



**INTERNATIONAL RICE RESEARCH INSTITUTE**

**Enhancing and Stabilizing the Productivity of Salt-Affected  
Areas by Incorporating Genes for Tolerance of Abiotic Stresses  
in Rice**

(IRRI Ref. No.: DPPC2007-09)

Final Report

submitted to the

**German Federal Ministry for Economic Cooperation and Development  
(BMZ)**

June 2012

Contact:

**Corinta Quijano-Guerta**

Director for External Relations

Telephone: +63 (2) 580-5600 ext. 2513; Direct: +63 (2) 580-5621; Fax: +63 (2) 812-7689 or 580-5699

E-mail address: [drpc-irri@irri.org](mailto:drpc-irri@irri.org) Mailing address: DAPO Box 7777, Metro Manila, Philippines

Courier address: 10<sup>th</sup> Floor, Suite 1009, Security Bank Center, 6776 Ayala Avenue, Makati City 1226

Telephone: +63 (2) 891-1236, 891-1303



**1. Name of IARC:** International Rice Research Institute (IRRI)

**2. Project Title:** Enhancing and Stabilizing the Productivity of Salt-Affected Areas by Incorporating Genes for Tolerance of Abiotic Stresses in Rice

**3. GTZ Project Number and Contract Number**

Project number: 07.7860.5-001.00

Contract number: 81099952

**4. Reporting Period:**

January 2008 – December 2011 (with one-year no-cost extension)

**5. Project Coordinator and Project Scientists**

Dr. Abdelbagi M. Ismail

Principal Scientist

International Rice Research Institute

DAPO Box 7777

Metro Manila, Philippines

Tel: +63 2 580 5600; 845 0563; 844 3351 ext. 2843

Email: [abdelbagi.ismail@cgiar.org](mailto:abdelbagi.ismail@cgiar.org)

Dr. J. Damien Platten, IRRI

Dr. Michael J. Thomson, IRRI

Dr. Glenn Gregorio, IRRI

Dr. Hei Leung, IRRI

Dr. Thelma Paris, IRRI

**6. Collaborating Institutions and Staff including NARES and German Partners**

Drs. D.P. Singh, J.N. Reddy, Central Rice Research Institute (CRRI), Orissa, India

Drs. G. Singh, R.K. Gautam, B.K. Bandopadhyay, Central Soil Salinity Research Institute (CSSRI), Karnal, India

Dr. P.C. Ram, N.D. University of Agriculture and Technology (NDUAT), Faizabad, India

Dr. M.A. Salam, Bangladesh Rice Research Institute (BRRI), Gazipur, Bangladesh

Dr. Nguyen Thi Lang, Cuu Long Delta Rice Research Institute (CLDRRI), Vietnam

Dr. Detlef Weigel, Max Planck Institute, Tübingen, Germany

Dr. Jochen Reif, Dr. Hans Peter Maurer, and Prof. Albrecht Melchinger, University of Hohenheim, Stuttgart, Germany

**7. Project Description**

The project aims to develop rice varieties tolerant of salt and related stresses, along with suitable management practices, to improve food security and stabilize productivity on more than 20 million ha of rice land affected by salt stress. We propose to integrate genotyping array technology into our marker-assisted breeding program to expedite QTL (quantitative trait loci) fine-mapping and whole-genome selection and to accelerate the transfer of salt-tolerance QTLs

into high-yielding rice varieties and elite breeding lines. The project will also combine tolerance of salinity and submergence in popular varieties for coastal areas. Research activities will involve NARES scientists from Bangladesh, India, and Vietnam to provide capacity building, to ensure that new varieties are adapted to their environments and will meet farmers' agronomic and quality preferences, and to ensure continued development and promotion of improved varieties after conclusion of the project.

**Goal:**

Increase and stabilize food productivity and enhance livelihood and food security in salt-affected areas through salt-tolerant rice varieties.

**Purpose:**

- 1) To develop and distribute to NARES varieties containing QTLs for enhanced tolerance of salt stress together with accompanying management options, for dissemination, with the potential to double yield under stress conditions in target areas;
- 2) To provide NARES with varieties combining tolerance of submergence and salinity for coastal areas.

**Outputs:**

- Varieties possessing *Saltol* and a combination of *Saltol* and *Sub1*: introgression of *Saltol* into four popular varieties completed using marker-assisted backcrossing (MABC) for increased vegetative-stage tolerance and initially evaluated with NARES; lines combining *Saltol* with *SUB1* developed and tested for both salinity and submergence tolerance.
- Additional salinity-tolerance QTLs associated with reproductive-stage tolerance identified and two additional QTLs for vegetative-stage tolerance fine-mapped using microarray genotyping technology; an optimized MABC system developed for introgressing multiple QTLs.
- Genes and alleles associated with salinity tolerance discovered through candidate gene analysis and mutant screening combined with in-depth physiological characterization.
- NARES capacity for marker-assisted breeding, large-scale phenotyping, and participatory research strengthened through degree and non-degree training.

## **8. Major Research Findings**

*Highlight important achievements: technologies and products developed, and new or improved research methodologies. Compare the planned outputs with the actual achievements (auto-evaluation). Give reasons why certain outputs could not be achieved. What has been done to overcome constraints or unexpected problems? Detailed research reports should be added as annexes.*

Detailed (full) research reports can be found in Appendices 2 and 3. Selected figures and tables are presented in Appendix 1.

## **Activity 1. Marker-assisted backcrossing**

### **Achievements:**

#### **IRRI, Philippines**

- MABC of the *Saltol* QTL from the FL478 (IR66946-3R-178-1-1) genetic background has been completed. A MABC system was developed after fine-mapping of *Saltol*, and used for its introgression into the genetic backgrounds of the following mega-varieties:
  - BRR1 dhan28: most popular during the dry season in southern Bangladesh
  - IR64: the most popular variety in South and Southeast Asia
  - BR11: the most popular variety during the wet season in Bangladesh
  - BRR1 dhan29: popular during the dry season in southern Bangladesh
- Additionally, substantial progress has been made in introgressing *Saltol* into the genetic background of Azucena.
- Pyramiding of the *Saltol* and *SUB1* QTLs was completed in the genetic backgrounds of IR64, Swarna, and BR11.
- This work has been conducted by PhD scholars from Bangladesh (Akhlash Rahman, Ruhul Sarker, Armin Bhuiya), an MSc student from Vietnam (Ho Van Thè), and a PhD candidate from the Philippines (Marjorie de Ocampo).
- Field trials of BRR1 dhan28-Saltol, IR64-Saltol, and BR11-Saltol are being carried out at IRRI (Philippines) and sites in Bangladesh and India.
- Capacity for marker-assisted breeding has been greatly enhanced with the acquisition of an Illumina BeadExpress and a Fluidigm SNP genotyping platform at IRRI. These provide capacity for high-throughput genotyping of hundreds of SNP markers on hundreds of samples simultaneously, in various flexible designs. Both are already being used in breeding efforts and also in generating some of the data reported here.

#### **Cuu Long Delta Rice Research Institute (CLDRRI), Vietnam**

##### *Activity 1.1 Marker-assisted backcrossing of Saltol into popular Vietnamese varieties*

- Salt-tolerance genes were transferred into improved and recommended varieties through conventional crossing, with a stable yield of at least 4 t/ha, early maturity, acceptable grain quality, resistance to the major insect pests and diseases, and tolerance of submergence. Agronomic characters were scored under different saline field conditions (Tables 1.1.1 and 1.1.2, Appendix 1).
- Plant materials: Nine cultivars were selected to represent various degrees of resistance to salt stress and were crossed to sensitive cultivars, and advanced to the BC<sub>2</sub>F<sub>2</sub> generation. Transgressive segregation was observed for salt tolerance.
- Evaluation of salt tolerance of 230 BC<sub>2</sub>F<sub>2</sub> lines: The optimal electrical conductivity (EC) for screening was determined to be 18 dS/m based on observations at three EC levels: 6, 12, and 18 dS/m.
- Phenotypic variation in salinity tolerance: Salinity tolerance was evaluated for several populations at four EC levels (0, 6, 12, 18 dS/m). Distribution of salt tolerance (EC 18 dS/m) in the IR28/ Pokkali BC<sub>2</sub>F<sub>2</sub> bulk was continuous and nearly normal. For the cross IR28/Mot Bui Do, wide variation was observed for yield. The results demonstrated that the optimal EC was 18 dS/m.

### *Activity 1.2 Testing of salt-tolerant lines under different environmental conditions*

- Another useful gene for salt tolerance was transferred from high-yielding indica varieties by backcrossing to Om1706 as the recurrent parent and, with advances in cell culture and molecular markers, the potential for introgressing genes for salt tolerance is more promising than before. Some of these lines were recently released as commercial varieties in the Mekong Delta of Vietnam.

### **CRRI, Cuttack, India**

- An extensive crossing program was undertaken to incorporate salinity tolerance into popular rice varieties of different maturity durations using the salt-tolerant donors FL378, FL478, FL496, Pokkali, Orumundakan, and Hasawi, through conventional as well as molecular-marker-assisted approaches. See Appendix 1, Table 1.1.3 to 1.1.5.
- For developing rice varieties with wider adaptability, the most promising lowland genotypes from different eastern Indian states selected from the Lowland Shuttle Breeding Program were used in multiple crossing for incorporating salinity tolerance using IR71700-247-1-1-2 and IR72176-307-4-2-2-3 as salt-tolerant donors.
- Tolerance of salinity and submergence was combined into popular lowland rice varieties using FL378, FL496, IR71700-247-1-1-2, and IR72176-307-4-2-2-3 as salt-tolerant donors and Swarna-Sub1, Sambha Mahsuri-Sub1, IR49860-7, and IR82810-407 as submergence-tolerant donors. Several breeding lines were developed and are being further evaluated. See Figure 1.1.3.
- Four rice varieties, Luna Suvarnaa (CR Dhan 402), Luna Sampad (CR Dhan 403), Luna Barial (CR Dhan 406) for the wet season, and Luna Sankhi (CR Dhan 405) for the dry season, have been released by the state of Odisha.
- Nine advanced breeding lines are in the second and third year of testing for coastal salinity tolerance and two lines in the second year of testing for alkalinity and inland salinity under the All India Coordinated Rice Improvement Program (AICRIP).
- Five short-duration salt-tolerant rice varieties introduced from Vietnam through this project (OM5935, OM576, OM4498, OM6050, and OM6051) were found promising for coastal saline areas in the dry season, and they are being further evaluated to nominate them for release.
- Three breeding lines from IRRI, IR72593-B-13-3-3-1, IR70870-B-P-2-2, and IR77674-B-25-1-2-1-3-12-3-AJY, with significantly higher grain yield than IR72046-B-R-3-3-3-1 (Luna Sankhi) were found promising for the dry season. However, none of the IRRI lines tested in the International Rice Salt Stress Tolerance Network (IRSSTN) trials was suitable for the wet season because they are short and mature earlier (Fig. 1.1.1-1.1.2 and Table 1.1.6, Appendix 1).
- Breeding lines with combined tolerance of salinity and submergence being developed at CRRI are more promising for coastal Odisha than lines introduced from IRRI, because the latter are shorter, have photo-insensitivity, and have shorter duration, not suitable for the wet season.
- In on-farm participatory varietal selection (PVS) trials during the wet season, CR2092-141-2, CR2462-1-139-1-1, CR2462-1-154-1-1, CR2461-1-102-1-2, CR2468-1-319-3-1, and CR2464-1-203-1-1 produced significantly higher grain yield than SR26B. CR2092-141-2 performed

consistently well for four years. The first two genotypes were consistently selected by farmers and will be considered for release.

- Farmers' preferences of rice varieties were based not only on grain yield but also on other traits such as tolerance of insect pests and diseases, quality of straw, grain and cooking quality, lodging resistance, tillering ability, and panicle size.
- One breeding line, IR42016-B-R-3-3-3-1, was performing well during the dry season and was compared with farmers' varieties at 21 sites in 2008 and at 300 sites in 2009, with average yield of 4.8 to 4.9 t/ha compared with 3.5 to 3.8 t/ha for the farmers' varieties. This line is also being considered for release.

#### **CSSRI Regional Research Station, Canning Town, India**

- BMZ-20 (IR84649-275-3-2-B) and BMZ-23 (IR84649-292-3-1-B) lines were found best in terms of grain yield in moderate-sodic (pH<sub>2</sub> approx. 9.5) environments and BMZ-17 (IR84649-95-1-1-B) was best in high-sodic (pH<sub>2</sub> approx. 9.9) environments. BMZ29 (IR84649-320-21-1-B) and BMZ24 (IR84645-305-6-1-B) were found best after the check varieties in saline conditions (EC<sub>iw</sub> approx. 10.0 dS/m) during 2009. See Table 1.1.7 and Figure 1.1.4, Appendix 1.
- The lines BMZ23, BMZ28, BMZ29, and BMZ31 performed better in terms of grain yield under high-salinity conditions (EC<sub>e</sub> 6.7 dS/m) at Canning Town station. See Table 1.1.8 and Figure 1.1.5, Appendix 1.
- Among Saltol derivatives, BMZ24 and BMZ20 were quite promising under moderate-sodic conditions (pH<sub>2</sub> 9.5). However, the derivatives IR84649-21-20 and IR84645-281-10-B-B were better in saline areas because of their higher tolerance than other genotypes. Under moderate sodicity, IR84649-275-3-2-B proved better also. These lines are being further evaluated before recommendation for release.

#### **N.D. University of Agriculture & Technology (NDUAT), Faizabad, India**

- Two breeding populations at F<sub>2</sub> and F<sub>3</sub> (Sarjoo 52 × CSR30 and IR64 × IR66946-3R-178-1-1) were evaluated during 2009 and 2010 for sodicity tolerance at the reproductive stage.
- Forty entries of IRSSTN (WS 2008, 2010) and 43 entries (WS 2009), including two checks (CSR30 and Narendra Usar Dhan 3), were evaluated at pH 10. Most of the lines had low to medium yield under saline/alkaline conditions. Promising lines were further characterized for use in breeding. See Table 1.1.9 and Figure 1.1.6, Appendix 1.
- Twenty lines selected previously were evaluated for sodicity tolerance and zinc efficiency at pH 9.5 and pH 10.2 in two different sets during three consecutive years, 2008 to 2010. Higher yield in sodic soils was identified as the best selection criterion.
- Fifty advanced breeding lines were evaluated during the wet season of 2009 and 2010 at pH 9.5. Most of them performed well at this sodicity level.
- Thirty-seven promising breeding lines were received from Karnal and evaluated for yield and its contributing traits along with salinity/alkalinity tolerance scores (SES) during the 2009 and 2010 WS. Good genetic variability was observed for yield and its components along with duration.

- Twenty IRRI-derived F<sub>6</sub> lines (Swarna-Sub1/-Saltol) were evaluated in three replications at pH 9.5 during kharif 2009 and 2010 for their tolerance of sodic soil. High heritability was observed for plant height, effective tillers, and panicle length.
- Two QTL mapping populations of 225 lines each from the crosses CSR27 × MI 48 and CSR-11 × MI 48 developed at Karnal were evaluated at pH 9.7 during 2008. Out of these RILs, 50 were selected and evaluated in 2009 and 2010. These RILs will be further evaluated and selected in subsequent years.
- Single cross combinations were made between CSR36 and local popular varieties such as Sarjoo 52 and NDR359 using CSR36 as a donor parent during the wet season of 2009 and 2010. F<sub>2</sub> plants were selected on the basis of salinity/alkalinity tolerance scores and other agronomic traits.
- In addition, 30 segregating populations (F<sub>2</sub>, F<sub>3</sub>, BC<sub>1</sub>F<sub>2</sub>, and topcrosses) were developed and evaluated for sodicity tolerance at pH 9.5. Selections were made in the F<sub>2</sub> and BC<sub>1</sub>F<sub>2</sub> generations, while topcrosses were purified for evaluation at higher sodicity.

#### **State Plant Breeding Institute, University of Hohenheim, Germany (Appendix 2)**

- We extended the software package Plabsoft and implemented a module facilitating the simulation of marker-assisted introgression of multiple genes.
- We used the developed module and compared alternative breeding plans and selection strategies with respect to (1) the required population size, (2) the number of marker data points, and (3) the percentage of the recurrent parent genome recovered in the final breeding product.

#### **Evaluation:**

Marker-assisted introgression of *Saltol* into four popular varieties and pyramiding of *Saltol* and *SUB1* in several genetic backgrounds have been completed at IRRI. Field evaluations have been carried out, both at IRRI and with partner institutes in Bangladesh. Evaluation of *Saltol-SUB1* pyramided lines has been carried out in several genetic backgrounds. Significant progress was also made at NARES partners in developing and testing of salt-tolerant material and several lines were either released or are being nominated. Thus, all aspects of the output have been achieved. In addition, partner institutes in Vietnam and India have made significant progress in introgressing *Saltol* and pyramiding *Saltol* and *SUB1* in locally preferred varieties, extending the work achieved by many-fold.

#### **Activity 2. Identification and fine-mapping of additional salinity-tolerance QTLs**

##### **Achievements:**

##### **IRRI, Philippines**

##### *Discovery of novel QTLs*

- A number of QTL mapping populations have been constructed and analyzed (Appendix 1 Table 1.2.1):
  - Cheriviruppu × Pusa Basmati 1
  - Capsule × BRR1 dhan29
  - FL478 × Azucena

- FL478 × IR29
- Chikiram Patnai × Azucena
- Kala Rata × Azucena
- In addition, the Pokkali × IR29 RIL population has been phenotyped in greater detail at the seedling stage, and is currently being phenotyped for reproductive-stage tolerance.
- Thirteen additional QTL regions, located on most chromosomes, have been identified from these populations. Major QTLs on chr 1L (31–35 Mb and 37–43 Mb), chr 3S (0–5 Mb), chr 3L (25–36 Mb), and chr 12S (4–10 Mb) have been identified from multiple mapping populations and appear robust (Appendix 1 Table 1.2.1 and Fig. 1.2.1).
- Three reproductive-stage QTLs have been identified from the Cheriviruppu × Pusa Basmati 1 population; however, all co-locate with vegetative-stage loci (Appendix 1 Fig. 1.2.1).
- A total of 184 SNP markers were identified from the Illumina indica-indica OPA set that showed consistent polymorphism between many parental genotypes. Using these markers, it should be possible to produce a linkage map of 100–140 markers between most parental combinations. These were thus designed as Fluidigm SNP assays, which should make linkage map construction (and thus QTL discovery) significantly faster and cheaper, with a much higher marker density than is currently typical.

#### *Fine-mapping and NIL development*

- The qSur1 QTL (chromosome 1, 37–43 Mb) has been targeted for fine-mapping in the FL478 × IR29 BC<sub>3</sub>F<sub>4</sub> population. This has been carried out using Fluidigm SNP markers. Forty markers in the region of 37–41 Mb on chromosome 1 were designed from the McCouch SNP diversity panel. A survey of these for polymorphism revealed six polymorphic SNPs in the 39–41 Mb region. Work is ongoing to identify further polymorphic markers in the QTL region. These markers will help in MABC to combine this QTL with *Saltol*.
- QTLs on chr 1L and chr 3L have also been targeted for NIL development from the Capsule × BRR1 dhan29 population. A BC<sub>2</sub>F<sub>1</sub> population was genotyped to confirm the target QTL introgressions at chromosomes 1 and 3 from Capsule in the background of BRR1 dhan29. These lines will be made available for further fine-mapping efforts.

#### **Cuu Long Delta Rice Research Institute (CLDRRI), Vietnam**

- RM315 (chromosome 1) and RM223 (chromosome 8) were found to be linked to seedling salinity-tolerance loci at EC 18 dS/m in the IR28/AS996 and IR68552-55-3-2/OM1706 populations, respectively.
- Using a selective genotyping strategy combined with the parental survey, 11 varieties were identified with polymorphism in RM223: Doc Phung, IR28, OM2395, OM1706, Soc Trang 5, AS996, OM4900, OM6162, Pokkali, Mot Bui Do, and Doc Do. These markers will be useful for introgressing this locus into these varieties.

#### **Evaluation:**

A large number of QTL populations have been developed and analyzed during the course of the project. Although these have mostly identified QTLs common to several populations, a number of unique QTLs have also been identified. One additional QTL for vegetative-stage



tolerance is being fine-mapped (genetic materials and marker systems have been developed; analysis ongoing). Work on a second QTL has not progressed due to the additional ongoing work on MABC, but, since candidate genes have been identified for the chr 3L QTLs (and several others), fine-mapping may not be necessary. The Fluidigm and Illumina BeadExpress SNP assay systems are uniquely suited to MABC of multiple QTLs, with their combination of high-throughput and custom assay designs. The set of 184 SNP markers designed from the indica-indica OPA will provide a high-density set for both foreground and background selection in future MABC approaches, and it has wide applicability in many genetic backgrounds. Considerable progress was also made at all partner locations. Thus, the milestones for this activity have been accomplished.

### **Activity 3. Gene and allele discovery**

#### **Achievements:**

#### **IRRI, Philippines**

##### *Discovery of novel donors*

- Existing donor germplasm for salinity tolerance has been derived almost solely from the region around eastern India and southern Bangladesh. Thus, while many very useful QTLs have been identified from these donors, there is a strong possibility that additional major salinity-tolerance mechanisms could exist in different parts of the world.
- A pilot screening program was carried out. Approximately 550 accessions were chosen from diverse geographic areas that could plausibly experience salinity stress, particularly areas adjacent to mangrove swamps.
- From this screen, about 105 accessions with moderate to high salinity tolerance were identified.
- In addition, six accessions of *Oryza glaberrima* with moderate to high salinity tolerance were identified, a first for this species.
- These accessions came from areas as disparate as the Philippines to China, the Middle East, Madagascar, and East and West Africa (Appendix 1 Fig. 1.3.1). Analysis of genome-wide SNP polymorphism showed that these accessions were derived from diverse genetic backgrounds, including all recognized subspecies. As expected, many came from the indica subspecies, but, surprisingly, many others were from the aromatic subspecies (Appendix 1 Fig. 1.3.2).

##### *Physiological analysis of novel donor germplasm and genetic comparisons with tolerance from FL478*

- Selected accessions from the donors discovered through screening were used in a series of experiments aimed at determining their physiological basis of tolerance.
- Salinity-induced injury was strongly and quantitatively associated with leaf blade Na<sup>+</sup> concentrations for nearly all accessions. Thus, the majority of accessions are using Na<sup>+</sup> exclusion mechanisms (such as *Saltol*) to produce tolerance. However, comparison with alleles present at the *OsHKT1.5* gene (see below) shows that a number of these accessions are using mechanisms apart from *Saltol* to achieve low leaf blade Na<sup>+</sup> concentrations that are causing tolerance. In addition, several other mechanisms were discovered in rare cases (Appendix 1 Fig. 1.3.3).

- Two accessions were also found that show high tissue tolerance, one from Thailand and the other from Senegal in West Africa (Appendix 1 Fig. 1.3.4)
- In addition to the physiological characterization results, a quantitative computer model has been developed for simulating the effects of salinity treatment on plant Na<sup>+</sup> contents, chlorophyll contents, and SES. The model takes into account tolerance mechanisms such as block of Na<sup>+</sup> entry, Na<sup>+</sup> exclusion (sequestration), and tissue tolerance. Using this model, it is possible to investigate the effects of combining different tolerance mechanisms under different treatment regimes. This model also predicts that Na<sup>+</sup> exclusion mechanisms will produce the best tolerance of short-term stress conditions, which appears to be borne out by several lines of evidence. However, effective tolerance of long-term stress will require either tissue tolerance mechanisms or the combination of both Na<sup>+</sup> exclusion and tissue tolerance. The model also clearly shows that, although blocking of apoplastic bypass flow is already highly effective even in rice, improving this by even a few percentage points will have huge effects on tolerance. This could thus be an important target for future screening and QTL discovery work.
- Crosses have been made between a number of novel donor lines and IR29 and/or FL478 to determine the genetic basis for tolerance, and the interactions with tolerance derived from FL478. In some cases, such as in the F<sub>2</sub> generation between FL478 and the variety with tissue tolerance from Thailand, a significant fraction of the population outscored FL478 in long-term, severe salinity stress conditions (it survived 38 days at EC 21 dS/m; Appendix 1 Fig. 1.3.5). This donor also appears to have only a small number of loci conferring its tolerance, unlike FL478 with more than six loci identified.

#### *Allele mining of the Saltol candidate gene*

- There is a strong likelihood that the *Saltol* QTL is conferred by the *OsHKT1.5* gene. However, almost nothing was known about allelic variation in this gene, or how these alleles relate to activity at the *Saltol* locus.
- Fragments of the gene were cloned from diverse salt-tolerant and sensitive accessions. A total of seven major alleles were identified from various accessions, far higher than the average of 3.5 alleles per gene present in the genome as a whole. The level of sequence divergence of these alleles was also about 5 times higher than the genome-wide average. Sequences for these alleles have been deposited in the GeneBank under accession numbers JQ695808–JQ695818. A phylogenetic comparison of these alleles and correlation with salinity tolerance is found in Appendix 1 Fig. 1.3.6.
- Comparison of Na<sup>+</sup> concentrations in the leaves of accessions carrying these various alleles shows that the aromatic allele has by far the highest activity and the japonica allele the least (Appendix 1 Fig. 1.3.7). A tentative hypothesis can be made regarding the allelic activity series:
 

Aromatic > aus > Hasawi > IR29 ~ Daw > japonica ~ Agami
- A majority of the strong Na<sup>+</sup>-excluding lines (e.g., Pokkali, Kala Rata, Chikiram Patnai, Kuti Patnai, Nona Bokra) carry the aromatic allele. Surprisingly, however, FL478 carries the aus allele (despite its assumed derivation from Pokkali), and thus FL478 may not be the best donor for the *Saltol* QTL.

- Fluidigm SNP markers have been developed to distinguish all seven known alleles for use in future breeding and screening efforts.

#### *Identification of novel salinity-tolerance genes via whole-genome sequencing*

- It was decided to take advantage of the new low-cost, high-throughput sequencing technologies to facilitate sorting through genes in these QTL regions. DNA from selected donor lines (Pokkali, FL478, Capsule, Hasawi, and IR29) was resequenced on Illumina GAIIX or HiSeq2000 platforms (Appendix 1 Table 1.3.1 and 1.3.2). Sequencing data were mapped to the Nipponbare reference genome.
- To facilitate the discovery of polymorphism and compare this polymorphism between donor lines, a database tool was developed that allows:
  - (1) the identification and prioritization of candidate genes in a QTL region, based on several different sets of genome annotation models;
  - (2) identification of polymorphisms in each gene from each set of genome sequencing results;
  - (3) comparison of these polymorphisms between donor and recipient lines;
  - (4) recording of these results, together with other observations on gene expression, published functional studies, etc., back into the genome annotation information; and
  - (5) prioritization of genes for follow-up work based on these observed changes.
 The logical structure and workflow of this tool is presented in Appendix 1 Figure 1.3.8.
- Using this tool, it is possible to identify candidate genes in a 12-Mb QTL region within a few hours, a task that previously took about a week with other tools.
- Using this system, candidate genes for four QTLs on chromosomes 1 and 3 have been identified (Appendix 1 Table 1.3.3).
- Two of these have already been validated in additional genotypes, and cloning of the others is under way.

#### *Screening of IR64 EMS mutant populations*

- Several thousand M<sub>2</sub> families derived from EMS mutagenesis of IR64 seeds were screened for altered salinity responses.
- Additional M<sub>2</sub> families derived from mutagenesis of FL478 were also screened.
- Several dozen mutants were identified from the two populations, with either enhanced (IR64 lines) or reduced (FL478 and IR64 lines) salinity tolerance.
- Two mapping populations were derived from crosses between selected mutant families from the two populations.
- These populations were advanced to the F<sub>3</sub> stage, phenotyped, and genotyped.
- QTL analysis identified some loci controlling altered salinity responses, which are now being further evaluated (Appendix 1 Tables 1.3.4 to 1.3.6).

### **Cuu Long Delta Rice Research Institute (CLDRRI), Vietnam**

#### *Evaluation of diversity among various rice genotypes using candidate gene markers*

The differences in yield parameters among 85 diverse salt-tolerant rice genotypes were evaluated under saline conditions. Ten candidate gene primers were used to investigate

correlation with yield parameters. The results revealed that the difference between genotypes with all morphological traits was significant at 5% probability. By single marker analysis (SMA) and stepwise multiple regression analysis (SMRA), the candidate gene markers showed significant correlations with morphological traits at 5% and 1% probability. The genetic diversity of rice genotypes was identified by the Dice coefficient of similarity from 64% to 100% and many groups were established by clustering based on candidate gene marker data. The dendrogram obtained from the candidate gene markers was more discriminatory than the one obtained from morphological traits.

### **Evaluation:**

The focus on identifying candidate genes via whole-genome sequencing (WGS) has put this work on a much firmer practical and theoretical basis, and allowed significant advances in identifying and analyzing candidate genes in a very short period of time. Candidate genes for all known QTLs have thus been examined, and the causative genes for four QTLs in addition to *Saltol* may already have been identified. Multiple alleles for these candidate genes have been identified and cloned. Software tools have been developed to streamline the identification and examination of candidate genes via WGS, thus reducing future workloads considerably. Allele mining of the major *Saltol* candidate gene has identified significant genetic variation, and allowed a tentative ranking of allelic strength, which will be of significant importance in MABC approaches. Screening of mutant populations and the development of mutant QTL mapping populations has identified several genetic loci for follow-up candidate gene analysis. The physiological basis of tolerance in donor lines has been examined further.

Thus, all aspects of the milestone have been achieved and in many cases exceeded. In addition, a large number of additional donor lines have been identified and characterized, many of which display additional mechanisms complementary to those seen in current donor varieties. Genetic studies show that at least some of these mechanisms are additive or synergistic with tolerance from FL478. Together with the quantitative computer model of salinity-tolerance mechanisms, these should now allow rapid progress in developing varieties with reasonably higher and more robust tolerance than that of any currently known donors.

### **Activity 4. Training and capacity building**

**Achievements:** (Detailed lists provided in Appendix 1, Tables 1.4.1-1.4.2).

#### **IRRI, Philippines**

- Developed and held the inaugural training program “Phenotyping for abiotic stresses,” at IRRI, Philippines, 27 October-10 November 2011. Attended by 25 participants from South and Southeast Asia and Africa.
- Developed a phenotyping protocols manual (distributed at the training program in 2011) that for the first time brings together updated protocols for carrying out screening and characterization experiments for several abiotic stresses, including salinity and submergence.
- Donated equipment to partners: PCR machines and vacuum emasculators for crossing.
- Hosted 16 visiting scientists from 11 countries in Asia and Africa for various durations for hands-on training.

- Supported five PhD candidates and six MSc candidates from Vietnam, Bangladesh, and the Philippines to pursue their thesis research under this project.
- Supported the participation of five people from partner institutions in “Marker-assisted selection (MAS) in rice: theory, practice, and application” held from 24 November to 5 December 2008, facilitated by the IRRI Training Center. The course provided hands-on training and enhanced the capability of scientists to apply MAS in rice breeding programs:
  - S.M. Hisam Al Rabbi, scientific officer, Biotechnology Division, BRRI, Gazipur, Bangladesh
  - Mr. Chau Thanh Nha, junior assistant, CLDRRI, Vietnam
  - Dr. B.C. Marandi, senior scientist, Division of Crop Improvement, CRRI, Cuttack, Odisha, India
  - Dr. Neeraj Kulshrestha, senior scientist, CSSRI, Karnal, Haryana, India
  - Dr. S.P. Singh, assistant crop physiologist, NDUAT, Faizabad, Uttar Pradesh, India
- Five scientists participated in the molecular breeding course, 22 February to 5 March 2010 at the IRRI Training Center:
  - Nirmal Sharma, scientific officer, BRRI Regional Station, Barisal, Bangladesh
  - Ratna Rani Majumder, scientific officer, BRRI, Bangladesh
  - Bui Phuoc Tam, junior assistant, CLDRRI, Vietnam
  - Tran Binh Tan, junior assistant, CLDRRI, Vietnam
- Frequent exchange visits have been carried out with partner institutions in Bangladesh, Vietnam, and India.

#### **Cuu Long Delta Rice Research Institute (CLDRRI), Vietnam**

- Thirty-one researchers from An Giang Province attended a training course on PCR setup in March 2010 at CLDRRI.
- Three MSc and five BSc students completed their thesis research under this project (Appendix 1 Table 1.4.1).
- Three researchers (Tran Thi Nhien, Tran Binh Tan, Pham Thanh Toan) received on-the-job hands-on training for 20 days at IRRI.

#### **CRRI, Cuttack, India**

- Dr. B.C. Marandi, scientist, participated in training on “Marker-assisted selection in rice: theory, practice, and application” and “Screening rice genotypes for salinity tolerance” at IRRI, Philippines, from 24 November to 10 December 2008.
- Dr. Vijaya Bhattacharjee, senior scientist, attended training on “Phenotyping for abiotic stresses” at IRRI, Philippines, during 27 October-10 November 2011.

#### **Evaluation:**

Numerous students have been trained at levels ranging from short-term and longer-term hands-on short courses in specific areas to BSc, MSc, and PhD thesis research. Practical on-site and high-level interactions were also made possible through frequent visits by the PIs to project sites and interaction with researchers and farmers. Extensive collaborative networks have been established during the course of this research, which has greatly facilitated achievements and

progress. The creation of the phenotyping workshop series has been a great success, and provided a unique opportunity for exchange of knowledge on different approaches for phenotyping, which still remain the bottleneck for both conventional and molecular breeding progress.

## 9. Assessment of Research Findings

*Describe possible implications for the research institute, the collaborating NARES and (German) partners. Implications for potential users of research products such as farmers, agri-business, extension services, development projects, and implications on the policy level (legislation, safety regulations) should also be mentioned.*

- The *Saltol* locus is sufficient in areas affected by intermediate salt stress and appears to work best in specific genetic backgrounds. The identification of allelic variation at the *Saltol* locus and the ranking of these alleles in terms of allelic strength is a significant advance, enhancing the utility of the *Saltol* QTL in plant breeding efforts.
- The acquisition of flexible and high-throughput SNP genotyping platforms at IRRI will be a huge boost to both QTL discovery and marker-assisted breeding. The speed, reliability, and moderate cost of genotyping populations on these platforms should be considered when planning future work in these areas. Likewise, the cost of whole-genome resequencing is already comparable with the cost of cloning three individual genes, and is decreasing rapidly; using this technique through this project helped in speeding the identification of candidate genes in record time, and this should therefore be considered when planning gene discovery projects.
- An important lesson we learned: marker-assisted breeding efforts should, whenever possible, take into account the alleles of the candidate genes for target QTLs present in both the donor and recipient parents. Determination of the gene responsible for a target QTL, the allelic activity series for that gene, and the alleles present in both donor and recipient lines should be made a priority to maximize benefits and shorten the time for breeding.
- There is a strong need to determine epistatic interactions involving known and novel QTLs. The experience with *Saltol* shows that introgression of a single QTL is a significant step forward but additional QTLs are needed for higher tolerance in severe cases. The lack of tolerance in some *Saltol* introgression lines suggests that epistasis between *Saltol* and other loci is significant. This highlights the importance of identifying additional, highly effective QTLs complementary to *Saltol*, and the need to assess genetic interactions between QTLs prior to their use in breeding.
- The lack of significant interactions between *Saltol* and *SUB1* is of high significance and a step forward for developing more resilient varieties with multiple stress tolerance.
- The numerous rice breeding lines developed conventionally or through MABC carrying *Saltol* and both *Saltol* and *SUB1* are made available to NARES partners, and could be either used directly after testing or used as potential donors for developing locally adapted varieties.
- Numerous large-effect QTLs for vegetative-stage as well as reproductive-stage salinity tolerance were identified/fine-mapped by IRRI, and these would be of great help for developing varieties with much higher salinity tolerance by combining them at both stages. Deployment of such varieties would offer opportunities for rice cultivation even in marginal lands that otherwise remain unused.

- Simulations software has been extended and functions to optimize the introgression of the two genes (*Saltol* and *SUB1*). The functions have been employed to elaborate resource-efficient strategies in marker-assisted introgression of two unlinked genes.
- Crucial factors determining the efficiency of introgressing multiple genes into elite varieties have been identified through intensive simulation studies. In this context, multistage selection procedures have been elaborated, facilitating an efficient stacking of multiple genes in elite varieties.
- Progress made in this project has triggered the national programs to allocate more resources for research on abiotic stresses in rice and to give more attention to unfavorable areas, and a few projects are already under operation.
- The project also helped in strengthening the capacity of NARES institutions in participating countries through building their infrastructure and human resources, including MSc and PhD degree training. This remains a great asset for these institutions for future research programs, especially for effective phenotyping and the use of MABC breeding to enhance tolerance of abiotic stresses.
- Farmers have started reaping the benefits of stress-tolerant rice varieties and many more benefits are expected in the future with the release of lines that are at an advanced stage, and with further development of varieties tolerant of multiple abiotic stresses. Such varieties will ensure greater yield stability and could potentially encourage the involvement of private seed growers in seed production and marketing of these new stress-tolerant varieties. This would ensure a higher rate of seed replacement and better yield stability.

## 10. Knowledge Transfer

*Describe what has been done and what still needs to be done to ensure that the research findings (products and research methodologies) will be used and/or further developed by the various users groups, such as research institutes (IARC, NARES, ARO), extension and training institutions, farmers, agribusiness, policy makers.*

- Varieties with *Saltol* introgression and pyramided lines combining *Saltol* and *SUB1* have been made available to partner institutions in Bangladesh, Vietnam, India, and elsewhere. In some cases, salt-tolerant varieties developed conventionally have been released as approved varieties in their own right, and in many other cases elite lines have acted as donors in the development of locally adapted cultivars by partner institutions.
- Uptake of the SNP genotyping platforms for both QTL mapping and marker-assisted breeding is promising but still slow because of the relatively high cost. This is expected to change in the near future and there will be more uses, even by our partner institutions. The use of these techniques is now incorporated as a major part of IRRI training programs conducted annually to ensure further uptake and uses.
- Gene sequences, when relevant, have been published in GeneBank.
- Some of the research findings of the project have already been published (Section 15). Other publications, for example, on aspects of the technologies and methodologies, have been prepared (SNP genotyping, phenotyping) or are in the drafting stage (genome sequencing methodology, novel germplasm identified, various QTL mapping studies, and development of NILs).

- Advanced salt-tolerant breeding lines developed in the project were usually evaluated extensively in farmers' fields as demonstrations, and promising lines were further evaluated through farmers' participatory varietal selection (PVS) trials with improved crop management practices. Selected lines were then nominated for further testing in national networks and for release. This process also creates considerable awareness about the varieties, both at the policy level and among farmers. Through this approach, four varieties were recently released in Odisha, India, alone over the past year.
- Varieties and breeding lines developed through co-funding from this project also become part of ongoing/new IRRI activities to ensure continuity.
- Some of the salt-tolerant varieties released recently are gaining publicity and helping in expanding rice cropping in new areas, as in southern Bangladesh and eastern India (Odisha and West Bengal). More efforts are needed to ensure sufficient seeds of these varieties for large-scale dissemination. Leaflets published in local languages, training of extension personnel, and the use of mass media are helping as outscaling activities.

## 11. Training

*Describe the formal training and on-the-job-training provided during the project phase. Name persons trained, and where applicable, the grades obtained. Assess the success of the training, if applicable.*

This has already been summarized under Activity 4 (Training and capacity-building activities), and also as Appendix 1 Tables 1.4.1 and 1.4.2. Below is a brief summary:

- BMZ provided full/partial support for the participation of 16 scientists who participated in various training programs at IRRI.
- Five PhD (one completed) and six MSc (five completed) candidates were partially or fully supported for their thesis research at IRRI.
- Short-term research visits for an additional three scientists to IRRI co-funded by BMZ:
  - Sanjida Parveen Ritu (Bangladesh); 3 months; 1 July 2010. The purpose is to use ORYZA2000 to assess the performance of the aus-aman rice cropping system in coastal Bangladesh, finalize Ph.D dissertation, and draft a manuscript.
  - M.S. Anuradha Singh (India); visitor/collaborator; 5 August 2011, for one year. The purpose is to perform genetic diversity analysis and phenotyping for submergence and anaerobic germination for a set of rice germplasm from India and IRRI (GRC), and to investigate the correlation between phenotype and haplotype data for selected genes.
  - Mr. Felipe De Campos Carmona (Brazil, IRRI); intern, 17 May to 8 November 2010. To evaluate a set of germplasm from Brazil and IRRI for salinity tolerance during germination, investigate traits associated with tolerance during germination and early establishment, and become acquainted with activities at IRRI.
- Senior Scientist Dr. Neeraj Kulshreshtha from CSSRI participated in MAS training at IRRI in December 2008.
- Dr. Y.P. Singh (Lucknow), Dr. S. Mandal (Canning Town), and Mr. Sharik Ali, senior research fellow (Karnal), acquired training on "Experimental design and data analysis" conducted by IRRI under the STRASA project at the Central Rice Research Institute, Cuttack, Odisha, India, on 7-11 Sept. 2009.



- More than 40 scientists and students attended training courses at IRRI in various fields as summarized in Appendix 1 Tables 1.4.1 and 1.4.2.

## 12. Lessons Learned

*Give a brief description of lessons learned (as far as these are not already part of the implications), referring to the planning of the research project, the execution of the workplan, the collaboration with the various partners, and the training events. State reasons for success and failures.*

- Genetic interactions that take place between known QTLs appear to play a very large (even dominant) role in determining salinity tolerance, that is, any single QTL by itself seems to have minor effects in many genetic backgrounds, but the combination of two or three acts synergistically to produce high tolerance. This will help in designing future breeding strategies but requires the development of software and platforms that facilitate the introgression of multiple QTLs/genes.
- When considering marker-assisted introgression of a QTL into a recipient variety, the possibility that the recipient variety already contains the QTL needs to be considered, though it may lack tolerance because of other interactions. Cloning the gene responsible for the QTL will make this analysis possible by identifying and sequencing it in any recipient variety.
- There is considerable unreported variation in salinity tolerance in rice, and further screening efforts are likely to bear significant fruit. However, steps should be taken to identify germplasm with alternative mechanisms of tolerance, and one of these steps should be the genotyping of candidate genes at *Saltol* and other known trait loci. The Fluidigm SNP assays developed for *HKT1.5* will facilitate this.
- Whole-genome resequencing is an extremely cost-effective solution for identifying candidate genes for QTLs, and the cost for this is constantly decreasing. Combined with the QTLSeq tool, the identification of candidate genes should become routine, and focus should be given to validation efforts.
- Good gains are possible through conventional breeding and work should be carried out in parallel with any molecular breeding programs, especially those involving complex traits.
- Tremendous gains were made from working with partners at target sites where new salt- and submergence-tolerant varieties are required. This considerably helped strengthen their breeding programs besides providing feedback on the products of the project. This also helped identify agronomic traits desired at any specific site; for example, long-duration taller varieties for the wet season in coastal areas, but dwarf short-maturing varieties for the dry season in the same areas. Considerable variation also exists in what is considered high quality. Short-bold grain type varieties are preferred in coastal Bangladesh while only varieties with long slender grains can be accepted in Vietnam.
- With the availability of an array of stress-tolerance QTLs, focus should shift to enabling national programs to use MABC routinely as an integral part of their breeding programs to upgrade their popular varieties into stress-tolerant versions to maximize the benefits.
- Because unfavorable rice areas always face multiple stresses, future strategies should be directed toward incorporating multiple QTL/genes for any single stress and also for several stresses.

- Simulation studies conducted by our German partners were extremely helpful in deciding on future strategies for incorporating multiple QTLs/genes for the same stress or for different abiotic stresses. This is both for the breeding strategy to be used as well as the choice of an efficient and cost-effective genotyping platform.

### 13. Future Research Needs

*Give a brief description of the research needs (gaps of knowledge) identified and which could not be directly addressed during the project phase. These may be research methods which need refinement or new questions raised by potential users of the (planned) research findings.*

- There is a need to identify additional QTLs for seedling-stage tolerance that combine well with *Saltol*, and target these for marker-assisted selection and pyramiding.
- There is considerable scope for the identification of both additional donor varieties and novel, highly effective QTLs. The most effective method for producing highly tolerant materials would seem to be:
  1. Identify significantly new donor germplasm displaying novel mechanisms of tolerance and/or donors with high tolerance but not sharing a highly active allele of *Saltol*.
  2. Assess interactions with known sources, for example, in an F<sub>2</sub> population with FL478.
  3. Identify *large-effect* QTLs from these disparate germplasm sources.
  4. Identify candidate genes via whole-genome sequencing of high-value donor sources.
  5. Use this information in gene validation and the design of perfect markers for breeding.

Steps 4 and 5 have been substantially advanced by the current research funded through this proposal, for QTLs found in the FL478, Pokkali, Capsule, etc., populations. However, there is a strong need to identify additional highly effective QTLs (equivalent to *Saltol*) for higher tolerance in breeding lines. Screening efforts have identified many new potential donors and partially characterized them, but these need to be moved through the process of assessment of interactions with *Saltol* and other known QTLs, QTL discovery and the identification of causative genes, and the design of markers.
- Interactions between known QTLs (apparently including *Saltol*) is an important process, yet almost nothing is known about the interactions taking place. Determination of which QTL combinations perform best should be a research priority.
- There is a dire need to identify multiple QTLs for higher salt tolerance at both the seedling and reproductive stage, to be combined in suitable backgrounds. Efforts should also shift toward tolerance of multiple stresses commonly encountered in natural fields, such as submergence and salinity, salinity and drought, salinity and stagnant floods, etc. In most cases, tolerance of a single stress is not sufficient to ensure release of a variety that can replace existing yet low-yielding local landraces.
- More resources are needed to conduct a rigorous program of field evaluation with farmers, incorporating both varieties and best management practices. This should also include adjusting the cropping patterns for higher and more stable productivity. This is now made possible with the availability of shorter maturing, stress-tolerant varieties. More research is needed to re-structure the existing “old” and less productive systems.

#### 14. Summary

- Marker-assisted backcrossing of *Saltol* has been completed in a number of genetic backgrounds, at IRRI and at partner institutions.
- Pyramiding of *Saltol* and *SUB1* has been completed in two genetic backgrounds at IRRI and several others at partner institutes.
- Salinity tolerance of *Saltol* introgression lines is effective in areas affected by moderate salt stress, but additional QTLs need to be combined with *Saltol* for more resilient varieties.
- Seeds of lines developed have been disseminated to collaborating partners.
- The performance of *Saltol* introgression and *Saltol*-*SUB1* pyramided lines has been assessed in the field at IRRI and with partner institutions.
- Analysis of QTL populations has identified five robust QTL regions (apart from *Saltol*) that can be targeted for future fine-mapping and marker-assisted breeding efforts.
- One additional QTL for vegetative-stage tolerance is being fine-mapped.
- SNP genotyping platforms from Illumina and Fluidigm were developed and are being implemented to facilitate QTL discovery and marker-assisted breeding efforts.
- A set of SNP markers on the Fluidigm system has been developed that has wide applicability for many QTL populations, thus markedly reducing the time and cost involved in identifying QTLs from novel sources.
- Putative candidate genes for four QTLs (in addition to *Saltol*) have been identified and are being cloned for transgenic verification.
- Whole-genome sequences have been obtained for five relevant varieties to speed the process of QTL cloning and marker development.
- Software tools were developed for data mining of genome sequencing results, enabling the identification of highly likely candidate genes in a short period of time.
- Allele mining of the *Saltol* candidate, *OsHKT1.5*, identified seven major alleles, which have been ranked according to likely level of activity.
- Several alleles were sequenced for each of the newly identified candidate genes.
- Many additional donor varieties have been identified, and physiological mechanisms elucidated. These are potential donors for novel QTLs.
- First-level pyramiding crosses with new and existing germplasm/mechanisms show good promise for significantly extending salinity tolerance.
- Mutants with altered salinity responses have been identified, and loci mapped, paving the way for cloning candidate genes.
- Training of several PhD and MSc candidates and collaborating scientists has been accomplished.
- A phenotyping training module for major abiotic stresses in rice and protocols manual were developed and used.
- An extensive collaboration network for MABC and testing of varieties has been developed, and several salt-tolerant varieties released through co-funding of this project.

## 15. Publications, Papers, and Reports

List under this item all relevant documents which have been published during the project phase. Please send copies of any publications, papers and reports which have not yet been sent together with the progress reports to the GTZ.

- Nakhoda B, Leung H, Mendioro MS, Mohammadi-nejad G, Ismail AM. 2011. Isolation, characterization, and field evaluation of rice (*Oryza sativa* L., Var. IR64) mutants with altered responses to salt stress. *Field Crops Res.* 127:191-202.
- Thomson MJ, Zhao K, Wright M, McNally KL, Rey J, Tung CW, Reynolds A, Scheffler B, Eizenga G, McClung A, Kim H, Ismail AM, de Ocampo M, Mojica C, Reveche MY, Dilla CJ, Mauleon R, Leung H, Bustamante C, McCouch SR. 2011. High-throughput SNP genotyping for breeding applications in rice using the BeadXpress platform. *Mol. Breed.* DOI 10.1007/s11032-011-9663-x.
- Thomson MJ, de Ocampo M, Egdane J, Rahman MR, Sajise AG, Adorada DL, Tumimbang-Raiz E, Blumwald E, Seraj ZI, Singh RK, Gregorio GB, Ismail AM. 2010. Characterizing the *Saltol* quantitative trait locus for salinity tolerance in rice. *Rice* 3:148-160.
- Zhang ZH, Liu Q, Song HX, Rong XM, Ismail AM. 2011. Responses of different rice (*Oryza sativa* L.) genotypes to salt stress and relation to carbohydrate metabolism and chlorophyll content. *African J. Agric. Res.* (in press)
- Zhang ZH, Liu Q, Song HX, Rong XM, Ismail AM. 2011. Responses of contrasting rice (*Oryza sativa*) genotypes to salt stress as affected by nutrient concentrations. *Agric. Sci. China* 10:101-105.
- Alam R, Rahman MS, Seraj ZI, Thomson MJ, Ismail AM, Tumimbang E, Gregorio G. 2010. Investigation of seedling-stage salinity tolerance QTLs using backcross lines derived from *Oryza sativa* L. Pokkali. *Plant Breed.* 130:430-437.
- Sexcion FSH, Egdane JA, Ismail AM, Dionisio-Sese ML. 2009. Morpho-physiological traits associated with tolerance of salinity during seedling stage in rice (*Oryza sativa* L.). *Philipp. J. Crop Sci.* 34:27-37.
- Mahata KR, Singh DP, Saha S, Ismail AM, Haefele SM. 2010. Improving rice productivity in the coastal saline soils of the Mahanadi Delta of India through integrated nutrient management. In: Hoanh CT, Szuster B, Pheng KS, Ismail AM, Noble A, editors. *Tropical deltas and coastal zones: food production, communities and environment at the land-water interface*. CABI Publishers, Oxfordshire, UK. p 239-248.
- Mahata KR, Singh DP, Ismail AM. 2009. Crop and natural resource management for high and stable productivity in coastal saline areas. In: Haefele SM, Ismail AM, editors. *Natural resource management for poverty reduction and environmental sustainability in fragile rice-based systems*. Proceedings No. 15, International Rice Research Institute, Los Baños, Philippines. p 122-133.
- Lang NT, Buu BC, Ismail AM. 2008. Molecular mapping and marker-assisted selection for salt tolerance in rice (*Oryza sativa* L.). *Omon Rice* 16:50-56.
- Lang NT, Tu1 PTB, Thanh NC, Buu BC, Ismail AM. 2009. Genetic diversity of salt tolerance rice landraces in Vietnam. *J. Plant Breed. Crop Sci.* 1:230-243.
- Lang NT, Trinh Thi Luy, Bui Chi Buu, Ismail AM. 2010. The genetic association between the yield, yield components and salt tolerance in rice. *Omon Rice* 17:99-104.

- Lang NT, Bui Chi Buu, Ismail AM. 2011. Enhancing and stabilizing the productivity of salt affected areas by incorporating genes for tolerance of abiotic stress in rice. *Omon Rice* 18:41-49.
- Lang NT, Nguyen Van Tao, Bui Chi Buu. 2011. Markers-assisted backcrossing (MAB) for rice submergence tolerance in Mekong Delta. *Omon Rice* 18:11-21.
- Lang NT, Pham Thi Thu Ha, Chau Thanh Nha, Pham Thi Be Tu, Bui Chi Buu, Gregorio G, Ismail AM. 2011. Breeding rice varieties resistant to salinity for adaptation to climate change in Vietnam. *Vietnam J. Agric. Rural Dev.* 4:74-77.
- Lang NT, Tran Thanh Binh, Nguyen Van Bang, Tran Van Canh, Nguyen Duc Thuan, Bui Chi Buu. 2011. Study on breeding submergence tolerant rice varieties in Mekong Delta. *Vietnam J. Agric. Rural Dev.* 4:78-85.
- Paris TR, Saha S, Singh DP, Mahata KR, delos Reyes Cueno A, Zolvinski S, Ismail AM. 2010. Assessing needs, constraints and opportunities in coastal saline environments: a case in Orissa, India. In: Hoanh CT, Szuster B, Pheng KS, Ismail AM, Noble A, editors. *Tropical deltas and coastal zones: food production, communities and environments at the land-water interface.* Oxfordshire (UK): CABI Publishers. p 320-334.
- Saha S, Singh DP, Mahata KR. 2009. Coastal saline ecosystem in India. In: Haefele SM, Ismail AM, editors. *Natural resource management for poverty reduction and environmental sustainability in fragile rice-based systems.* Proceedings No. 15, International Rice Research Institute, Los Baños, Philippines. p 146-157.
- Sen P, Mahata KR, Singh DP. 2009. Development and evaluation of salt tolerant rice genotypes for coastal saline areas of eastern India. *J. Indian Soc. Coast. Agric. Res.* 27:38-40.
- Singh DP, Mahata KR, Saha S, Ismail AM. 2010. Crop diversification for improving water productivity and rural livelihoods in coastal saline soils of the Mahanadi Delta, India. In: Hoanh CT, Szuster B, Pheng KS, Ismail AM, Noble A, editors. *Tropical deltas and coastal zones: food production, communities and environment at the land-water interface.* Oxfordshire (UK): CABI Publishers. p 249-263.
- Singh RK, Redoña E, Gregorio GB, Salam MA, Islam MR, Singh DP, Sen P, Saha S, Mahata KR, SG, Sharma SG, Pandey MP, Sajise AG, Mendoza RD, Toledo MC, Dante A, Ismail AM, Paris TR, Haefele SM, Thomson MJ, Zolvinski S, Singh YP, Nayak AK, Singh RB, Mishra VK, Sharma DK, Gautam RK, Ram PC, Singh PN, Verma OP, Singh A, Lang NT. 2010. The right rice in the right place: systematic exchange and farmer-based evaluation of rice germplasm for salt-affected areas. In: Hoanh CT, Szuster B, Pheng KS, Ismail AM, Noble A, editors. *Tropical deltas and coastal zones: food production, communities and environment at the land-water interface.* Oxfordshire (UK): CABI Publishers. p 166-182.
- Singh DP, Mahata KR, Ismail AM. 2011. Challenges and prospects for enhancing and stabilizing rice productivity in coastal saline areas of the Mahanadi Delta, India. Paper presented at the Delta 2011 conference on "Deltas under Climate Change: The Challenges of Adaptation" held on 2-4 March 2011 in Hanoi, Vietnam.