Trials on Cross and Mutation Breeding of Rice for Adaptability to Nature Farming

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Summary

We have tried to develop rice varieties, through cross and mutation breeding for adaptability to nature farming in these past more than ten years. We succeeded in selecting breeding lines for adaptability to nature farming, in the hybrid populations from the cross of representative native varieties of Japan, 'Asahi' and 'Kamenoo'. Two breeding lines, namely AKH2 and AKH4, which were of high yield with high eating quality in nature farming, were selected from the cross breeding. However, the selected breeding lines were too late for heading date to be adaptable to wide range of Japan island other than southern part of Japan e.g. Kyushu district. We tried to improve the negative trait for late heading of the breeding lines through the use of induced mutations within five years. This paper refers to the breeding process and discuss on the merit of mutation breeding and on how to use the technique for gaining adaptability to low input sustainable agriculture.

Introduction

Conventional agricultural systems dependent on chemical fertilizer and agricultural chemicals should be converted to low input sustainable agriculture with less amount of chemicals in the situation of serious climate change covered in the world. It is reported that more than 20% of all greenhouse gases, e.g. CO_2, CH_4, and N_2O, might be emitted from the field of agriculture (Nagano et al., 2012, Skinner, 2014). We have tried to perform cross breeding combined with use of induced mutations of rice for adaptability to nature farming, aiming at developing the system of sustainable agriculture. Nature farming is defined in this paper as a farming system that uses only residue of rice plants as the nutrition with no agrochemicals and chemical fertilizer (Okada, 1953). We are reporting a case of the breeding performed in Yunomae-cho, Kumamoto-prefecture, Japan. The AKH2 breeding line was finally selected for high yield and high eating

quality in the nature farming condition, from the hybrid population derives from the cross between Japanese native varieties, 'Asahi' and 'Kamenoo'. However, the AKH2 breeding line was found to carry weak points for late heading and long culm. We have thereupon tried to improve the demerits of late heading and long culm through use of induced mutations. Accordingly, some breeding lines with earlier heading date and/or shorter culm length as well as higher yield in the low input conditions were selected. The selected MAKH2 breeding line was noticed as promising new rice variety adaptable to nature farming or low input sustainable agriculture.

This paper refers to the breeding process and discuss on the merit of mutation breeding and on how to use the technique for gaining adaptability to low input sustainable agriculture.

Materials and methods

In 2005, Japanese native varieties, Asahi and Kamenoo were crossed at the Ohito Experimental Farm located in Izunokuni-city, Shizuoka-prefecture located at almost center of the main island of Japan. The 1,638 F_2 plants derived from 16 F_1 plants were grown with planting space of 30 cm × 20 cm in the fields of nature farming of Ohito Experimental Farm in 2007. All the harvested rice straws and 100 kg rice bran were applied to the rice fields as nutritional materials. The 140 plants were selected by eye measurement from all the F_2 plants in the fields. In 2008, 140 F_3 lines originating from the selected F_2 plants were grown and 36 F_3 lines were selected by eye measurement in the field. The selected F_3 lines were measured for wight of grain and eating quality using the measuring instrument (Kett Electric Laboratory, Tokyo, Japan).

Table 1 shows the breeding process performed from 2009 to 2017 in Yunome-town, Kumamoto-prefecture in Kyushu-district, Japan. In 2009, seeds of the selected 36 F_3 lines were sent to a farmer, Mr. SHIIBA, Takema in Yunomae-town, Kumamoto-prefecture to be grown as F_4 lines in the fields of nature farming. In the selection tests, any nutritional matters were never applied to the breeding field.

Ten or less breeding lines were used for adaptation and yield tests from 2013 to 2015, after selection tests up to 2012. In the adaptation and yield tests, the selected 10 or less lines were grown in randomized block design with three replications (60 plants were grown per one line of one block). Grain yield and eating quality were measured as well as main agricultural traits, e.g. culm length, panicle length, number of panicles per plant and so forth. Two breeding lines, AKH2 and AKH4, were finally selected for registering commercial cultivar patent. AKH2 was, in fact, registered as commercial cultivar 'Kumaminori' in 2018.

Table 1. The process of cross breeding of native varieties, Asahi and Kamenoo, performed in the farmer's fields in Yunomae-town, kumamoto-prefecture, Kyushu-district (2009~2017).

Year	2009	2010	2011	2012	2013	2014	2015	2016	2017
Hybrid generation and number of lines grown	F_4 36	F_5 23	F_6 17	F_7 14	F_8 10	F_9 7	F_{10} 6	F_{11} 5	F_{12} 2

However, both breeding lines carry a week point for late heading not to be adaptable to wider range to the northern part in Japan. In such a situation, we tried to improve the demerit of the breeding lines through the use of mutation breeding. In 2013, about 5,000 F_8 seeds of AKH2 and AKH4, were irradiated by gamma-rays of 200 Gy at Japan Atomic Energy Agency (currently Takasaki Advanced Radiation Research Institute, National Institutes for Quantum and Radiological Science and Technology). About 4,000 M_1 plants each of AKH2 and AKH4 were grown in the fields at the Ohito Experimental Farm. Seed fertility was checked using randomly selected 50 M_1 plants. The selection process for early heading of the mutants is shown in Table 2. As shown in the Table 2, after 5 years since the irradiation time, two noticeable mutants were selected from the population of AKH2 in 2018.

Table 2. Selection process for early heading of rice mutants from the irradiated populations of AKH2 and AKH4.

Original line	Year and generation					
	2013	2014	2015	2016	2017	2018
	M1	M2	M3	M4	M5	M6
AKH2	4,428N* 48%***	15,840N	119L**	11L	6L	2L
AKH4	3,936N 45%***	14,400N	105L	14L	6L	0L

*N: number of plants. **L: number of lines.

*** average seed fertility for 50 M_1 plants.

Results

Fig.1 shows grain yield and eating quality of the breeding lines, AKH2 and AKH4, which were selected from cross breeding of Asahi and Kamenoo. In the figure, 'Hinohikari' was put as a control because of it being representative leading variety in Kyushu district. As seen in the figure, grain yield of the AKH2 was higher than AKH4 and Hinohikari. As for eating quality, significant difference was not found among the two breeding lines and Hinohikari.

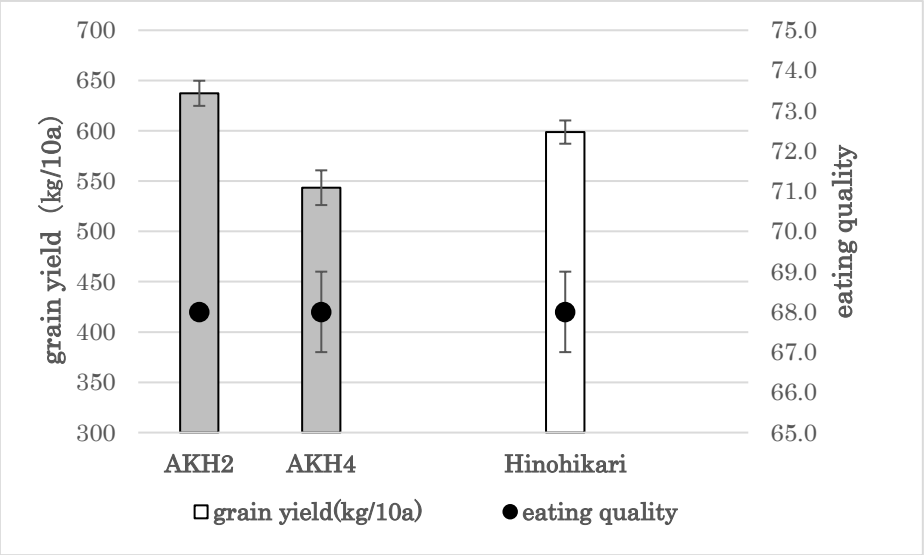

Fig.1. Grain yield and eating quality of the breeding lines, AKH2 and AKH4, in comparison with control (Hinohikari) in the nature farming (from results for adaptation and yield tests in 2014).

We requested some farmers, who were concerned with nature farming in the Kyushu district, to grow the three lines using farm machines. Fig.2 shows one of the results from the farmers on grain yield and eating quality. As seen in the figure, the AKH2 showed the highest yield. Eating quality of rice was clearly higher in the two breeding lines than in Hinohikari, suggesting that eating quality is to be higher in the selected breeding lines than in the popular leading variety, Hinohikari in the Kyushu district. We finally selected the AKH2 breeding line for registering commercial variety in 2018.

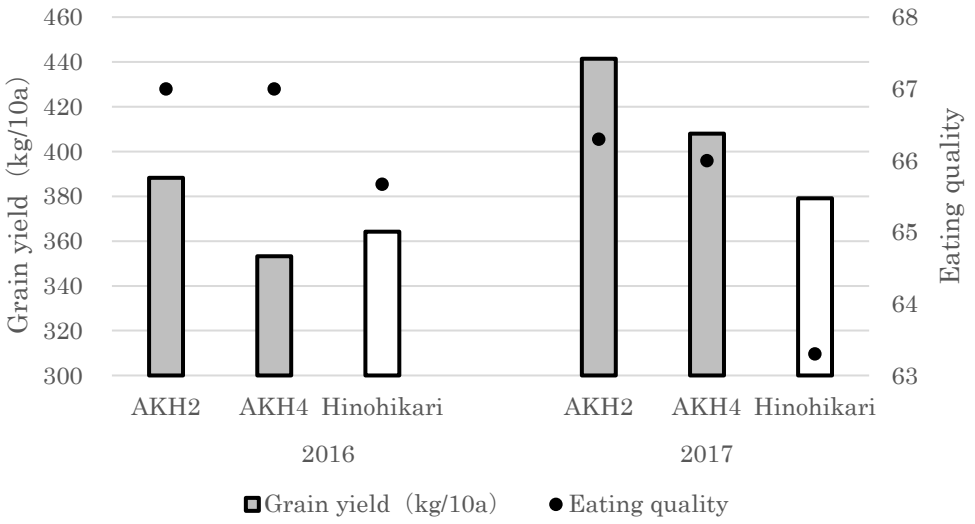

Fig. 2. Grain yield and eating quality of breeding lines, AKH2 and AKH4 selected from the cross breeding in case of being grown by planting machine by a farmer in Kumamoto prefecture in 2016 and 2017.

Fig.3 shows heading date and culm length of the breeding lines of AKH2 and AKH4 comparing with Ashahi and Kamenoo (parent varieties) and Hinohikari (a representative leading variety in Kyushu area). As seen in the figure, heading date of the both breeding lines was almost same as one of the parent varieties, Asahi, which was significantly later in heading date than Kamenoo and Hinohikari. It was noted that heading date of the selected breeding lines was significantly later than that of the local leading variety, Hinohikari. It may be inconvenient for farmers in the local area to grow the breeding lines because of it being late in heading date. Culm length of both breeding lines was almost same as that of the parent varieties, Asahi and Kamenoo. However, it was found that culm length of the breeding lines was significantly longer than that of Hinohikari. We tried to improve the weak point for late heading of the breeding lines to get wider adaptability to grow in northern part of Japan.

Fig.4 shows the frequency distribution for heading date of M_3 mutant lines originating from irradiation of gamma-rays to F_8 seeds of the breeding lines of AKH2 and AKH4. As seen in the figure, the earliest heading date of the mutant originating AKH4 was put in 21~31th in July. Heading date of total 15 mutant lines from both the breeding lines was put in 1 ~10th in September, whereas heading dates of AKH2 and AKH4 were respectively 8th and 6th in September. Heading date of the mutant lines put in 1~10th in September seen in the figure was earlier than that of both the breeding lines, AKH2 and 4. Anyhow, mutant lines put in 1~10th in September were not so

practically effective as breeding materials for early heading.

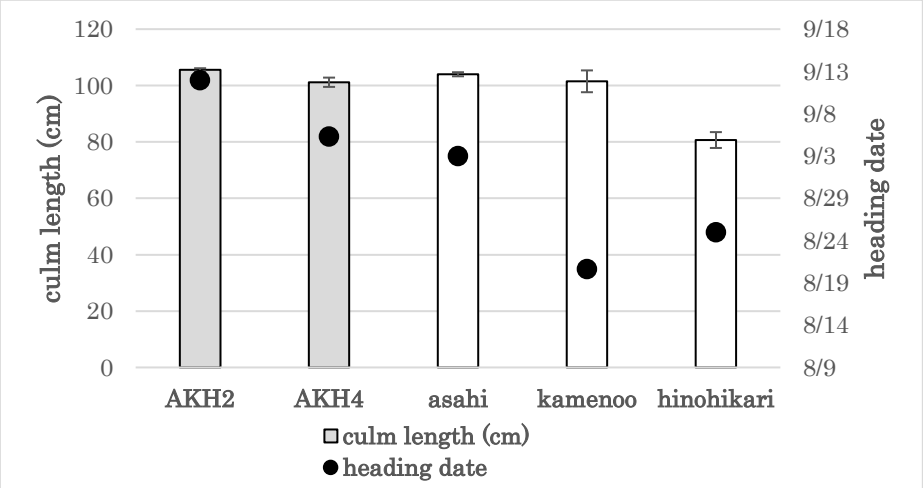

Fig. 3. Heading date and culm length of the breeding lines, AKH2 and AKH4 selected from the cross combination of Asahi and Kamenoo (2014).

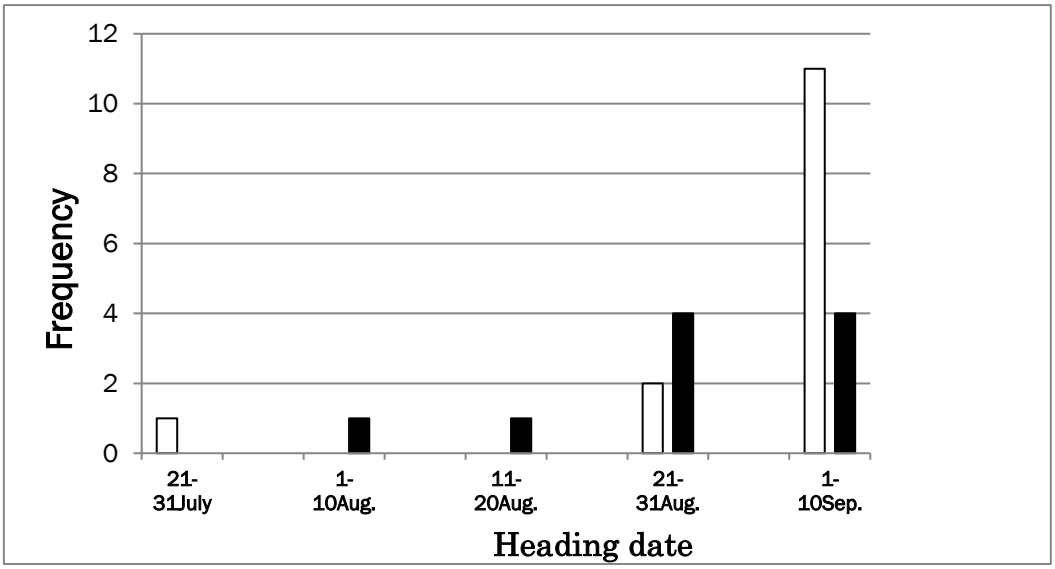

Fig. 4. Frequency distribution of heading date of the mutant lines originating from irradiation of gamma-ray of 20 krad to F_8 seeds of AKH4 (white bar in the figure) and AKH2 (black bar in the figure).

Fig.5 shows culm length and heading date of 6 mutant lines selected as breeding materials for the relevant breeding purpose, which were originating from irradiation of gamma-rays to F_8 seeds of AKH2. As seen in the figure, heading date of all the mutant lines selected was significantly earlier than that of control, AKH2. Heading date (12th in August) of the mutant line, MAKH2, was about one month earlier than AKH2. In case of MAKH2-7, heading date was about one week earlier than the control (AKH2). Culm length of the mutant lines was significantly lower than that of the control, excepting one case of MAKH2-7. We, after all, selected two mutant lines, MAKH2-6 and MAKH2-7, of which heading date are earlier than control, AKH2, with same or more grain yield as the control.

Fig.6 shows plant type of the selected M5 mutant lines, MAKH2-7 and MAKH2-6 comparing with control, AKH2. It was found that MAKH2-7 carried much higher number of panicles comparing with the control, though the culm length was as high as the control. We have finally selected the MAKH2-7 mutant line as a candidate for registering new rice cultivar for adaptability to nature farming or low input sustainable agriculture.

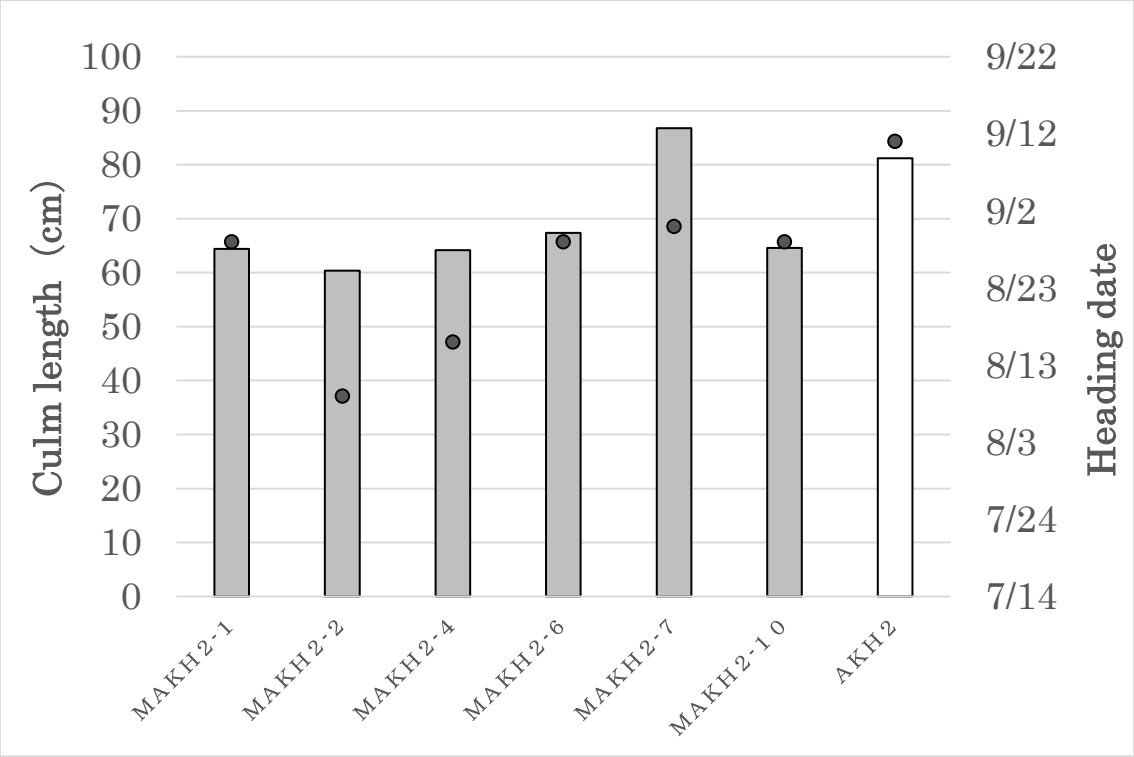

Fig.5. Culm length and heading date of M_4 mutant lines (2017) originating from irradiation of gamma-rays of 20 krad to F_8 seeds of AKH2.

Fig.6. Plant type of the selected mutant lines, MAKH2-7 and MAKH2-6 and control, AKH2.

Discussion

We succeeded in development of new rice variety, Kumaminori, through cross of native varieties, Asahi and Kamenoo, which shows higher grain yield and better eating quality in the nature farming or low input sustainable agriculture. The new variety has been released to farmers to grow in Kyushu district, Japan. However, heading date of the variety is too late to be adaptable to wider range toward northern part of Japan. It is noted that farmers are not to be suffering from lodging due to long culm length of the new variety, because of its strong culm. Farmers are, in general, used to be suffering from lodging of rice in Kyushu district, where is a route of typhoon in autumn season. It was practically proofed for strong culm of the new variety, Kumaminori that plants of the variety were not fell down when the typhoon came in 2008, while the modern leading variety, Hinohikari was done (Fig.7).

We have also succeeded in improving late heading date of the breeding line (AKH2) or new variety, Kumaminori within only five years through use of induced mutations. It is, in general, to take more than ten years in case of cross breeding for completing breeding purpose. In fact, we have taken more than 13 years to develop the new variety, Kumaminori.

Fig.7. Comparison of scenery of the rice fields of Kumaminori in nature farming and Hinohikari in conventional one when attacked by the typhoon in 2018.

The other merit of mutation breeding is to improve a target character without altering other desirable traits of the original variety. It is supposed that the selected mutant line, MAKH2-7 is to be inherited with desirable characters from the original variety, Kumaminori, e. g. high grain yield and eating quality in the condition of nature farming or low input sustainable agriculture. It was especially noted that the original variety, Kumaminori (AKH2) has been proved from epidemiological survey to carry a healing function to atopy and allergic disease. It was found earlier by Hiroshi HASEGAWA that the Yukihikari variety developed in Hokkaido was to carry healing function to atopy or allergic disease, whereas the representative leading variety, Kirara397 in Hokkaido tended to cause the disease (Miura et al., 2003; Hasegawa, 2019).

Representative native variety of Japan, Kamenoo and Ahahi were found to carry healing function to atopy or allergic disease (Sakuma and Komatsu, 2018, The Japan Agricultural News, 13th December 2019). The varietal difference of rice for ability to react to the disease was earlier found to be controlled by gene systems (Monnma, 2005; Sonoyama et al., 2009). The results of epidemiological tests suggest that Kumaminori or the AKH2 breeding line could be inherited healing function to allergic disease from

mother varieties, Asahi and Kamenoo. We would expect that the MAKH2-7 mutant line would carry the healing function. Epidemiological tests for allergic disease of the mutant line are in progress. We are now taking a step for registration of the mutant line, MAKH2-7 for commercial variety.

We have found through the breeding of rice adaptable to nature farming that native varieties could be effective materials for breeding of rice for nutritious foods, as well as for adaptability to low input sustainable agriculture. We also would pay attention to the idea proposed by Indonesia and the Philippines in the sub-project "Mutation Breeding of rice for Sustainable Agriculture (2013~2017)" that native varieties can be effective breeding materials for mutation breeding of rice adaptable to low input sustainable agriculture (Nakai, 2018).

In conclusion, we propose that mutation breeding would be useful tool for development of rice varieties for adaptability to low input sustainable agriculture. In addition, we want to suggest that the native varieties be used as effective breeding materials for mutation breeding of rice for adaptability to low input sustainable agriculture, contributing to rehabilitation of the earth environment and human health.

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References

NAGANO, Hirohiko; KATO, Shigeru; OHKUBO, Shinnji and INOBUSHI. Emission of carbon dioxide, methan, and nitrous oxide from short- and long-term organic farming Andosols in central Japan, Soil Science and Plant Nutrition 58, 793-801, 2012.

SKINNER, Colin, GATTINGER, Andreas;, MULLER, Adrian;, MADER, Paul, FILESSBACH, Andreas; STOLZE, Mattias; RUSER, Reiner and NIGGLI, Urs. Greenhouse gas fluxes from agricultural soil under organic and non-organic management-Aglobal meta-analysis, Science of The Total Environment, 15, 553-563, 2014.

OKADA, Mokichi. Shizenn Nouhou Kaisetu (in Japanese) ("A commentary on Nature Farming" translated by the author), Eikou Shuppann Co. Ltd., 1953.

MIURA, Takako; MIURA, Shunnsuke; Matsuda, Michio, HASEGAWA, Hiroshi and YANAGIHARA, Tetsuji. Examination of different reaction between rice strains on

rice-allergy patients with atopic dermatitis, Arerugi no Rinnshou 23-14, 44-50, 2013 (in Japanese).

Hiroshi Hasegawa, Yukihikari to komearerugi (http://hasekuri.a.la9.jp/yukihikari.htm, 2019/03/20) (in Japanese).

SAKUMA, Masaru and KOMATSU Miyuki. Turuokasi no Sizennnouhou Jissenn ni yoru Kennkouna Machidukuri(in Japanese) (Performance of nature farming for promoting healthy town in Turuoka-city, translated by the author) Koukai Sinnpojiumu Yuukinougyou・Sizennnouho kara hirogaru Machidukuri in Tokyo (in apanese)(Symposium on organic・nature farming held in Tokyo, translated by the author) 17 February 2018.

The Japan Agricultural News. 13th December 2019.

MONNMA, Michiko. Characteristics of protein profile of low allergenic rice variety, "Yukihikari", Rep. Nat'l. Food Res. Inst, No69, 7-12, 2005.

SONOYAMA, Kei; OGASAWARA, Toru; GOTO, Haruka; YOSHIDA, Tomoyo; TAKEMURA, Naoki; FUJIWARA, Reiko; WATANABE, Jun; ITO, Hiroyuki; MORITA, Tatsuya; TOKUNAGA, Yoshinari and YANAGIHARA, Tetsuji. Comparison of gut microbiota and allergic reactions in BALB/c mice fed different cultivar of rice, British, Journal of Nutrition 103, 218-226, 2009.

NAKAI, Hirokazu. Project Review on FNCA Mutation Breeding Project in 2017, in FNCA 18th Coordinator Meeting 2018.