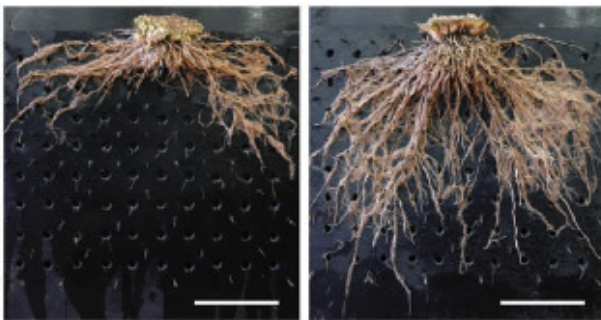


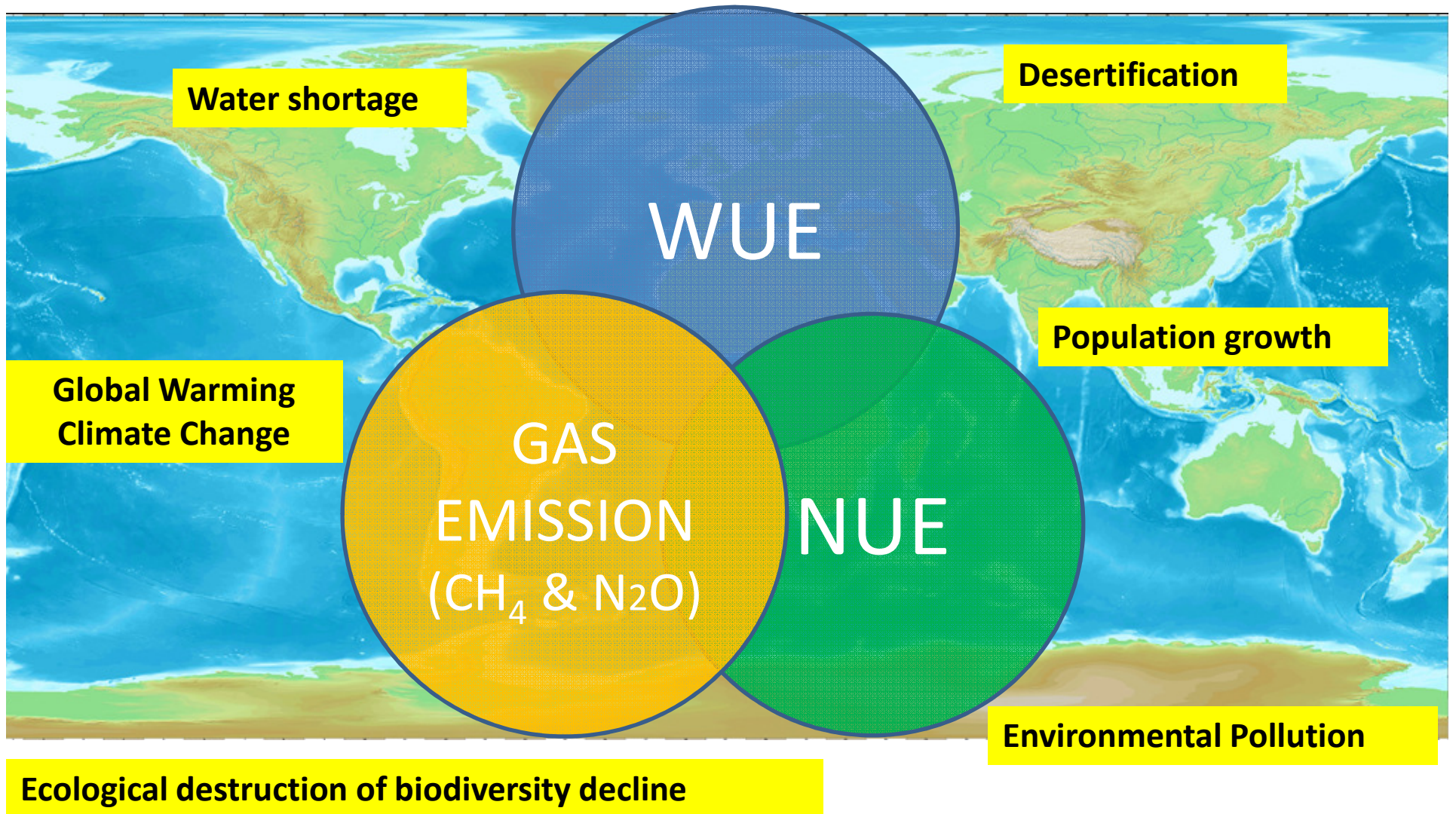
Rice Root physiology work at CIAT: Identification of ideal root system to improve water and Nitrogen uptake under stress conditions

Satoshi Ogawa

JSPS Post-doctoral fellow



Our challenging



These problems are further exacerbating

Underground Revolution

NEWS FEATURE FOOD

NATURE | Vol 466 | 29 July 2010

AN UNDERGROUND REVOLUTION



Plant breeders are turning their attention to roots to increase yields without causing environmental damage. Virginia Gewin unearths some promising subterranean strategies.

Tangled, dirty and buried underfoot, roots are a mess to study. Digging them up is a time-consuming and sometimes back-breaking process. The shovel must be wielded with care to preserve the roots' delicate branching patterns, the root hairs and the microbes that cling to them. All of this explains why roots have been largely out of mind, as well as out of sight, for agricultural researchers — until now.

Many scientists are starting to see roots as central to their efforts to produce crops with a better yield — efforts that go beyond the Green Revolution. That intensive period of research and development, starting in the 1940s, dramatically boosted food production through the breeding of high-yield crop varieties and the use of pesticides, fertilizers and more water. But the increases were accompanied by a depletion of groundwater and, by 1998, an eightfold increase in nitrogen-based fertilizer usage, bringing environmental problems such as polluted waterways. The leaps in yield have still left many hungry. And the revolution missed many developing nations, some of which have poor soils and limited access to irrigation and expensive fertilizers. "Those strategies of the past aren't working now to meet growing food needs," says Jonathan Lynch, a plant nutritionist at Pennsylvania State University in University Park.

"Roots are the key to a second green revolution — one that doesn't rely on expensive inputs," says Lynch. Roots deliver water and nutrients, two of the most essential, often-limiting, factors that a plant needs. Why keep putting in more water and fertilizers, he and others reason, when they might instead improve roots' ability to use what's already there and, in the process, help to convert 'marginal' lands into productive ones.

There is room for improvement. Although plant breeders have already made huge gains by manipulating 'above ground' traits — for example, by breeding dwarf plant varieties, which put more energy into producing grain rather than the stalk — the same is not true for root traits. "One reason we now have any potential

to increase yields is because the tremendous genetic variation trapped in roots has been neglected," says Lynch. Here, *Nature* reports on four of the most promising leads for boosting food production through roots.

Designer roots

Roots are most efficient when their architecture is tailored to their environment. Deep roots can tap water beneath parched soils, whereas fine, shallow roots can exploit soils in which limiting nutrients are trapped at the surface.

Michelle Watt, a plant biologist at the Commonwealth Scientific and Industrial Research Organisation (CSIRO) in Canberra, is working to produce varieties of wheat that are better suited to drought-prone areas. In a recent study of wheat lines, Watt's team found that the roots of some lines penetrate 25% deeper than others'. The team crossed lines that had deeper, faster-growing roots with widely used cultivars to develop 400 new wheat lines, which are now being field-tested in India and Australia.

Watt is also taking advantage of new genetic tools. Rather than wade through the 17 billion base pairs of the bread wheat genome, though, her group is searching for genetic markers that are associated with deep roots in the much smaller (271 million base pair) genome of *Brachypodium distachyon*, a temperate grass in the same subfamily as wheat whose genome was sequenced earlier this year. The team hopes that the markers will make it possible to identify, from seeds, which wheat varieties are likely to have deep roots, without going through the laborious process of growing the seedlings, digging them up and measuring their roots.

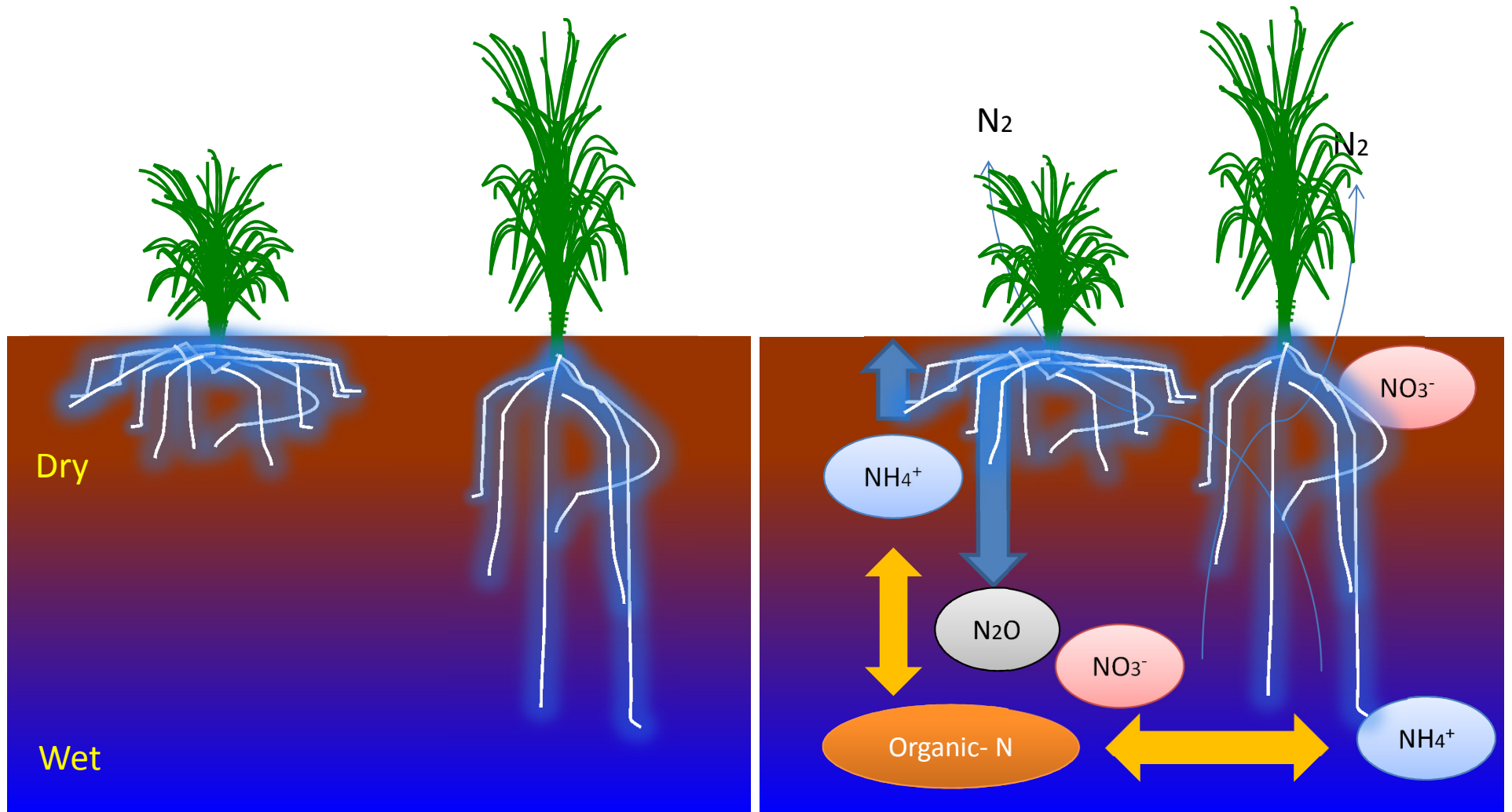
At Penn State, Lynch has found that, when water is limited, maize lines that incorporate a large amount of intercellular air space in root tissue have an eightfold higher yield than plants without this ability'. When stressed, it may be that plants reduce the metabolic costs of



Increased productivity without environmental damages

- ✓ Water
- ✓ Plant nutrition
- ✓ Soil–plant–microbe interactions

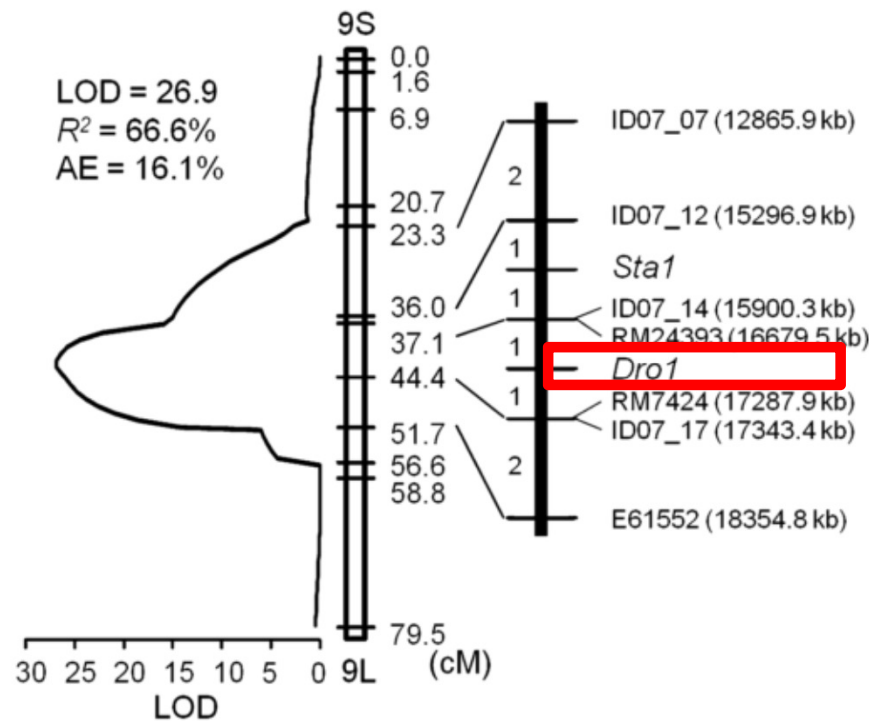
What is an Ideal Root Type in Rice?



Water Use Efficiency (WUE)

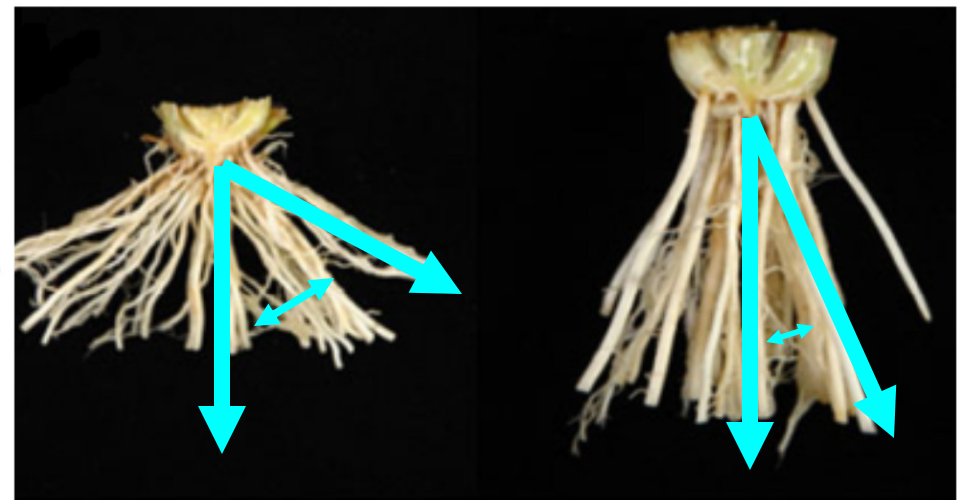
Nitrogen Use Efficiency (NUE)

Identification of Deeper Root Gene: Root Angle Concept



IR64

Kinandang
Patong(KP)



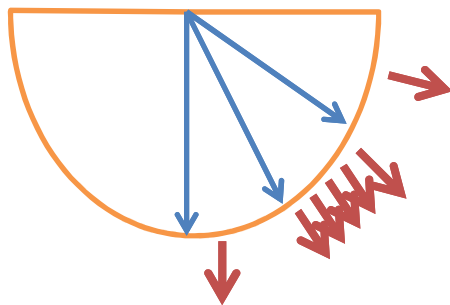
“*DRO1*, a major QTL involved in deep rooting of rice under upland field conditions” (Uga, 2013)

Effect of *DRO1* Gene on Root Angle



IR64

**RDR:
10.2±4.8**

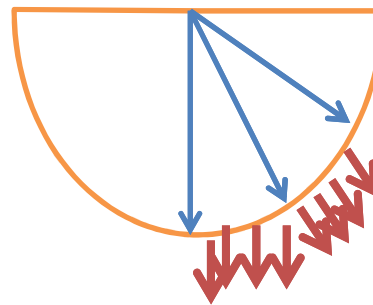


10%



DRO1 NIL

**RDR:
47.1±10.2**

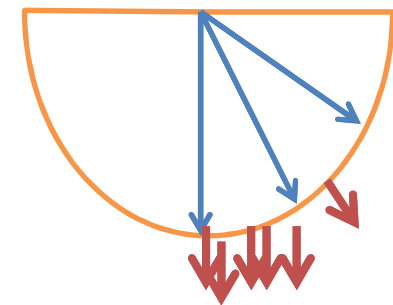


50%



KP

**RDR:
77.8±9.6**

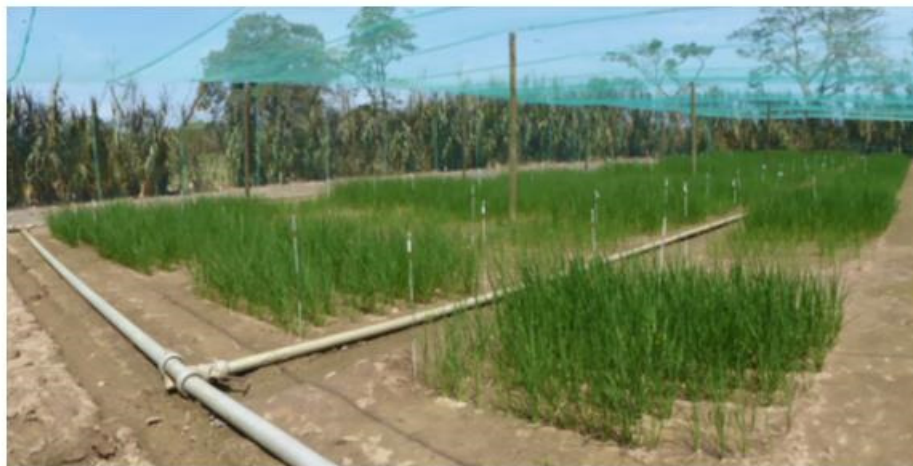
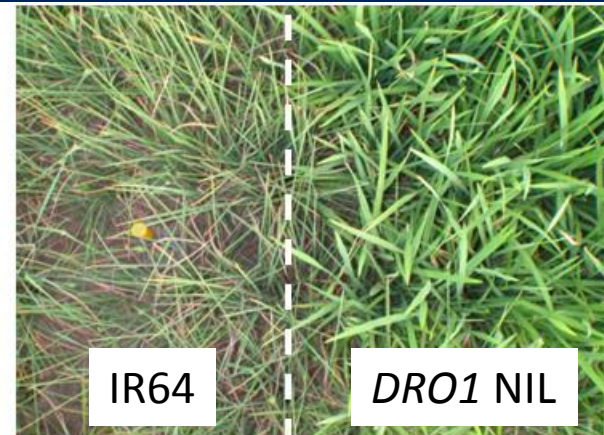


80%

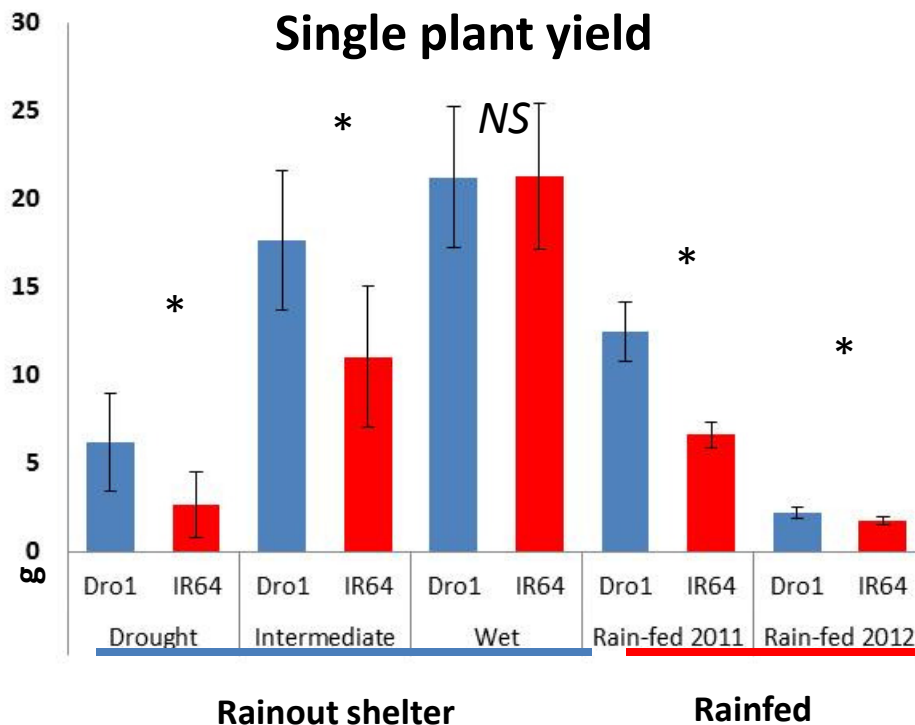
DRO1 QTL Gene Improved Grain Yield under Water Limited Conditions



Rainout Shelter drought experiments



Santa Rosa rainfed experiment



Ref: Uga et al. (2013)

N Field Experiments at CIAT



Measured traits

At flowering period

N content of flag leaf (Kjeldahl), leaf chlorophyll content (SPAD value), Plant height

At harvesting period

Number of reproductive tiller

At post-harvesting (After drying)

Individual grain yield, individual plant biomass, panicle length and 1000 grain weight

NDT traits

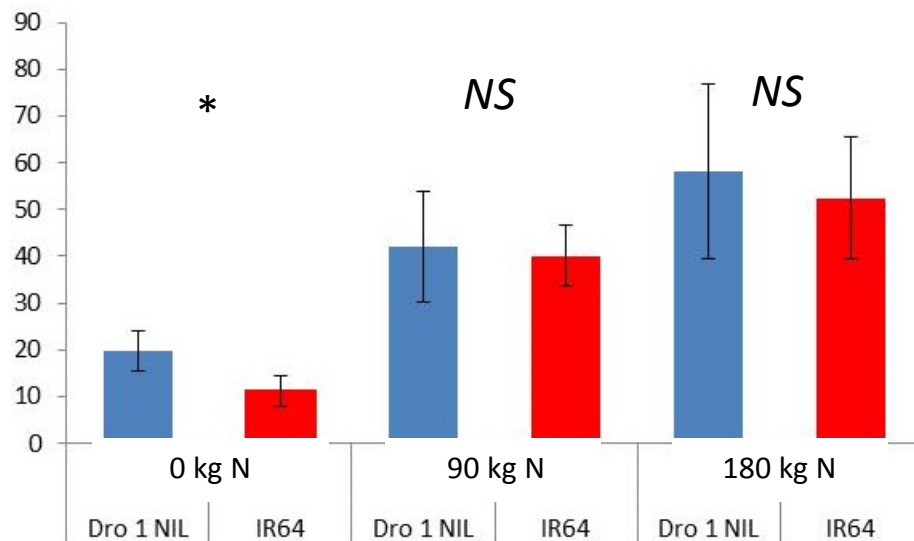
NDT traits (Relative value) = measured value_{native} / measured value_{FP}

E.g.) RGY (Relative grain yield) = Individual grain yield_{native} / Individual grain yield_{FP} (Wei et al. 2012)

DRO1 QTL Gene Improved Grain Yield under N Deficient Conditions



Single plant yield (g)



N experiments

Table 2 | Mean dry matter accumulation, grain yield, and harvest index in IR64 and Dro1-NIL lines as affected by line and fertilizer treatment

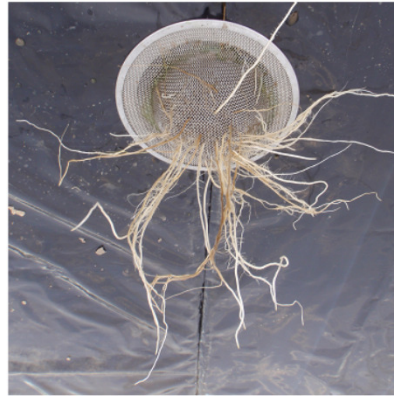
Line (L)	Fertilizer treatment (F)	DWH ^a	DWM ^a	Yield ^b	HI ^c
		(t ha ⁻¹)	(t ha ⁻¹)	(t ha ⁻¹)	
IR64		10.92	15.47	6.15	0.43
Dro1-NIL		11.24	16.27	6.80	0.45
	No treatment	8.86	12.21	4.71	0.43
	Treatment	13.30	19.52	8.24	0.45
ANOVA					
L		ns	*	**	**
F		**	**	**	ns
L×F		ns	ns	ns	ns

^aDWH, dry weight at heading; DWM, dry weight at maturity.
^bYield is given for unhulled rice with water content of 15%.
^cHarvest index (HI) was calculated by dividing the dry weight of panicle by the dry weight of the above-ground parts.
 *Significant at $P < 0.05$; ** significant at $P < 0.01$; ns, not significant at $P < 0.05$. P values are based on ANOVA.

N experiments

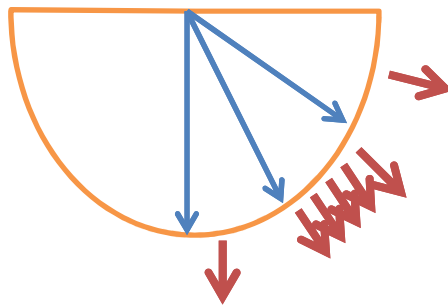
Ref. Arai-Sanoh et al. (2014)

Will Stronger Deep Root Contribute plant Performance?



IR64

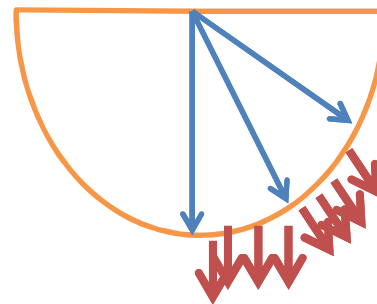
**RDR:
10.2±4.8**



10%

DRO1 NIL

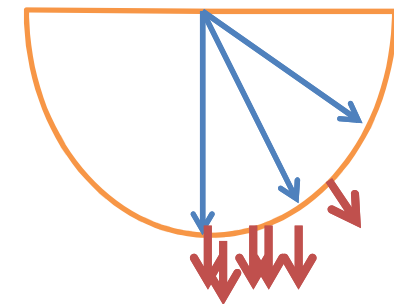
**RDR:
47.1±10.2**



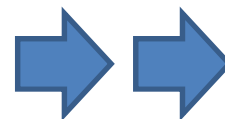
50%

KP

**RDR:
77.8±9.6**



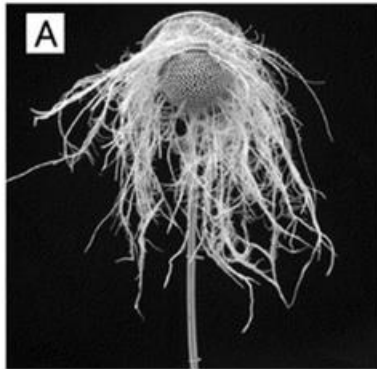
80%



?

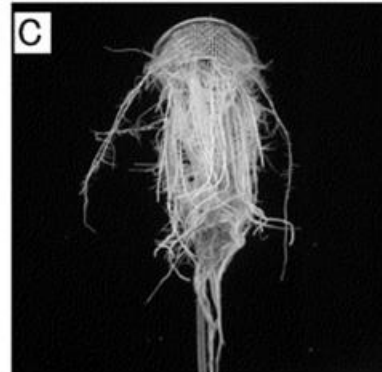
Multi-Environmental Testing of Different Root Angle Variation

Fuente: Patent of Dr. Uga WO2011078308 A1



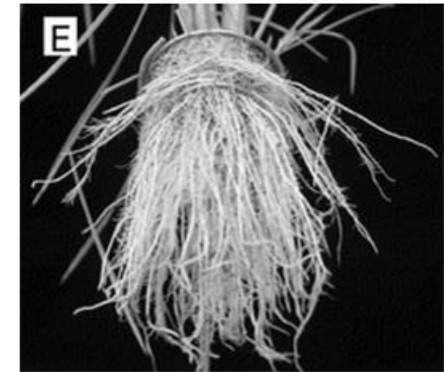
IR64

2; Vector control line



DRO1 NIL

2; *DRO1* transgenic lines (single copy)



3; Multi-copy

DRO1 transgenic lines



WUE



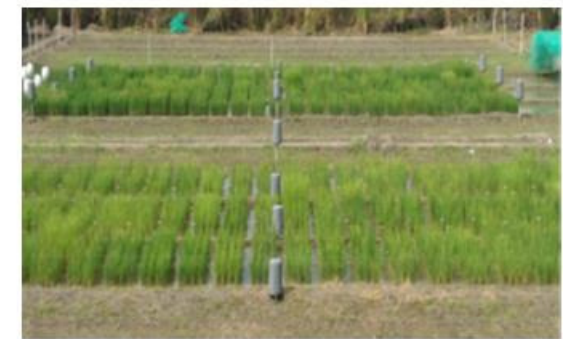
NUE



Transgenic rain-out shelter



Santa Rosa rainfed station



Nitrogen deficient field

Does Other Rooting Patterns Contribute?

Diversity in Rice Rooting pattern



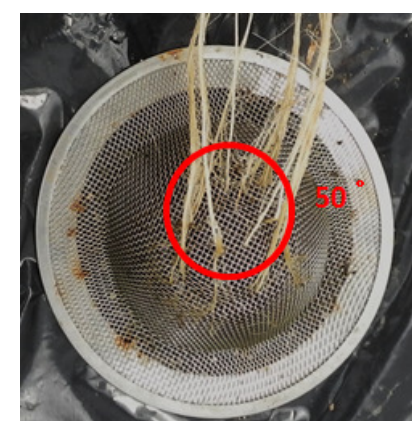
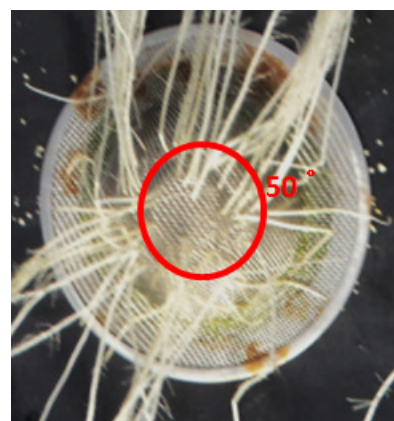
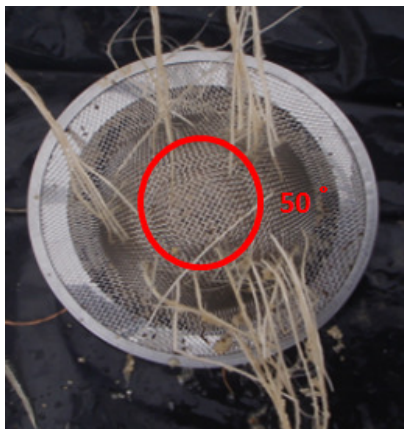
Monomorphic-Shallow (IR64)
RPV = 20.67



Dimorphic (*O. rufipogon*)
RPV = 2.75

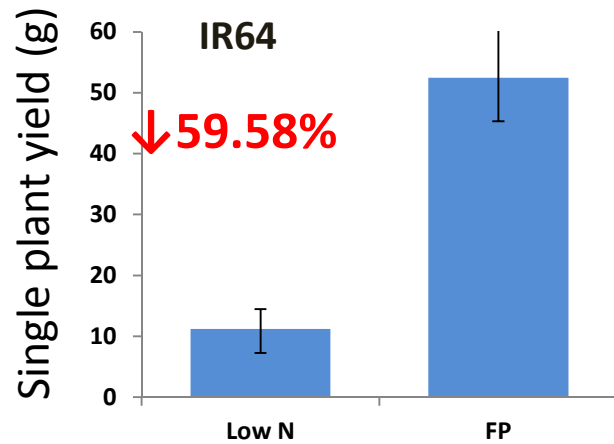


Monomorphic-Deep (Curinga)
RPV = 12.33

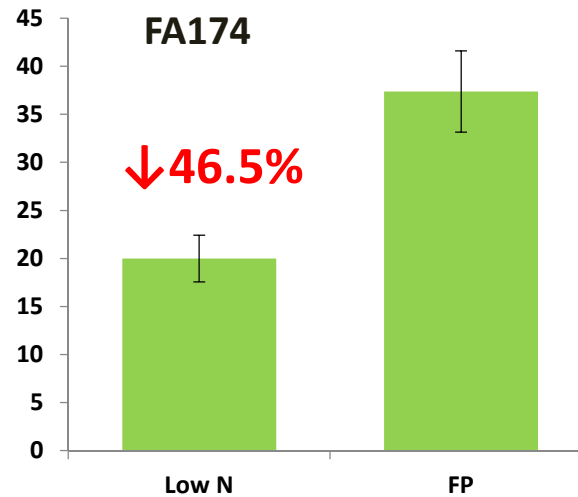


Dimorphic is an Ideal Rooting Pattern for NUE

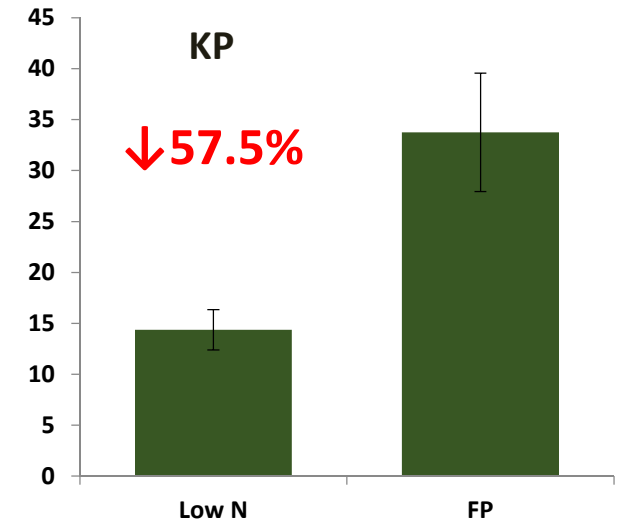
Monomorphic-Shallow



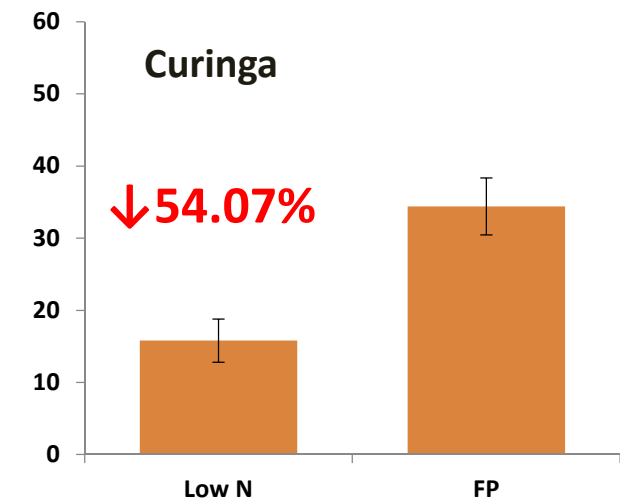
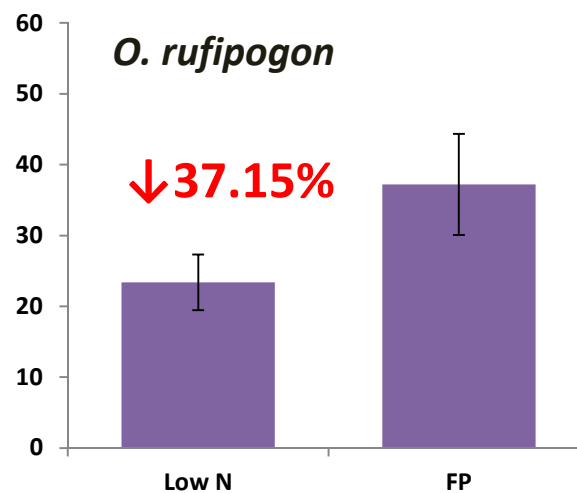
Dimorphic



Monomorphic-deep

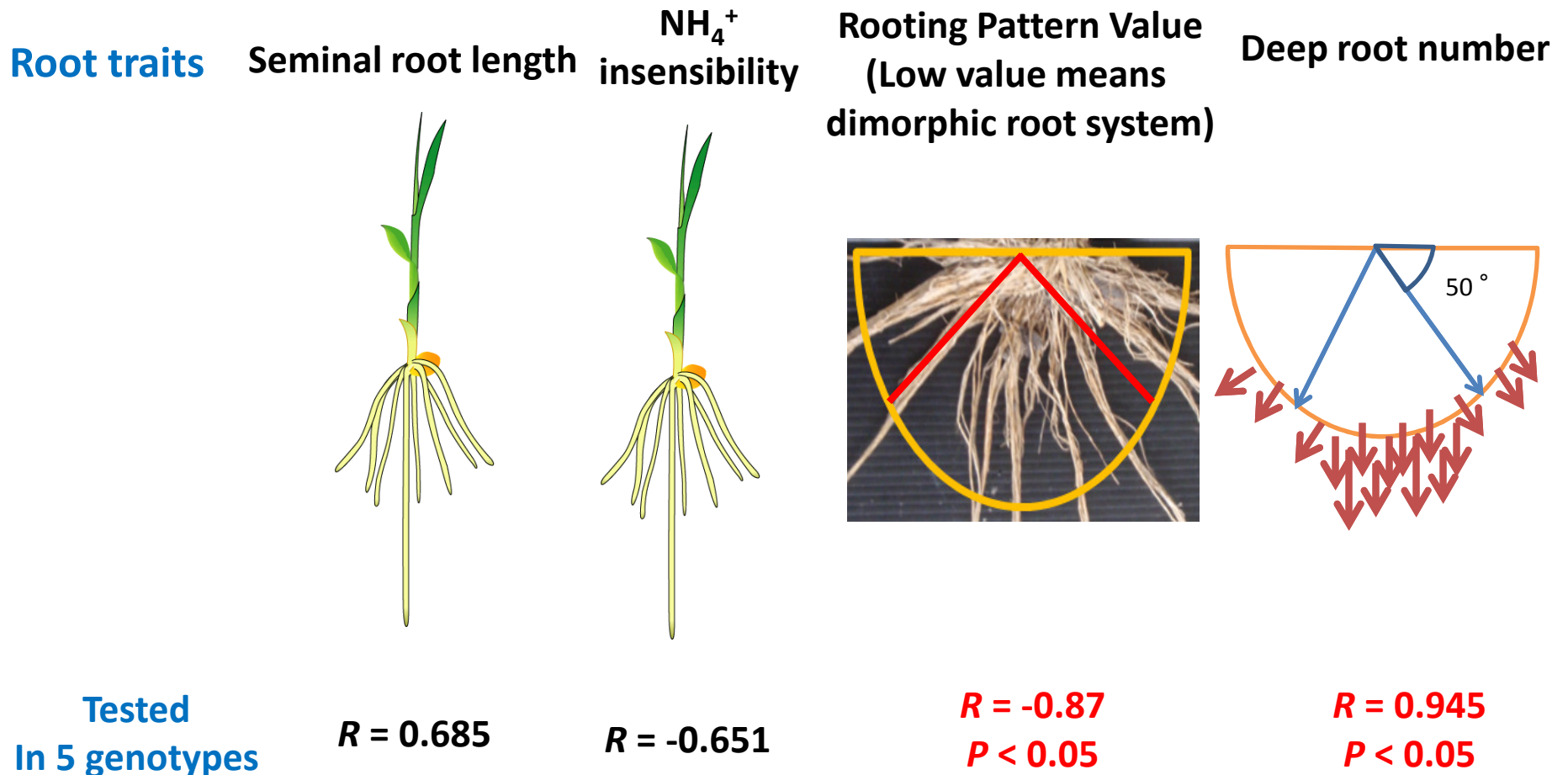


N experiments
under lowland
conditions



Dimorphic Root Contributed to Grain Yield under N Deficient Conditions

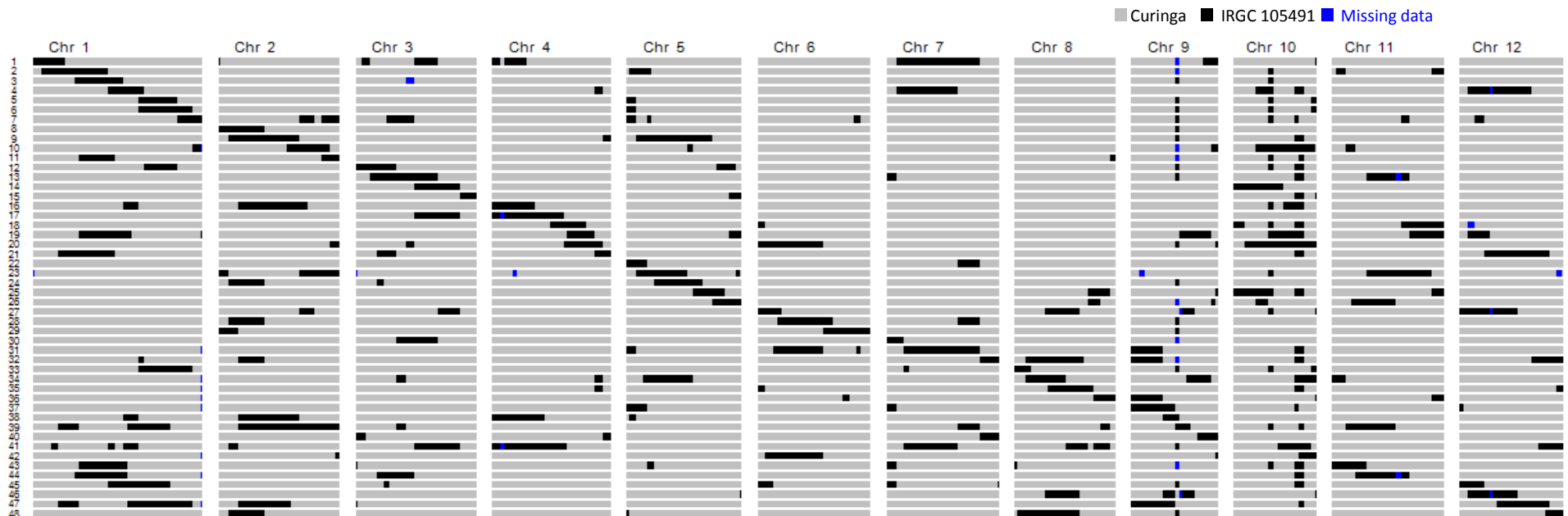
Correlation between root traits and relative grain yield



Identification of QTLs Regulating Root System Architecture for NUE

Materials

48 CSSLs between Curinga x IRGA105491 (*O. rufipogon*)

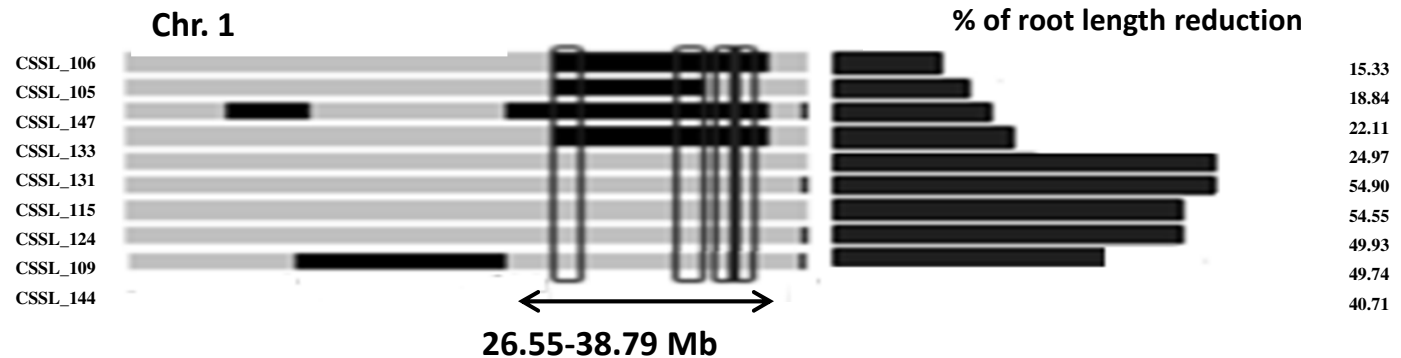


QTLs analysis: CSSL finder (Lorieux M, 2005)

QTLs Identified from *O. rufipogon* Regulating Root Traits

Seminal root elongation

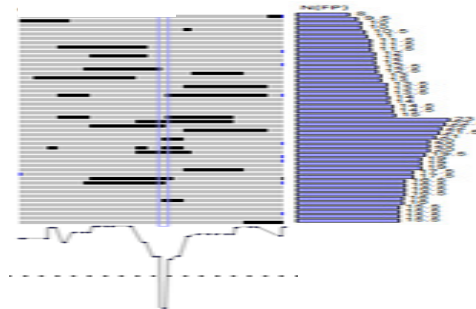
Seminal root length under 500 μM NH_4^+
 Relative seminal root length (500 μM NH_4^+ / 5 μM NH_4^+)



Root growth angle

Deeper root number

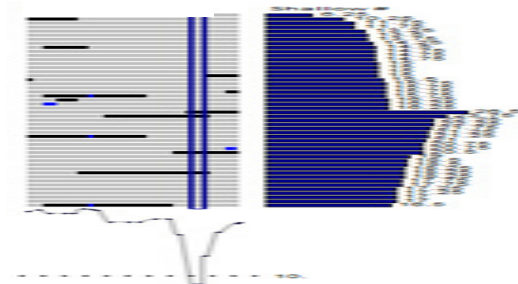
Chr.1



23.45-36.46 Mb

Shallow root number

Chr.12



19.93 - 24.85 Mb

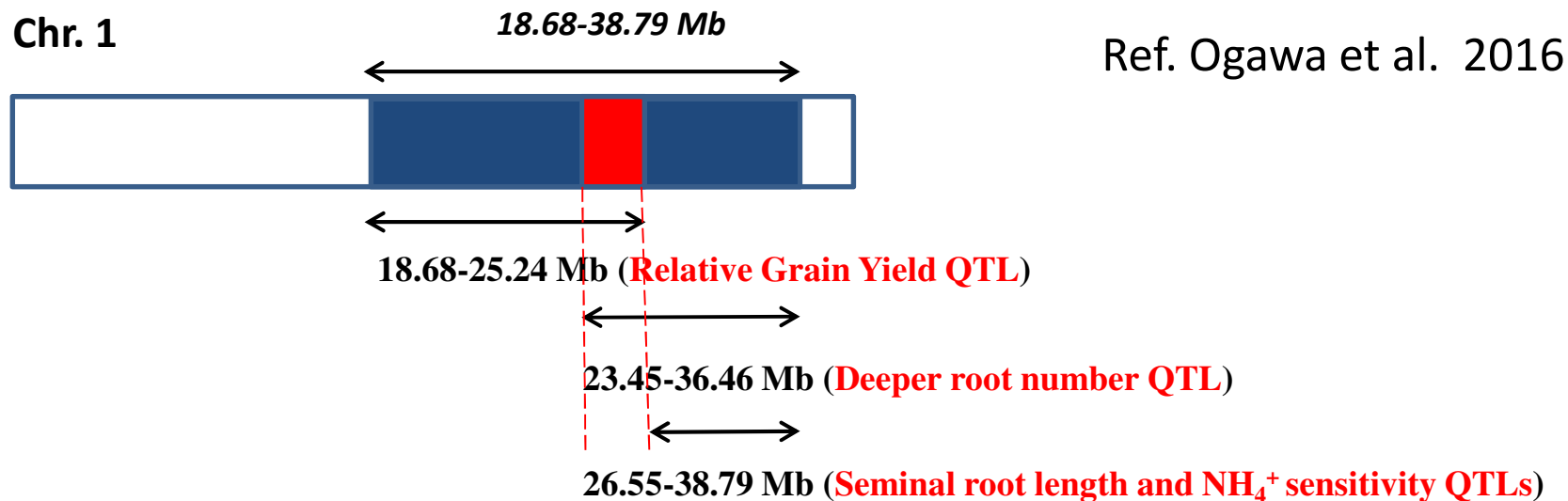
QTLs Identified from *O. rufipogon* Regulating Agronomic NUE

8 agronomic QTLs and 3 Nitrogen Deficiency tolerance QTLs

Trait	Condition	Chr.	Marker	Position(Mb)	Positive allele	Season
Relative grain yield	NDT trait between Native and FP	1	id1010490-id103568	18.68-25.24	<i>O.rufipogon</i>	Feb.- Jun.
Single plant yield	FP	3	id3002476-id3004123	4.32-7.68	<i>O.rufipogon</i>	Both trials
biomass	Native	4	id4005120-id4007907	17.68-24.36	<i>O.rufipogon</i>	Feb.- Jun.
1000 grain weight	Native and FP	5	Id5006603-id5012179	16.45-25.79	<i>O.rufipogon</i>	Both trials
Low SPAD value	FP	7	id7000142-id7000609	0.74-4.66	<i>O.rufipogon</i>	Both trials
Low Nitrogen content	FP	7	id7000142-id7000609	0.74-4.66	<i>O.rufipogon</i>	Feb.- Jun.
Relative N content	NDT trait between Native and FP	7	id7000142-id7000609	0.74-4.66	<i>O.rufipogon</i>	Feb.- Jun.
Relative SPAD value	NDT trait between Native and FP	8	id8000171	0.53	<i>O.rufipogon</i>	Feb.- Jun.
Higher tiller number	Native	9	id9000233-id9000580	0.88-10.75	<i>O.rufipogon</i>	Feb.- Jun.
Higher tiller number	FP	10	id1005370-id1006910	18.66-22.34	<i>O.rufipogon</i>	Aug. - Dec
Early flowering	Native and FP	12	id12003803-id12005677	9.54-16.74	<i>O.rufipogon</i>	Aug. - Dec

Ref. Ogawa et al. 2016

Colocation of Three Agronomic NUE and Root Trait QTLs Identified in This Study



Reported QTLs in the same regions from other studies

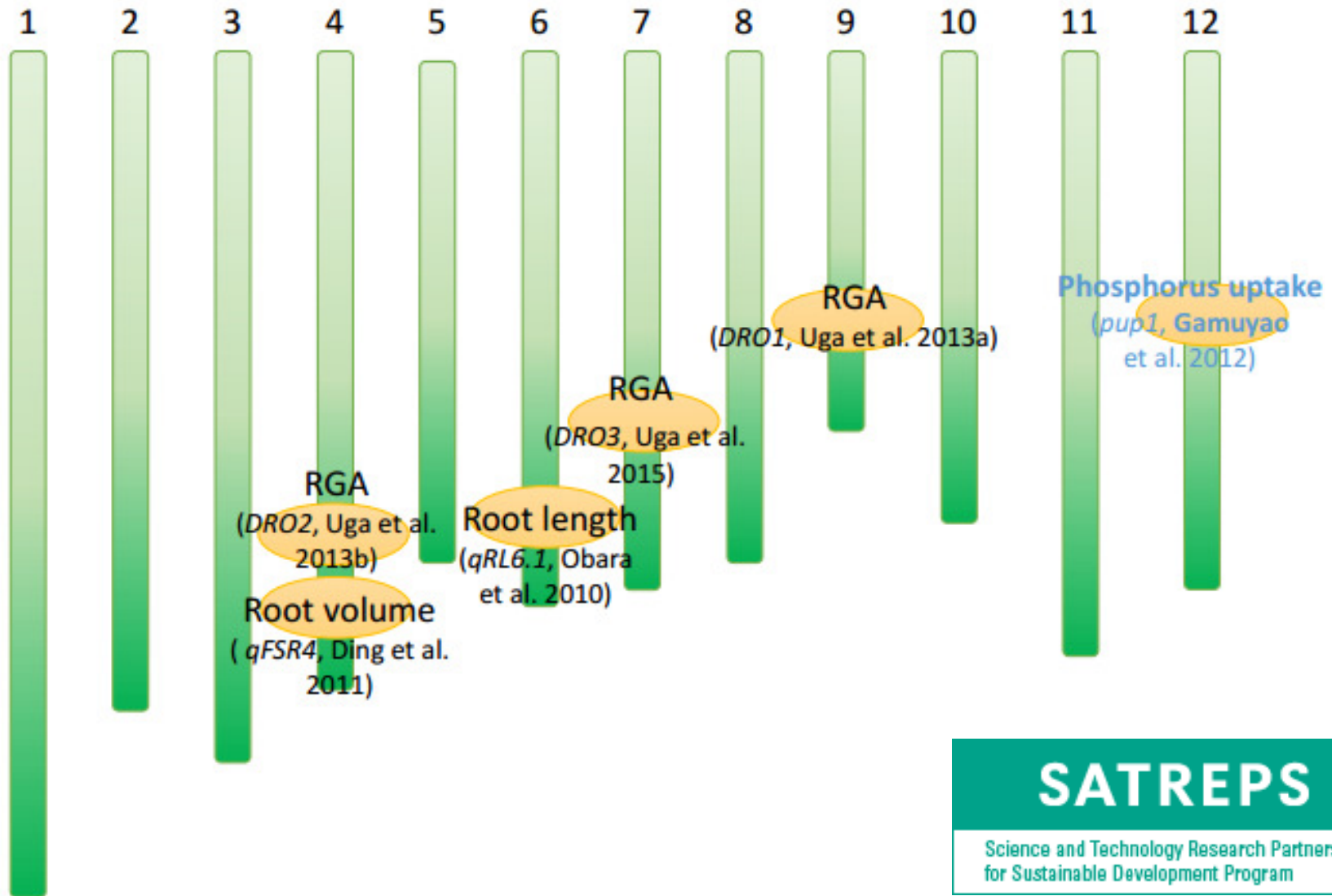
Candidate gene name	Gene function	The Rice Annotation Project Database gene position	Reference
PIN	Auxin efflux carrier component	Os01g0455500	Carraro et al. 2012
IAA	Amino acid hydrolase homolog precursor (involved in auxin homeostasis)	Os01g0510600	Ding et al. 2008
Tat protein	Twin-arginine translocation pathway signal domain containing protein	Os01g0456400	Fukao et al. 2011
ARFs	Auxin responsive factor 3	Os01g0480600	Wang et al. 2009
IAA8	Auxin-responsive protein	Os01g0484500	Groover et al. 2003
IAA8	Auxin-responsive protein	Os01g0488500	Groover et al. 2003
XPL1	Phosphoethanolamine N-methyltransferase	Os01g0500300	Luo et al. 2012
OsGLT1	NADH-glutamate synthase	Os01g0681900	Goto et al. 1998
OsAAT2 1 D14673	Aspartate aminotransferase	Os01g0760600	Song et al. 1996

Conclusions

- ***DRO1*** gene can be ideal candidate for marker assisted breeding to improve water and Nitrogen use efficiency.
- **Dimorphic root system** showed better adaptation under water and N deficient conditions compare to monomorphic root systems

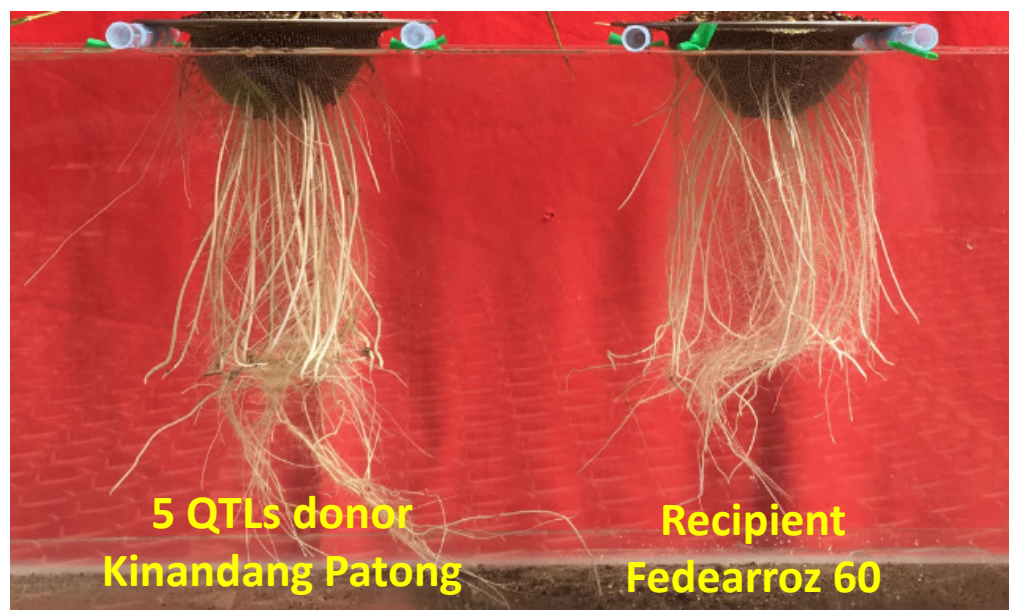
SATREPS Rice Project:

Ideal Root development by QTL pyramiding approach



Current Status of Root QTL Pyramiding

	KP
Tiller number	4.0±0
Height	73.2±3.0
Deep	15.7±2.6
Shallow	13.5±2.8
RDR	53.8±3.3
MRL	26.8±2.3
Total	29.2±5.1
Shoot weight	1,150.0±151.0
Root weight	363.0±112.0
S/R	3.39±0.80
TRL	427.2±53.7



	FA60
Tiller number	5.7±0.8
Height	72.3±0.7
Deep	15.0±1.5
Shallow	45.0±4.8
RDR	25.1±2.8
MRL	23.2±2.0
Total	60.0±5.1
Shoot weight	1555.3±100.2
Root weight	501.2±122.0
S/R	3.30±0.82
TRL	762.2±99.8

Cross	F1	BC1F1	BC2F1	BC3F1	BC3F2	BC3F3
F 60	10	278 → 4	162 → 7	285	285	?
CT21375	10	239 → 3	162 → 4	258	→ ?	?
F 174	10	199 → 4	162 → 5	360	→ ?	?
F 473	10	267 → 4	162 → 7			

Acknowledgement



Dr. Kensuke OKADA



Dr. Joe Tohme, Dr. Fernando Correa, Dr. Manabu Ishitani, Dr. Michael Selvaraj,
Alba Lucía Chávez MSc. Dr. Edgar Torres, Milton Valencia MSc.,
Angela Joseph Fernando MSc.



Dr. Yusaku Uga
Dr. Yuka Kitomi



Dr. Susan McCouch
Dr. Juan David Arbelaez



Natalia Espiñoza MSc



Dr. Mathias Lorieux



独立行政法人
JSPS 日本学術振興会
Japan Society for the Promotion of Science





Thank you for your attention

