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# 水稻 高蛋白 突然變異系統에 關한 研究

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# Studies on the High Protein Mutants of Rice

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## SUMMARY

Several high protein mutant lines($M_4$ plant generation, 1974) obtained from X-ray irradiated Jinheung variety were examined at three different locations for their agronomic characters, protein and grain yields. On the other hand, high protein-short culmed-early maturity mutant line No. 398 ($M_{10}$ plant generation, 1974) induced from Hokwang was crossed back to its mother to investigate the gene(s) controlling protein and its pleiotropic relation to other mutated characters.

Although variation of protein percent of mutant lines from Jinheung was comparatively large depending on year and location, most of the high protein mutant lines had higher protein yield per unit area than the mother variety and their grain yields were equal to or better than the mother, being resistant to both leaf and neck blast. They were several days earlier-maturing and had shorter-culm except one mutant line.

The culm length and heading date of $F_1$ between high protein mutant 398 and its mother Hokwang were intermediate. Accurate assessment of segregation of culm length and heading date in $F_2$ generation and protein percent in $F_3$ seeds will be conducted in 1975.

## INTRODUCTION

Since the discovery of mutant genes controlling lysine content in maize endosperm by Mertz *et al.*[9] and Nelson *et al.*[10], intensive works for improving the amino acid balance and nutritive value of various crops have been made both by exploring high protein genes in the existing varieties[1,2,5,6] and by employing induced mutations[3,4,11].

On the other hand, emphasis has been given to the development of better analytical and nutritional screening techniques to provide the breeders means of more rapid and accurate assessment of the protein quantity and quality of their breeding materials[7,8].

Although rice has very low protein percent, rice protein have the highest biological value among the cereals. The glutelin fraction accounts for more than 70 percent of the total protein. This fraction has a balanced amino acid composition containing fairly large amount of lysine as compared with other fractions. Thus, it may be quite feasible by simply increasing the protein content of rice to obtain a balanced and yet raised amino acid distribution. Therefore, in the mutation breeding for rice protein primary step is to obtaining mutants with high quantity of protein.

Rice seed of Jinheung variety was X-ray irradiated and mostly normal-looking or several days-earlier mutants were selected in $M_2$. In later generations they were screened for their protein percent and several lines of high protein mutant were obtained.

On the other hand, high protein mutant No. 398 was selected from among the drastic mutants of

Hokwang variety. This mutant is, besides the trait of high protein, also early-maturing and short-culmed.

Present report deals with the performance of several high protein lines from Jinheung variety and the result of the crossing between the high protein mutant 398 and its mother variety Hokwang.

## MATERIALS AND METHODS

Materials used were six high protein mutant lines and three lines of fairly good grain yield ($M_4$ plant generation in 1974) derived from Jinheung and high protein mutant line 398($M_{10}$ in 1974) from Hokwang variety.

The agronomic characters of the mutant lines from Jinheung were analyzed, and the grain and protein yields were examined at different locations. For grain and protein yield test, plot sizes were 4–8m$^2$, designed randomized, replicated three times, and mother varieties were included. Nitrogen content was determined by the conventional micro-Kjeldahl method and it was converted into protein using the factor 5.95. Moisture contents ranged from 12–13% at the time of analysis.

Mutant 398 was crossed back to its mother variety Hokwang and mutated characters were examined in $F_1$ and $F_2$ generations.

## RESULTS AND DISCUSSION

### 1. Performances of high protein mutant lines in different locations

In 1973 from about 3,000 mutant lines 150 lines

**Table 1.** Protein percent of grain and protein yield per unit area of high protein mutant lines from variety Jinheung cultivated under different locations.

| Mutant | Year and location | Protein (%) | Single grain wt (mg) | mg protein per grain | Grain yield/10a (%) | Protein yield/10a (%) |
|---|---|---|---|---|---|---|
| Mother variety | 1973, KAERI[a] | 6.4 | 25.0 | 1.59 | | |
| | 1974, 〃 | 7.7 | 22.7 | 1.75 | 100 | 100 |
| | 1974, Loc. 1[b] | 7.6 | 25.0 | 1.90 | 100 | 100 |
| | 1974, Loc. 2[c] | 7.1 | 24.2 | 1.72 | 100 | 100 |
| Mutant No. 919 | 1973, KAERI | 8.5 | 24.7 | 2.10 | | |
| | 1974, 〃 | 8.7 | 25.3 | 2.20 | 113.3 | 128.1* |
| | 1974, Loc. 1 | 8.7 | 25.0 | 2.18 | 101.8 | 116.6* |
| | 1974, Loc. 2 | 7.6 | 24.0 | 1.82 | 108.6 | 116.2* |
| No. 1964 | 1973, KAERI | 9.3 | 24.6 | 2.30 | | |
| | 1974, 〃 | 8.6 | 25.0 | 2.15 | 108.6 | 121.3* |
| | 1974, Loc. 1 | 8.5 | 25.0 | 2.13 | 111.7 | 130.9* |
| | 1974, Loc. 2 | 7.8 | 24.5 | 1.91 | 110.1 | 121.0* |
| No. 1685 | 1973, KAERI | 8.4 | 24.5 | 2.06 | | |
| | 1974, 〃 | 8.4 | 22.8 | 1.92 | 107.8 | 117.7* |
| | 1974, Loc. 2 | 7.6 | 24.1 | 1.83 | 107.8 | 115.4* |
| No. 1953 | 1973, KAERI | 8.6 | 29.3 | 2.53 | | |
| | 1974, 〃 | 8.3 | 22.5 | 1.87 | 114.1* | 123.0* |
| | 1974, Loc. 2 | 7.6 | 24.4 | 1.85 | 106.5 | 113.9* |
| No. 2305 | 1973, KAERI | 9.0 | 22.8 | 2.04 | | |
| | 1974, 〃 | 8.3 | 22.2 | 1.84 | 114.1* | 123.0* |
| | 1974, Loc. 2 | 7.2 | 23.8 | 1.71 | 113.7 | 115.4* |
| No. 2772 | 1973, KAERI | 8.6 | 26.7 | 2.31 | | |
| | 1974, 〃 | 9.4 | 26.3 | 2.47 | 96.1 | 117.3* |
| | 1974, Loc. 1 | 8.5 | 27.0 | 2.30 | 110.8 | 123.9* |
| | 1974, Loc. 2 | 8.7 | 27.8 | 2.42 | 119.3* | 146.2** |

a Institute Farm, Korea Atomic Energy Research Institute, Seoul.
b Kyonggi-do, 20 km South-west of Seoul.
c Kangwon-do, 150km North-east of Seoul.
* Significant at 5%.
** Significant at 1%.

were selected based on their agronomic traits and afterward they were screened for their protein content. Several mutant lines of very high protein percent as well as good grain yield were obtained. In 1974 these materials were grown at three different locations to further confirm the results of previous year and to test preliminary grain and protein yield per unit area.

Generally the results in 1974 were not so different from those in 1973(Table 1). Most of them had higher protein than the mother variety and their grain yields were equal to or better than the mother. As compared with other varieties so far used for the same purposes, this variety produced unusually many high protein mutants with fairly good agronomic traits or yield. They were several days earlier-maturing and had shorter culm except mutant No. 2772 (Table 2).

**Table 2.** Agronomic characteristics of high protein mutant lines from variety Jinheung examined at Institute Farm.

| Mutant | Protein (%) | Heading date | Culm length (cm) | No. of panicles per plant | No. of grains per panicle | 1000-grain wt (%) | Ripened grain (%) | Degree of blast[a] susceptibility Leaf | Neck | Grain yield/10a |
|---|---|---|---|---|---|---|---|---|---|---|
| Mother variety | 7.7 | 28 Aug. | 75.1 | 12.9 | 105 | 22.7 | 87.8 | M | S | 100 |
| Mutant No. 919 | 8.7 | 16 〃 | 73.9 | 13.4 | 95 | 25.3 | 88.3 | R | M | 113.3 |
| 1064 | 8.6 | 15 〃 | 72.9 | 13.9 | 87 | 25.0 | 93.9 | 〃 | R | 108.6 |
| 1685 | 8.4 | 22 〃 | 72.4 | 15.1 | 86 | 22.8 | 91.4 | 〃 | 〃 | 107.8 |
| 1953 | 8.3 | 22 〃 | 71.5 | 20.1 | 88 | 22.5 | 92.1 | 〃 | 〃 | 114.1 |
| 2305 | 8.3 | 22 〃 | 72.0 | 17.1 | 88 | 22.2 | 91.8 | 〃 | 〃 | 114.1 |
| 2772 | 9.4 | 15 〃 | 91.4 | 12.3 | 96 | 26.3 | 94.2 | 〃 | M | 96.1 |

a R: Resistant, M: moderate, S: susceptible.

Variation of protein percent of mutant lines was comparatively large depending on year and location. In general results at the Institute Farm and Kyonggi-do were rather similar, while those at Kangwon-do were lower. The protein percent of No. 2772 was higher than the mother in both years and at three locations, while the other lines were variable depending on year and location.

Nos. 1064, 1685, 1953 and 2305 were similar in their agronomic traits, being short culmed, early maturing resistant to blast at Institute Farm and Kyonggi-do. At Kangwon-do No. 2772 was better than the other lines, being earlier, stiff-strawed, higher yielding and blast-resistant, but disease-resistance and yield were variable depending on location. This line is peculiar in that despite of the longer culm length its heading date was earlier. In most of the mutant materials obtained so far, the two characters were generally negatively correlated.

## 2. Mutant lines with good agronomic traits

Besides the selection of high protein mutant men-

**Table 3.** Mutant lines with ideal plant type and good yielding characters from variety Jinheung.

| Mutant | Heading date | Culm length (cm) | No. of panicles per plant | No. of grains per panicle | 1000-grain wt(g) | Ripened grain (%) | Degree of blast susceptibility Leaf | Neck | Grain yield/10a |
|---|---|---|---|---|---|---|---|---|---|
| Mother variety | 28 Aug. | 75.1 | 12.9 | 105 | 22.7 | 87.8 | M | S | 100 |
| Mutant No. 2 | 22 〃 | 70.9 | 16.0 | 93 | 21.9 | 95.0 | R | R | 114.9 |
| 1761 | 24 〃 | 70.7 | 15.7 | 91 | 21.9 | 93.1 | S | 〃 | 106.9 |
| 2578 | 18 〃 | 75.1 | 10.9 | 111 | 25.0 | 94.2 | R | 〃 | 106.1 |

tioned above, a few lines selected in 1973 and 1974 based on the morphological or agronomic traits were ideal in their plant type. They were short-culmed, earlier heading, blast-resistant, and higher yielding as compared with mother variety. Among them mutant No. 2 appears to be most promising (Table 3).

Their protein percents were nearly equal to the original variety, but varied with the year.

### 3. Test for blast disease

Leaf blast was examined according to the standard method practiced in International Rice Research Institute (IRRI). Neck blast was tested by raising the mutants at the corner of the Institute Farm under disease-inducing conditions of dense planting, heavy nitrogen application and without fungicide application.

Most of the mutant lines were resistant to both leaf and neck blast(Tables 2, 3). Subsequently further tests are considered necessary before confirmation of the resistance, because it has often been experienced that the high protein mutant lines were tended to be rather blast-susceptible or manifestation of resistance was different depending on year and location. The same is true in most of the other characters in case of mutant lines.

### 4. High protein mutant No. 398

398($M_{10}$ plant generation, 1974), selected from Hokwang variety, has two drastically changed characters of short culm and early maturity besides the 30% increase of protein content. The grain yield is comparable to mother or less, depending on years. Grain size and shape is similar to those of mother (Tables 4, 5).

**Table 4.** Agronomic characteristics of high protein mutant No. 398. *

| | Year | Heading date | Culm length (cm) | No. of panicles per plant | No. of grains per panicle | 1000-grain wt(g) | Ripened grain (%) |
|---|---|---|---|---|---|---|---|
| Mother variety Hokwang | 1971[a] | Sep. 4 | 85.8 | 16.6 | 87.8 | 19.9 | 92.1 |
| | 1972[b] | Sep. 18 | 84.1 | 18.7 | —[d] | — | — |
| | 1973[c] | Aug. 22 | 78.2 | 13.7 | 88.8 | 21.9 | 97.8 |
| Mutant No. 398 | 1971 | Aug. 3 | 61.4 | 16.0 | 90.6 | 20.5 | 78.9[e] |
| | 1972 | Aug. 6 | 60.8 | 21.9 | 105.2 | 20.3 | 91.2 |
| | 1973 | July 28 | 55.3 | 13.2 | 81.9 | 21.8 | 97.2 |

* $M_9$ plant in 1973.
a Ordinary weather.
b Year of unusual cold weather.
c Hotter than average year.
d Poor maturity due to cold weather.
e Susceptible to neck blast.

**Table 5.** Crude protein, grain weight, and weight of embryo and endosperm of high protein mutant No. 398*.

| | Year | Protein (%) | 1000-grain wt(g) | mg protein per grain | Wt of embryo & endosperm per grain(mg) | | Endosperm wt / Embryo wt | Prot. amount of emb. & endosp. per grain (mg) | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Embryo | Endosperm | | Embryo | Endosperm |
| Mother variety Hokwang | 1971 (Heavy N-fertilizer) | 8.63 | 20.78 | 1.793 | 0.507 | 20.27 | 39.98 | 0.101 | 1.697 |
| | 1973 (Normal N-fertilizer) | 6.22 | 21.92 | 1.363 | 0.568 | 21.35 | 37.57 | 0.107 | 1.243 |
| Mutant No. 398 | 1972 (Heavy N-fertilizer) | 11.24 | 21.21 | 2.384 | 0.612 | 20.60 | 33.45 | 0.128 | 2.213 |
| | 1973 (Normal N-fertilizer) | 8.42 | 21.77 | 1.833 | 0.537 | 21.23 | 39.51 | 0.142 | 1.686 |

* $M_{10}$ seed in 1973.
Poor maturity due to cold weather in mother variety in 1972 and no result due to neck blast in 1971.

It was crossed back to mother in late summer of 1973. The purpose of backcross was (1) to introduce the high protein to mother, (2) to examine the gene(s) controlling the protein, (3) to clarify the linkage or pleiotropism between the high protein and culm length or earliness. Being anxious to know how the mutated characters segregate in $F_2$, half of $F_1$ seeds was sown in late autumn that year, $F_1$ generation was raised through the winter of 1973 − 1974, harvested in late June 1974 and the $F_2$ seed was sown immediately(this experiment is designated tentatively as EXPT A). The rest of the seeds was sown in the spring of ordinary sowing season in 19 74 and harvested in the autumn the same year (EXPT B).

The culm length and maturity of $F_1$ in the test B were shown in Table 6. Unlike our expectation of complete dominance, both characters were intermediate showing no dominant-recessive relationship.

**Table 6.** Culm length and heading date of $F_1$ and its parents, Hokwang and mutant No. 398.

| Trait | Hokwang | $F_1$ | 398 |
|---|---|---|---|
| Culm length (cm) | 84.3±2.16[a] (20)[b] | 76.3±2.96 (35) | 65.7±0.95 (20) |
| Heading date | 28 Aug. | 14 Aug. | 5 Aug. |
| (Days from sowing to heading) | 129.9±1.69 (20) | 116.5±2.14 (35) | 107.0±1.39 (20) |

a Standard deviation.

b No. of individuals examined.

The segregation of culm length and heading date in $F_2$ generation and protein percent in $F_3$ seed in the test A was unclear. As the $F_2$ seeds were sown at the end of June (ordinary sowing time is mid-April), both growth and maturity were extremely poor and $F_3$ seeds of most of the segregants became small-sized or immature. Although it is hardly possible or premature with these inaccurate results to assess the number of genes controlling the abovementioned characters or to ascertain their linkage or pleiotropic relations, it is interesting to find, among the segregants, individual with short culm-early maturity-low protein percent, indicating that protein is inherited independently with the other two mutated characters. Long culm-late maturity-high protein segregant was also obtained, but its high protein was considerd possibly due to the small grain and insufficient maturity caused by late maturity which was the result of forced heading in the green house. The accurate assessment of the segregation in $F_2$, however, will be conducted in 1975 using plants of $F_2$ generation which are raised in normal conditions.


## 摘　　要

(1) 振興에 放射線을 處理하여 얻은 高蛋白突然變異系統(1974年에 $M_4$世代, 種子는$M_5$)들에 對해 3個 地城에서 特性調査, 單位面積當蛋白質收量, 種子의 收量調査等을 實施하였고, 한편 (2) 湖光에서 얻은 高蛋白이면서 短稈, 早熟인 變異系統 398(1974年에 $M_{10}$, 種子는 $M_{11}$)에 對해 變異形質들을 支配하는 因子와 이의 多發性(또는 連關)如何를 究明하기 爲해 이를 母品種과 交雜하여 $F_1$, $F_2$世代에 있어서 變異形質들의 發現 및 分離를 調査하였다.

1. 振興由來의 高蛋白變異系統들의 蛋白質含量은 年次 및 地城間에 變異는 있으나 母品種에 比해 모두 높고 種子收量은 母品種과 비슷하거나 增加되었다.

2. 이들의 熟期는 5-10日 단축되었고, 稈長은 1系統을 除外하고는 短稈化되었고, 葉 및 穗首稻熱病의 抵抗性은 比較的 强한 편이었다.

3. 高蛋白變異系統 398과 母品種 湖光과의 $F_1$에서 稈長, 熟期는 兩親의 中間型으로 나타났다. $F_2$에서 短稈, 早熟이면서 低蛋白인것이 分離되어 高蛋白因子와 기타 變異形質과는 獨立的으로 遺傳되는것 같으나 $F_2$世代와 $F_3$種子가 非正常的 環境下에서 生育 및 成熟했기 때문에 確實치 않고, 正確한 것은 正常環境에서 자란 $F_2$世代에서 밝혀질 것이다.


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