# A Short Report on Gamma Ray Induced Rice Mutants Having High Protein Content

SACHIHIKO TANAKA* and SINPACHIRO TAMURA**

* Chief, First Breeding Technique Laboratory, Institute of Radiation Breeding
** Chief, Food Analysis Division, Food Research Institute

## Introduction

In Japan, people take more than two-thirds of their total protein intake from plant foods and one-third of the total protein is supplied by rice. Not only for Japanese, but also for other people whose main diet is rice, quantity and quality of the rice protein have very important nutritive significance. Protein of polished rice, although it has less quantity than that of wheat flour, has the best nutritive quality among grains. Thus, if we succeed in increasing the content of two essential amino acids, i. e., lysine and threonine, rice could be utilized as a rich source of protein like that of animal protein.

Apart from this aspect, it may be possible to protect the damage of stored rice from insects by breeding rice varieties which do not contain some of the essential amino acids needed by noxious insects. It is hoped to breed such rice varieties having no arginine and histidine which are essential for the life of insects and not for human beings. If we succeed in developing a rice variety lacking in amino acids vital for both human beings and insects, it could be conveniently and safely stored and we can take the deficient amino acids by supplementing our diet with industrially produced ones.

Thus, in rice breeding, one of the important objects is to increase the protein content and to change the constitutive ratio of amino acids. Many breeders have succeeded in improving the chemical components in many agronomic plants and, moreover, many scientists have reported on their successes of inductions in chemical components by ionizing radiations. Therefore, it is expected that induction of mutations on protein content and constitutive ratio of amino acids in rice might be detected in mutant lines with or without visible change of characters.

In the present experiment, the analyses of amino acids were made in mutant lines induced by chronic gamma irradiations in consideration with the visible characters. We found some mutant lines which show two times higher protein content than the original variety. Although we have only a few data up to date, the interesting results will be discussed here.

## Materials and methods

At the Institute of Radiation Breeding, during 1963-1967, we selected about 700 mutant lines on the basis of visible characters from the progenies of Norin No. 8 rice after chronic gamma irradiations and they are being preserved as fixed lines. In the spring of 1968, out of these mutant lines, 71 lines showing such as early heading date : 15, short clum : 15, dwarf : 14, chlorophyll variation : 17, blast resistance : 10 were analysed concerning their amino acids contents at the Food Reseach Institute.

For the analysis, one panicle was harvested from each testing line in the fall of 1967 and one grain from each paincle was used in April, 1968. But, to secure precision, one panicle each was taken from three control plants and from two plants of the line No. 5 which was found to have high protein content. The following analytical method was employed: one brown rice grain was hydrolyzed in 6 N HC1 at 110° degrees C for 24 hours and then, it was analyzed with an Hitachi Model 3B amino acid analyser. No precise analysis was made on the amino acids, tryptophan, cystine, tyrosine, and methionine for the reason that these may be destroyed by the hydrolysis. Protein content was estimated from the sum of amino acids.

Mutant lines which were found to have high protein content in April, 1968 were multiplied together with the original variety. All panicle progenies of these lines were harvested in the fall of 1968 and three seeds from each line were sampled at random as a material for electric microscopic observations. One preparation from each endosperm was used for the observations on protein.

## Results and discussion

A. Variation of protein content among the mutant lines.

Protein content of the tested mutant lines varied largely. If we take the percentage of the protein content of the original variety as an index(100), the contents of the mutant lines varied between 72.4 - 185.4% and this indicated that some of the mutants which showed change in visible traits had also changed in their protein content. Owing to insufficient number of tested lines, at present, no clear correlation between protein content and mutated agronomic traits such as earliness, short culm, etc. was observed. In Table 1, frequency of the mutant lines having varied protein content is presented in connection with the respective mutated traits.

B. Origin and specific traits of the mutant lines having high protein content and variation in constitutive ratio of amino acids in brown rice.

Among the tested lines, brown rice of the line No. 4 and No. 5 had almost doubled protein content and that of the line No. 177 had 1.7 times protein content in comparison with that of the original variety. The lines of No. 4 and No. 5 were selected from $M_2$ progeny of the plants of Norin No. 8 which were irradiated chronically with gamma ray of 150 R/day (20 hours) through the whole growth period, seedling stage to maturity. In 1968, they arrived in $M_7$ and it was observed that all of their visible traits were stable.

The lines No. 4 and No. 5 had earlier maturity by 60 days and 50 days respectively when compared with that of the original variety. Both the lines had short culms, and moreover, the height of the line No. 4 was shorter than that of No. 5. Growth habits of these lines were similar to that of the original variety. The line No. 177 was selected from the $M_3$ progeny of plants of Norin No. 8 which were irradiated recurrently during 1962 and 1963 with a dose rate of 150 R/day through the whole growth

**Table 1.** Variation of Ratio of the Protein Content in Mutant Lines Classified into Five Groups.

| Mutant trait | Ratio of percentage of protein content (%)* | | | | | | | | | | | | Number of |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 72.4 | 80 | 90 | 100 | 110 | 120 | 130 | 140 | 150 | 160 | 170 | 185.4 | mutant lines |
| Earliness | 0 | 0 | 2 | 0 | 1 | 2 | 2 | 2 | 4 | 0 | 1 | 1 | 15 |
| Short culm | 0 | 0 | 0 | 1 | 2 | 6 | 2 | 0 | 1 | 3 | 0 | 0 | 15 |
| Dwarf | 0 | 1 | 0 | 0 | 0 | 2 | 3 | 2 | 1 | 5 | 0 | 0 | 14 |
| Chlorophyll | 1 | 0 | 2 | 1 | 2 | 4 | 2 | 3 | 1 | 1 | 0 | 0 | 17 |
| Blast resistance | 0 | 0 | 2 | 2 | 4 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 10 |
| Number of mutant line | 1 | 1 | 6 | 4 | 9 | 15 | 10 | 7 | 7 | 9 | 1 | 1 | 71 |

* Ratio of percentage of protein content : $\frac{\text{Protein content of mutant line (\%)}}{\text{Protein content of original variety (\%)}} \times 100$

Range of the ratio: For example, 100% has a range of 95.1-105.0%

Table 2. Agronomic Characters of High Protein Rice Line*

| Variety and lines | Heading date | Length of culm (cm) | Length of panicle (cm) | No. of panicles | Wt. of panicles per plant(gr) | No. of grains per panicle | Wt. of one grain (mg) |
|---|---|---|---|---|---|---|---|
| Norin No. 8 (Original) | 28, Aug. | 95 | 19.8 | 8.7 | 25.7 | 124 | 23.8 |
| No. 4 (Mutant) | 25, June | 45 | 16.4 | 11.6 | 19.8 | 67 | 20.6 |
| No. 5 (Mutant) | 5, July | 58 | 18.2 | 11.0 | 23.8 | 101 | 22.9 |
| No. 177 (Mutant) | 26, Aug. | 42 | 11.4 | 8.0 | 14.3 | 111 | 13.4 |

* Observations in 1965

Table 3. Amio Acid Content in the Brown Rice of the Mutant Lines of No. 4, No. 5 and No. 177 (mg/gr of brown rice for respective amino acid).

| | Original variety | | | | Mutant line | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Replication | Norin No. 8 | | | | No. 4 | No. 5 | | | No.177 |
| Amino acid | I | II | III | Mean | I | I | II | Mean | I |
| Lysine | 2.91 | 2.71 | 2.52 | 2.71 | 4.15 | 4.76 | 4.02 | 4.39 | 5.22 |
| Threonine | 2.20 | 2.26 | 1.87 | 2.11 | 4.22 | 3.65 | 4.37 | 4.01 | 3.26 |
| Arginine | 4.65 | 6.54 | 4.72 | 5.30 | 10.52 | 10.49 | 10.64 | 10.57 | 8.57 |
| Histidine | 1.61 | 1.55 | 1.56 | 1.57 | 2.78 | 2.94 | 2.94 | 2.94 | 2.70 |
| Others | 41.86 | 42.19 | 39.61 | 41.23 | 80.83 | 73.78 | 85.36 | 79.57 | 69.32 |
| Total | 53.23 | 55.25 | 50.28 | 52.92 | 102.50 | 95.62 | 107.33 | 101.48 | 89.07 |
| Ratio in comparison to original variety (100 of original var. as an index) | — | — | — | 100 | 194 | 181 | 203 | 192 | 168 |

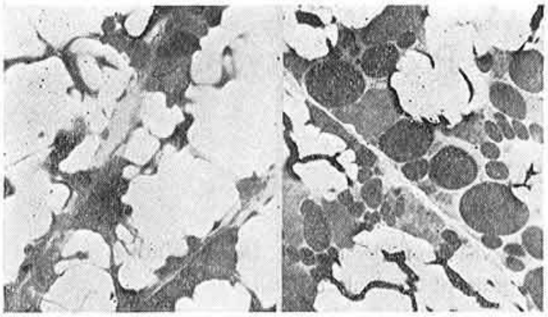
period and it arrived in $M_6$ in 1968. Although this line showed the same maturity date as the original variety, the growth habits showed a marked change as characterized by dwarf type. Heading date, length of stem, length of panicle, number of panicles, weight of panicles per plant, number of grains per panicle and weight of one grain are shown in Table 2.

The contents of amino acids: lysine, threonine, arginine, histidine and others (total of asparagic acid, glutamic acid, proline, glycine, alanine, valine, isoleucine and phenylanine) are shown in Table 3. As indicated in this table, the lines No. 4, No. 5 and No. 177 increased remarkedly, not only in their protein content but content of lysine and threonine, which are responsible for the higher nutritive value, increased in the same level as that of others. However, the content of essential amino acids for noxious insects such as arginine and histidine were also boosted.

C. Electric microscopic observation on protein in endosperm of the high protein mutant lines and the original variety.

Because of the shortage of polished rice of high protein lines, an electric microscopic observation was made to have data concerning the protein content of polished rice and distribution of protein between polished rice and rice bran.

Photographs showing localization and content of protein in a starch layer of endosperm of mutant line No. 5 and the original variety are shown in Fig. 1. Structures of a starch layer of the mutant lines No. 4 and No. 177 were similar to that of the mutant line No. 5 but, the structures of high protein lines were markedly



A. Original Norin No. 8 B. Mutant line No. 5

Fig. 1. Cross-section of starch layer of rice endosperm showing compound starch granules and protein bodies. (×2720)

different from that of the original variety.

It is certain that the mutant lines which were found to have high protein content by analysis of brown rice increased their protein content not only in rice bran but also in polished rice.

As we have obtained some mutant lines with somewhat lower or higher protein content from a small number of visible mutant lines induced by chronic gamma irradiations, it suggests the effectiveness of radiation breeding for the improvement of protein content in rice.

**References**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