# PUP1 AND BEYOND: NEW IDEAS, TRAITS AND GENES FOR HIGHER PHOSPHORUS EFFICIENCY


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***Matthias Wissuwa*** holds a Doctorate degree in Plant Sciences from the University of Arizona (USA). His research focuses on plant adaptation to abiotic stresses with particular focus on nutrient uptake and utilization in rice. He has previously worked at the International Rice Research Institute (IRRI) and is currently a principal scientist of the Crop, Lifestock and Environment division at JIRCAS where he maintains close collaborations with scientists at IRRI and AfricaRice

# ABSTRACT

Selection of modern varieties has typically been performed in standardized, high fertility conditions with a primary focus on yield. This could have contributed to the loss of plant genes associated with efficient nutrient acquisition strategies and with adaptations to soil-related stresses. Given the necessity to use scarce and increasingly costly fertilizer inputs more efficiently, while also raising productivity on poorer soils, it will be crucial to reintroduce traits and genes associated with efficient P acquisition and utilization into elite crop cultivars. JIRCAS together with collaborators within the global rice research community has screened diverse sets of rice gene bank accessions with the aim to identify new donor varieties for P efficiency traits and genes. After mapping of P efficiency loci, candidate genes have been identified and characterized and tolerance mechanisms were investigated physiologically. At various stages findings have been confirmed by field experiments to assure practical relevance of research endeavors. One successful project recently identified the *Pup1* gene enhancing P uptake from low-P soils (Gamuyao et al. 2012). Molecular markers diagnostic of the superior *Pup1* allele have been identified (Pariasca-Tanaka et al. 2014) and shared with breeding partners throughout Asia and Africa, enabling them to conduct their own marker assisted selection program with locally preferred varieties. Similar efforts are ongoing for novel P uptake and P utilization genes. How such improved varieties may affect phosphate flows in agricultural systems will be discussed.

**KEYWORDS**

Phosphorus acquisition, phosphorus utilization, plant breeding

**REFERENCES**

Gamuyao, R., Chin, J.H., Pariasca-Tanaka, J., Pesaresi, P., Catausan, S., Dalid, C., Slamet-Loedin, I., Tecson-Mendoza, E.M., Wissuwa, M. and S. Heuer, 2012: Nature, 488: 535-539.

Pariasca-Tanaka, J., Chin, J.H., Drame, K.N., Dalid, C., Heuer, S. and M. Wissuwa, 2014: Theoretical and Applied Genetics, 127: 1387-1398.

# Pup1 and beyond:
# New ideas, traits and genes for higher phosphorus efficiency

Matthias Wissuwa

JIRCAS

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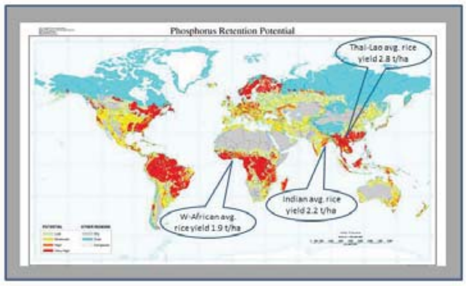
## P availability – a problem of P fixation in soil




- P fixation in soils is one of the stresses encountered in many rainfed environments
- This fixation also means P fertilizer is used inefficiently by crops, if applied
- Resource-poor farmers often have no access or cannot afford P fertilizer

**Breed P efficient varieties or just apply the needed P fertilizer?**

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## Phosphorus fertilizer prices


US Farm price Triple-Super-Phosphate (source: USDA)

- Currently raw material and processed fertilizer prices at 3-4 times the level of 1990-2006.


- Either input prices for farmers increase and profitability of rice decreases
- Or government budgets for subsidies 'explode'

➢ Changing policies and recommendations

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## Target: Develop new cultivars with improved yield under P deficiency

P efficiency: 3 basic components

1. grow to where the P is
   → **Root growth**
2. extract P efficiently
   → **Root efficiency**
3. Produce max. biomass per unit P
   → **P utilization efficiency (PUE)**

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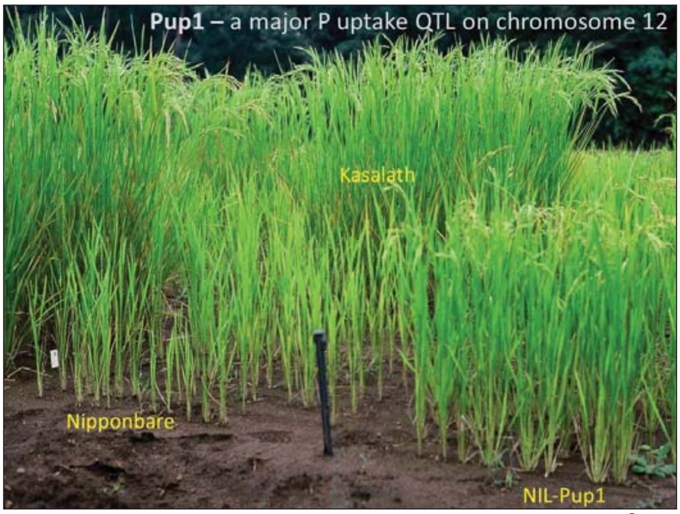
## Pup1 – a major P uptake QTL on chromosome 12

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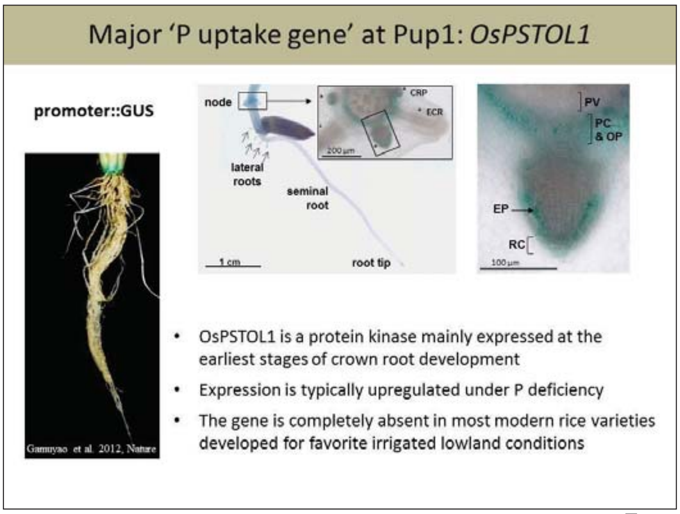
Major 'P uptake gene' at Pup1: *OsPSTOL1*
promoter::GUS
node
lateral roots
seminal root
root tip
1 cm
CRP
ECR
200 µm
PV
PC & OP
EP
RC
100 µm
Gamuyao et al. 2012, Nature
OsPSTOL1 is a protein kinase mainly expressed at the earliest stages of crown root development
Expression is typically upregulated under P deficiency
The gene is completely absent in most modern rice varieties developed for favorite irrigated lowland conditions

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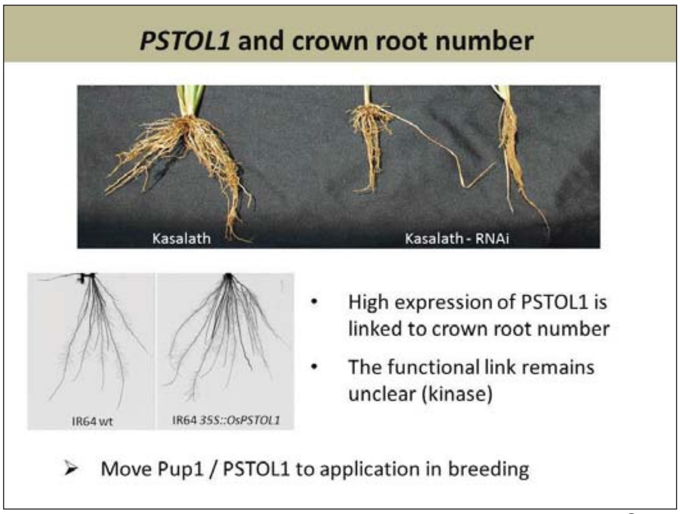
*PSTOL1* and crown root number
Kasalath
Kasalath - RNAi
IR64 wt
IR64 35S::OsPSTOL1
High expression of PSTOL1 is linked to crown root number
The functional link remains unclear (kinase)
Move Pup1 / PSTOL1 to application in breeding

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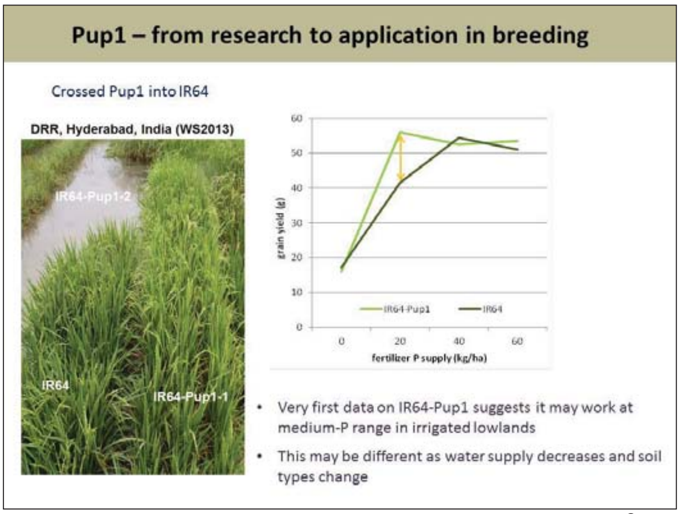
Pup1 – from research to application in breeding
Crossed Pup1 into IR64
DRR, Hyderabad, India (WS2013)
IR64-Pup1-2
IR64
IR64-Pup1-1
grain yield (g)
fertilizer P supply (kg/ha)
IR64-Pup1
IR64
Very first data on IR64-Pup1 suggests it may work at medium-P range in irrigated lowlands
This may be different as water supply decreases and soil types change

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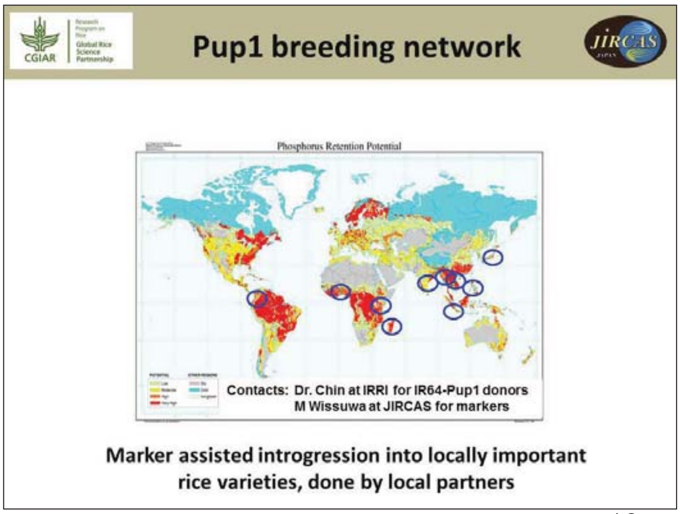
Pup1 breeding network
CGIAR
JIRCAS
Phosphorus Retention Potential
Contacts: Dr. Chin at IRRI for IR64-Pup1 donors
M Wissuwa at JIRCAS for markers
Marker assisted introgression into locally important rice varieties, done by local partners

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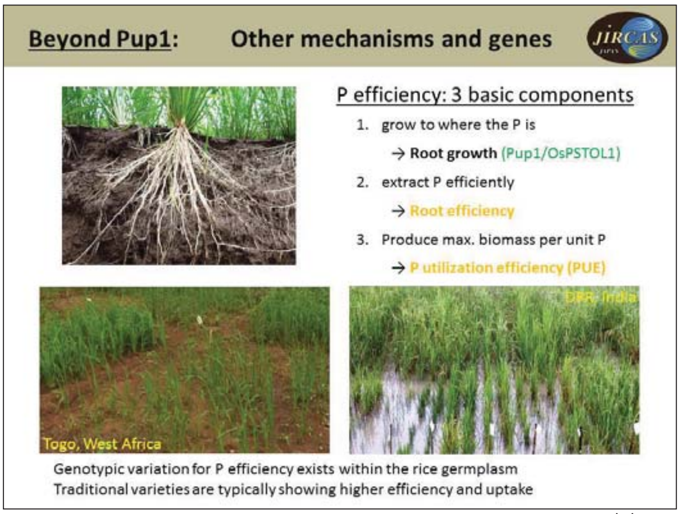
Beyond Pup1: Other mechanisms and genes
JIRCAS
P efficiency: 3 basic components
1. grow to where the P is
→ Root growth (Pup1/OsPSTOL1)
2. extract P efficiently
→ Root efficiency
3. Produce max. biomass per unit P
→ P utilization efficiency (PUE)
DRR, India
Togo, West Africa
Genotypic variation for P efficiency exists within the rice germplasm
Traditional varieties are typically showing higher efficiency and uptake

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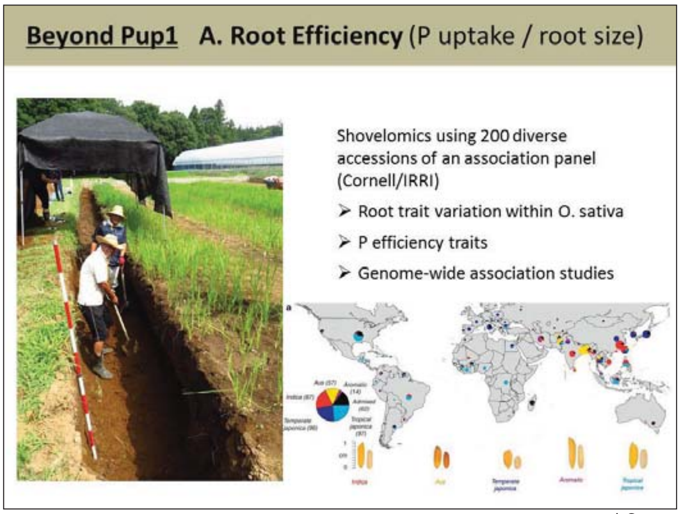
Beyond Pup1 A. Root Efficiency (P uptake / root size)
Shovelomics using 200 diverse accessions of an association panel (Cornell/IRRI)
Root trait variation within O. sativa
P efficiency traits
Genome-wide association studies

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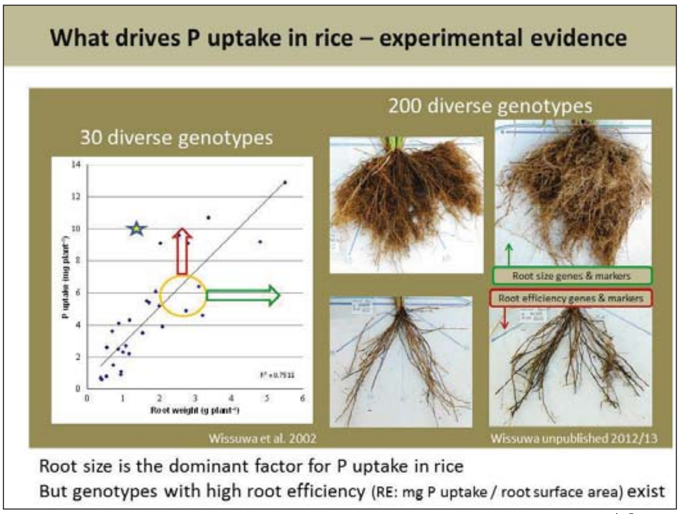


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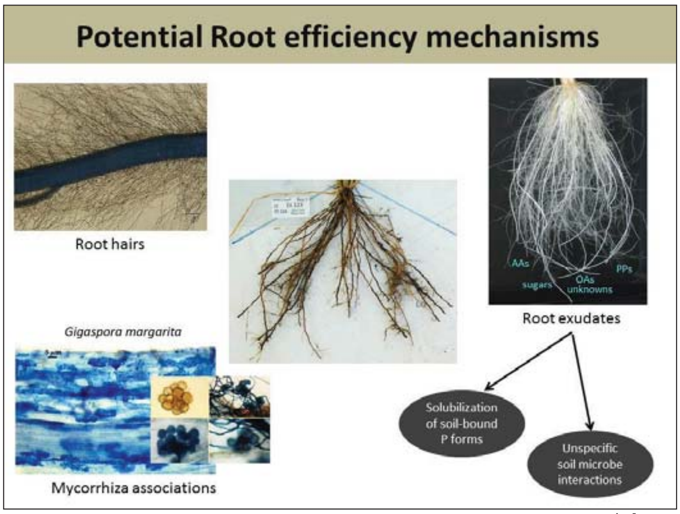


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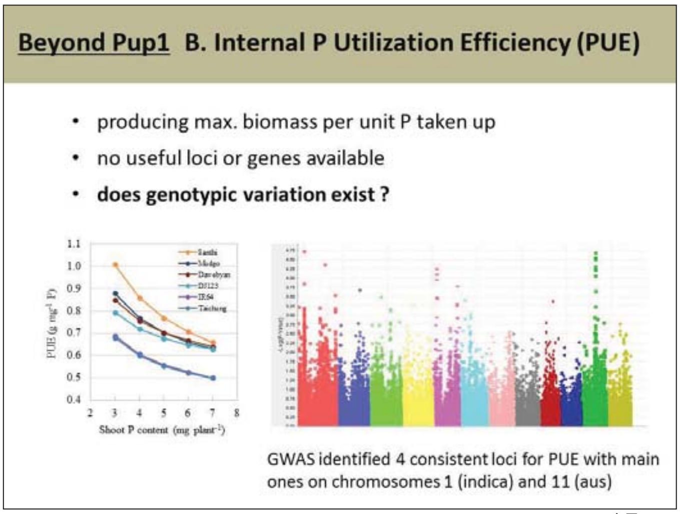


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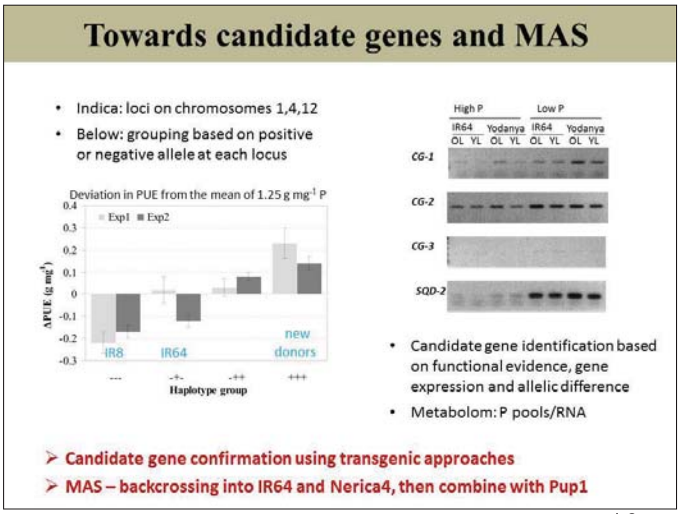


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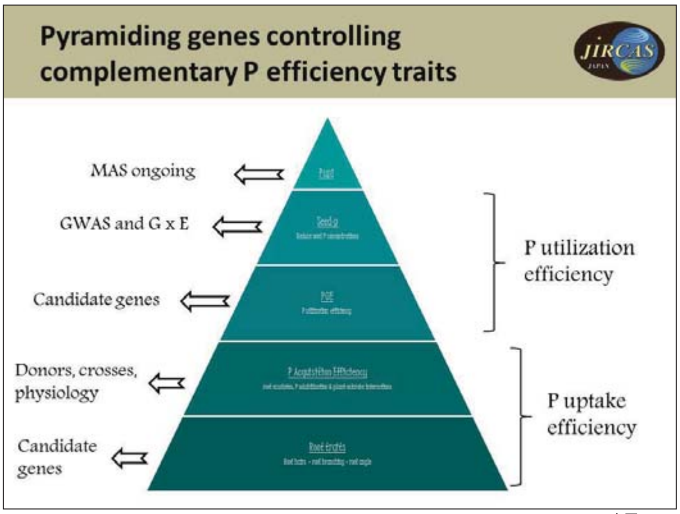


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**Chair Matsumoto:** Next speaker is Dr. Matthias Wissuwa from JIRCAS. He holds a doctorate degree in Plant Sciences from the University of Arizona. His research focuses on plant adaptation to abiotic stresses with particular focus on nutrient uptake and utilization in rice. He has previously worked at the IRRI and is currently a principal scientist of the Crop, Livestock and Environment Division at JIRCAS. He maintains close collaborations with scientists at IRRI and AfricaRice. The title is “Pup1 and Beyond: New Ideas, Traits and Genes for Higher Phosphorus Efficiency.” Dr. Wissuwa, please.

**Dr. Matthias Wissuwa:** Thank you, Mr. Chairman. Ladies and gentlemen, it’s my pleasure to be here and introduce you to some of the work we’ve done and some of the ideas we’ve developed in relation to improving phosphorus efficiency in rice.

Here I have listed a few average rice yields we can observe around the rice growing area; and while typically it’s recognized that the potential rice yield is upwards of 6 tons, even 8 tons on ideal conditions, very often national average yields are about 2 tons. So, we do have a very substantial yield gap and unavailability of phosphorus to the plant is one of the component stresses that reduce yields at the farm level. It might not be the most important stress, but it is one of the components of the overall stress. And partly to blame are soils that fix phosphorus quite tightly. As you can see here indicated in the red areas, these are soils where the total amount of P might be quite high, but the availability to the plant is very low because of this fixation or binding of P in the soil. How to improve the situation for farmers? The question is do we just focus on breeding efficient varieties that take up more P and use it more efficiently or do we just apply the needed fertilizer? The answer to this question is probably we should do both; but whereas in the past most of the emphasis was on applying more P fertilizer, recently a little bit more emphasis has been put on breeding new varieties. The reason is that the fertilizer prices have increased a lot and these price increases have put P fertilizer out of reach for many resource-poor farmers.

What effects do these high fertilizer prices have on a farm level? Either it increase the input cost and thereby reduces the productivity or the profitability of rice production or in many countries governments actually do put a fixed price on fertilizer inputs by subsidizing it. In scenarios where we have this very steep price increases, this is no longer sustainable and so we’ve seen government after government has reduced the subsidies, as you can see, for example, here where it is very rapid increase in fertilizer prices in India.

As a result, phosphorus has actually made it into the news. Here is an article talking about improving P efficiency in Sri Lanka and then there’re several other articles that stress that fertilizer prices have increased a lot because the supply of very high-grade phosphorus rock is slowly decreasing, which means the process of mining and shipping is getting a bit more expensive, and the other factor is that the subsidies have been slashed.

The research that my group and my partners are involved with is developing new cultivars, rice cultivars in the sense that have improved yield under P deficient conditions. There are basically three components of improving P efficiency in any crop, one is to improve root overall growth because phosphorus is relatively tightly bound in the soil: it doesn’t move in the soil so the plant root has to grow towards the phosphorus. There are two other additional strategies that we could follow, one is to develop plants that extract phosphorus that is tightly bound in the soil more efficiently so to breed for root efficiency. And the third part is to breed for better utilization of P that is taken up.

I want to give you one example of breeding for higher root growth and higher phosphorus uptake based on our own work that is related to the Pup1 locus. Pup1 stands for P uptake. It’s a major QTL on chromosome 12, identified in rice from the donor variety Kasalath that grows very well and extracts a lot of P from a P-fixing soil, as you can see here. This is a picture from experiments done in JIRCAS on a soil that is highly P fixing. You can see another variety Nipponbare here is hardly able to produce any yield under these conditions. We have identified this QTL on chromosome 12. Through breeding and marker-assisted selection, we can bring it into a Nipponbare background. And the resulting plant would be this near-isogenic line here that’s 98% identical to Nipponbare, but it has 2% introgessions from Kasalath from the donor. You can see this restores the ability to take up P and to use it well under these conditions.

Together with colleagues at IRRI, we have then done additional work to identify the genes that are behind this particular QTL. The main gene that we found we call PSTOL1 for P starvation tolerance and it is a gene that is mostly expressed in developing root primordia that you can see here. The greenish color means the gene is expressed in this particular tissue. And in a matured plant, you can still see that the main area of expression of the gene is at the base of a stem where the crown roots develop. So, this is a gene that enhances root growth. There is two interesting points about this gene; one, it is a protein Kinase so it is not a functional gene itself. It just senses phosphorus deficiency and relays a signal to other genes that then do the work and lead to more root growth. The second important or interesting point is that this gene is completely absent in most modern irrigated rice cultivars. So Nipponbare doesn't have the gene and IR64 doesn't have the gene. Many other rice cultivars grown around the world under ideal fertilized irrigated conditions don't have the gene. The varieties that do have the gene are typical land races or varieties bred for stressful environments.

We have then confirmed that this gene actually does affect root number and root growth so we have knocked the gene out in the donor Kasalath and so these two plants are Kasalath transgenic plants that don't have the gene function anymore. You see the root number decreases. Then similarly, if you overexpress this gene in IR64 you see that root growth is enhanced. And with more roots developing, there is more phosphorus uptake.

So now together again with partners at IRRI and partners throughout the world, we are trying to enter from the scientific gene discovery phase into the plant breeding phase and we try to bring Pup1 into other rice varieties, like IR64, that are then tested with partners throughout the world. And here I have one preliminary piece of data where you see that the yield of the new IR64-Pup1 line is improved at a very low fertilizer input rate in this experiment.

At this point, we have established some sort of informal Pup1 breeding network with other CG Centers and national partners that are interested in marker-assisted introgression into the locally important rice varieties that are grown in those countries. And this should be done by local partners with a bit of support from my colleague at IRRI and from myself in terms of marker development and other logistic support.

Now this gene, the Pup1 gene or PSTOL gene, as I said, enhances root growth and with that we have a good candidate gene to tackle this first mechanism. But as I said there are other mechanisms as we could develop plants that extract P more efficiently from the soil. Whenever we do screening experiments, whether it is in West Africa on an upland soil or in India on an irrigated soil, we do see there is a lot of genotypic variation in rice for the ability to take up phosphorus. And very typical it is traditional varieties that are far superior to modern varieties. So for example, here all these are commonly grown Indian modern varieties and these are land races collected in the hills of Northeastern India. These rice varieties obviously have genes that are very interesting and that should be targeted in finding new sources of tolerance, either for higher root efficiency or better internal utilization, and that is what we're doing at the moment.

To look at root efficiency, we look at variation for root traits. So, we do a lot of digging and characterizing roots in the soil. And rather than using a QTL mapping population, as we've done before, that is just a cross between two parents, we're now using a process called Genome-wide association, where we use gene bank accession, maybe 200-300 different accessions that have all been characterized with molecular markers. And we then link their performance to the genotypes that we identified. And typically when we do these experiments, we still see that root size is a dominant factor that improves phosphorus uptake.

In this sense, the bigger the root weight the higher the phosphorus uptake. And if you take an average variety that would be around here, having medium root weight and medium phosphorus uptake. In our screening, we have identified this type of genotype that has very small roots compared to the normal type, but shows very high phosphorus uptake. This is now a very good candidate for this novel trait what we call root efficiency. And so the target is to develop or to identify genes and markers that control this particular trait to improve phosphorus uptake at a given same root size and then combine that possibly with Pup1 or other novel genes that we might identify that increase the root weight and thereby increase P uptake.

Potential mechanisms that we look at currently to understand what drives root efficiency are things like root hairs or mycorrhiza. The previous speaker has given a very good introduction on the importance of these two factors. We're also looking at solubilization of soil-bound P forms through the excretion of organic acids or other substances or through other unspecific soil microbe interactions.

Then we take a third component of the overall P efficiency scenario and that is the increased internal P utilization efficiency. So, the idea is that once the phosphorus is taken up into the plant, there still is a factor of how efficient this P is used in terms of biomass production. And there is no useful gene or any known locus available that controls the trait. We don't even know whether genotypic variation existed when we started this experiment. The good news is that genotypic variation does exit. As you can see here, at a given shoot P content, genotypes differ in how much biomass they can make, which relatesto their internal P use efficiency. Again, it is typically traditional varieties that are superior. We've done this Genome-wide association where we identified loci, for example, on chromosomes 1, that increase use efficiency in rice.

We can then again go on a candidate gene finding expedition and we look at haplotypes or the genetic makeup at these particular loci. We see that modern varieties typically lack positive genes for P use efficiencies where they have reduced use efficiency. And traditional varieties that we treat as new donors for this particular trait, they have several positive alleles or positive genes for this trait. Now, we're trying to identify candidate genes and markers for this particular trait. And we would confirm the candidate genes using experimental transgenic approaches, but the target is to identify in the end markers and use these markers to transfer P utilization genes into mega varieties like IR64 and Nerica4; and in the end, to combine them with more efficient P uptake, as we had for the Pup1 locus.

So the final strategy we have in our project is pyramiding genes controlling P efficiency traits that are complementary to each other, combining bigger root growth with very efficient phosphorus uptake per root size with very efficient or improved utilization of the P taken up. And as you can see, we are at different stages in our particular project. The marker-assisted selection for Pup1 is ongoing. For other traits, we have candidate genes that we evaluate and in many cases we already have made crosses with particular donors. In the end, we try to combine all positive traits in certain very important varieties and test them throughout the world.

With that, I just want to acknowledge some of my collaborators that is; first of all, my own group at JIRCAS. Then I work very closely with several people at IRRI and in Africa Rice, and we have a host of other partners that contribute significantly towards this type of work. Thank you very much.

**Chair Matsumoto:** Thank you, Dr. Matthias. Dr. Matthias's team tried to breed the P-efficient variety to adapt to low P soils. And, we know the Pup1 to contribute the P uptake. It is very powerful in low P soil area. Now, we have also few minutes, but do you have any question and comments?

**Dr. Dinesh Sharma:** Okay, thank you for the presentation. I have two points to discuss. One is about the root growth pattern. So, I am wondering the Pup1 gene is having any relation with the root angle or I can say any hormonal relation like auxin or something.

**Dr. Matthias Wissuwa:** It doesn't have any relation to root angle. The hormonal regulation we don't know yet because we don't really know what the downstream genes are, and this is one of the research topics we are trying to sort of reviving in the next few years.

**Dr. Dinesh Sharma:** Thank you. The second one is about the GWAS. So in the GWAS, you have the nice panel, association mapping panel, and also you found some loci, nice loci or linked markers. My point is do you find any kind of relation of geographical location of those association panel with relation to the phosphorus uptake, because sometimes association panel might have the land raises also. I am just wondering any geographical location of those land raises having related to the phosphorus uptake.

**Dr. Matthias Wissuwa:** There is a general effect that lot of very good varieties are belonging to the <u>aus</u> subspecies of rice and most of them come from Northeastern India, Bangladesh, that region. So there is a hotspot of improvised soils, acidic soils, and the rice varieties developed there or landraces developed there typically have some tolerance mechanism. Other than that, when we look into the indicas, there is no clear indication which is more efficient. There is no geographical hotspot anymore.

**Dr. Dinesh Sharma:** Thank you.

**Chair Matsumoto:** Another question and comment? Okay. Thank you, Dr. Matthias.

**Chair Tobita:** Now the Session 1: Improvement of Crop Productivity in Infertile Soils, we had seven presentations from this morning to till now. So, I think it's good to have presentations not only dealing with natural resource management and also we have some presentations in the view from the crop improvement. So, I think this is a good or better fit to the aim of this symposium theme and we have several presentations which is describing about the results or achievements in the developed countries or in Japan or East Asian countries. And the presenters showed us some perspectives, these results or achievements to utilize these results in the crop productivity improvement in the developing regions. Okay, thank you very much.

And this would be transferred to the general discussion after the Session 2. Okay. So, thank you very much for your cooperation. Now the Session 1 is finished. Thank you very much.

**Chair Matsumoto:** Thank you very much.