Grain Characteristics and Inheritance of Green-kerneled Rice (*Oryza sativa* L.)

Sang-Ho Chu¹, Ho-Hoon Lee¹, Su-Noh Ryu², and Hee-Jong Koh¹*

¹School of Plant Science, Seoul National University, Seoul 151-742, Korea  
²Korea National Open University, Seoul 110-791, Korea

Abstract : This study was conducted to characterize and to elucidate the inheritance mode of green-kerneled rices which were selected in rice germplasms collected in China. Three green-kerneled varieties (Hexi 41, Beijingluimi-1, and Jilinluimi-1), three regular white varieties (Ilpumbyeo, Dasanbyeo, and Hwacheong *wx sgr*) and 11 hybrid populations crossed between them were used. To investigate the environmental variation, plant materials were grown under two levels of nitrogen-fertilizer and under different ripening temperatures. The green-kerneled rices maintained green color in pericarp even after physiological maturity stage because the chlorophyll was faded away slowly. Hexi 41 showed the highest green color among green-kerneled germplasms, and followed by Beijingluimi-1. The greenness of dehulled rices of Hexi 41, Beijingluimi-1 and Ilpumbyeo were not significantly affected by different nitrogen-fertilizer levels. High temperature during ripening significantly decreased the greenness of brown rices of Hexi 41, Beijingluimi-1 and Ilpumbyeo. The greenness of brown rices was found to be inherited in a quantitative fashion implying that polygenes were involved. The broad-sense heritabilities ranged from 0.51 to 0.92, suggesting that selection for greenness in early generations might be feasible. However, there was a strong correlation between the greenness and days to heading, indicating that the greenness was largely affected by air temperature during ripening as well as by the genotype. The strategy for breeding green-kerneled rice varieties in relation to heading date was discussed.

Key words : Green-kerneled rice, greenness, chlorophyll, heading date

INTRODUCTION

The endeavors to enhance the grain quality in rice were steadily continued during 1980s ~ 90s to meet the increasing market demand of high-quality rices (Choi 2002). With the elevation of living standard, consumption per capita of rice is being decreased, but the trend in rice consumption becomes more diversified and quality-oriented. In addition, internationally growing pressure for free trade of rice has been threatening our rice production industry due to the relatively low competitiveness of domestic rice in terms of price. For these reasons, rices with diversified quality for special purposes, such as rices of special-taste, functional rices to human health and rices for processing, have been extensively studied.

The various colored rices from white to red/black are reported, which contained more protein, vitamins, minerals, and antioxidants than white rice (Choi & Oh 1996, Gu & Xu 1992, Kim *et al.* 1994, Koh *et al.* 1996). And they are expected to be useful in breeding programs for quality diversification. We identified some green-kerneled rices in germplasms introduced from China. The green-kerneled rices have green color in brown rice even after physiological maturity due to retained chlorophyll in the pericarp (Hoshikawa 1989). However, the green-kerneled rices have not been payed attention until recently as a novel source for diversified qualities in rice.

This study was carried out to characterize and to elucidate the inheritance mode of green-kerneled rices to obtain basic information for breeding green-kerneled rice varieties.

MATERIALS AND METHODS

Plant materials and cultivation

The green-kerneled rices used in this experiment were Hexi 41, Jilinluimi-1 and Beijingluimi-1, which were identified in germplasms introduced from China. They were crossed with regular white rices, Ilpumbyeo, Dasanbyeo, and Hwacheong *wx sgr* and then the F₂ populations were used for inheritance study.

The parents and F₂S were seeded on plastic-tunnel nursery bed in Experimental Farm of Seoul National University at Suwon on April 27, and transplanted on May 31 in one plant per hill with a distance of 30 × 15 cm. Fertilizers were
applied at N-P₂O₅-K₂O=100-80-80 kg/ha. P₂O₅ and K₂O were applied as basal fertilization only, while N was applied with a ratio of 0.50 : 0.25 : 0.25 (basal stage : tillering stage : panicle-initiation stage). Field management and chemical inputs for disease and pest control followed the standard methods of the Experimental Farm.

To know the effect of N fertilizer on greenness, two levels of N fertilizer, 100 kg/ha and 50 kg/ha, were applied. For the treatment of different temperatures at ripening stage, the plants of four varieties were placed in both greenhouse and field. To minimize the possible error by the difference of nutrition status of the plants between in the field and greenhouse, plants grown in the field were moved into the greenhouse at panicle initiation stage.

**Measurement of characters**

Panicles of five varieties in two nitrogen levels were labeled at heading date with tape binder, sampled from 20 days after heading (DAH) to 45 DAH with a five-day interval, dried in glass house for 3 days and dehulled. One panicle of each plant from eleven F₂ populations was labeled at heading date with tape binder and sampled at 40 DAH.

The greenness of dehulled rices was measured by colorimeter (MINOLTA CR-200, Japan) with three replications. Hunter values were L (lightness), a (from red to green; +100 (red) ~ -80 (green)), and b (+ (yellow) ~ - (blue)). Hunter ‘a’ (red ~ green) value of each sample was adopted to evaluate the greenness of samples.

To measure the chlorophyll content, the 100 dehulled rice grains were ground into fine powder in a cyclone sample miller. Chlorophyll was extracted from the powder with 5 ml of 80% acetone for 10 minutes in 50 polypropylene tube. The tube was centrifuged 30 min at 3000 rpm., and the absorbance of the upper phase was measured by spectrophotometer (BECKMAN COULTER Du 800, Germany).

The broad-sense heritability ($h²_B$) for greenness in F₂’s was calculated as follows:

$$h²_B = \frac{VF₂ - 1/3(Vp₁ + Vp₂ + VF₁)}{VF₂}$$

($Vp₁$, $Vp₂$ = parental variance, $VF₁$ and $VF₂$ = $F₁$ and $F₂$ variance)

**RESULTS**

**Varietal variation of greenness**

The greenness of dehulled rices harvested at 40 DAH of Hexi 41, a germplasm selected as a green-kerneled rice, was compared with a regular white rice cultivar, Ilpumbyeo. As shown in Fig. 1, visual appearance of Hexi 41 was remarkably green. Chlorophyll pigmentation which maintained the kernels green remained in pericarp as in Fig. 2. Fig. 3 shows the changes in greenness of selected green-kerneled rice germplasms during grain filling, compared to regular white varieties, Ilpumbyeo and Dasanbyeo. The greenness of dehulled rices before 20 days after heading (DAH) appeared similar among germplasms, however, after 20 DAH, the difference in greenness became larger. At 40 DAH, the greenness of dehulled rices almost disappeared in Jilinluimi-1 and Dasanbyeo, remained a little in Beijinguimi-1 and Ilpumbyeo, and was largely sustained in Hexi 41. Hexi 41 seemed to be a good genetic resource for breeding green-kerneled rice varieties.

Chlorophyll was extracted from dehulled rices of four varieties and the absorbance was measured with spectrophotometer. Only one peak at 663 nm, which corresponded to chlorophyll $a$, was observed in all of the samples (Fig. 4).
The reason why chlorophyll b was absent in pericarp remained to be studied. There was a highly positive correlation ($r^2=0.9042$) between the greenness (a) value measured by colorimeter and the absorbance value measured by spectrophotometer, indicating that the darker the greenness, the more the chlorophyll concentration (Fig. 5).

**Environmental variation**

The heading dates of Hexi 41 and Beijingliumi-1 varied a little under different N fertilization. The greenness of kernel appeared not to be significantly affected by N levels (Fig. 6).

Though heading dates of the varieties in greenhouse were similar to those in the field, the greenness of the varieties in the field and greenhouse was significantly different from each other except Dasanbyeo (Fig. 7). The absorbance data by spectrophotometer confirmed the remarkable difference of greenness between in the field and in greenhouse (Fig. 7). Hexi 41 showed the highest greenness value among varieties at 25 DAH both in the field and greenhouse, although the greenness of Hexi 41 grown in the field was much higher than that grown in the greenhouse. Particularly in Dasanbyeo, greenness was almost faded out at 30 DAH even in the field, suggesting that grain filling period was shorter or that degradation process of chlorophylls in pericarp was more rapid than other varieties.

**Inheritance of green-kerneled rice**

The seven F2 populations were made from the crosses between green-kerneled varieties and regular varieties and four F2 populations were made from the crosses among three green-kerneled varieties. F3 seeds harvested at 40 DAH from each F2 plant were scored for greenness using colorimeter. Frequency distributions of greenness degree (a) in F2 populations are shown in Fig. 8. In every cross, the greenness was segregated in a quantitative fashion, indicating that the greenness might be controlled by polygenes. Even F2 populations between three green-kerneled varieties exhibited the similar segregation pattern to those between green-kerneled varieties and regular varieties. This also means that two germplasms, Jilinliumi-1 and Beijingliumi-1, might not be valuable as germplasms for breeding green-
Grain Characteristics and Inheritance of Green-kerneled Rice (*Oryza sativa* L.)

kerneled rices in Suwon area. Transgressive segregants toward dark greenness were observed in some F2 populations. However, only a few transgressants showing darker greenness than Hexi 41 could be selected. The broad-sense heritabilities ($h^2_B$) in F2 for greenness ranged from 0.51 to 0.92 as in Fig. 9, suggesting that selection for greenness in early generations might be effective.

There were significant correlations between heading date and the greenness of rice kernel in all of the F2 populations tested (Fig. 10). The later the heading date, the darker the greenness. However, large variations even among the plants showing the same heading date were also observed, indicating that greenness of grain was affected by genotypes as well as temperature during ripening. At any rate, we may infer from these results that late-heading genotypes should be selected in breeding programs for greenness.

Dark green-kerneled plants in F2 populations which significantly deviated from the regression line in Fig. 10 were selected for generation advancement. Some of the selected plants exhibited darker greenness than Hexi 41 and were expected to be promising (Table 1).

**DISCUSSION**

The green-kerneled rices maintained green color in pericarp even after physiological maturity stage because chlorophyll in the seed coat faded away slowly. Hexi 41 showed the darkest green color among green-kerneled germplasms. Beijinlului-1 also showed the green color. However, Jilinluimi-1 originally collected as a green-kerneled rice germplasm from Northern China, seemed a regular white variety, probably due to that fact that early heading in Suwon might caused the rapid degeneration of chlorophylls on pericarp by high air temperature during grain filling.

The greenness of rice kernels was not significantly affected by the different levels of nitrogen fertilization. This may correspond to the previous reports that grain filling duration and rate were not affected by nitrogen levels but by genotypes (Cho *et al*. 1987). The greenness of rice kernels grown in the field remained longer during ripening period.

---

**Fig. 6.** Changes on greenness (a) of two varieties by DAH grown under medium N (100 kg/ha) and low N (50 kg/ha) fertilizer.

**Fig. 7.** Comparative changes on greenness (a) value (left) and absorbance ($A_{663}$) of four varieties by DAH in the field (F) and greenhouse (G).
Fig. 8. Frequency distribution for greenness (a) of grains in F₂ populations.

than in the greenhouse except Dasanbyeo. This might be attributable to the relatively higher temperature in the greenhouse which shortened the ripening period as previous reports (Ahn 1973, Lee 1983, Kwon and Park 1988, Lee et al. 1996) and subsequently cause the faster degradation of chlorophylls in pericarp. However, as seen in Fig. 6, shorter duration of ripening period might not be the unique factor which affected the rapid degradation of chlorophyll, because the greenness was almost disappeared even in 25 - 30 DAH in the plants grown in greenhouse, which was not enough period of time for full ripening (Kwon & Park 1988). In Dasanbyeo, the greenness almost faded out at 30 DAH even in the field, suggesting that grain filling period was shorter or that degradation process of chlorophylls in pericarp was faster than other varieties. This may correspond to the reports that, in Tongil type cultivars, the ripening period was shorter and the ripening speed was faster than in japonica cultivars (Cho et al. 1987, Choi 1986, Kwon & Park 1988).

Fig. 10. Regression graphs between days to heading and greenness (a) of grains in F2 plants (doted line : ±σ).
Greenness of brown rices was found to be controlled by polygenes, however selection for greenness in early generations might be feasible inferring from the relatively high heritabilities in F2.

Greenness of rice kernels are largely affected by the air temperature during ripening because degradation process of chlorophyll might be accelerated in higher air temperature. So, in order to optimize temperature range at ripening stage to keep the kernels green and fully riped, optimum heading date and harvesting time have to be decided in terms of both breeding and cultivation.

The green-kerneled rices used in this study were affected by not only meteorological factors but also genetic factors. And we could select some dark-green rice lines which showed transgressive segregation. Thus, breeding program for the green-kerneled rice should be considered in relation to the environmental conditions, particularly temperature where the varieties will grow. And the production method should be set up for each green-kerneled variety to maximize the greenness.

**ACKNOWLEDGEMENTS**

This work was supported in part by a grant (LS0101) from Rural Development Administration and in part by a grant (201030-3) from Agricultural R&D Promotion Center (ARPC), Ministry of Agriculture & Forestry.

**LITERATURES CITED**


<table>
<thead>
<tr>
<th>Cross combination</th>
<th>Average greenness of grains harvested from F2 plants</th>
<th>Selected F2 plants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a&lt;sup&gt;1&lt;/sup&gt;</td>
<td>No.</td>
</tr>
<tr>
<td>Hexi 41 / Ilpumbyeo</td>
<td>-5.03</td>
<td>121</td>
</tr>
<tr>
<td>Hexi 41 / Hwacheong wx sgr</td>
<td>-4.42</td>
<td>134</td>
</tr>
<tr>
<td>Jilinluimi-1 / Ilpumbyeo</td>
<td>-0.54</td>
<td>117</td>
</tr>
<tr>
<td>Jilinluimi-1 / Hwacheong wx sgr</td>
<td>-1.20</td>
<td>124</td>
</tr>
<tr>
<td>Beijingluimi-1 / Ilpumbyeo</td>
<td>-2.99</td>
<td>139</td>
</tr>
<tr>
<td>Beijingluimi-1 / Hwacheong wx sgr</td>
<td>-3.28</td>
<td>114</td>
</tr>
<tr>
<td>Hexi 41 / Beijingluimi-1</td>
<td>-3.43</td>
<td>125</td>
</tr>
<tr>
<td>Jilinluimi-1 / Beijingluimi-1</td>
<td>-2.37</td>
<td>179</td>
</tr>
<tr>
<td>Beijingluimi-1 / Hexi 41</td>
<td>-3.77</td>
<td>133</td>
</tr>
<tr>
<td>Hexi 41(P)</td>
<td>-5.50</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>The greenness (a) of dehulled rices was measured by colorimeter (MINOLTA CR-200, Japan). Hunter value ‘a’ represents the greenness of grain samples; +100(red) ~ -80(green).