Investigations on the red rice pigmentation have been carried out for about forty-five years since the time of Kikkawa (1912). In respect to the colored layers of the red rice, several reports on the histological researches have been published, but there are some disagreements among them. For examples, Kikkawa (1912) and Parnell et al. (1917) reported that pigments of the red rice were found in the pericarp, Kondo (1917) and Ishikawa and Shibuya (1930), however, reported the colored layer was the seed coats; and furthermore, Nagao (1951) observed the pigments in both those two parts.

The present authors considered that it was necessary to investigate the pigment of the red rice from the view point of biochemistry in order to make clear which observation was correct and what was the cause of the disagreements among those earlier investigators.

Although reports of chemical research on the red rice pigments were published by Nagai (1921, 1926), it can not be recognized that the experiments performed by him were enough to make the properties of the red rice pigments clear, especially because he did not attempt to make any identification with any known substances. Consequently, the name "protocyan" which was proposed by him as a chemical name for the red rice pigments is not based on any fully reliable background. Recently, one of the present authors detected catechol tannin from the red rice (Miyamoto 1954), and after that, the authors have got some results in their experiments concerning the chemical properties and the location of the pigments. It is an aim of this paper to report on their accounts and, at the same time, to make some considerations about the relationship between the pigments and the genes, $Rc$ and $Rd$, which are concerned with the pigmentation.

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Materials

Samples used in this research were the materials that had been collected in the Institute of Plant Breeding, Hokkaido University and were derived from plants bred true over many years by members of the staff there.

It was reported by Nagao and Takahashi (1947) and Nagao (1951) that $Rc$ and $Rd$ are the genes being concerned with the red rice pigmentation and $Rc \, rd$ with the brown rice pigmentation respectively, so the following varieties or strains having genes of $Rc \, Rd$, $Rc \, rd$ and $rc \, rd$ were tested.

<table>
<thead>
<tr>
<th>Genes</th>
<th>Varieties or Strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Rc , Rd$</td>
<td>Akamuro, Tokachikuromomi</td>
</tr>
<tr>
<td>$Rc , rd$</td>
<td>Funentairyu, H-20$^1$</td>
</tr>
<tr>
<td>$rc , rd$</td>
<td>Chabo, Murasakiiine, O-aoge</td>
</tr>
</tbody>
</table>

The pericarp and the seed coats (including aleuron layer) were used for qualitative and quantitative analyses. They were separated from the starch layer in the green neck stage because when the grain is ripen, they stick together and it becomes hard to remove them separately.

Experiments

A warm water extract of the sample was cooled down and qualitatively analysed according to Klein’s procedure. Results obtained by that procedure are shown in the table 1.

The results in table 1 reveal that pericarp and seed coats of the red rice and the brown rice contain a considerable amount of catechol tannin, and the main pigments causing these pigmentation have to be regarded as a series of catechin, catechol tannin and phlobaphene.

In order to verify that view, the authors estimated the catechin + catechol tannin contents in the samples according to the method which was described in their previous paper (1956: unpublished). Phlobaphenes were not estimated because they were insoluble in warm water, but the content of phlobaphenes in those samples seemed not to be very large.

Table 2 shows (1) the parallelism between the intensities of pigmentation and the catechin + catechol tannin contents of the red rice and the brown rice, and (2) that only traces of the pigments are localized in the white or ordinary rice. In order to gain further evidence that the pigments of the red rice are series of catechol tannin and phlobaphene, the pigments were hydrolysed with potassium

$^1$) A bred true progeny of a hybrid.
hydroxide. As catechol tannin is known to be converted into protocatechuic acid and phloroglucin when hydrolysed with potassium hydroxide, it is necessary, if the above view is correct, that these two substances should occur when the pigments are hydrolyzed with potassium hydroxide. In other words, the following formula must be satisfied.

$$\text{KOH} \rightarrow \text{protocatechuic acid} + \text{phloroglucin}$$

About 60 cc of warm water extract from the seed coats of Tokachikuromomi (3 gm) was added 20 cc of 20% potassium hydroxide and boiled, then solution colour changed from brownish yellow to red and distilled phloroglucin which was detected by d-xylose and hydrochloric acid. As protocatechuic acid is known to show a
colour reaction turning red with hot potassium hydroxide, this may be another positive proof for authors' views concerning the chemical properties of the pigments.

**Discussion**

The chemical properties of protocyan, the pigment of red rice, were described by Nagai (1926) as follows: (1) protocyan was easily soluble in a hot 5% potassium hydroxide, and (2) an alcohol solution of the chromogen of protocyan changed red when hydrochloric acid was added to it and heated.

So, if the pigments were composed of various series of catechin, catechol tannin and phlobaphene as the authors proposed in the present paper, the above mentioned properties of protocyan had to be checked whether they could be explained by the properties of the series. Property (1) was actually recognized upon the hydrolysisation of the pigments with potassium hydroxide. The second (2) was also observed and described in the table 1. That reaction was based on the fact that the colourless and water soluble catechin and brownish yellow catechol tannin which is also water soluble, are polymerized into brownish red and water insoluble phlobaphene when they are heated with hydrochloric acid.

The series of substances were found in both pericarps and seed coats (including aleuron layers) of the red rice and the brown rice. It could be a chemical proof in support of the opinion of Nagao (1951) in respect to the pigment localization of the red rice and the brown rice. The disagreements among the results obtained by the previous investigators should be explained by the differences in length of the observations, because in vivo, the polymerization from catechin to phlobaphene proceeds slowly, furthermore red phlobaphene is visible but colourless catechin is invisible.

There are many Hay's russet spots on the pericarp of the brown rice, and sometimes those spotted sites are sunken. Probably, those spots are caused by the precipitation, in other words, the extreme accumulation of the pigments which coagulate the proteins of living cells around them.

In respect of the influences of gene Rc and Rd, the data presented in the table 2 reveal that Rc is responsible for accumulation of the pigments in pericarps and seed coats of the brown rice: further, the red rice needs another gene, Rd, to increase content of the pigments, so Rd has a quantitative effect. The pigments seem to be accumulated from other parts of the rice plant in which the authors (1956, unpublished data) have found a considerable amount of the pigments.

**Summary**

The chemical properties of the pigments occurring in the surface layers of the

1) Name of Ridgway's Color standards and color nomenclature.
brown rice and the red rice were studied. From the results of the qualitative analyses on warm water extract of the pigmented parts, pericarps and seed coats (including aleuron layers), the authors have arrived at the conclusion that the pigments are series of catechin, catechol tannin and phlobaphene.

Then, catechin+catechol tannin contents of those parts were estimated. A greater amount of the catechin+catechol tannin was estimated from the red rice and the smaller from the brown rice. Consequently, the brown rice gene, \( R_c \), is regarded to have an effect of accumulating the pigments in the pigment layers, and \( R_d \), which is a gene of the red rice when \( R_c \) is present, is regarded as causing a greater accumulation of the pigments than in case of the brown rice.

**Literature Cited**


Klein, P. 1932: Handbuch der Pflanzenanalyse Bd. 3.


