SESSION 1

Yoshimichi Fukuta: Good afternoon again everybody. We would like to start soon this session, because there is really limited time. I would like to provide each presenter、 20 minutes。We would like to discuss something in general discussion later. Please keep it within 20 minutes, and at least one or two questions or comments should be received directly in the presentation.
At first, I would like to introduce Dr. Kato. He is a scientist in National Institute of Crop Science. The specialty, he is focusing on today is 'Breeding of High Yielding Rice Varieties in Japan.' Please start Dr. Kato.

Hiroshi Kato: Thank you for your introduction Dr. Fukuta, and thank you for giving me a chance to make a presentation here. The title of my presentation is "Breeding of High Yielding Rice Varieties in Japan." The idea of "high yielding" you here is probably different from ours a little bit, so I'm going to discuss this first.
This slide is Japanese rice production. Our maximum rice production was 14 million ton in 1967, it was 40 years ago. At that time, our rice production was like this high. But now our rice production is only 8 million ton, almost half of the maximum amount.
This slide is the rice cultivated area of Japan. The rice cultivated area 40 years ago was roughly 3 million hectare, but now we have only 2.3 million hectare, and our cultivation need is only 1.6 million hectare only. This difference between 2.3 million hectare and 1.6 million hectare has mainly come from the result of adjustment of the production.

Japan has been suffering from the overproduction of rice in the past 40 years. This is mainly because the per capita rice consumption has decreased. Forty years ago, the per capita rice consumption was around 110 kilograms. Now it is only less than 60 kilograms. The per capita rice consumption has almost decreased to half in this period. This phenomenon is not only seen in Japan. These days there is also a decrease in rice consumption in Korea and it's the same in China, too. In these countries, the average income per person has been increasing, leading to a change in the pattern of food consumption. The grain-eating habit has shifted in part to meat or dairy products like milk, cheese and other kinds of foods.
Recently, there is an overabundance of paddy field in Japan and in these countries. This slide shows a conference of animal feed rice in China. A lot of conferences for animal feed rice are held in these countries. Because of this excess in paddy fields, paddy field can be used for animal feed production. Generally yield of forage maize is a better yielding forage crop than rice, but in this area, East Asia, we have much rainfall in summer. Upland crops are not suitable with this kind of climate, especially in the lowlands or plains. Rice is a suitable crop for wetlands and we can grow rice as animal feed crop. This policy is good for food security. This is because we can maintain the paddy fields so that they can be used for the production of our staple food in case of food shortage. In this sense, the cultivation of animal feed rice is a very important policy. So the high yield we are talking about is high yield rice for animal feed.

Next, I want to explain the history of high yielding rice breeding in Japan. Forty years ago, we suffered from a worldwide shortage of animal feed grain. At that time, we tried to use foreign genetic resources to make high yield rice. For example, we used Arborio which is an Italian big grain, or Milyan23 which is a Korean Indica variety. But most of these varieties are not so strong in lodging tolerance and some of them shatter very easy. We cannot use them as they are.
We started a project called "Super Rice Project" in 1982 using a wider genetic background and we bred high yielding varieties like Akenohoshi, Habataki and Takanari. But in 1994, this project ceased and there was no more national high yielding project. But in the year 2000, high yielding project was revived for the breeding of animal feed rice. At that time, our target was whole crop silage only. I will explain about this whole crop silage later. We bred varieties like Hoshiaoba, Kusahonami and Yumeaoba.
In the year 2008, in addition to whole crop silage we started grain feed rice breeding. This is the ordinary sense of high yielding. You may remember that in the year 2007, the US started the ethanol policy. They started to use grain for fuel production. As a result grain feed price rose very much. Because of that, we have started to breed high yielding grain feed rice.

We use grain for cattle, birds and pigs and we use rice straw also for cattle feed. Rice straw is very important for making high fat Japanese beef cattle. Also, maybe this is not so familiar, we use rice as a whole crop silage. We call it WCS. We harvest the upper part of whole rice at the yellow ripening stage, which is 2 weeks earlier than normal ripening. The grain is slightly soft and easy to digest for cattle.
This machine is specially designed for harvesting whole crop rice. By using this improved machine we can harvest tall rice plant too, and we can make very good silage. It can be used for the harvesting forage maize, wheat and barley as well. We also make rice rolls by spraying the plants with lactobacteria for making good quality silage rice. One roll is roughly 300 kilograms. We roll the crop up with the plastic film, and we can keep it in good conditions for at least a year.
The cultivation of whole crop silage and grain feed has increased a lot these days. These bars show grain feed and whole crop silage. In total there are more than 50,000 hectare of animal feed rice and rice is the second largest forage crop, next to forage maize in Japan. This is because we have big subsidies for WCS and grain feed. The

amount is 80,000yen per 10a which is equivalent to US $10,000 per hectare. I know this may sound unbelievable, but it is true. That's why the area has increased a lot recently.
For the past 10 years, we have bred around 20 varieties for whole crop silage and grain feed. These varieties are cultivated all over Japan these days. The common trait of these animal feed rice is strong lodging resistance. Also, because this project is a collaboration with the animal industries, we can get a lot of manure.
Let's compared the lodging resistance of these varieties with Koshihikari which is the most famous high quality rice variety with very low lodging resistance. Compared to Koshihikari, these forage rice varieties are very strong, except Kitaaoba from Hokkaido, which is the northernmost island. In Hokkaido, there is almost no damage due to typhoon, so highly lodging resistance is not required there.

I want to explain about our animal feed rice. We have actually two types, for grain feed and for WCS. We have grain feed only varieties. They have short plant height and the grain yield is high. This type is called "grain yield type." The other type is "stem and leaf yield type." In this case, the grain yield is lower but the whole crop yield is high. In between, we have the all-round type. We can use them for both grain feed and WCS. I am going to explain the grain yield type first and there are some unique traits about them.
Our breeding target is 10 ton per hectare for brown rice. This is a rather challenging target. I'm not sure if we can achieve this target or not.
This is one of the grain yield type, Bekoaoba. You can easily see that the size of the grain is quite large, and the 1,000 grain weight is 30 gram in Bekoaoba. Compared to 20 gram for the usual rice, it is 50% lar. Another variety is Hokuriku193, and this is also a high yielding rice. The rough rice yield is 780 kilogram per 10a, and this is the panicle of the high yielding rice Hokuriku193. You can see its panicle is very long and thick. Anyway, the high grain yield type has big grain or large panicle. With large panicle or big grain, the panicle weight is heavy. This trait is in common with grain yield type. We can categorize a plant type into panicle weight type and panicle number type in general. All of animal feed high grain yield type varieties are panicle weight type.

Another variety I want to explain is Momiroman which is grain yield type and it is also high yield. The unique trait of this variety is maturity duration, which is the duration from heading date to maturity date. It takes around 55 days in Momiroman. Ordinary variety usually takes 40 days, so it is 2 weeks longer. Longer maturity duration is another common trait of this kind of high grain yield type.
This slide shows another trait of the Momiroman, its brown rice and this is ordinary rice variety. You can see the chalkiness of this variety. This chalkiness is usually considered as an inferior trait. We think this is good trait as animal feed rice. By using these grain traits, we can distinguish animal feed rice from rice for human consumption. This is good because government give big subsidies for animal feed rice. Farmer can sell rice for human consumption for ¥200 per kilogram. For animal feed, a farmer can only sell at ¥20 per kilogram, almost one-tenth.
For preventing varietal contamination, we have herbicide sensitivity of rice. Benzobicyclon is a rice herbicide. By using this herbicide, we can destroy volunteer rice plant. This is a tolerant line. We sprayed the benzobicyclon here and this variety is intact. But this variety is destroyed here, this variety, too. By using this herbicide, we can completely kill the undesirable rice plants. This is good for preventing contamination. After making rice for animal feed, the next year, farmers may want to make rice for human consumption. In that case, farmers needs to clean up their fields. Even if a small amount of contamination occurrs in the farmers' products, the selling price of rice of human consumption decreases a lot, so this trait is very useful.

This is a summary of our grain type and we have to strengthen the insect pest and the lodging resistance and the cold tolerance by using a wider genetic background. We use Indica varieties a lot in our breeding program. Indica varieties are generally weak for white backed plant hoppers and rice stripe too, so we have to tackle with these problems. Rice stripe is important for succeeding crops of barley and rice double cropping system. In that case, rice stripe is usually very serious, so we have to introduce resistance to it.
Usually high temperature damages the quality of the grain. In our case the quality of the grain is not of much concern, but high temperature causes sterilities. I think it is causes serious damage to high yielding rice, so we have to tackle that too.
For higher grain yield, we enhanced the panicle weight, long growth duration and strong culm. Also, for additional value for the grain feed, we are breeding black and red rice for animal feed. These traits can enhance the nutritional value for animal feed.

Let's move along to another part. The breeding target of WCS is completely different from grain yield type. I think this is new for you. Our target is on total digestible nutrient. This is a very important trait for the animal industries. Our target is o13 ton per hectare. This value is almost the same as that of forage maize. Rice is much shorter than forage maize but the actually yield may not be very much different. We try to achieve high WCS yield similar to forage maize.
This is a variety for WCS and this is a variety for grain feed. You can see the difference in plant height. For grain feed the usual height is 90 cm, but for WCS it is over 110cm, and the TDN yield is quite high, 1.2. This is close to the target, 1.3. We're almost there. This is Tachiaoba, another variety. We introduced thick and long root system for preventing lodging. This is also a good yield variety.
This is a unique variety Tachisuzuka. The panicle is quite small. Because of this the panicle is light and so the lodging resistance is quite high and the whole crop yield is quite good. This kind of small panicle is good because we can give this kind of rice to dairy cattle. Their digestive system is usually weak, so they cannot digest the grain

well. Fewer grains is good for dairy cattle. In this kind of rice plant, there is higher non-structural carbohydrate in the stem. So even with small panicle, the nutritional value of the whole plant is not inferior to ordinary rice plants. We have sufficient nutrition in the stem, so this is good enough.
We also bred Leaf Star, and this is also a unique variety. In this variety, the lignin content is low. You can see the brownness in the panicle and the stems. This brown color comes from low lignin. This is a pleiotropy expression of low lignin. This is good for cow and maybe also good for bioethanol production, too.

This is the summary. I have already introduced this one. Beta-carotene is also important.
Up until now we are using traditional breeding methods, but now we are studying these varieties using SNP markers in collaboration with Dr. Yano, who is our next speaker. We analyze our varieties and some of them are Indica. The yellow part is Japonica. We separated Indica and Japonica. Japonica and Indica are not equally distributed, some big bias exists. Except Tachisugata, even though we made Indica-Japonica cross hybridization so often, intermediate type rarely appeared. Almost all of them are categorize into Japonica type or Indica type with the exception of Tachisugata, which is just half Indica-half Japonica. This is actually very strange. This is very difficult to produce. In the future we are going to use these SNP markers and try to identify the contributing factor of the genome for high yielding rice. In this case high yielding include not only the grains, but also the including whole plant too. I hope we can show better results in the future. That's all.

Yoshimichi Fukuta: Thank you for interesting presentation. We can receive one or two direct question or comment.

Jagdish Ladha: Do you also get a variation in grain filling duration in temperate condition, from heading to maturity I think you mentioned some varieties have got very large differences. I thought it's pretty much controlled by temperature and you don't get much variation at least in temperate, also in tropical condition we don't get variation, but do you get variation?

Hiroshi Kato: The variation of the growth duration ?

Jagdish Ladha: Yeah, grain filling from heading to maturity.

Hiroshi Kato: Yes, we do. In our material for analyzing the SNP markers we also have some different lines, long maturity duration and short ones, I think that does not depend on the temperature. Actually at high temperature, maturity duration becomes shorter. At low temperature, maturity duration becomes longer. But I think we can exclude that.

Yoshimichi Fukuta: Yeah, last one please. Dobermann.

Achim Dobermann: Yeah, thank you, a very interesting presentation. We have recently, well maybe 2 years ago started to screen modern Indica varieties with regard to fodder value traits, straw digestibility. We are screening modern rice varieties in India for digestibility of the straw for cattle feeding.
We are finding, to our surprise I suppose, a large variation in digestibility of the straw, even though we have never bred for that on purpose. That is of interest in that part of the world because this could essentially be dual purpose rice variety, so grain for human and straw for feeding cattle. Have you conducted any work in Japan on looking at straw, not the green biomass but straw from paddy as a source for cattle feeding? Is there a genetic variation that you have there?

Hiroshi Kato: I think at least the lignin content is different. Other variation I think, may not have been studied well, not yet.

Yoshimichi Fukuta: Thank you for discussion. I would like to move to the next presenter, Dr. Masahiro Yano from the NIAS. His presentation is 'Genomics-Assisted Allele Mining and its Integration to Breeding in Rice.'

Masahiro Yano: Thank you very much for introduction. First of all, I would like to thank the organizer for giving me this opportunity to present our recent activity on this particular occasion.
Today, I'm going to talk about more specific research area, rice genomics, and also some of the result we are trying to integrate into rice breeding. As many of you know, whole rice genome has been decoded by International Rice Genome Sequencing Project. This big achievement has been done by the multinational collaboration. After this achievement so many rice scientists are focused on the gene discovery using this big information. I will introduce briefly the activity in Japanese research.
Many Japanese rice scientists are focused on the cloning of the gene and this is the list of the gene in the last decade. Some of those gave a big impact for the plant biology just in the understanding of the fundamental phenomenon in the plant. But also some of these gave a very big impact in terms of breeding. I'll just show you that some of the genes are located on the genome, so this gene has already been used or will be used in the rice breeding by the marker-assisted selection. Not only Japanese activity, just here I put a very big gene sub1 gene. Actually some of those are disease resistant and some of those are insect resistant and other traits.
Today I will just briefly introduce this trait, deeper rooting. Today's topic is to just briefly show you the root morphology of deeper rooting, so discovery of the gene and also introduction of this gene into elite cultivar. Also, I will briefly show you our development of the platform for the future gene discovery and breeding. One is plant

material development and one is establishment of the platform of genome-wide SNP typing for genetic analysis and marker-assisted selection.

For deeper rooting. If you look at the global scene, actually the shortage of water is one of the biggest problems to the production of the plant. Actually drought damage in the rice-growing area in many developing countries that rely solely on rain to grow the rice, so therefore, breeding the drought-resistant lowland rice is becoming important research topic.
We focus much more on the root system. Deeper and thicker root system is an important component associated with avoiding the drought stress in rice. We just embarked on the QTL cloning and its utilization of the deeper rooting.

Here, you can see the two parental lines we used. One is IR64; this is the mega-variety in lowland rice in South Asia, and high yield and shallow root system. You can see here this basket and many roots are coming downward, and various thin roots. If you look at here, Kinandang Patong is a Philippine upland rice and this is a tropical Japonica and showing the deeper rooting.
This is a very interesting characteristic to know what gene is involved. Actually I have to mention that this kind of work has been done mainly by Dr. Uga, he may be now in this room, and he developed this kind of screening systems. Actually the phenotyping is the most important thing to perform the genetic analysis, and he designed a very small metal sieve and then just checking the deeper or shallow rooting system in so many plants and very quickly.
This kind of screening system allowed us to do the genetic analysis and this is the result of the QTL analysis and we detected a major QTL on chromosome 9 and we did a fine mapping and defined a very narrow region, and then we further defined the region more fine, and finally we cloned the gene.
Today, I don't want to go into the molecular aspect of this gene, maybe Dr. Uga will give you some other opportunity to get more information about Dro1 gene. But I have to mention today more breeding aspects.
Once we identify the Dro1 on chromosome 9, so we introduced Dro1 into the IR64 from the Kinandang Patong. Here, this is IR64 and this is NIL Dro1. Dro1-NIL is a very small segment that has been introduced from Kinandang Patong. Then, you can see here very clear differences. This IR64 is shallow rooting and only small segment integration resulted in deeper rooting.
This is a very good sense in terms of the breeding and we would like to do further analysis. In greenhouse we checked the differences. Next question is what about the differences in the real soil, and Dr. Uga has checked the performance.

Then, this is the result. This is IR64 recurrent parent and this is Dro1-NIL and this is also donor parent Kinandang Patong. Although you can clearly see the differences between Kinandang Patong and Dro1-NIL, so it's in terms of the deeper rooting. But also you can clearly distinguish between IR64 and Dro1-NIL in terms of deeper rooting. The root has reached to the more deeper area in Dro1-NIL than that of the IR64.
This kind of deeper rooting can be achieved without root elongation or without root biomass increase, just as biomass of the root is same. That indicated angle of the elongation might be different. Then, we just check the performance for the drought avoidance.
In the greenhouse, we developed the artificial screening system and checking several performances and canopy temperature and stomatal conductance and also photosynthesis rate. All of the traits are much improved in the Dro1-NIL compared with the IR64. Finally we checked productivities or just the culm length and the panicle length, there are no differences and shoot biomass is increased and also panicle and also panicle weight is also increased. Particularly, if you look at the number of filled seed per plant it just strongly increased in the Dro1-NIL.

In the artificial condition, we check a good performance in the Dro1 gene in terms of the drought avoidance. The next question is what happened in the real field, so just in the artificial condition. We now set up the two collaborations. One is the collaboration with International Center for Tropical Agriculture, CIAT. Dr. Ishitani is in the room, so we collaborated very much and are checking the performance in the Dro1 in the more practical condition. Dro1-NIL has been tested under different levels of the drought stress using the experimental field with rain-out shelter.
Now, we get good results, so maybe this kind of data will be also presented by Dr. Uga or Dr. Ishitani in some places. Also, this year we set up the additional collaboration with International Rice Research Institute. In this program Dro1-NIL will be tested under the lowland condition and multiple locations in IRRI, IRRI's drought breeding networks. The Dro1 will be introduced into other mega varieties if it showed a very good performance to study the expression of drought tolerance in different genetic background.
We are very much interested to know the result of this kind of collaboration, then more precisely we know the performance of Dro1 in terms of the drought avoidance.

I'll move on to the next topic. To discover the gene with agricultural importance one of the important factors is material, genetic analysis required several type of materials and actually the material development takes quite a long time. We have to design the early times to start and then produce several types of materials. Then, this is what kind of material can be used.
In general, primary mapping population, F2, or recombinant inbred line, and BIL, backcross inbred line can be used for the detection of QTL. But only use of the primary mapping population allowed us to define the approximate

region. If you go into the more detailed analysis, we need more good materials, so I mean the advanced mapping population, advanced backcross progeny, making the background more uniform for making the introgression line or nearly-isogenic line.
This kind of material development allowed us detection of the minor QTL or validation of the major QTL or more fine mapping and sometimes genetic interaction, and finally, just the cloning and also making the near-isogenic line or combining the two or three QTL into the one particular background.
To this end, actually we set up a large-scale development of the chromosome segment substitution line or other materials and picking up the Koshihikari and Nipponbare, other Japanese temperate Japonica as the recurrent parent, and also we selected several types of the donor cultivar distributed in Asia. The red means the accessions were selected based on the trait of interests, and blue means that the accessions were selected based on the sequence variation, just sequence variation produced at the several clusters, so we selected a representative accession of each clade as the donor line. Now, we are still continuing the plant material development, but after developing the plant material we just deposited this material to the Rice Genome Resource Center in our institute. Actually the material is the number of line or you can get the genotype data by the number of markers. Once you've obtained this kind of material, maybe you can check the phenotype at your own site or your own way and then you can find unique region of the genome for the further analysis.
Anyway, this is ongoing though not all of them. Now, if we develop the additional one maybe we can straightforward deposit it at this site and researchers can obtain those materials.

In addition to the material development, we also launched a re-sequencing of the rice diverse accession by next generation sequence system. Actually, this is about the Asian accession and also these varieties have been used for the donor of the material development. We also sequenced temperate Japonica, our tropical Japonica cultivar from Japan or China.
Actually this kind of sequence data is more powerful too. Because only sequence data is not so powerful but actually this sequence allowed us to – once you defined some particular region of interest using the materials, I mean the CSSL or other plant materials, then you can easily see what is the difference in the sequence level. Actually combination of the sequence information and the plant materials, and also good phenotyping skill will facilitate the discovery of the new gene of the future needs. Maybe we have to increase the number of accessions for this kind of information in the future and I hope to complete soon making the alignment.
Also, another thing is the marker analysis. More than 10 years ago, marker analysis is a very, very tough work, not so many people can do easily. But now there is so much progress, one is the next generation sequencing and also additional progress is actually microarray system for the SNP typing.

Using the next generation sequence system, we have performed a genome-wide discovery of the SNPs, and actually so many SNPs were detected and validated. But if you have a large number of SNPs, still we cannot do typing easily, some big facilities are required, so then we decided to make a smaller number of core set. These core sets have been selected from the more large number of mother set, and in terms of the chromosomal location and also allele frequency.
This set provided us more and more information on the diverse germplasm analysis. But also if you think about Japanese rice breeding, in Japan, breeders are using many Japanese temperate Japonica, so temperate Japonica showing the very narrow genetic diversity and this type of the marker cannot be used directly. Then, we design for the Japonica cultivar. Still a large number of SNPs can be detected and also making the core SNPs.
This kind of marker system is very powerful for shortening the time of the experiment for marker typing. I'll summarize my talk today, here again if you have a mapping population, sometimes we developed chromosome segment substitution line or other groups are also developing these types of materials. Now, the material is already obtained and some people are doing the phenotyping, so phenotyping allowed us to define some particular region and sometimes you can go into the cloning. But without cloning you can go into the development of the near isogenic line and checking the performance and sometimes the combination of them.
These kinds of activities are very good in the plant breeding. Actually, the facilitation of this kind of process is inevitable in the future needs. I have to emphasize one thing, phenotyping. Now, we have materials and we have a good marker system. We don't want to care much about the genotyping.
Now we have to pay much attention on the phenotyping. So maybe we set up the different methods and the different conditions and also more high throughput and reliable, unreliable phenotyping does not provide us with any good results. Now, we have to pay much attention on the phenotyping in case of the integration of those genomics to the plant breeding or rice breeding.

Now, I'll just show you the contributors in the NIAS. There are so many contributors. But I'll just say that Dr. Yusaku Uga is mainly involved in the cloning and also integration of the Dro1 into the IR64, and also now he is the main player for the collaboration, so maybe he will get more good results in the future. I would like to thank the Ministry of Agriculture, Forestry and Fisheries, Japan. Thank you very much for your attention.

Yoshimichi Fukuta: Thank you for the introduction of a powerful research work and a really strong work in Japan. Any direct question or comment? Later on, we will discuss about it in the general discussion. I would like to change the chairman from now, so Dr. Virk from IRRI.

Parminder Virk: Thank you Dr. Fukuta. The third speaker this afternoon is Dr. Hei Leung, he is the principal scientist at IRRI and also Dr. Dobermann showed one slide this morning, in GRiSP there are six themes, one of the

themes has to do with genetic resources and he is also a global leader for that theme as well, Dr. Leung.

Hei Leung: Thank you Dr. Parminder Virk for the kind introduction. First of all, let me thank the organizer to give me the opportunity to give you an overview of the GRiSP Theme 1 on Genetic Diversity and Gene Discovery, which is integrated with Theme 2 on Developing New Varieties. These two themes represent a continuum of research activities that span conservation and utilization of genetic diversity, discovery of gene functions, and plant breeding. Today, my talk will focus on the development of genetic diversity platform that supports this agenda. Dr. Yano has already discussed a similar topic from the perspective of Japan rice research programs. I will emphasize on the use this platform to improve rice breeding.

Research contents of GRiSP Theme 1

Theme 1 on genetic diversity and gene discovery has the following elements. One is to conserve, manage, efficiently use the largest collection of rice germplasm that are entrusted to IRRI. The international Gene Bank has about 120,000 accessions. We have a serious responsibility to take good care of this invaluable resource. I will discuss later an initiative to reveal the diversity of the Gene Bank that will enable practical breeding.

The second point of my talk is about the importance of connecting genotypes and phenotypes. Given sequencing technology is getting less costly and more efficient, we have an opportunity to decode the a large collection of germplasm at sequence resolution. However, that's not enough to just have genome sequence information. We need to know the biological characteristics, or phenotypes, of the germplasm. The critical challenge is to establish the causal relationships between genes and phenotypes. One of the main business of GRiSP Theme 1 is to close the gap between the gene and phenotype. Because phenotyping is such a diverse activity, this needs to be done through global collaboration.

Understanding the available genetic variability of tolerance to environmental stresses and their underlying genetic basis is critical towards developing climate-ready rice. Although we cannot directly alter the course of climate change, at least in terms of rice production we can apply the best science to develop rice varieties resilient to the climatic extremes as much as possible.

Finally, under the Theme 1 of GRiSP, we include exploratory research of a long-term nature—that we call "Frontier Research" or "Blue-Sky Research". Here, we identify research agenda that does not necessarily yield payoff in 2 or 3 years. Rather a longer-term investment. I'll come back to briefly mention the idea of understanding yield ceiling and C4 rice as examples of Blue-Sky Research.

Throughout my talk, I will also emphasize the collaborative framework by which we have benefited from collaboration with Japan, and also highlight new opportunities for more collaboration.

Genetic diversity as foundation

The diagram depicted in this slide shows that genetic diversity is the foundation of all plant breeding activities. Much of the rice diversity are stored as germplasm accessions in national gene banks, and in the International Gene Bank at IRRI.

Considering the importance of conserved germplasm for future plant breeding we must make sure that we do well in conservation, as well as disseminating the materials for public use. We need a public platform by which we can increase efficiency of conservation and distribution. Most importantly, we need to unlock this diversity so that they can put it to use for dealing with current constraints or unexpected problems in the future.

Under the International Rice Genome Sequencing Project (IRSGP), Japan took the lead in producing a complete genome of the japonica variety Nipponbare. Since then, we have detailed sequence information about a few more additional varieties. Yet, relative to the total diversity of rice, we know very little.

Historically, we have been using passport data of germplasm accessions as our baseline information. These include varietal names, sites of collection, and sometime with locations specified by longitude and latitude. Yet, considering the potential value of these conserved germplasm, we are under investing in this treasure trove. Given genome sequence provides the "universal codes" for comparative analysis and the potential to infer biological function, it is compelling to argue that the "minimal information" for all conserved accessions in the Gene Bank should be genome sequence. The small size of rice genome, makes technically and financially feasible to launch the project of sequencing rice Gene Bank.

Rice SNP Consortium

Currently, the IRRI's Gene Bank has about 120,000 accessions. We consider 10% will be a reasonable representation of the total. We understand that sequence data of 10,000 accessions will be a major undertaking—from producing to analyzing the data, finally putting the synthesized information to use. We therefore adopt a step-wise process by beginning the analysis of a smaller collection (approximately 2,000) under a Rice SNP Consortium. Through the Rice SNP Consortium, we generated enough SNP data so that we can design a chip to interrogate a collection of rice accessions. Working with a small collection, it will pave the way to conduct a large scale gene-phenotype relationship study.

The Rice SNP Consortium started with generating and receiving donated sequence information of diverse varietal groups. This rich pool of sequence information enabled the design and construction of a Affymetrix chip to interrogate 1 million SNP in a genotype. At the same time we have also assembled about 2000 lines as our starting collection with which we will genotype with the chip and phenotpye for multiple traits.

The 2000 lines come from a diverse collection of rice germplasm as depicted in the dendrogram. There are in fact about 3000 lines represented in the diagram. These were genotyped and grouped by SSR markers. This collection consists of japonica, indica, tropical japonica, aus, and aromatic and so on. The Affymetrix chip was designed by

Dr. Susan McCouch's laboratory at Cornell University. It was based on SNP data from about 150 genomes. Through hybridization of germplasm DNA with the with 25-mer features on the chip, one can determine the 13th base of the 25 mer. This technology has been validated using a 44K chip by Dr. Susan McCouch at Cornell University. In using the 1 million SNP chip, we expect to detect, on average, 1 SNP for every 500 base pair, providing sufficient resolution to determine haplotypes and genome-wide association analysis. Also, the chip contains methylation sites that will allow investigation of epigenetic phenomenon as well. We are currently processing about DNA samples on this chip.
In summary, through the Rice SNP Consortium, we concentrate on genotyping 2000 lines using the Affymetrix chip. The 2000 lines represent broad diversity, with indica constituting the largest proportion. We are particularly interested in the aus because many of the useful agronomic traits are coming from the aus group. We often do not know whether some traditional varieties belong to aus or indica groups until we have DNA fingerprinting data. We also include *O. glaberrima* from Africa and some wild species as well.
As illustration, this slide shows some of the results from using the 44K chip published recently in Nature Communications. By generating phenotypes on about 400 lines that have been genotyped with the 44K chip, they are able to establish a relationship between genomic regions and phenotypes (e.g. plant height and so on). This represents only the tip of the iceberg as the lines have not been extensively investigated for economically important or complex traits. In sum, the procedure allowed you to associate which chromosome regions are determining what phenotypes at a reasonable resolution.

Need for reference genomes for pan-varietal groups
Now, a digression here. Dr. Yano mentioned about efforts in Japan to produce a high quality sequence of the aus variety Kasalath. Through the Consortium, we have gathered sequence data from the variety IR64. IR64 is unique that it is not only a popular variety for many years but it is a genotype widely used in genetic studies. Using improved de novo genome assembly algorithms, we are beginning to assemble IR64 sequence. We have aligned about 80% of IR64 sequence to Nipponbare genome (look at the last column). But it also means that the 20% are "homeless". We are working with colleagues at Cold Spring Harbor to connect the pieces. We would like to invite the community to contribute to the completion of the IR64 sequence assembly. We are hopeful that we can have IR64 as the reference indica genome soon. We also hope that we are able to collaborate with colleagues in Japan to produce a pan-Aus genome molecule in the near future.

Gene bank sequencing. Only a few years back, the idea of sequencing a large collection of rice lines would seem prohibitive. The thinking among conservationist and gene bank curators have changed. Instead of having passport data, we are considering DNA sequence data as the basic information.
We are working with Beijing Genomics Institute (BGi) and Chinese Academy of Agricultural Sciences to begin sequencing 3000 lines. Our strategy is to do it in stages. If 3000 genomes is sufficient in cover most the rice diversity (discovering rare alleles). we don't want to go beyond 3000 even though our project target is 10,000, approximately 10 % of the Gene Bank. The main objective is to have a complete catalogue of genetic variation available in the rice Gene Bank. One may get to the point of diminishing return. However, if the diversity is deep, one would need to go beyond 3000. As sequencing costs continue, sequencing additional varieties or accessions will be not be an issue in the near future.

Phenotyping network
To make use of the sequence information, we must invest in parallel the biological characterization of germplasm. We are organizing a phenotyping network – a self-organizing network that welcome participation. This network will first concentrate the 2000 lines and selected lines actively used in breeding. Through a network of phenotyping experts, we can subject same materials to different phenotypic characterization. For instance, some of them could look at root traits, and some could look at yield potential, and some may can look at molecular variation such as transcriptomes or proteomes.
By working on a common collection of lines, it is possible to associate the new phenotypes with the genome sequence variation. In this way, the phenotyping data can be accumulated over time, producing database where SNP variation or narrow regions of chromosomes are associated with phenotypic characteristics.

Novel genetic populations
Future discovery of gene function and novel phenotypes must rely upon the ingenuity in creating new genetic resources. Dr. Yano has already highlighted the importance of making specialized genetic stocks, such as chromosome substitution lines. As an example, I'd like to describe our effort in making genetic populations for fine mapping as well as for plant breeding. The population is called MAGIC. It's a good name because it captured people's attention.
MAGIC stands for Multiparent Advanced Generation Inter-Cross. It involves intercrossing multiple parents, about 8 or 16, to make F1. The F1s are then inter-crossed to scramble the genomes. This cycle of crossing the F1s is repeated several generations (3-5 times). We take about 2-3 years to complete depending on how fast one can advance the population.
By the end, you have a large number of recombinant inbred lines that capture the recombined chromosomes of eight different genomes. In addition, you can capture the interactions between different genomes. Because they go through many recombinations, the mapping resolution is high, so that you can fine map the gene at a high resolution. We estimate that that if we do five rounds of intermating, you can get it down to about 3 kilobases resolution.

These slides show a few snapshots of the MAGIC populations grown in the field in the wet season of 2011. Although these are unreplicated trials, we observed signs of transgressive segregation. i.e., some lines have phenotypes outside the range of the parental lines. For example, of the 400 indica MAGIC lines evaluated, some exceeded the yield of the parental lines and check varieties (based on the single plot).
By next year (2012), replicated field evaluation will be conducted to determine how this population generates new variation and allow you to detect multi-genome interactions and transgressive segregation, which has not been exploited in our current breeding program.
To fully use this population, we have applied a low-cost sequencing technology called Genotyping by Sequencing (GBS) to generate partial sequence data of the MAGIC lines. The technology involves making a library of restriction enzyme-digested fragments and then sequence the library. The short sequences are then assembled using

bioinformatic algorithm.
We apply GBS to 200 MAGIC lines to obtain SNP data per line. After filtering, we obtained about 7000 SNP per genotype which is sufficient to explore genotype-phenotype association. We treat the MAGIC population as a collection "natural recombining germlasm" and apply GWAS algorithms to associate chromosome regions with a trait of interest. As an example, we were able to locate genomic regions that are known to harbor resistant genes against bacterial blight and blast. These preliminary results come from analysis of only 200 lines. We expect the resolution will increase as we analyze a larger population.

Climate-ready rice
I would like to briefly describe IRRI's breeding efforts towards climate-ready rice. The big four climate-related production problems are: drought, submergence (flooding), salinity, and heat waves. One of the recent successful story is the deployment of submergence tolerance varieties in the flood-prone environments. The spread of submergence tolerant varieties has been rapid and benefited millions of farm households. Dr. Dobermann was in Eastern India recently and he said "Well, we need more of these varieties".
We are actively combining different stress tolerance genes to produce "two-in-one" or three-in-one" varieties to provide security against salinity, submergence, as well as drought. It is, however, important to make sure that combining multi-stress tolerance genes do not lead to yield penalty. For heat tolerance, we have some candidate donors, and our heat team has begun to discover some QTL for tolerance to sterility under high heat. We are getting close to fine mapping these QTL.
For the biotic stress, I would like to highlight the collaboration on virus resistance through the BRAIN Project in Japan. This work led to the successful cloning the tungro-resistant gene. It is noteworthy that the research is in collaboration with Korea as well. Thus, is a result of three-party collaboration: IRRI, Korea, and Japan. Rice viruses problems are potentially tied to climate change because many rice viruses are transmitted by insects (brown planthopper and Greenleaf hopper), the populations of which are sensitive to climatic factors.
The GRiSP Program will allow us to develop more inter-country collaborative relationships. For example, the yield potential workshop recently held in Colombia has identified inter-disciplinary topics for collaboration. We are exploring ways to work with Japan research institutions to address some of these research questions.

Frontier research
I will briefly mentioned the C4 Rice project as an example of frontier research that is high risk and high pay-off. A key to the C4 Rice project is to create genetic variation that enable detection of C4-like characteristics. In this case, I would like to express our gratitude to Dr. Ichikawa of National Institute of Agrobiological Sciences (NIAS). He generously provided a large collection of full length cDNA, over-expressing lines (the FOX lines) to IRRI to screen for C4-features. Thus, many of the valuable genetic resources produced with foresights by Japan research institutions are now being put to good use.

Conclusion
I'd like to use a list of GRiSP products to end my talk. We have existing work now that are listed here, and it's not meant to be a comprehensive list. Dr. Dobermann has emphasized that GRiSP is product-driven. These are all products that we wanted to get the results for. These are individual activities that are underpinning these outputs.
During this symposium, we are discussion with the research community in Japan, aligning research collaboration under this GRiSP platform so that we can bring complementary research expertise together. Much of the work cannot be done by single institution alone. We need collaboration with others so that everyone can contribute their talents and expertise. For each of the GRiSP Programs we can actively develop a table like this. It is an open platform that anyone interested in rice research are welcome to make the contribution under this GRiSP Program.
I have a long list of colleagues and collaborators to thank and acknowledge. It's a large team of people and I have not even listed any external collaborator. Within IRRI we hope that we continue to build this team, interacting with the international community interested in the GRiSP Program. After listening to all the presentation today, it is clear that there are mutual interests and opportunities to harmonize collaboration. Over next year, we will gradually populate the GRiSP website with contents and information on GRiSP activities, so that people will know about the opportunities as well as excitement to work together. Thank you very much. Sorry to take a little bit more time.

Parminder Virk: Thank you Hei. We will take questions for Hei in general discussion as we are short of time. I will move on to the last presentation which will be done by Dr. Matthias Wissuwa. He is a scientist in JIRCAS and he will be talking on the 'Utilization of Abiotic Stress Tolerant Genes in Rice.'

Matthias Wissuwa: Thank you for the introduction and thank you for giving me the chance to introduce some of our work and our ideas here. I will talk about abiotic stress and after this very fast high-flying presentation I will bring you down to earth a little bit in more than one way. When we talk about abiotic stress, it is largely about closing the yield gap. The potential rice yield in tropical countries is about 7 to 9 tons and the average yield worldwide in the tropics is just over half of that and in some cases, in India and Sub-Saharan Africa, it is even less than half of the potential yield.
To improve tolerance to biotic and abiotic stresses will be a key to overcome this yield gap. The main abiotic stresses are nutrient deficiencies, particularly for phosphorous and nitrogen, and when we think about minor nutrient, zinc is rather important and quite often deficient. Then there are the climate-related abiotic stresses; drought, heat, and flooding, and finally a few more soil-related stresses; salinity, sodicity, aluminum, or iron toxicity.

Breeding strategies in the past have usually involved a selection in target environments, and that is successful and can improve tolerance. But if you want to make full use of all the genetic resources and new technologies that have been developed, we actually have to go use QTL mapping or association mapping in combination with marker-assisted selection. I want to very briefly mention the Sub1 locus for submergence tolerance - we've already heard about SWARNA-Sub1 - because it really is the model for a successful introgression of an abiotic stress tolerance gene via marker-assisted selection.
Here are some photos from IRRI. We have young rice seedlings that are then fully submerged and after the water is gone most of the plants die with only those seedlings having the Sub1 gene surviving. This is really a very nice case of an abiotic stress tolerance gene with a big effect. Originally QTL mapping was done in 1994 I think, but it still took more than 10 years before Sub1 was actually cloned.
It was a big story, published in Nature, gaining a lot of public interest, but in addition, cloning Sub1 helped to develop the gene-specific markers that are needed to do the marker-assisted selection in its most effective way.
Here is just a brief outline of how marker-assisted selection for Sub1 worked. Initially the Sub1 donor (IR49830) was crossed with SWARNA, the variety lacking Sub1. This was followed by three back-crosses to SWARNA and following each backcross step we have a marker-assisted selection step. In the end, you have SWARNA reconstituted but with a Sup1 allele from the donor.

This has a huge effect in farmer's field. As you can see here in the foreground are farmer's varieties that do not have Sub1 and that have poorly survived submergence. As a result yield will be very low on this field. Behind we have SWARNA Sub1 plus some other Sub1 varieties, and you can easily see the big difference and a huge effect of Sub1 in comparison to farmer's varieties. Apparently Sub1 varieties are now tremendously popular in Bangladesh and India, so much so that seed production can't keep up with demand.
A lot of us are working on abiotic stress use Sub1 as our model – a success story that we would like to repeat. But, I think I should stress that Sub1 is probably a rather special case and that it will not be easy to achieve success to that extent with many other traits. First of all, we typically focus on truly quantitative traits or QTL, while Sub1 actually almost qualifies as a qualitative trait in the sense that plants either survive or they don't survive. That makes a trait much easier to handle.

Furthermore, it is also very simple to screen for Sub1. One just submerges plants at the seedling stage, and within 2 weeks or so, one knows whether a given plant has the Sub1 gene or not. Finally, Sub1 seems to be a surprisingly rare gene. Most of the modern varieties lack Sub1, which means the potential to have impact is huge because almost every variety with high yield potential doesn't have the gene and would thus benefit from Sub1.
Now, the situation with most other QTLs is probably really quite a bit different and more complicated. Most traits are truly quantitative which means that if we look at the variation present in a population of lines or a mapping population, the QTL might explain 30% of the whole variation which still leaves 70% of the variation as is either due to other genes or due to background noise or environmental effects.
The phenotyping may also be much more difficult. Very often it has to be done in the field over an entire season. One has to wait until yield data is gathered to make an assessment and that complicates matters since we cannot easily eliminate a large number of plants early on during the selection process. Last but not least, the presence and absence of the target gene is often not very clear based on the phenotype. If we take variety IR64 with a phenotypic mean close to the middle of a distribution of genotypes, and if the QTL effect is only about 30%, one doesn't know whether a genotype that is in the middle of a distribution already carries this particular gene and lacks every other beneficial locus or carries other beneficial loci but lacks the gene of interest.

Now, I want to switch from Sub1 and talk a bit about my own project which is a much more typical QTL called Pup1, which stands for phosphorous uptake. It confers tolerance to P deficiency in rice, particularly on upland conditions. It was mapped here in Tsukuba in 1998 in a population derived from Kasalath and Nipponbare that we got from Dr. Yano's group. Kasalath is the tolerant donor while Nipponbare hardly produces any grain here on this P deficient soil. A few years after mapping this QTL we were able to provide the conceptual proof that marker-assisted selection for Pup1 works by developing a near-isogenic line that is 98% identical to Nipponbare, but that contains the Pup1 gene from Kasalath. Just like for SWARNA-Sub1, this Nipponbare-Pup1 showed a large positive effect in the field.
Yet it still took quite a long time to accomplish the map based cloning of Pup1 since it turned out that the Nipponbare model genome did not contain Pup1. Only after sequencing the entire Pup1 locus in Kasalath, again with the help of Dr. Yano's group, were we able to make some progress.

On this slide showing the alignment of the Kasalath (bottom) and Nipponbare (top) sequences, red bars signify very high sequence similarity while yellow is still good similarity. As you see most of the region doesn't show any similarity. It almost looks like we sequenced two different species. Particularly here, this part is a big 100 kilo base insertion-deletion (INDEL) that is completely absent in Nipponbare. Obviously, there are many genes present in Kasalath that were not present in Nipponbare. This really highlights the need to sequence tolerant donor varieties.
Yet even after we got this Kasalath sequence there was no obvious candidate gene involved in phosphorous uptake or P physiology. We identified several candidate genes based on expression patterns - I indicated them with arrows - and followed a transgenic approach to confirm their effect under P deficiency.
One approach used knockout lines in Kasalath and as you see, if you knockout this particular gene (blue graph), P uptake and plant performance completely breaks down. For the other candidate genes we didn't see that dramatic effect. Here on the right hand side we have over-expressed Pup1 in Nipponbare (green graph). We get about a 60% increase in P uptake and a 60% increase in grain yield in these transgenic lines. Based on these results we are confident to have cloned the major gene at this Pup1 locus. The possibility remains, though, that a second candidate gene plays a positive role since our main candidate apparently interacts with this second candidate, potentially producing an even stronger phenotype. We have to further test that. We also have to go back to gene bank accessions and look for novel and possibly stronger Pup1 alleles.
Nevertheless we are already at the point where we can do marker-assisted selection because we have gene specific or allele-specific markers. My colleagues at IRRI are now mostly using IR64 as a recipient parent. After introgressing Pup1 into IR64 and IR74 we see some positive phenotypic effect on tiller number under P deficiency. These are still very small scale trials, but in next year we anticipate that we will have the first real yield trials in the field using this IR64 and IR74-Pup1 breeding material. Similar activities are ongoing at AfricaRice but are at a much earlier stage. We have to identify suitable donors and agree on which ones to focus on.

Now, I want to talk a little bit more about what comes after Pup1, and for that let us look at QTL effects in general. Here on the left, we have the unusual case of a QTL (Sub1) acting almost like a single gene. If you look at the performance of number of genotypes or a mapping population, we detect a bimodal distribution that indicates a single gene largely determines the phenotype. In contrast most QTLs result in a normal distribution (right hand side) and we assume that multiple genes affect the phenotype. This is not an entirely hypothetical distribution, but partially based on data that we have gathered for many rice accessions. We know that Kasalath is not the most P deficiency tolerant variety ever, yet it performs very well in Japan, whereas Nipponbare is rather poor. The Nipponbare-Pup1 near-isogenic line is a little better than average, and this blue arrow could represent the Pup1 effect.

Now, based on this very initial data that we have on IR64 we would assume that IR64 already is quite a lot better than Nipponbare and that the Pup1 effect in the IR64 background is quite a bit smaller than in Nipponbare. One point I want to make with this graph is that Pup1 obviously depends on the genetic background, but the second point I want to make is that there is still some room for potential further improvements. Using Pup1 we have only improved performance by roughly 40% (in IR64) and further improvements are possible unless P deficiency was controlled by just one major gene, which it is not. Therefore this story can continue and we can look for additional QTLs that would be used in a QTL pyramiding strategy.

We are following two approaches to find these additional QTLs. One is to just look at new germplasm to find novel donors. We worked with AfricaRice, conducting a screening experiment in Togo on a low P soil. In the photo of that trial one can detect quite a bit of variation and the genotypes that really stands out is an African rice, an Oryza glaberrima. We have tested three different Glaberrimas so far and they all look very promising. They all are also very efficient in phosphorous uptake here in Japan on our soil.
By now we have started to do some QTL mapping in NERICA populations and this is a photo taken only a month ago showing how much better the glaberrima parent CG14 performs compared to the sativa parent WAB 104. Left and right one sees lines of our mapping population. We detected quite a bit of variation and a few lines are actually performing much better than WAB 104. At this point we are optimistic to find a new strong QTL and based on our preliminary results we know that it would not be identical to Pup1. All QTL detected were on a different chromosome compared to Pup1.

The second approach to detect additional QTLs uses plant physiology and our understanding of the trait to look for additional mechanisms that might be important for plant performance under P deficiency. With this little cartoon I want to emphasize which general mechanisms may be crucial to turn a plant that is sensitive to P deficiency (left) into a tolerant one (right).
Based on our understanding of phosphorous uptake and how plants perform under P deficiency, we know one of the most important traits is root growth. Root size, possibly root fineness, and root hairs all improve P uptake and we believe Pup1 affects this particular aspect. But there are other aspects that are important and one could be root exudation or in other words, the release of substances like organic acids that can solubilize phosphorous from the bound forms in the soil. Such solubilized phosphorous would then become available for plant uptake.
A third mechanism could be very efficient utilization of P inside the plant. Efficient translocation of P from roots to shoots, remobilization of P from old leaves and transport to young leaves are important aspects of P utilization, and we intend to look at genes linked to any of these particular processes. Looking for mechanism-specific genes would require designing screening experiments capable of revealing genotypic variation linked to a specific

mechanism. Designing experiments capable of doing so will be a key step to unravel the genetic variation that so far remains locked up in gene banks.

To do so we would want to evaluate a representative sub sample of the rice gene pool such as the set of rice lines that Susan McCouch's group developed and that were already introduced by Hei. These lines have been genotyped using a very large number of SNP markers, allowing us to do genome-wide association mapping, hopefully identifying novel alleles present in a very broad subsample of the rice gene pool as opposed to the very narrow genetic base present in a biparental cross that we typically used for QTL mapping up to now.
We focused on internal P utilization efficiency (PUE), evaluated as the amount of biomass produced by genotypes per limited amount of P. We grow plants in individual bottles, each bottle containing 0.8 milligram P, and PUE is estimated based on the total biomass a genotype can produce from this fixed P supply. These are some of the results that we got. The experiment was done last year in collaboration with colleagues at IRRI. Most of the modern varieties were very inefficient: All IR varieties tested (IR64, IR36, IR8) as well as Tequing and Koshihikari were at the low end in terms of PUE. The Aus varieties, this rather strange subgroup of the Indicas, seem to be the most efficient. Not all Aus varieties are efficient, but they were definitely over-represented here in this very efficient group beyond a PUE of 2.5.

I further want to stress that phenotyping really becomes a big bottleneck now that population sizes of 350, Hei talked about 2000 lines, have been fully genotyped and are available for association studies. Our experiment on PUE involved 350 genotypes, evaluated under two levels of P supply with 3 replications each. That equated 2100 experimental units. 2100 bottles that need to be filled, refilled and pH adjusted etc; and for a relatively small research group like mine, this represented a logistical challenge and clearly showed us the limits of phenotyping. Strong collaborations, such as our with IRRI, will be crucial to conduct these sorts of experiments.
Here are some preliminary results from this genome-wide association mapping involving 350 gene bank accessions. We found several putative loci of interest on chromosomes 1, 2, 11 and 12. The peak one on chromosome 11 is a completely novel QTL that has never been identified in relation to phosphorous, so we are really quite excited about these results. From here to application in marker assisted breeding will definitely be a long way, but possibly still much shorter than if we had started with the old traditional QTL mapping as in the past. I'm really quite excited about these new ways of doing mapping.

Let me finish with some conclusions and outlook. I think at present the bottleneck in the molecular breeding for abiotic stress tolerance is the limited number of precisely mapped high impact genes. I have mentioned Sub1 and Pup1 and we heard about saltol and the drought-tolerance gene. I know IRRI has an anaerobic germination gene in the pipeline, but considering that a large number of groups have done work over a decade on genes controlling abiotic stress tolerance, we do not have a lot of QTLs in the breeding pipeline at this point.
Yet due to advances in technology that provided high throughput pipelines for marker-assisted selection it would be possible to handle a lot more QTLs and to combine two or three QTLs in marker-assisted selection or marker-assisted pyramiding. My observation, from a person that is more involved in phenotyping and in the original mapping, is that the genomics advances have really outpaced our phenotyping ability over the last couple of years. To make full use of all these new technologies that are available, we really need to place more emphasis on phenotyping. With such enhanced focus on phenotyping the potential to have impact in the field will be considerably advanced by the new techniques available.

Let me finish by this little cartoon here. We definitely target impact in farmer's fields, and donors are very much interested in this. The scientific community, the big universities in US, Hei and other 'genomics' people have been working a lot on the high throughput pipelines in the past. The idea is that high throughput pipelines and sequencing capacities will allow us to tap the genetic diversity present in gene banks. Through phenotyping we would then tag the QTLs, genes and alleles needed to fill this pipeline.
Again, my observation is that there is a lot of emphasis these days on the impact - rightfully so. The scientific community has very strongly emphasized the development of technology but so far I think the poor cousin in this whole picture still remains the phenotyping. Unless the community is putting a little bit more emphasis on phenotyping we will have created this bottleneck up here that will limit how much impact we can achieve at the end of our pipeline. We have to remember it takes about 10 years from mapping loci to impact in farmers fields, so avoiding the bottleneck I see up at phenotyping should be an urgent task now. With that I thank you for your attention.

Parminder Virk: Thank you Matthias. First of all on behalf of JIRCAS and on behalf of Dr. Fukuta and my personal behalf, I would like to thank all the four speakers for this session.
We have about 15 to 20 minutes on all the four presentations for open discussion and we will invite questions for all the speakers please. Thank you.

Yoshimichi Fukuta: In section 1, the theme is from genome research to rice breeding. Molecular geneticists or physiologists made nice presentations and they mentioned the importance of phenotyping, and IRRI and NIAS are developing some useful material for genetic analysis.
But the important point is that these information and materials are how to associate to the breeder. It's a point that's a little bit lagging I think in the whole discussion. I would like some comment from especially the breeder, some suggestion to us. Firstly, I would like to ask Dr. Kato, do you have any comment for the future work for the

association between the breeder doing something there with geneticist, if it is possible to make some comment?

Hiroshi Kato: Now, we have collaboration between Dr. Yano and I. We have QTL analysis for yield, and we also include the material transfer or those kinds of things. We just started that collaboration. Actually I'm very much expecting – because we could not do such kind of analysis until now. We have materials, SNPs and our animal feed rice varieties with wide genetic variations. I think we can do it using them, soon.

Yoshimichi Fukuta: Which means that this new technology will be useful in the breeding program in Japan?

Hiroshi Kato: I think so.

Yoshimichi Fukuta: How about IRRI? Dr. Parminder Virk is rice breeder in IRRIs, how about the situation in IRRI, Dr. Virk, can you make comment?

Parminder Virk: Yeah, actually as Dr. Kato has said, technology has moved quite a bit now. In the past, breeders were not able to use gel-based assays, they were very slow as well as it used to be very expensive to adopt to breeding programs. But now we have techniques like SNP assays, some examples have been shown in three presentations today and, in fact, Dr. Yano also showed Dro1 as an example. But in one of his slides he showed so many genes have been cloned by Japanese scientists and also scientists from other countries like China and other places. Now, we have lots of genes for traits of interest to breeders which can be actually followed through the SNP analysis.
In IRRI we have invested in two kinds of SNP platforms, Illumina as well as the new platform on nanotechnology-based platform, which we talked this morning. Actually now we will be able to use forward selection for many of the cloned genes and where SNPs are available, and also we'll be looking into converting many of the markers which are like SSR or SDS-based markers into SNP-based assays for agronomical useful traits for breeders.
By next year we will be launching a forward trade-based SNP chip which probably more than 50 genes will be available for SNP assays to be followed, not only for marker-aided back-crossing but also marker-aided selection, forward selection for biotic stresses like BA genes, XA genes, for grain quality genes, for abiotic stress resistance genes and so on.
I think probably next year will be very exciting and in case of high yield potential breeding that we are going to launch a big project, where a Japanese scientist will also contribute. We are looking at several genes which Dr. Yano showed an example for gene-enhancement loci will be pyramided in different backgrounds using SNP assays, using those cloned gene information.
I think now there will be lot of interactions between gene cloners and using the modern technology like SNP assays in order to implement those in the breeding programs and IRRI will be taking the same course as Dr. Kato has said.

Yoshimichi Fukuta: Okay, I understand, so it's mainly – okay.

Masahiro Yano: I totally agree with you and also we have a chance to collaborate now currently. But Matthias mentioned some success story is depending on the trait differences, so clear differences. If you think about yield performance, sometimes it's a very continuous, a very small effect QTL. We have to think now about establishment for the new method or new single way to connect between such a small effect QTL into the breeding. Simple marker-assisted selection cannot be applied, so that's the most important thing in the future, so people in genomics and also people in physiology and also the breeders have to jointly think about new methods I think.

Yoshimichi Fukuta: Regarding minor genes for accumulation for the breeding it may be one of the solutions, as Dr. Hei mentioned some MAGIC. I think these technologies are powerful tools for the accumulation of the unknown genes or the target genes. At the same time, it's necessary to do the selection of plant. Do you have any comment?

Hei Leung: I want to be guilty talking about MAGIC all the time but I do like it. I think the association genetics really works. For a long time we are doubting that association genetics is just detecting this major - like plant height and things like that, and people say why do you need to go through all the trouble to find out plant height and so on. But I think I've received more and more data, the association genetics, if your genotyping resolution is high and you still need very good phenotyping obviously. Because of the scale of the diversity looking at, it does allow you to get down to a very good resolution in terms of detecting.
Now, I don't believe in one population or one approach probably to get there, but what I'd like to hear so far is this, everyone has something to bring to the table, like we may be making a MAGIC population. There are many people making the chromosome substitution line. I always believe that it's a convergence of these multiple resources that really crack open. I think the key in the future is, one, we need to make sure that genotyping is becoming very cheap, so we can actually afford to do high-resolution genotyping.

Second is having a very common phenotype database so that when you do the two things together, as many people put in more information on the public domain, now we can still maintain your intellectual IP and so on, but eventually you can actually have some kind of public domain by which people can look in the computer and see this relationship. Then, you can go home and do your fine tuning.
But I think the resolution would be still high if you combine all the dataset and that you can actually get down to it

at a gene level, almost like the maize. Because the reason why rice is still a problem is t the Linkage Disequilibrium (LD) is very big, right, and maize is down to the gene, but we have a lot of resources available, a lot of recombination happening. I think you get down to the gene level. Then, you go back to Parminder's point about you can use functional SNP to detect your locus. Once you have that then I think the selection, even the minor effect QTL will be feasible.
But it's true, though; I agree with Matthias, there are some traits that will always be interacting on the background like drought. That will be one example. That was something that there is no substitution for good genetic work due to Saltol, but I think more and more people now will be sharing data like this, then you do have to work and you get the whole community chipping in. I think that may be a way, I'd like to call it participatory or collaborative phenotyping. If we do that, then I think you can have a very quick impact.

Matthias Wissuwa: Okay, thank you very much. I'm clearly not a breeder, but I must admit I enjoyed the presentations very much. They were really great. So much information, so I hope you will arrange that we will get copies of these presentations. We'd really like to have them. I'd just like to have three comments. The first is on the O. glaberrimas.
I'm very happy to see actually that the O. glaberrimas are a source of genes that are of interest, I think, beyond Africa. From the last presentation, we learned that it is perhaps a new source to stimulate P uptake in these glaberrimas. From my own work I know that screening glaberrimas we discovered are certainly new genes, other than Saltol, that will be of importance for salinity tolerance, and work on rice yellow mottle virus also already illustrated the richness of resistance to that particular disease in glaberrima. I think we should really explore that in greater detail. What is there for Africa and for the world.
The second one is on Aus varieties. The work of our agronomists, Katsuki Saito, clearly showed that Aus varieties in Africa and upland rain-fed systems outyield NERICAs consistently. The problem there is lodging, disease resistance, and grain quality. But clearly, they consistently outperform upland NERICA, so we have a clear possibility to break the yield ceiling there I think, and it's probably then related to phosphorus use efficiency.
The last comment I have is that the presentation is focused on abiotic stress which I think is logic because it's probably the easiest. The Sub1 gene is of importance for Bangladesh and in Asia, anywhere it's going to work as well and we have already evidence in Africa, and there is huge need for that in countries like Sierra Leone and Liberia. It looks as AfricaRice is always following like 3 years later and it will be nice if it could catch up immediately. Clearly, all these abiotic genes conferring resistance to abiotic stresses are probably of importance immediately.
For biotic stresses I don't know, and that's going to be a lot more difficult I think and I know that Japan has done a lot of work on BLASTs and we really need to see how we can work more to get around that. I just had a question on whether there is any possibility to learn from what has been done for the tungro virus. Is there any link we can make with the work we do on rice yellow mottle virus? I don't know if anybody can answer that question.

Hei Leung: I'm no expert on the yellow mottle, but then I talked to Dr. Choi, our virologist. There is some evidence in the same gene family the elongation factor may be involved in both tungro and they are testing that. But there is nothing conclusive. I was very excited too that they are two things doing the same thing for the two viruses. The second thing is, just quickly, I just ran out of time. I did want to say about the impact of climate change on biotic stress, and one slide which I didn't have a chance to show is the idea – I think Fukuta-san already started the same from the Blast network – of creating some kind of bio station.
Bio station in a sense that you have well situated geo-climatic region, different biotic stress environment and putting some common, and I think the Blast people have already done that differential, but they don't have to be differential lines, they could be elite breeding lines so that you directly get into the breeding program. But they should be in common so that you can actually get a data and get an assessment of which location the hot spot and how does the variety of the genotype react to it. I think that's something we used to do about 15 years ago, ran out of support, and then we assumed that we already know the pathogen but in fact we don't.
I think if we can revive this idea about what the Blast network has done, but really look at the biotic stress by multiple diseases, by putting this well-known genotype, the well-characterized genotype, I think then we can make maybe actually better than 15 years ago when we just were not knowing the genotype of the plants. Right now, we may know the genotype of the plant and put them in different sites. India will be a good place. Africa will be a good place and different regions. I think we need some coordination and setting itself but I think the Blast network has already done a part of that idea.

Yoshimichi Fukuta: Thank you. Dr. Kumashiro, any comment?

Takeshi Kumashiro: Thank you very much. Regarding the differential, actually the Blast differential is a powerful tool, but I think we also have a differential for bacteria leaf blight and now we are using the differential variety probably developed by IRRI to just find out prevalent races in Africa bacteria blight. Also for R&D which I will talk tomorrow, we are developing a sort of differential for each trait.

Yoshimichi Fukuta: Okay. Another question or comment about this discussion? I think regarding some IRRI or Japanese institute like Advanced Institute, this is easy to discuss about it or some collaboration in the scientific matter. But regarding AfricaRice, CIRAD is maybe in a little bit different situation; because NARS and these institutes really need some technology, direct access to the farmer, something very different which isn't there. In most of the situations it is common, Dr. Hei suggested, so something we need as a platform to discuss it, so bring

together the materials and the idea together or something.
Maybe there was a request on virus analysis because no scientist is in Japan anymore but something will be necessary to do jointly with some other countries. We really need some platform. I guess this session is really first time for the platform, so hopefully I would like to continue it as a discussion supported by the Ministry of Agriculture in Japan. Any other comment or something, how about Virk-san, do you have a final comment?

Parminder Virk: Yeah, I think Dr. Yano showed a very good genetic resource, CSSL lines and RILs and others in one of his slides. I think that will be a very good material and in GRiSP also we are looking at high throughput phenotyping platform even for those traits which we never measured before also. I think that resource will be extremely useful which can be phenotyped very precisely and then we have the genotypic information already, so we can learn a lot.
On top of what Hei said we have association mapping panel, we have MAGIC population, RILs coming through, so this resource which is CSSL line is very powerful resource for phenotyping for those traits where we know not a lot.
High throughput phenotyping will be very useful which is being established, as Hei has shown once or two slides on that. I think we are looking at very exciting times and tomorrow afternoon in the open session we will have a very good discussion on that.

Yoshimichi Fukuta: Finally, I ask Dr. Dobermann to give some comment in this session.

Achim Dobermann: Yeah, I'm not sure whether it's a comment or a question, you can decide for yourself. I was actually quite intrigued by all presentations but Dr. Kato's presentation, I found that very intriguing because of the very different concept for yield potential or yield in Japan now, and of course you may think that well, you know, that is a very Japanese thing, Korea and Japan and maybe China. But I think the materials that you have developed there and the traits that you are looking at in those, and also even the protocols for those traits, I could see a lot of potential spill over or application potential for our interest in terms of breeding for grain yield potential in other parts of the world.
The plant types that you are looking at for grain feed, rice, or whole silage crop price are quite intriguing from the way they look, the strengths, how tall they are; some of the traits like the non-structural carbohydrates are also something that we are interested in, in terms of screening for translocation efficiency. I think there is a lot of interesting knowledge that has been accumulated in this line of research just in the last 5, 6, 7, 8 years in Japan, which we need to mine for that of our purpose.
I was even thinking about some of these varieties that are probably how ultimately in 20 years the C4 rice might look like. Just there is more grain on it. There is a lot of very interesting research that I think we need to think through how it could also be applicable for other environments. I think it was more of a comment, but if either Dr. Kato or Parminder want to further comment on this then please do.

Parminder Virk: Yes. On Dr. Kato's presentation, it was also interesting. I think Achim also raised a question of dual purpose rice for example which we are looking at in Asia, looking at straw as a fodder and then grain as for human consumption. I see we are going to look at very seriously for high yield potential. But one thing that was very sort of interesting from my point of view was, many of your lines are showing very good lodging resistance and that trait will be very important to look at very carefully.
Of course we have gene clone SEM2 from Habataki but then your resource is much wider, you have many lines which are showing good tolerance to lodging, which will be very important for high yield potential, there we are looking at large panicles and larger grains for high yield potential. It's a very interesting material. J.K., do you want to say something?

Jagdish Ladha: I just wanted to ask Dr. Kato-san. I thought you showed the biomass of rice, some of the varieties you could get as much as what you will get with the maize C4. Is it a true statement? You can get similar biomass, that's very interesting because that means rice is capable of producing that much of biological productivity.

Hiroshi Kato: Actually the TDN yield of rice is – our target is 1.3, actually I omitted some part. The TDN yield of forage maize is 1.3 but that is achieved by farmer's field, our achievement target is our field, so those differences exist. If we compare directly, I think in the upland the forage maize is better, but in the condition of the paddy we convert paddy field for the animal feed production. In that case, rice is good and we can keep the paddy field in a good condition.

Jagdish Ladha: My question is more for comparison with C4 like maize.

Hiroshi Kato: Yes, yeah, maize is better in a straight sense.

Yoshimichi Fukuta: Okay, thank you so much for attending this session. Unfortunately, time is over. The discussion will be continued in the reception hall and today we recognized we need to exchange information, maybe the collaboration in the future. Thank you so much.