GENOMICS-ASSISTED ALLELE MINING AND ITS INTEGRATION TO BREEDING IN RICE

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ABSTRACT

Since whole genome sequence of rice has been decoded, it has been more than 6 years. Rice sequence information has contributed on innovation of selection strategy in rice breeding (marker assisted selection) as well as functional analysis of rice genes. Elucidation of the association between nucleotide and phenotypic changes is inevitable to this end and has been a big challenge in molecular genetics and breeding of rice. Toward this goal, we have been involved in the genetic dissection of natural phenotypic variations in rice and have identified several genes involved in complex traits, including heading date, pre-harvest sprouting, root morphology, disease resistance and eating quality. To enhance the power of genetic dissection of complex phenotypes, we are developing several mapping populations, such as recombinant inbred lines and chromosome segment substitution lines, which will allow us to extract the useful alleles from natural variants. Recently, QTL for durable resistance to rice blast has been cloned from Japanese upland rice. This finding has opened new opportunity to introduction of the unique blast resistance gene without a linkage drag of low eating quality. We have also detected a major QTL for deeper rooting on chromosome 9. Based on the MAS, near-isogenic lines of IR64 with deeper root has been developed and is now under evaluation of its potential of drought avoidance. To facilitate allele mining using novel plant materials, we have also embarked on the genome-wide discovery of single nucleotide polymorphisms (SNPs). These SNP explore new strategy of genetic and breeding study. We have already revealed genomic sequences of several cultivars to detect SNP and definition of haplotype block of Japanese rice cultivars and high-yielding cultivars for forage use. Currently association between these particular haplotypes and phenotypic performance is investigated. These progresses have gradually acted as a force to change a landscape of selection in rice breeding.

KEYWORDS

Single nucleotide polymorphism (SNP), marker assisted selection, quantitative trait locus (QTL), mapping population

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Genomics-assisted allele mining and its integration to breeding in rice

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Whole rice genome has been decoded by International Rice Genome Sequencing Project (IRGSP)

Nature 436: 783-800 (2005)

ARTICLES

The map-based sequence of the rice genome

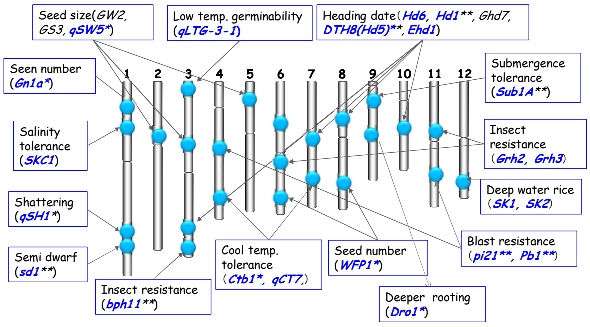
International Rice Genome Sequencing Project*

Genes identified in Japanese research activities

Hd1, *Plant Cell* (2000)
Hd6, *PNAS* (2001)
Hd3a, *PCP* (2002)
Ehd1, *Genes Dev* (2004)
Ehd2, *Plant Physiol* (2008)
Ehd3, *Plant J* (2011)
PAIR1, *Plant Cell* (2004)
MEL1, *Plant Cell* (2007)
MSP1, *Plant Cell* (2003)
RMS, *PNAS* (2009)
Xa1, *PNAS* (1998)
Pib, *Plant J* (1999)
OsRac1, *PNAS* (2006)
WRKY45
Plant Cell (2007)
RACK1A,*Plant Cell* (2008)
pi21, *Science* (2009)
Spl7, *PNAS* (2002)
qUVR10, *Genetics* (2005)
OsDREB1A-D, *OsDREB2A*,
Plant J (2003)
POLLUX, *CYCLOPS*,
Plant Cell (2008)

qSW5, *Nat genet* (2008)
qSH1, *Science* (2006)
Gn1, *Science* (2005)
qLTG3-1, *PNAS* (2008)
Sdr4, *PNAS* (2010)
SSI, *SSIII*, *Plant Physiol*
(2006, 2007)
d1, *PNAS* (1999)
d11, *Plant Cell* (2005)
gid1, *Nature* (2005)
gid2, *Science* (2003)
OsBR6ox, *Plant Physiol* (2002)
P450, *Plant Physiol* (2004)
LOG, *Nature* (2007)
OsGA2ox1, *Nature Biotech* (2003)
SNORKEL1, *SNOKEL2*, *Nature*
(2009)
Lsi1, *Lsi2*, *Nature* (2006) (2007)
Lsi6, *Plant Cell* (2008)

Genes contribute on improvement of rice cultivars



Seed size(*GW2*, *GS3*, *qSW5**)

Low temp. germinability (*qLTG-3-1*)

Heading date(*Hd6*, *Hd1***, *Ghd7*, *DTH8(Hd5)***, *Ehd1*)

Seen number (*Gn1a**)

Submergence tolerance (*Sub1A***)

Salinity tolerance (*SKC1*)

Insect resistance (*Grh2*, *Grh3*)

Shattering (*qSH1**)

Deep water rice (*SK1*, *SK2*)

Semi dwarf (*sd1***)

Blast resistance (*pi21***, *Pb1***)

Insect resistance (*bph11***)

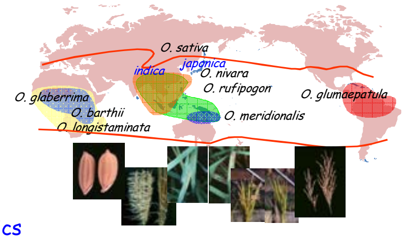
Cool temp. tolerance (*Ctb1**, *qCT7*,)

Seed number (*WFP1**)

Deeper rooting (*Dro1**)

*: Marker assisted breeding (MAS) has been progressed.
**: New cultivars has been developed by MAS

Natural variations in rice



Topics

- ➢ Discovery of useful allele and its introduction to elite cultivars (root morphology)
- ➢ Plant materials development for the allele mining
- ➢ Establishment of a platform of genome wide SNP typing for genetic analysis and marker assisted selection

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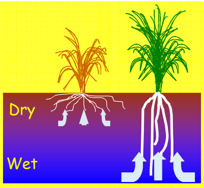
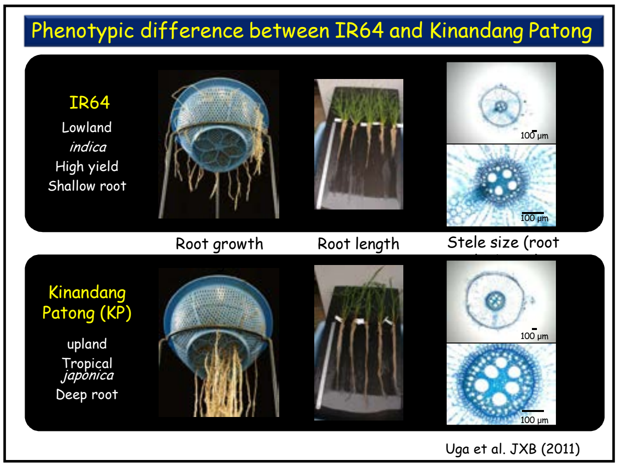
Drought avoidance using root traits QTL in rice

Global warming and spreading desertification are causing serious drought damage in rice-growing areas in many developing countries that rely solely on rain to grow rice. Therefore, breeding of drought-resistant lowland rice is becoming an important research topic.

- ➢ A deeper and thicker root system is an important component associated with avoiding drought stress in rice
- ➢ QTL cloning and its utilization may be useful to improve drought avoidance in rice





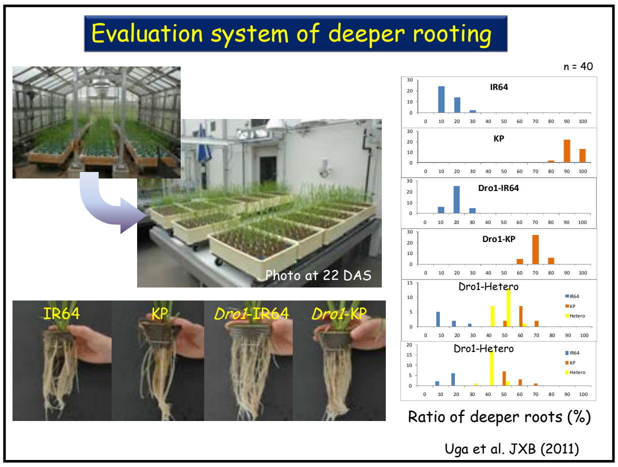
Phenotypic difference between IR64 and Kinandang Patong

IR64
Lowland
indica
High yield
Shallow root

Kinandang Patong (KP)
upland
Tropical *japonica*
Deep root

Root growth
Root length
Stele size (root

Uga et al. JXB (2011)

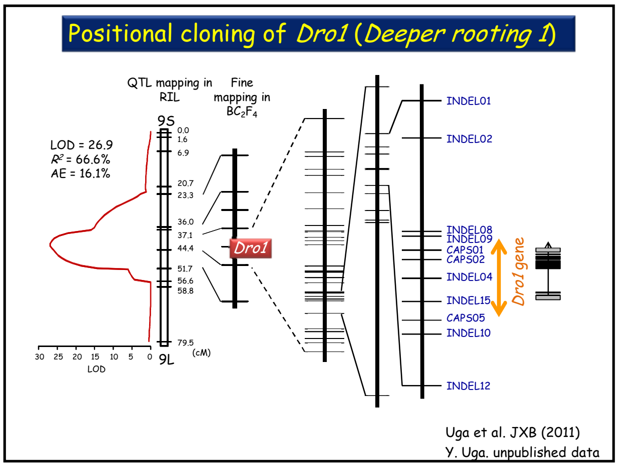


Evaluation system of deeper rooting

Photo at 22 DAS

Ratio of deeper roots (%)

Uga et al. JXB (2011)

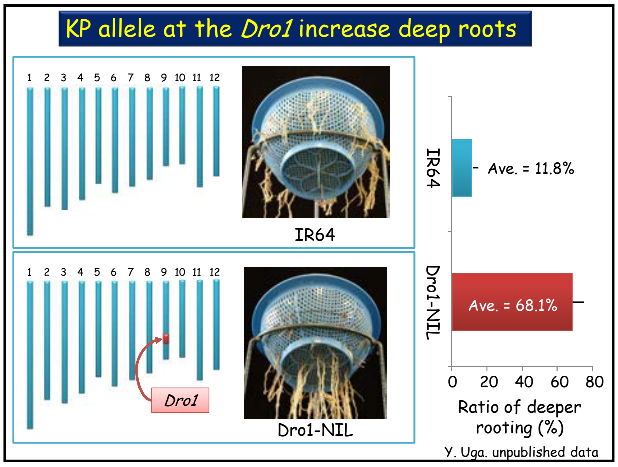


Positional cloning of *Dro1* (*Deeper rooting 1*)

QTL mapping in RIL
Fine mapping in BC_2F_4

LOD = 26.9
R^2 = 66.6%
AE = 16.1%

Uga et al. JXB (2011)
Y. Uga. unpublished data



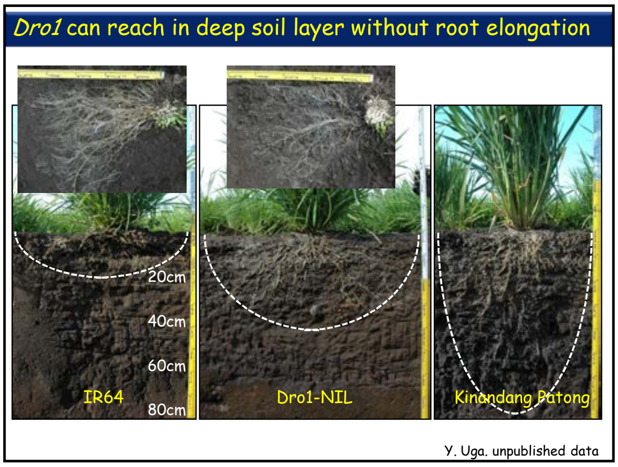
KP allele at the *Dro1* increase deep roots

Ratio of deeper rooting (%)

Y. Uga. unpublished data

Survey of diversity in root morphology in rice

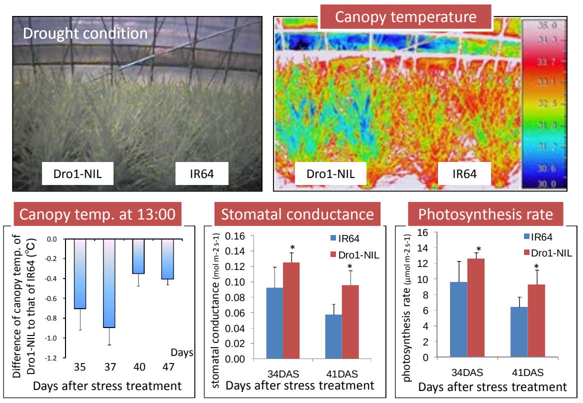
'Trench method' is an appropriate way to directly measure root distribution in the field.



Dro1 can reach in deep soil layer without root elongation

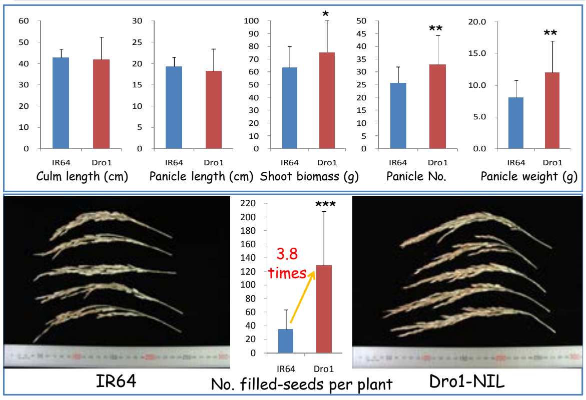
Y. Uga. unpublished data

Dro1-NIL well-photosynthesized under drought stress



Y. Uga. unpublished data

Dro1-NIL increased fertile seeds under drought condition



Y. Uga. unpublished data

Ongoing research activities on evaluation of performance of draught avoidance by *Dro1*

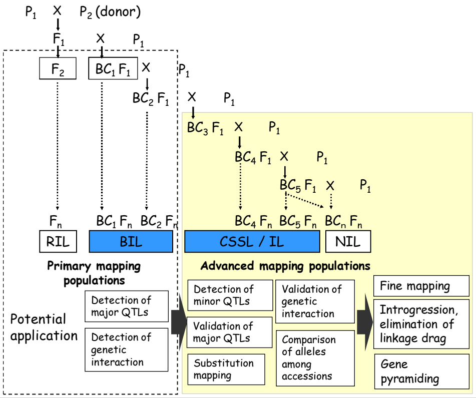
The International Center for Tropical Agriculture (CIAT) in Colombia

Dro1- NIL has been tested under different level of draught stresses using experimental field with rain-out shelter.

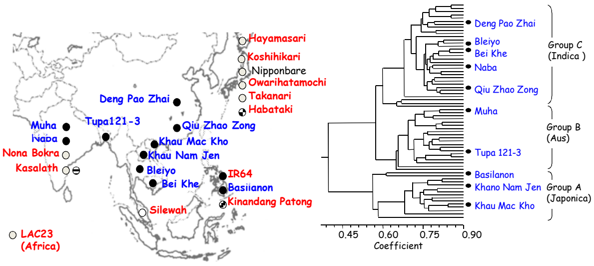
The International Rice Research Institute (IRRI) in Phlippines

Dro1- NIL will be tested under lowland condition and multiple locations in IRRI's drought breeding network. *Dro1* will be introduced into other mega varieties to study the expression of drought tolerance in different genetic backgrounds.

Plant materials and their utility in genetic mapping



Origins and genetic relationship among the donors used to develop CSSLs



Accessions were selected based on trait of interests

Accessions were selected based on sequence variations

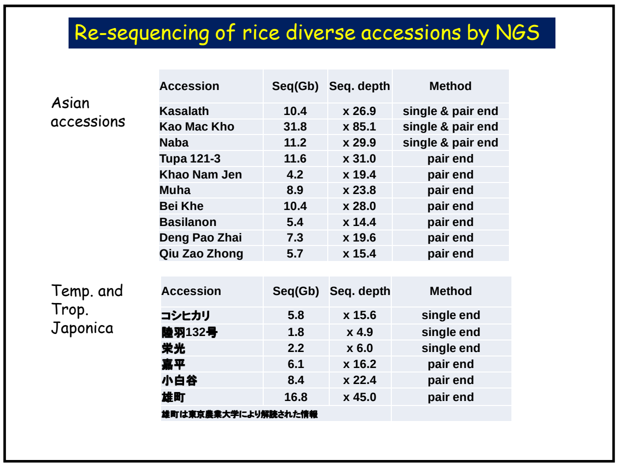
Mapping populations available for genetic research

Distributed from Rice Genome Resource Center (RGRC) at NIAS

http://www.rgrc.dna.affrc.go.jp/

Cross	Pop. structure	Recurrent	No. lines	No of markers
Nipponbare/Kasalath	BIL	Nipponbare	98	245
Nipponbare/Kasalath	CSSL	Nipponbare	54	204
Koshihikari/Kasalath	BIL	Koshihikari	182	162
Koshihikari/Kasalath	CSSL	Koshihikari	39	130
Sasanishiki/Habataki	BIL	Sasanishiki	85	236
Sasanishiki/Habataki	CSSL	Sasanishiki	39	166
Akihikari/Koshihikari	DHL		210	169
Koshihikari/NonaBokra	CSSL	Koshihikari	44	145
Nipponbare/Koshihikari	BIL	Koshihikari	79	111
Nipponbare/Koshihikari	BIL	Nipponbare	127	111
Nipponbare/Koshihikari	CSSL	Koshihikari	41	108
Nipponbare/Koshihikari	CSSL	Nipponbare	48	108
Koshihikari/Jarjan	BIL	Koshihikari	95	149
IR64/Kinandang Patong	RIL		116	131

BIL: Backcross inbred line, CSSL: Chromosome segment substitution line. DHL:Doubled haploid line, RIL: Recombinant inbred Line



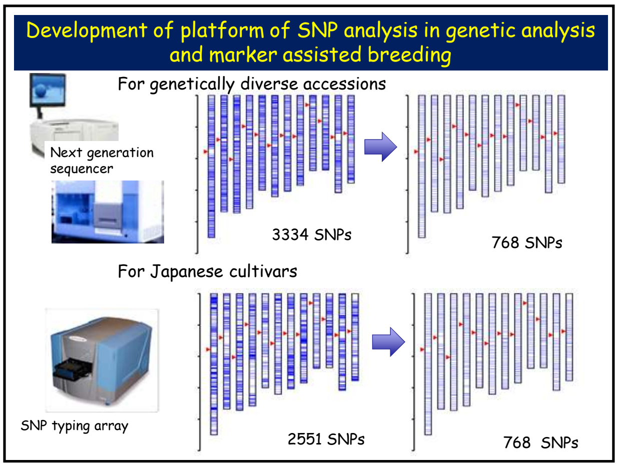
Re-sequencing of rice diverse accessions by NGS

Asian accessions

Accession	Seq(Gb)	Seq. depth	Method
Kasalath	10.4	x 26.9	single & pair end
Kao Mac Kho	31.8	x 85.1	single & pair end
Naba	11.2	x 29.9	single & pair end
Tupa 121-3	11.6	x 31.0	pair end
Khao Nam Jen	4.2	x 19.4	pair end
Muha	8.9	x 23.8	pair end
Bei Khe	10.4	x 28.0	pair end
Basilanon	5.4	x 14.4	pair end
Deng Pao Zhai	7.3	x 19.6	pair end
Qiu Zao Zhong	5.7	x 15.4	pair end

Temp. and Trop. Japonica

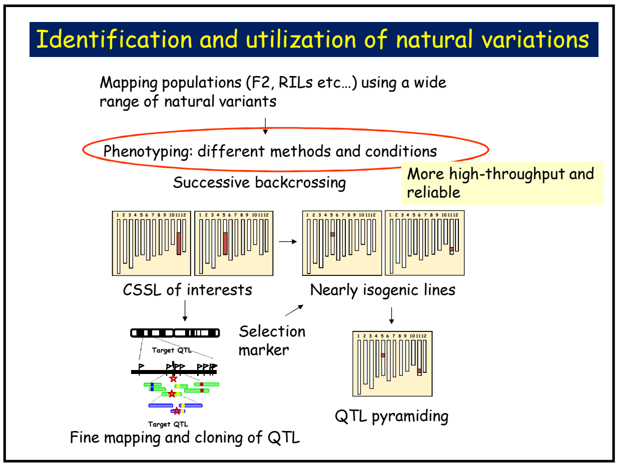
Accession	Seq(Gb)	Seq. depth	Method
コシヒカリ	5.8	x 15.6	single end
陸羽132号	1.8	x 4.9	single end
栄光	2.2	x 6.0	single end
嘉平	6.1	x 16.2	pair end
小白谷	8.4	x 22.4	pair end
雄町	16.8	x 45.0	pair end
雄町は東京農業大学により解読された情報			



Development of platform of SNP analysis in genetic analysis and marker assisted breeding

For genetically diverse accessions

For Japanese cultivars



Identification and utilization of natural variations

Mapping populations (F2, RILs etc…) using a wide range of natural variants

Phenotyping: different methods and conditions

More high-throughput and reliable

Successive backcrossing

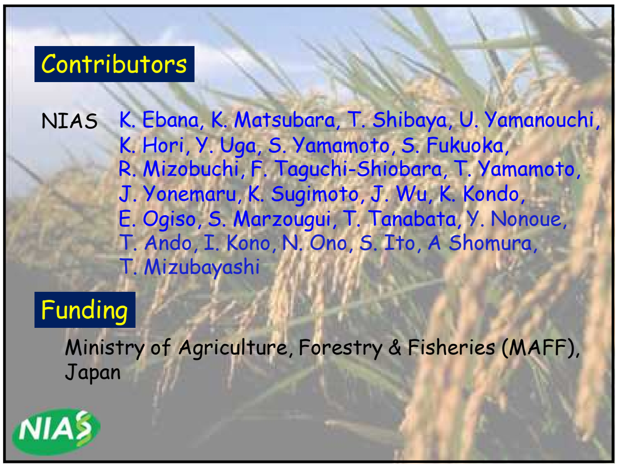
CSSL of interests

Nearly isogenic lines

Selection marker

Fine mapping and cloning of QTL

QTL pyramiding



Contributors

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