Towards innovations in agrobiological sciences and biotechnology: Status and prospects

Review Report

The reviewers provided the report on the NARO Research Program "Towards agrobiological sciences and biotechnology: Status and prospects". NARO scientists responded the report as follows;

The evaluation committee consisting of

- Prof. Dr. S. B. Gelvin, Purdue University, USA
- Prof. F. Marec, Institute of Entomology, Biology Centre, Czech Academy of Sciences, Czech Republic
- Prof. H. Niemann, Institute of Farm Animal genetics, FLI Mariensee, Germany
- Dr. R. Rodriguez-Cerezo, European Commission, DG Joint Research Centre, Spain

visited the Institute of Agrobiological Science (NIAS) of the National Agricultural Research Organisation (NARO) in Tsukuba on Nov. 20th and 21st, 2017. On 20th Nov. 2017, two site inspections were made, one to visit the experimental fields of the Institute used for testing genetically modified crops, predominantly rice, and the second to inspect the facilities related to research work on genetically engineered silk worms. On Tuesday 21st November 2017, a symposium was held in which the seven projects that were under review, gave their presentations followed by extensive discussion with the peer reviewers group.

General comments

1. The committee thanks NARO for perfect organization and providing extensive material prior to and during the evaluation sessions.
2. NIAS-NARO has to be congratulated for an impressive list of achievements in basic science. NIAS has a regular flow of publications in peer reviewed scientific journals with a remarkably high proportion in leading journals of the field. The reviewing committee recognised differences with regard to the number of high ranking papers between the various research projects. The peer reviewing committee recommends that NIAS-NARO should continue this successful path of conducting internationally highly competitive and acknowledged research.
3. The committee appreciates the applied aspects of all the projects. We wish to remind NARO, however, that excellent applied research rests on a firm basis of fundamental discoveries. Without these discoveries, novel ideas for scientific applications will diminish and eventually evaporate. We urge NARO to continue support for an appropriate mixture of fundamental and applied research.
4. The evaluation committee encourages NIAS-NARO to seek more contact with domestic and foreign Universities to attract talented students and Postdocs from all over the world to foster the process of internationalization of the research Institute.

NARO responses to the comments of the Evaluation Committee

1. NARO aims to promote R&D on agriculture, food and the environment from the global viewpoint and to bring back the results of such efforts to society. In order to maximize our research achievements, NARO is organized and operated to enable us to integrally pursue research areas ranging from the fundamental, applied and dissemination aspects. NARO recognizes the importance of potentially expanding high-quality basic science. NIAS, which plays the central role in the basic and fundamental science and

technology in NARO, will continue to expand and deepen the research programs in this direction.

2. NARO aims to contribute to the solution of various domestic and global issues with regard to agriculture, food, and environment on the basis of high-quality basic science. Thus, we will continuously place emphasis on basic science.
3. Considering the importance of basic science, NARO has special research funds to support particularly promising research projects which are selected by the NARO president. We also take various appropriate measures, including human resources, to support good basic research projects.
4. Global viewpoints are indispensable to solve various problems in current agriculture. To this end, NARO will increase the recruitment of foreign researchers. In addition, we will try to increase the publication of research articles in internationally renowned journals as well as the presentation of recent research results in international meetings. We will also exert more efforts to gain international recognition by promoting exchanges with international research organizations and improving our English website to disseminate the research achievements of NARO and NIAS more effectively.

Feedback on NARO responses

The Evaluation Committee acknowledges the detailed responses to all comments made by committee members to the general performance of NIAS as well as to individual research projects. Here is our feedback on these responses.

To general comments, the Committee welcomed NARO's positive response to our general comments and is very pleased that NARO is aware of the critical importance of basic research and will further continue in supporting scientific excellence and projects on fundamental questions of current agrobiology and on research of new technologies. The Committee also highly appreciates that NARO will take the necessary steps to promote the internationalization of research at NIAS.

Project 1 – Research to utilize useful function of plants and microorganisms for agriculture

This research group consists of 34 scientists from various backgrounds of plant and microbial biology, and reflects the highly heterogeneous nature of the numerous disparate projects. These include manipulation of plant defense genes (WRKY45, BSR1, etc.) to breed novel pathogen resistances into plants, chemical control of plant diseases, control of virus infection of plants, identification of plant-based antimicrobial compounds, generating a transposon-tagged *Lotus japonicus* library, analysis of the mechanism of rice infection by the rice blast fungus, utilization of rhizosphere plant-associated microbes, and acetate-mediated protection of plants against drought. This group has been, with some exceptions (the virus research), highly productive, with many publications in high-profile journals (Nature Plants, Plant Cell, Plant Journal, PLoS Pathogens, PNAS, Plant Biotechnology Journal, etc.). However, this project suffers from a lack of coherence, with too many disparate projects that do no naturally fit together. The research group as a whole more resembles a traditional university Plant Pathology department rather than a cohesive, goal-oriented group. The basic research is great, and we encourage continuation of explorations of fundamental problems that may yield practical applications. However, we recommend the (politically difficult) strategy of focusing this group on two or three major projects. For example, it is not really clear why, with a transposon library already extant in *Medicago truncatula*, it is so important to establish yet another legume gene knock-out model system (despite the fact that these species differ in some aspects of their symbiotic relationships).

Because of the high quality of science and publications, **we rate this project A with a tendency towards S – good quality with only minor revisions needed no changes needed.** However, **we rate the organizational aspects of this project B/C – fair quality and moderate/major revision needed.**

NARO responses to the comments of the Evaluation Committee

We would like to express our gratitude for highly evaluating the research content of this project. Especially it is an honor to mention about publications of research results in prominent research journals. Even the research on virus that was treated as an exception has the result published in PNAS (publication list no.7) and we are proud that we have been producing a high level of research results throughout this project.
Project 1 is mostly composed of seed researches that correspond to the most fundamental part of the researches in NARO. At the beginning of the 4th midterm, we evaluated the research proposals and selected those with potential impact on agricultural production. Therefore, the process of planning research subjects is obviously different from the basic researches in universities. In addition, we review the progress of each research subject in evaluation meetings held three times a year and make substantial changes. In FY 2016, for example, we terminated or combined 10 research subjects out of 18 by the end of the fiscal year. We initiate a substantial number of research subjects at the beginning to ensure the diversity of research seeds. However, we make rather dynamic changes even after the project has been started for proper research management. I was not able to explain this during the review meeting because of time limitation.

We have encouraged collaborations between different research subjects when synergistic effects are expected. For example, we are testing the combination of effective plant-activating agents and pathogenic-microorganism-controlling microorganisms, which were developed in different research subjects, to see possible synergistic effects in disease control.

Regarding the transposon mutagenesis, transposon-tagged lines of *Medicago truncatula* have been utilized worldwide primarily in the studies of symbiosis in pasture as pointed out. However, *Medicago truncatula*, which is taxonomically closely related to pea and alfalfa, is substantially different from many important leguminous crops such as common bean and soybean, in several aspects including the mode of symbiosis. On the other hand, *Lotus japonicus* is more closely related to common bean and soybean than *Medicago truncatula* and is therefore used as a model plant for the studies of symbiosis in these leguminous crops in many countries including Japan. The transposon-tagged lines we developed are non-transgenic and thus serve as a powerful tool for accelerating those studies without strict regulations as compared with genetically modified organisms.

Feedback on NARO responses

As stated in the response to our evaluation, we were unaware that numerous projects had been combined or terminated. We encourage NIAS-NARO to continue to evaluate projects and modify them as needed.

Project 2 – Development of research strategies for agricultural insect pest management: functional analyses of insect genes and behavior

This research group consists of 34 scientists and focuses on deep understanding of several aspects of insect biology that have a potential to be used either for the improvement of current pert management technologies or for the development of new strategies of insect pest control. These include (1) understanding the fundamental mechanisms of insect metamorphosis aiming to developing new insecticides, (2) identifying genes responsible for insecticide resistance to facilitate their early detection in populations of insect pests, (3) identifying genes involved in plant defense against insets and their use to protect the crop, and (4) understanding the visual cues of light in insect predators to find out alternative methods of insect pest control. The group uses up-to-date methods of molecular biology, reverse genetics, and genomics including NGS technologies in combination with broad knowledge of model systems under study. In each topic, scientists achieved significant results, mostly published in highly respected journals (e.g. PNAS, J. Biol. Chem., DNA Research, Scientific Reports, Proc. Biol. Sci. B). In particular, their contribution to understanding the JH

signaling pathway and the molecular mechanisms that regulate the metamorphosis of holometabolous insects is among the highlights in the fields of insect physiology and developmental biology. Based on very solid basic research, scientists of this group have also made considerable progress in applied research. They developed promising tools applicable in agriculture and vector control. For example, their high-performance screening system of JH-related chemicals can identify new insecticides acting as growth regulators. Also, their PCR-based detection system for insecticide resistance in pest populations has shown a potential for the early detection of resistant insects which should allow one to choose alternative control methods to prevent damage. They also acquired important knowledge on the molecular interactions between rice and the brown planthopper (BPH), a pest of rice plants, which helps to select new rice cultivars resistant to BPH-infestation. Finally, their finding of the specific-light preference of insect parasitoids and predators, verified in field tests, led to the development of a unique strategy for insect pest management through attracting the natural enemies to agricultural fields by crop illumination with violet LED-light. Overall, the work of this group is scientifically sound and the research program consistent and well targeted on current pest control issues. The rating is: **S – high quality and no revision needed.**

NARO responses to the comments of the Evaluation Committee

We are grateful for the high evaluation of the Research Project 2. We will continue our effort to introduce advanced technologies in the area of pest management.

Feedback on NARO responses

We have no further comments.

Project 3A – Clarification and utilization of animal immune system

This research group consists of 6 scientists, which mainly work on unravelling basic molecular mechanisms related to immunology with potential impact for practical application in pig breeding. The major focus is on characterizing porcine pattern recognition receptors (PRRs) of various genes which are involved in the recognition of these molecules at early staged of infections. The results of their research are published regularly in peer reviewed journals in the field of *Immunology*, but also in general journals. Many of the articles are in the top journals (first 1/3 of the respective field). A specific highlight has been a recent publication in the journal "Scientific Reports". The leader of the group has been a member of the consortium that published the porcine genome sequence in 2012. This is the major basis for in-depth genetic studies into the the immunological capability of domestic pigs. A major goal is to use the SNPs and polymorphisms to breed pigs with a higher resistance against common porcine diseases with high economic importance. The group has discovered several PRR polymorphisms and is currently underway to test their suitability under production conditions in several farms in Japan. The research of the group is important, both for gaining a better understanding of immunology in domestic pigs, but also for developing new strategies to increase disease resistance. In this respect, the group is encouraged to use novel breeding technologies (genome editors) with the specific purpose to developing pigs with specific disease resistant traits. Overall, the work of this group is scientifically solid and critically important for both, basic and applied aspects of the Japanese pig industry. The overall rating is: **A – good quality with only minor revisions needed.**

NARO responses to the comments of the Evaluation Committee

Distribution of the meat products derived from pigs generated by genome editing in the Japanese market will face many obstacles and is practically impossible at present. However, we agree that improvement of

pig phenotypes by genome editing is useful in terms of basic research investigating mechanisms of disease resistance and future perspectives of the research. We think that there may be research fields suitable for analysis by using genome editing, such as examination of allelic influence in the populations with the uniformed genetic background. In near future, we will consider introduction of the genome editing method to our research for the appropriate area such as mutations of viral receptors in pigs.

Feedback on NARO responses

We encourage NIAS-NARO to exploit the great potential of the new breeding techniques to improve disease resistance and thus the health status of commercial pig production in Japan.

Project 3B – Development of biomedical pig models using genetic engineering techniques

This group consists of 10 scientists and has developed a number of genetically modified porcine models for human diseases. A major highlight has been the development of pigs with a compromised immune system by knocking out the IL2 and RAG genes, respectively. This results in a publication in "Cell Stem Cell" with a very high impact factor of 23.394. The group is using up-front technologies, including genome editors and advanced cloning protocols to produce valuable pigs for the biomedical community in Japan and abroad. More recently, the group has also produced pigs with an agriculturally important trait, with the aid of gene editing, via knockout of the Myostatin gene (MSTN) which show enhanced muscle growth and could thus help to increase efficiency of Japanese pork production. The group has also started to get into stem cell research which would be an ideal complementation to the ongoing activities. Overall, this group is a valuable asset to NARO that provides the critical bridging into biomedicine. The domestic pig increasingly gains importance in biomedical research and the work that is dome in this project is of critical importance for the Japanese biomedical research community. With the initiation of agricultural research projects (MSTN) a fresh start has been made to introduce valuable new genotypes into the Japanese pig production. Overall, this group is fully competitive at the international levels. The rating is: **A with a tendency towards S.**

NARO responses to the comments of the Evaluation Committee

Thank you for giving the good evaluation for our project 3B. We will promote the development of biomedical porcine models. We are studying the model pigs for the use in Japan at present. However, we want to promote the use of our model pigs in the future in foreign countries as well as Japan.

Feedback on NARO responses

We appreciate the positive response to our comments and encourage NIAS-NARO to strengthen international visibility via new biomedical pig models.

Project 4 – Social implementation of vaccine rice for Japanese cedar pollinosis and the development of novel genetic engineering technology to create innovative industries

The group has 36 scientists and has two distinctly different, but related activities with the overall goal of using rice as a molecular farming crop.

The first activity is a vey applied and practical project with the ambitious goal of producing an edible vaccine against pollen allergy. The topic selected is extremely relevant, given the problems associated with current allergy vaccines (frequent visits to hospital for injections, high share of patients discontinuing the

treatment).

The group has created a synthetic peptide used as an antigen based on epitopes derived from major pollen allergens. GM rice plants expressing this peptide are used now in clinical trials, so the project is at a stage where soon the proof of concept can be established. Using model mice the oral rive-based vaccine has shown development of immune reaction and symptom reduction. Moreover, a phase I clinical trail in humans was concluded in 2012/13 and no adverse effects found with doses up to 80 g of rice. A Phase II clinical trial to assess efficacy was conducted in 2013/14. Although allergen-specific immunotolerance was elicited in patients given the rice, the clinical symptoms were not different from those of controls. New Phase II clinical trials with 2 years of vaccine administration are underway. Results (2018) will determine the future of the vaccine.

Overall, the vaccine project is very promising and some recommendations could be made. First is to try include in clinical trails some sort of standard (injected purified antigen or commercial injectable vaccine if available) against which the efficacy of the *oral* rice vaccine can be benchmarked (currently tested only against a placebo). Second, if phase II is successful and the NARO embarks into large phase III trials, it is very important to discuss with stakeholders of the rice supply chain the issue of coexistence of the "vaccine rice" and food rice in the same country. Although the vaccine rice – if commercial – will be cultivated in controlled greenhouses, the fact that is indistinguishable from normal rice makes it important to develop awareness and to obtain the opinion of the entire rice sector before final steps of release, in order to prepare best practices for cultivation and handling to avoid unwanted admixture with normal rice.

The second part of this project involves developing novel technologies for plant genome engineering. This latter project is and extremely highly competitive international endeavor, and the NARO group should be commended for maintaining a leading role in the development of these technologies. The groups have published a very large number of papers in high level journals (New Phytologist, Scientific Reports, Genome Biology, Nature Plants, Plant Physiology, Plant Journal, etc.). The Plant Genome Engineering Unit has presented numerous seminars at international meetings, giving high visibility to work conducted at NARO. The rating is: **A with a tendency towards S.**

NARO responses to the comments of the Evaluation Committee

1. Include in clinical trials some sort of standard
   Thank you for your valuable comments and useful suggestions. We also think that such kind of benchmark is desirable to clearly demonstrate the efficacy. Clinical trials are now being conducted by the two hospitals in Japan. Although NARO collaborates with these hospitals, clinical research programs were designed by the hospitals and approved by the IRB in each organization. Therefore, we cannot directly respond to the suggestion but will have a discussion with the hospitals to take account of the suggestion.
2. It is very important to discuss with stakeholders of rice supply chain the issue of coexistence of the vaccine rice and food rice in the same country. We entirely agree with your comment. If the clinical studies move to a next large-scale level, we must discuss with various stakeholders about producing substantial amounts of vaccine rice. To accommodate such situations, we need to develop the systems of coexistence of GM rice and food rice. We have a rule for the cultivation of GM rice in the confined fields within our institute. But, unfortunately, there is no rule of the coexistence of GM rice and food rice for the commercial cultivation, let alone supply chains, in Japan. Therefore, we are trying to lobby with the MAFF to consolidate a rule regarding the coexistence.
3. It is important to develop awareness and to obtain the opinion of the entire rice sector before final steps of release. We agree with this comment from the reviewers. Through the science communications with

various stakeholders, we have been developing awareness about the vaccine rice. We also think that if commercial cultivation is realized, it is important to gain acceptance from the entire rice sector and the general public before releasing vaccine rice.

Feedback on NARO responses

We welcome the commitment for discussing with the two hospitals the design of the clinical trials. We also take note of the plans to push for the development of guidelines for coexistence between GM and non-GM rice cultivation.

Project 5 – Revolutionize the silk industry through the use of GM technology

This research group consists of 48 scientists and has developed a variety of transgenic silkworm lines for the production of recombinant proteins and functional silk of different properties with the overall objective to restore the tradition of sericulture in Japan. For the production of recombinant proteins, such as antibodies, enzymes, and diagnostic reagent proteins, they use a GAL4/UAS system with the expression in middle silk glands of the silkworm. Several products have been already commercialized, for example, the antibody Rituximab which is used as a drug for hematological cancers. Even more successful is the use of constructed transgenic silkworms and designed technologies for the development of new silk materials including fluorescent silk, spider silk thread as the strongest natural fiber, ultra-thin silk for extra fine dresses, affinity silk and the so-called clickable silk which allow adding any desired functional molecule through a simple chemical reaction and is thus very promising for its applications such as a drug delivery system in the veterinary and human medicine.

It should be emphasized that all these successful achievements have been made possible by the excellent results of silkworm research at NIAS over the last 20 years. NIAS scientists played a leading role in insect transgenesis and performed the first successful germline transformation of the silkworm already in 2000 (Tamura et al. 2000, Nature Biotechnology). They also participated in sequencing the silkworm genome (second genomic sequence in insects – see Mita et al. 2004, DNA Research), and in integrating the genomic sequence with linkage map of the silkworm (Yamamoto et al. 2008, Genome Biology). These achievements also provided powerful research tools for other insects and established the silkworm as a model system for carrying out cutting-edge research. Recently, scientists of this group established several methods of genome editing in the silkworm with the aim to improve the genome engineering technology. The methods include knock-out using ZFN (Takasu et al. 2010, Insect Biochem. Mol. Biol.) and TALENs (Sajwan et al. 2013, IBMB; Takasu et al. 2013, PLoS ONE; Takasu et al. 2016, IBMB) and knock-in using TALENs and CRISPR/Cas9 (Daimon et al. 2014, Dev. Growth Differ.; Nakade et al. 2014, Nat. Commun.). Surprisingly, the articles cited are not given in the written report of this group.

The group has also made a significant progress in the implementation of farm-rearing of transgenic silkworms. Scientists identified risks and benefits of farm-rearing and presented research data clearly demonstrating relatively high safety of handling transgenic silkworms under mass rearing conditions. Thanks to this effort, farm-rearing of GM silkworms has been approved in Japan since September 2017. Overall, the considerable achievements in genome engineering of the silkworm and its application would justify the ranking S – high quality and no revision needed.

However, the publication list in the project presenting mostly articles in specialized journals with narrow scope does not fully reflect a high quality of research preformed by this group. Therefore, the overall rating is: **A with a tendency towards S.**

Note: during the Reviewer meeting in Tsukuba, a new relevant article has been published by this group in a highly recognized general journal:
Sato M, Kitani H, Kojima K (2017) Development and validation of scFv-conjugated affinity silk protein for specific detection of carcinoembryonic antigen. *Scientific Reports* **7**: 16077. DOI:10.1038/s41598-017-16277-6

NARO responses to the comments of the Evaluation Committee

We greatly appreciate your valuable comments about our achievement in this project, which is encouraging for us. In the pre-explanatory material, the description was limited to our research outcomes that are potentially and practically utilized in society, and we did not describe our efforts to develop basic technologies used for the applied research due to space limitation. We also omitted genome editing because this basic technology is still in the development phase in silkworm and thus has not yet reached the phase of practical utilization in the "silk industry". In addition, only the publications that are strictly relevant to the researches described in the pre-explanatory material were listed.

On the other hand, in the oral presentation, we also introduced our ongoing basic researches that will support social implementation of our research products in the future. Consequently, there was a discrepancy in the contents between the pre-explanatory material and my oral presentation due to time limitation, which may have led to the criticism of the reviewers.

We will continue our efforts towards the development of new silk industry based on basic and applied researches including the improvement of transgenic/genome-editing techniques and the application of these techniques to elucidate insects' unique functions. We will also continue to apply for the approval of the farm-rearing of various new GM silkworms.

Feedback on NARO responses

We are pleased to accept your explanation of the discrepancy between the printed material and oral presentation of this project, and actual achievements of this research group. We also thank you for providing us the impressive list of publications of the whole group. Perhaps, in order to avoid similar misunderstandings in future, we recommend that you provide the next evaluation committee a complete publication list, along with the project and the relevant publications cited therein.

Project 6 – Promotion of biotech researches and social Implementation of their outcomes

This project is being carried out by the section of Biotech Research Promotion and Public Affairs that has been newly established at NIAS-NARO (the exact number of staff allocated to this section was not known to the panel). The new Section has the mission to (i) supporting scientists doing research with GM organisms to comply with Japanese regulations and (ii) promoting public understanding of GM technologies and products with outreach activities.

The panel found the establishment of this section to be an excellent initiative and to be extremely helpful for the all research projects of NIAS-NARO. Centralizing expertise in regulatory affairs and helping scientists navigate the difficult transition from the laboratory to field/human testing is an achievement of this group. This conclusion is supported by the number of applied projects (vaccine rice, GM silkworms) that are in advanced stages of commercial development. The presentation was convincing and professional.

With regards to the second mission of this group, the panel found a dedicated and enthusiastic team that prepared imaginative activities to promote understanding of GM technologies among interested stakeholders,

interested adults and children. Relationships with the media are also responsibility of this group, and the projects on GM silkworms and vaccine rice have been selected as top stories of NIAS-NARO for the press. In general, the panel found the work to be conducted professionally and offered the suggestion to target teachers from primary and secondary schools specifically when promoting understanding of biotechnology. The raring is: **S – high quality and no revision needed.**

NARO responses to the comments of the Evaluation Committee

We believe that it is necessary to bridge our efforts at various levels in order to maximize the practical application of innovative research results using genetic recombination technology. We are grateful for the high evaluation of the activities introduced in Project 6. We will continue to promote and strengthen such activities in the future.

Feedback on NARO responses

We have no further comments.