

## Reviewers' comments and our responses

The result of the evaluations of four reviewers, and the responses of the program director to each reviewer's comments are as follows.

### 1) Action class assessment of the overall research program

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### 2) Comments and suggestions for the research program

The research program meets the national priorities and policies. Of the four crops reviewed there is overproduction of rice and shortage of other three commodities in the country. Since there is overproduction of rice, breeders have successfully developed varieties for alternate uses such as for making bread and noodles. Goals for increasing production of wheat, barley and soybean are reasonable and breeding targets are appropriate. <sup>a)</sup> The breeding programs should expand the collaborations with industry especially for quality testing. Industry is more likely to actively promote adoption of new varieties. Efforts should be made to broaden the germplasm base of these crops. Since CGIAR centers are repository of world germplasm, additional germplasm should be introduced from these centers. <sup>b)</sup> A couple of areas of research that could use more attention are comparative genetic analyses of traits across species especially for the rice, barley and wheat programs. While minor comments for improvement are suggested for specific projects in the comments above, the seven research projects were impressive in their scientific rigor, clearly identified objectives, well-established and appropriate methodologies, and measureable outcomes.

(Responses to the underlined comments)

<sup>a)</sup> We will further enhance cooperation with the food industry, particularly at the earlier stages of the breeding program, to promote the acceptance of new varieties by consumers.

<sup>b)</sup> Because genome synteny has been identified in many chromosomal loci, and mass-sequence data are determined daily for rice, barley and wheat, we will continue to conduct comparative genetic analysis to evaluate important agricultural traits through collaboration with other programs and institutions.

### 3) Comments and suggestions for the seven research projects

#### (1) Rice breeding and quality research for development of suitable varieties for the food industry

The rice breeding and utilization project is very well organized. This project is expanding its focus on foods to include more functional ingredients and maintenance of grain quality under increasing temperatures. This expansion is warranted, provided the project can continue to meet

the needs for improved varieties with high quality and adaptation to changing environmental and disease pressures. Tremendous progress has been made in developing varieties with high grain quality. A unique project aim is to develop rice varieties for making bread and noodles. It is clear these studies are innovative, forward looking. <sup>a)</sup>By the development of the molecular markers and the expansion of the gene source, the further acceleration of the studies can be expected.

(Response to the underlined comment)

<sup>a)</sup>We regard the development of DNA markers as an important research aim in this project. At present, MAS (marker assisted selection) is performed for eating quality and resistance to disease and insects. In addition, our project is attempting to identify gene(s) and quantitative trait loci (QTLs) involved in important agricultural traits, for future MAS. Expansion of gene diversity is also sought. For example, chromosomes of wild relatives are recurrently introduced into cultivated rice.

## **(2) Physiological and genetic research on rice yield and temperature stress**

The rice yield physiology project on yield enhancement and high temperature tolerance is excellent. Cooperation between plant physiologists and breeders/geneticists is admirable. The development of chromosome segment substitution lines (CSSL) in both “Koshihikari” and “Takanari” backgrounds is a powerful tool that will likely have great utility in identifying molecular markers associated with physiological traits that can be used for marker assisted selection. <sup>a)</sup>However, it must be noted that the CSSL will not be able to account for traits with quantitative inheritance, involving multiple genes of small effect and gene interactions including epistasis. Studies on high temperature tolerance are equally important. Temperatures higher than 26°C during grain filling cause chalky grains and chalky grains have lower market value. The analysis of metabolites and hormonal control under investigation will clarify the key processes during the grain development and will help identify the biomarkers for high temperature tolerance. In particular, use of knock-out lines to study high temperature tolerance is an excellent approach.

(Response to the underlined comment)

<sup>a)</sup> This project will advance the detection of QTLs using CSSL, and attempt to accumulate useful QTLs in order to demonstrate their effects. The identification of multiple genes with limited effects, and their gene interactions, is considered an important research aim. We will examine these subjects using BILs (backcrossed inbred lines) and association analysis through collaboration with other projects and institutions.

### **(3) Development and utilization of genetic technology for improving productivity of rice**

The rice biotechnology project is very impressive. This project has a well-defined focus on three key issues, improvement of yield, cold tolerance and cleistogamy to reduce the likelihood of gene escape from genetically modified rice. For yield enhancement, a gene from cyanobacteria was introduced to improve photosynthesis. Photosynthetic activity of transgenic rice as assessed by CO<sub>2</sub> fixation was increased 20%. The research approach to introduce the *APXa* (ascorbate peroxidase) gene to decrease oxidative stress and to introduce the *1-SST* (sucrose:sucrose 1-fructosyltransferase) gene to reduce water stress may help reduce low temperature damage. Incorporation of a gene for cleistogamy in transgenic rice should inhibit the pollen flow from transgenic to non-transgenic plants which is one of the concerns of those who are opposed to genetic engineering.

### **(4) Wheat breeding for high quality and yield, and development of utilization technology**

The wheat breeding and utilization project is a productive project that has released several varieties with disease resistance and novel starch properties and close collaboration with industry is essential to ensure acceptance of new varieties. The engagement with prefectural experiment stations and flour companies will provide advanced knowledge and serve to ease the acceptance of new varieties. A major bottleneck to wheat production is pre-harvest sprouting as the wheat harvest coincides with rainy season. The wheat and soybean biotechnology project has cloned the *MFT* gene on the chromosome 3A and collaborates with this wheat project.<sup>a)</sup> Also there are recent reports of new genetic sources of *Fusarium* head blight (FHB) resistance that could be exploited in the breeding program. This is a productive project that publishes many papers in high quality journals.

(Response to the underlined comment)

<sup>a)</sup> Major genetic resource for FHB resistance is found only in “Sumai 3” in this project. We regard the suggested new sources of FHB resistance as useful, and will evaluate the resistance of the new resources under Japanese weather conditions.

### **(5) Barley breeding for high yield, and quality and development of utilization technology**

The barley breeding and utilization project is an unusually creative breeding program that is producing barley varieties for a wide range of products some of which are unique to Japan. The project is addressing major quality issues (e.g., low browning after boiling), disease resistance (e.g., *Fusarium* head blight (FHB), barley yellow mosaic virus (BYMV)), improved baking quality, culm strength, and yield. They are developing varieties with novel starch properties and high  $\beta$ -glucan content as well as new cultivars that have the proanthocyanidin-free mutation

which reduces the browning reaction. Unfortunately, this also results in susceptibility to pre-harvest sprouting (PHS). However, <sup>a)</sup> there have been several QTLs reported in the literature for seed dormancy in barley and these could be exploited to reduce the susceptibility to PHS. Quite a few varieties have been released that have novel characteristics. However, the high  $\beta$ -glucan variety will require an intense marketing campaign to educate people about its benefits. <sup>b)</sup> It may need to increase the diversity of germplasm fed into the project to meet longer-term productivity goals. <sup>c)</sup> Another suggestion is to incorporate FHB resistance from U.S. barley varieties such as Pinnacle (2-row) and Quest (6-row).

(Responses to the underlined comments)

- <sup>a)</sup> We consider PHS resistance of proanthocyanidin-free barley cultivars as a central issue. We will evaluate the reported QTLs, and introduce them into proanthocyanidin-free barley cultivars.
- <sup>b)</sup> We recognize that it is essential to increase the diversity of germplasm to improve varieties. We will continue our collaborative research with Okayama University, which is the center for barley and wild plant bioresources in Japan.
- <sup>c)</sup> We regard the suggested new sources of FHB resistance as useful, and will evaluate the resistance of the new resources under Japanese weather conditions.

## **(6) Soybean breeding for high yield and quality, and development of utilization technology**

The soybean breeding and utilization project is making good progress in improving soybean varieties for Japan. A major challenge for the soybean breeding project is the acceptance of improved varieties by industry and consumers. Modern selection methods (e.g.MAS) are being employed in accelerating the development of improved cultivars for specific, targeted traits which affect production. The near-term goals are well-matched to production issues. <sup>a)</sup> It also needs to increase the diversity of germplasm and not hamper genetic gain by the reliance on backcross breeding with the narrow genetic base. Broadening the genetic base will be an essential foundation for the improvement of yield potential, and this is clearly a longer-term project. <sup>b)</sup> Yield improvement will likely require innovative changes in management to match particular environments and to match given varieties. <sup>c)</sup> In addition to marker assisted backcrossing, the breeding program might consider forward breeding or genomic selection. The quality requirements are particularly demanding for soybean. <sup>d)</sup> For soybean food consumption, a metabolomics approach may be of use to identify key compounds necessary for the final products in addition to the QTL analysis of recombinant inbred lines (RILs).

(Responses to the underlined comments)

- a) We understand that it is indispensable for extending the genetic diversity of a breeding material to improve varieties. Our activities as part of the national genebank program are effective in utilizing genetic resources. We also conduct mutual exchange of material with the world network involved in soybean research.
- b) In the search for superior yield characteristics, we have this year (in 2012) begun a joint research program with related researchers, including agronomists. We expect to achieve remarkable yield results by utilizing the combined technologies of cultivation and cultivars. Much higher yields may be achieved through a continuous integrated approach.
- c).d) We actively explore all forms of advanced technology in the search for solutions, within the limits of available research resources. We will promote research activities in collaboration with universities and institutions.

#### **(7) Molecular biological and genomic approaches for improvement of wheat and soybean traits**

The wheat and soybean biotechnology project is making good progress in developing and utilizing biotechnology tools to address chilling and flooding stress and winter hardiness and pre-harvest germination in wheat. This research is particularly important because of the close association in working with respective breeders in identifying QTLs. One of the important recent discoveries was the cloning of the *MFT* gene which regulates seed dormancy that is essential for pre-harvest sprouting (PHS) resistance. This is a major accomplishment since this trait is difficult to improve. In soybean, the over expression of alcohol dehydrogenase 2 (*ADH2*) gene looks promising for flood tolerance. <sup>a)</sup> To take advantage of the extensive work on cloning genes and transcription factors, they should use the sequences to survey natural variation in the genes. This is a very productive project that publishes many papers in high quality journals. Another interesting discovery is the RNA chaperone that reduces secondary RNA structure under cold conditions. Similarly, the project on over expressing of the *1-SST* (sucrose:sucrose 1-fructosyltransferase) gene is important and may increase winter hardiness and snow mold tolerance of wheat.

(Response to the underlined comment)

- a) We will use the sequences to investigate natural variation in the genes with respect to *MFT*, *ABA 8'-ox*, and so on. In addition, we characterize the natural variations in terms of their usefulness as breeding material.