

PHILIPPINE RICE R&D HIGHLIGHTS 2012

THESIS



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Physiological and genetic analysis of the functional roles of root plasticity in growth and yield under transient soil moisture fluctuation stress in rice

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Soil moisture fluctuation (i.e. transient waterlogging to drought and vice versa) is frequently reoccurring in the rice field, which adversely affects plant growth and development. Roots play a vital role to cope with the negative effects of fluctuating soil moisture stresses in the field. Root plasticity has been suggested plays significant roles in plant adaptation, in such environment. Root plasticity is the ability of the plants to change its root phenotype and functions in response to changing environmental conditions such as soil moisture stress. OurThe research aimed to (1) elucidate the functional roles of root plastic development on yield under field condition of continuous cycle of transient soil moisture stresses, and (2) identify QTLs responsible on controlling the plastic root system development responses in transient moisture fluctuation stress.

Findings:

Morphological and physiological analysis

- In continuous waterlogging (CWL) conditions, the two genotypes (Nipponbare and CSSL47) showed no significant differences in most of the traits examined. In contrast, under continuous cycle of alternate waterlogging and drought (CAW-D) conditions, CSSL47 showed greater shoot dry matter production than Nipponbare, which was attributed to its higher stomatal conductance and photosynthetic rate, which then led to higher grain yield.
- Before heading stage, CSSL47 root plasticity was expressed as enhanced aerenchyma formation based on root porosity, which was associated with the promotion of lateral root production, elongation, and branching and the eventual increase in total root length.
- After heading stage, compared with Nipponbare, CSSL47 continued to produce more nodal roots from newly produced tillers, thus maintaining leaf photosynthesis and eventually resulting in heavier panicles.
- We were able to demonstrate that plastically promoted root growth, specifically in lateral root (LR) production, and could increase shoot biomass and grain yield under transient soil moisture fluctuation (SMF) stress.

QTL mapping analysis

- The QTLs that regulate LR production and aerenchyma developments were mapped on chromosome 12 and that regulate photosynthate allocation in roots were found on chromosome 6.
- Interesting QTLs with enhanced effect from Kasalath allele are clustered and overlapped on the short arm of chromosome 12 regions using the F2 CSSL population derived from Nipponbare and CSSL47. These QTLs controls the plasticity on lateral root and aerenchyma development.
- The presence of these QTLs on chromosome 12 and chromosome 6 had significant effects on plant adaptation in response to transient SMF, due to higher plastic root system development. This higher root plastic response attributed by these QTLs was for aerenchyma development, L-type LR production, and partitioning of assimilates to the roots.
- The QTL effects on plasticity in aerenchyma and lateral root development were significantly associated with the promotion of root production, elongation and branching, and the eventual increase in total root length under fluctuating soil moisture stress conditions, consequently, higher biomass production.
- The significant function of QTLs on chromosome 12 was further validated using the CSSL52 (derived from Nipponbare X Kasalath crosses), which contained substituted segment of Kasalath allele on chromosome 12 regions only. This genotype showed the enhanced aerenchyma formation (based on root porosity), which was contributed to the promotion of lateral root production, elongation and branching, and the eventual increase in total root length. Such plastic root system responses resulted into increased stomatal conductance and photosynthesis, and eventually increased shoot dry matter production under soil moisture fluctuation.

Conclusion

- Genetic variation in the expression of root plasticity is essential in improving the adaptive capability of rice plants grown under fluctuating soil moisture environment as in rainfed lowlands.
- The presence of QTLs on chromosomes 12 and 6 contributed to better adaptation to SMF owing to greater plastic root system development than those genotypes without the QTLs or containing only one QTLs on either chromosome.

- Greater plasticity in aerenchyma and LR development is the key traits for the development of root system structure that is more adaptive to SMF conditions. The allocation of more assimilate to the roots contributes to the maintenance of root system development and functions to support greater shoot growth and higher total dry matter production.
- Expression of these root plasticity QTLs under transient SMF seems to be necessary for improving the adaptive capability of rice plants to maintain growth and yield under fluctuating soil moisture environment.

Aroma Loss in Rice as Affected by Different Conditions during Postproduction Operations.

Mary Ann U. Baradi

One of the attributes that contribute to the overall rice quality is its aroma. Fragrant rice has become popular and continues to command higher price than ordinary rice because of their distinctive pleasant scent that makes them special. Freshly harvested fragrant rice exhibits strong aromatic scent but decreases with time and conditions during drying, storage, and milling. Of the many volatile compounds present in aromatic rice, 2-acetyl-1-pyrroline (2AP) is a major compound that gives rice its popcorn-like aroma.

The loss of 2AP in rice at various conditions during drying, storage, and milling were investigated. The mass diffusivity of 2AP in palay increased with increasing ambient air temperature. Palay dried at 55oC air temperature had greater aroma loss than those dried at lower air temperature. Samples stored at higher temperature (27oC) recorded higher 2AP loss than those kept at lower temperature (14oC).

The amount of 2AP in rice decreases with time. The free 2AP, being volatile, is lost due to diffusion. Experimental data for drying air temperature of 35oC exhibited increased aroma loss because of the long drying duration compared with higher drying air temperature set-up (45oC). Storage experiments indicated a rapid loss of 2AP during the first five weeks of storage and subsequently leveled off afterwards, attaining the level of starch bound 2AP.

As in most literature, the free 2AP are easily lost while bound 2AP are left, only to be released upon exposure to high temperature such as cooking. Bound 2AP are mostly found in the endosperm while free 2AP are in the bran. Removal of bran during milling reduces the 2AP level.

Some semi-empirical equations that explain the loss of aroma as affected by temperature and duration were also developed.

The study should be able to guide processors in understanding and controlling the parameters in drying, storage, and milling operations to produce high quality rice.

Genetic Analysis for Heat Tolerance and Early Morning Flowering at Flowering Stage in Rice (*Oryza Sativa* L.)

Neil Nemesio A. Baliuag

High temperature stress is a serious threat in rice production, agricultural productivity, farm incomes, and global food security in general. In China, temperature beyond 38°C which lasted for more than 20 days and coincided with the flowering stage have damaged three million hectares in the rice-growing region of Yangtze River Valley in 2003 which led to a huge yield lost of about 5.18 million tons of paddy rice (Lin et al., 2004, Xia and Qi 2004, Yang et al., 2004). It was partially blamed on susceptible hybrid rice cultivars. In Pakistan, a heat wave reduced rice yields of IR6 by 30% and 70% in hybrids (MARCO, 2009). Likewise, severe yield losses were experienced in 2006 and 2007 in South China (Zou et al. 2009) and the Kanto and Tokai regions of Japan during the summer of 2007 (Hasegawa et al., 2009).

In this study, genetic factors for heat tolerance and early morning flowering (EMF) in rice were evaluated as part of rice genetic improvement under heat stress condition. Quantitative trait loci (QTL) linked to heat tolerance and early morning flowering (EMF) were analyzed by selective genotyping of phenotypic extremes in 235 BC1F1 plants (derived from the cross PSB-Rc82//PSB-Rc82/WAB56-125) under high temperature and 684 BC1F2 plants (derived from 235 BC1F1) under glasshouse conditions using single marker analysis (SMA). For heat tolerance, data of pollen fertility and spikelet fertility of BC1F1 plants under high temperature were gathered, analyzed, and compared to parents. For EMF, four parameters of flower opening were determined, namely: time when flowers start to open (FOT), time of peak flowering (POF), time when most of the flowers are closed (FMC), and time when all of the flowers are closed (FCT).

Findings:

- Results of SMA revealed three markers on chromosomes 2, 3, and 8 were possibly linked to pollen fertility, while two markers on

chromosomes 7 and 8 were possibly linked to spikelet fertility under high temperature.

- SMA for EMF revealed three markers on chromosomes 5, 7, and 9 were possibly linked to FOT, while six markers on chromosomes 5, 7, 8, 9, and 11 were possibly linked to POF, FMC, and FCT.
- In general, markers on chromosomes 7 and 8 could be linked to QTLs controlling both heat tolerance and early morning flowering at flowering stage in rice.
- The results could be useful for further fine mapping and eventually for marker-aided selection for heat tolerant and heat escape in rice genotypes in the future.

Phytochemical and functional properties of Philippine sprouted brown rice (*Oryza Sativa* L) and its potential as base ingredient for functional beverages

Rodel M. Bulatao

This study aimed to evaluate the potential of sprouted brown rice (SBR) for the production of functional beverages. Rice samples were composed of NSIC Rc15, PSB Rc82, black rice, and brown rice. SBR samples were characterized in terms of their nutritional, phytochemical, and functional properties. Germination rate, length of sprouts, texture of cooked rice, and total soluble solid (TSS) content were also determined.

Black rice and PSB Rc82 produced the highest germination rate and longest sprouts. Sprouted black rice had the highest protein and fiber contents while sprouted PSB Rc82 obtained the highest carbohydrate content. Sprouted black rice also contained the highest level of phytochemicals except for the antioxidant scavenging activity. Sprouted black rice and brown rice produced higher water absorption index, water solubility index, and swelling power. Meanwhile, sprouted waxy rice had higher TSS content and softer cooked rice producing smoother texture of rice beverages than nonwaxy rice. All beverages except from those of NSIC Rc15 were perceived to have flavorful and sweet taste. The acceptability of rice beverages was comparable to each other. The study suggested that sprouted pigmented rice could be a potential source of natural antioxidants. Moreover, SBR from both white and pigmented rice varieties could be used to improve the nutritional and functional qualities of beverages.

Functional analysis and molecular breeding for improving stress tolerance using FOX-hunting System in rice (*Oryza sativa* L.)

Sailila E. Abdula

The study focused on the development of transgenic rice with full-length (FL) cDNA gene from Chinese cabbage (*Brassica rapa*) and identification of agronomically important genes. Along these lines, two selected genes, CBL-interacting protein kinase 1 (BrCIPK1) and UDP-glucose-4-epimerase I (BrUGE1) were characterized and studied.

Using *Agrobacterium*-mediated transformation, a total of 1,150 transgenic rice lines were developed comprising of 250 independent genes from Chinese cabbage. Genomic PCR analysis indicated that the average length of FL-cDNAs introduced into individual lines was 900~1200 bp. BLAST functional analysis of FL-cDNA genes showed 35.5% unknown function which contributed the highest number. Moreover, most of the randomly selected transgenic rice lines showed overexpression (92%) and single copy number. Cold and salinity evaluation of the transgenic rice lines showed moderately tolerant (94%) to cold but highly susceptible to salinity (85%). Field evaluation of transgenic rice lines showed minimal phenotypic alterations (with a mean of 12%) in all agronomic traits measured. The most commonly observed alterations were in the chlorophyll contents (40%), followed by the days to heading (26.4%). Other traits showed only minimal alterations with value ranging from 2.1 ~ 11.3%.

From the full-length cDNA overexpression (FOX) rice lines, a clone encoding CBL-interacting protein kinase 1 (CIPK1) was studied which consisted of 1,982 bp long with 216 bp of the 5'untranslated region (UTR), 1,509 bp of the coding region and 257 bp of the 3'-UTR. The ORF encodes a polypeptide of 502 amino acids with a calculated mass of 57.19 kDa. The regulatory domain of BrCIPK1 is highly conserved CBL-interacting module, NAF with seven residues (N, A, F, L, F, I, S) were absolutely conserved among the 15 amino acid NAF domain of the 15 related genes. Southern blot analysis showed a single copy number. mRNA expression analysis showed that BrCIPK1 was highly expressed in seedling of rice and in the shoot and pistil of *Arabidopsis*. Further, BrCIPK1 gene was localized in the cytoplasm and peripheral region in the plant cell. Moreover, analyses of gene expression on Ubi-1::BrCIPK1 rice lines showed that it was differentially expressed by cold, salinity and drought. Furthermore, BrCIPK1 interacted with OsCBL5 by yeast two-hybrid analysis, suggesting that the BrCIPK1 indeed hijack the rice CIPK-CBL-mediated signaling pathways. Additionally, improved proline and free sugar contents were observed in the course of time when tested in abiotic stresses. The improved proline was further supported by the higher mRNA expression of P5CS1, a gene that regulates proline biosynthesis in

plants, relative to the wild type. The significant improved proline and free sugar contents of Ubi-1::BrCIPK1 rice lines under abiotic stresses, along with the elevated P5CS1 mRNA expression may explain the improved tolerance to cold, salinity, and drought stresses.

Another gene studied is UDP-glucose 4 epimerase 1 gene. The gene name BrUGE1 had a total length of 1,328 bp that contains a single open reading frame of 1,056 bp, which encodes a polypeptide of 351 amino acid residues with a calculated mass of 39 kDa. Sequence analysis of BrUGE1 protein has the characteristic of an active site tetrad and NAD-binding motif of the extended short chain dehydrogenase/reductase (SRD) superfamily. Expression analysis showed that BrUGE1 is highly expressed in stem of rice. Interestingly, BrUGE1 mRNA was highly produced by drought stress and it enhanced tolerance. When soluble sugar was measured under drought condition, a significant increase in sugar was observed starting at six hours after imposition of stress. Furthermore, morphological evaluation of transgenic lines showed an increase in yield by 27%, accompanied by an increase panicle length, number of productive tillers/hill, and filled spikelets with advantage value ranging from 17 ~ 20% compared to the wild type Gopum. Furthermore, the growth of the wild type seedlings on galactose was increasingly inhibited with a decrease in UDP-glc epimerase 1 expression compared to the Ubi-1::BrUGE1 rice plants. In the transgenic lines, the increase of UDP-glc epimerase 1 expression was apparently sufficient to overcome the toxic effects of galactose. Therefore, the UDP-glc epimerase 1 is crucial enzyme in rice for growth in the presence of galactose. Taken together, Ubi-1::BrUGE1 rice lines from Chinese cabbage increased yield and improved other agronomic traits, probably by increasing the rate of filled grains. The enhanced drought tolerance may be due to the induction of soluble sugar which may act as osmolyte to compensate dehydration during drought stress.

Abbreviations and acronymns

ABA – Abscicic acid	EMBI – effective microorganism-based inoculant
Ac – anther culture	EPI – early panicle initiation
AC – amylose content	ET – early tillering
AESA – Agro-ecosystems Analysis	FAO – Food and Agriculture Organization
AEW – agricultural extension workers	Fe – Iron
AG – anaerobic germination	FFA – free fatty acid
AIS – Agricultural Information System	FFP – farmer's fertilizer practice
ANOVA – analysis of variance	FFS – farmers' field school
AON – advance observation nursery	FGD – focus group discussion
AT – agricultural technologist	FI – farmer innovator
AYT – advanced yield trial	FSSP – Food Staples Self-sufficiency Plan
BCA – biological control agent	g – gram
BLB – bacterial leaf blight	GAS – golden apple snail
BLS – bacterial leaf streak	GC – gel consistency
BPH – brown planthopper	GIS – geographic information system
Bo - boron	GHG – greenhouse gas
BR – brown rice	GLH – green leafhopper
BSWM – Bureau of Soils and Water Management	GPS – global positioning system
Ca - Calcium	GQ – grain quality
CARP – Comprehensive Agrarian Reform Program	GUI – graphical user interface
cav – cavan, usually 50 kg	GWS – genomwide selection
CBFM – community-based forestry management	GYT – general yield trial
CLSU – Central Luzon State University	h – hour
cm – centimeter	ha – hectare
CMS – cytoplasmic male sterile	HIP - high inorganic phosphate
CP – protein content	HPL – hybrid parental line
CRH – carbonized rice hull	I - intermediate
CTRHC – continuous-type rice hull carbonizer	ICIS – International Crop Information System
CT – conventional tillage	ICT – information and communication technology
Cu – copper	IMO – indigenous microorganism
DA – Department of Agriculture	IF – inorganic fertilizer
DA-RFU – Department of Agriculture-Regional Field Units	INGER - International Network for Genetic Evaluation of Rice
DAE – days after emergence	IP – insect pest
DAS – days after seeding	IPDTK – insect pest diagnostic tool kit
DAT – days after transplanting	IPM – Integrated Pest Management
DBMS – database management system	IRRI – International Rice Research Institute
DDTK – disease diagnostic tool kit	IVC – in vitro culture
DENR – Department of Environment and Natural Resources	IWM – in vitro mutagenesis
DH L– double haploid lines	IWM – integrated weed management
DRR – drought recovery rate	JICA – Japan International Cooperation Agency
DS – dry season	K – potassium
DSA - diversity and stress adaptation	kg – kilogram
DSR – direct seeded rice	KP – knowledge product
DUST – distinctness, uniformity and stability trial	KSL – knowledge sharing and learning
DWSR – direct wet-seeded rice	LCC – leaf color chart
EGS – early generation screening	LDIS – low-cost drip irrigation system
EH – early heading	LeD – leaf drying
	LeR – leaf rolling
	lpa – low phytic acid
	LGU – local government unit

LSTD – location specific technology development	PI – panicle initiation
m – meter	PN – pedigree nursery
MAS – marker-assisted selection	PRKB – Pinoy Rice Knowledge Bank
MAT – Multi-Adaption Trial	PTD – participatory technology development
MC – moisture content	PYT – preliminary yield trial
MDDST – modified dry direct seeding technique	QTL – quantitative trait loci
MET – multi-environment trial	R – resistant
MFE – male fertile environment	RBB – rice black bug
MLM – mixed-effects linear model	RCBD – randomized complete block design
Mg – magnesium	RDI – regulated deficit irrigation
Mn – Manganese	RF – rainfed
MDDST – Modified Dry Direct Seeding Technique	RP – resource person
MOET – minus one element technique	RPM – revolution per minute
MR – moderately resistant	RQCS – Rice Quality Classification Software
MRT – Mobile Rice TeknoKlinik	RS4D – Rice Science for Development
MSE – male-sterile environment	RSO – rice sufficiency officer
MT – minimum tillage	RFL – Rainfed lowland
mtha ⁻¹ – metric ton per hectare	RTV – rice tungro virus
MYT – multi-location yield trials	RTWG – Rice Technical Working Group
N – nitrogen	S – sulfur
NAFC – National Agricultural and Fishery Council	SACLOB – Sealed Storage Enclosure for Rice Seeds
NBS – narrow brown spot	SALT – Sloping Agricultural Land Technology
NCT – National Cooperative Testing	SB – sheath blight
NFA – National Food Authority	SFR – small farm reservoir
NGO – non-government organization	SME – small-medium enterprise
NE – natural enemies	SMS – short message service
NIL – near isogenic line	SN – source nursery
NM – Nutrient Manager	SSNM – site-specific nutrient management
NOPT – Nutrient Omission Plot Technique	SSR – simple sequence repeat
NR – new reagent	STK – soil test kit
NSIC – National Seed Industry Council	STR – sequence tandem repeat
NSQCS – National Seed Quality Control Services	SV – seedling vigor
OF – organic fertilizer	t – ton
OFT – on-farm trial	TCN – testcross nursery
OM – organic matter	TCP – technical cooperation project
ON – observational nursery	TGMS – thermo-sensitive genetic male sterile
OPAg – Office of Provincial Agriculturist	TN – testcross nursery
OpAPA – Open Academy for Philippine Agriculture	TOT – training of trainers
P – phosphorus	TPR – transplanted rice
PA – phytic acid	TRV – traditional variety
PCR – Polymerase chain reaction	TSS – total soluble solid
PDW – plant dry weight	UEM – ultra-early maturing
PF – participating farmer	UPLB – University of the Philippines Los Baños
PFS – PalayCheck field school	VSU – Visayas State University
PhilRice – Philippine Rice Research Institute	WBPH – white-backed planthopper
PhilSCAT – Philippine-Sino Center for Agricultural Technology	WEPP – water erosion prediction project
PHilMech – Philippine Center for Postharvest Development and Mechanization	WHC – water holding capacity
PCA – principal component analysis	WHO – World Health Organization
	WS – wet season
	WT – weed tolerance
	YA – yield advantage
	Zn – zinc
	ZT – zero tillage

We are a chartered government corporate entity under the Department of Agriculture. We were created through Executive Order 1061 on 5 November 1985 (as amended) to help develop high-yielding, cost-reducing, and environment-friendly technologies so farmers can produce enough rice for all Filipinos.

We accomplish this mission through research and development work in our central and seven branch stations, coordinating with a network that comprises 58 agencies and 70 seed centers strategically located nationwide. To help farmers achieve holistic development, we will pursue the following goals in 2010-2020: attaining and sustaining rice self-sufficiency; reducing poverty and malnutrition; and achieving competitiveness through agricultural science and technology.

We have the following certifications: ISO 9001:2008 (Quality Management), ISO 14001:2004 (Environmental Management), and OHSAS 18001:2007 (Occupational Health and Safety Assessment Series).

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