). NERICA 1, 2, and 7 were less affected by cold stress, with a 30 % mean reduction in FGR, while NERICA 6, 15, and 16 were greatly affected, with their FGRs being reduced by more than 80 %. NERICA 3 and 4 were moderately affected by cold stress, with about 45 % reduction rate in FGR. FGR significantly influenced the grain weights of the varieties with strong positive correlations (r = 0.83–0.91; $P < 0.001$), and thus, similar trends in grain weights were observed. Grain weights were reduced by 61.7–96.4 % under cold stress. NERICA 1, 2, and 7 showed significantly better performance than NERICA 3 and 4, while NERICA 6, 15, and 16 performed poorly under cold water irrigation. The Japanese varieties Koshihikari (very tolerant) and Ozora (moderately tolerant) were more affected by cold water irrigation than NERICA 1, 2, and 7. On the basis of the mean reduction rate (%) in FGR under cold stress, the varieties were classified as follows: NERICA 1, 2, and 7 as tolerant; NERICA 3 and 4 as moderately tolerant; and NERICA 6, 15, and 16 as susceptible to cold stress. However, NERICA 7 grain yields were lower under cold stress due to both greatly reduced number of panicles per plant and number of spikelets per panicle. Therefore, NERICA 1 and 2 were found to be suitable candidates for production in the highland regions of East Africa and should be promoted for production. WAB56-104 had superior performance to all other varieties in terms of yield as well as FGR under cold water conditions. WAB56-104 showed possibility of harboring cold tolerance genes and serves as good genetic resource with potential for utilization in improvement of CT of rice in breeding programs.

In Chapter 3, CT at booting stage on basis of spikelet fertility after cold water irrigation was evaluated using F_2 population derived from a cross between temperate *japonica*, Hananomai and tropical *japonica*, WAB56-104 (parent of NERICA1 to NERICA11). The agronomic performance of the F_2 population was evaluated at Aichi Prefecture Mountainous Agricultural Research Institute. Quantitative trait locus (QTLs) for CT and other agronomic traits were identified by composite interval mapping (CIM). Two QTLs for CT were detected on chromosome 8 (*qCTB-8*) and 10 (*qCTB-10*) with enhanced effects on the trait coming from Hananomai and WAB56-104 allele respectively. The QTLs explained 30% and 33% of phenotypic variation in spikelet fertility respectively. For the agronomic traits, 11 QTLs were detected as follows under cold water irrigation: two for panicle length (*qPL-1*, *qPL-3*), three for spikelet number per panicle (*qSNP-1*, *qSNP-3*, *qSNP-6*), three for culm length (*qCL-3*, *qCL-7*, *qCL-11*), and three for panicle weight (*qPW-1*, *qPW-3*, *qPW-7*) and their total contribution to the phenotypic variation was 22%, 88%, 34% and 85% respectively. No QTLs for the agronomic traits were identified in the same region with QTLs for CT suggesting that these traits and CT may be controlled by different genes. Moreover, correlation analysis showed that the traits were not related to CT except for panicle number and panicle weight. CT was negatively correlated with panicle number (r = -0.35, $P < 0.01$) and positively correlated with panicle weight (r = 0.61, $P < 0.001$). QTL analysis was also performed for the agronomic traits under normal water irrigation and a total of 19 QTLs for the various traits were detected in chromosome 1, 2, 3, 6, 7 and 10 and the phenotypic variation explained by individual QTL ranged from 5-88%. Furthermore, the genetic effects of the CT QTLs was evaluated using backcross genotypes selected using linked SSR marker assisted selection (MAS) under natural low temperature conditions in Kenya (BC_1F_4 genotypes) and under cold water water irrigation in Japan (BC_1F_5 genotypes). QTL validation results using selected BC_1F_4 and BC_1F_5 genotypes revealed that genotypes having homozygous alleles with enhanced effects for both CT QTLs showed higher spikelet fertility and thus improved yields under cold stress. The identified QTLs for CT and other agronomic traits will be useful in development of cold tolerant and high yielding varieties for production in high altitude areas such as in highlands of East Africa through marker-assisted selection (MAS).

In chapter 4, in a bid to develop a new cultivation technology for cold stress adaptation (cold escape), the functional role of a mutation allele in flowering time control (delay of heading) of rice grown under alternate wetting and drying (AWD) water saving technique was evaluated in a cold-prone environment. Heading date and grain weights of F_2 population derived from cross between mutant rice and WAB56-104 were evaluated under AWD (-30 kPa) and continuously waterlogged conditions (CWL). The F_2 population was genotyped and plants were grouped as mutant-type (23 lines) and WAB-type (28 lines). Mutant-type F_2 lines showed delayed heading by an average of 11 days (4-18 days) compared to only 4 days (0-9 days) in WAB-type F_2 lines. Three F_2 lines were identified that showed delay of heading by 11-17 days and maintained more than 95% grain weights under AWD. The three F2 lines could be useful as adaptable lines in breeding for a cold escape adaptation strategy. Results of this study showed that the mutation gene was effective in causing delay of heading time under moderate water stress. By practicing AWD technique of water management during rice cultivation, rice plants introgressed with the mutation gene could escape cold periods by delaying heading time and thereby reducing the risk of yield loss from cold damage.

The findings of this dissertation revealed that NERICA 1 and NERICA 2 are most suitable candidates for production in highlands regions of East Africa since they can maintain high filled grain ratio and high yields under cold stress and should be promoted for production. WAB56-104 was identified as a good genetic resource for breeding and improvement of cold tolerance of rice cultivars. Through QTL mapping using a population derived from cross between Hananomai and WAB56-104, two QTLs for CT based on spikelet fertility were detected on the short arm of chromosome 8 and 10 with enhanced effects on the trait coming from Hananomai and WAB56-104 allele respectively. The QTLs provide useful information applicable in the development of marker-based breeding for cold tolerant, high yielding varieties suited to the cold-prone regions of East Africa via marker assisted selection. Due to the variations in cold stress intensity, duration and occurrence in different seasons and years in cold-prone environments, a new approach for cold stress management is required. To maintain stable rice yields under such unpredictable conditions, crop adaptation strategies for cold stress management that complements breeding or varietal development with crop management practices may offer great potential. Such a strategy was developed and summed up as a cultivation technology for cold stress adaptation through flowering time manipulation (delayed heading), and included breeding of an adaptable variety and water management practice by alternate wetting and drying (AWD) water saving technique. By adopting the new cultivation technology, there is potential to minimize risks of cold damage during reproductive growth stage by escaping cold periods and thereby achieve sustained and improved rice yields in cold-prone environments.