

Variation of 2-Acetyl-1-Pyrroline Concentration in Aromatic Rice Grains Collected in the Same Region in Japan and Factors Affecting Its Concentration

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Abstract : Aroma strength of aromatic rice varies with the genetic and environmental conditions. We determined the concentration of 2-acetyl-1-pyrroline (2AP), a key compound of the aroma of aromatic rice, in 62 samples of rice grains (brown rice) from 'Hierì' produced by 17~24 farmers in 3 years in the Kubokawa area of Kochi Prefecture, Japan. Many of them showed similar values and the standard deviations were 27~31%. However, a few samples showed extremely high (200%) or low (60%) 2AP concentrations compared to the individual year averages (100%). The influence of harvest time and temperature during ripening on the 2AP concentration in the brown rice was also examined using two cultivars. During grain development in an early-heading cultivar 'Miyakaori', the 2AP concentration in the brown rice reached a peak at 4 or 5 weeks after heading (WAH) and then decreased rapidly to 20% of the maximum at 7 or 8 WAH. In a late-heading cultivar 'Hierì', the 2AP concentration peaked at 4 WAH then gradually decreased to 40% of the maximum at 8 WAH. The 2AP concentration was higher in brown rice ripened at a low temperature (day : 25°C/night : 20°C) than that ripened at a high temperature (day: 35°C/night: 30°C) in both a short-grain cultivar 'Hierì' and a long-grain cultivar 'Sari Queen'.

Key words : Aromatic rice, Harvest time, *Oryza sativa* L., 2-Acetyl-1-pyrroline, Temperature, Variation.

Aromatic (scented, flavored, fragrant, or pecan) rice, a rice type that emits a special aroma when cooked, has been cultivated mainly in South and South-East Asian countries from ancient times and is highly prized and deeply tied to folk customs (Itani, 1993). Among aromatic rice cultivars, 'Basmati' is the best known in the world rice market. It is said that 'Basmati' is not aromatic when grown outside the Punjab in Pakistan and India, and that in addition to the genetic factors the environmental condition, i.e., the Punjab climate and/or soil, is important for producing a strong aroma (Efferson, 1985). 'Khao Dawk Mali 105' (or 'Jasmine Rice'), the most important aromatic rice cultivar in Thailand, is reported to have the strongest aroma and the best quality when grown in the Tung Kula Rong Hai region in Northwest Thailand (Yoshihashi, 2001).

In Japan, aromatic rice has been cultivated on a small scale for religious purposes, festivals, special guests or daily use in many regions for over a thousand years. However, as improved non-aromatic rice cultivars with good quality and high yield have spread, small-scale production has disappeared, resulting in a decline in the number of aromatic rice

cultivars. Aromatic rice cultivars are now cultivated only for household use in the inner-mountainous areas in Japan. In Kochi Prefecture the farmers are systematically cultivating aromatic rice, 'Hierì' or 'Sawakaori' (Nakamura et al., 1996), for marketing under the direction of the Agricultural Cooperative Association (Nomura, 1985; Miyagawa, 1986; Itani, 1993, 2000), because farmers and consumers have learned that an improved taste can be obtained by blending 5~10% aromatic rice with the non-aromatic cultivars (Itani and Yamazaki, 1994). Thus, standardization of aroma quality of aromatic rice is required. Kubokawa area in Kochi Prefecture is the most active area for aromatic rice production in Japan (Itani, 1993; Fushimi and Ishitani, 1994). JA-Kubokawa (the Agricultural Cooperative in the Kubokawa area) has tried to convince farmers to produce aromatic rice with sufficient aroma quality and has developed guidelines for cultivars, fertilizer application, and drying methods (Itani, 1993).

In the present study, we focused on 2-acetyl-1-pyrroline (2AP) the key compound responsible for the specific aroma of aromatic rice (Buttery et al., 1983; Paule and Powers, 1989; Lin et al., 1990; Laksanalamai

Table 1. The profiles and characteristics of the brown rice of three aromatic rice cultivars used in this experiments.

Cultivars ^a	Origin	Heading date ^b	1000-grain weight ^c (g)	Length/width ratio ^c	Thickness ^c (mm)	Amylose Content ^c (%)	Protein Content ^{c,d} (%)
Hieri	selected from a local aromatic line in Kochi Pref.	8.22	25.2	1.49	1.9	20.5	8.5
Miyakaori	bred from a cross between a non-aromatic cultivar Ouu 282 and a local aromatic line in Miyagi Pref.	8.01	21.3	1.74	1.8	16.2	11.2
Sari Queen	bred from a cross between a non-aromatic cultivar Nipponbare and Basmati 370	8.25	15.8	2.76	1.5	18.8	9.9
Koshihikari (reference)	non-aromatic leading cultivar	8.06	22.2	1.72	1.8	18.0	9.2

a : All the cultivars originated in Japan. b : Average month and day in 1992-1996. c : Data in 1992 from Itani et al. (2002). d : On dry basis.

and Ilangantileke, 1993). 2AP is a chemical that emits a buttered-popcorn-like aroma at specific concentrations and is reported to be an important component of the toasted or sweet odor of wheat bread, popcorn, boiled beef and so on (Schieberle, 1991).

A distinct difference in aroma was recognized by farmers or merchants who dealt with aromatic rice in Kochi Prefecture. Therefore, we investigated the variations of 2AP concentration in the brown rice of the aromatic rice 'Hier' produced in Kubokawa area in Japan from 1992 to 1994. In addition, we examined the influence of harvest time and temperature during ripening on the 2AP concentration in the brown rice. Here we report interesting findings about these two factors among several factors suspected to affect the 2AP concentration from the JA guidelines.

Materials and Methods

1. Plant materials

We used mainly three aromatic rice cultivars that were the most popular in Japan (Table 1). 'Hier' is a local, late-heading, japonica-type, aromatic rice cultivar and 'Miyakaori' is an improved, early-heading one. 'Sari Queen' is a Japanese/Indian-'Basmati'-related, late-heading aromatic rice cultivar bred by Japanese Ministry of Agriculture, Forestry and Fisheries (Ando, 1991; Kaneda, 1992).

(1) Variation of 2AP concentrations in the aromatic rice samples collected in Kubokawa (Experiment 1)

Between 1992 and 1994, we collected brown rice grains of aromatic rice (cultivar : Hier) from farmers in the Kubokawa-area, Kochi Prefecture. In 1992, we obtained 19 samples produced by different farmers, in 1993, 17 samples from farmers and one sample mixed and dried in the country-elevator of JA-Kubokawa, and in 1994, 24 samples from farmers and one sample from the country-elevator. The total number of samples was 62. The brown rice samples were stored in a refrigerator before 2AP analysis every year.

(2) Change in 2AP concentration after heading (Experiment 2)

In 1994, we cultivated Miyakaori in the rice fields of Hiroshima Prefectural University and harvested panicles from two hills per plot at 26 to 56 DAH (days after heading). In 1996, we cultivated Miyakaori and Hier and harvested the former at 3 to 14 weeks after heading (WAH) and the latter at 3 to 10 WAH. The panicles harvested were dried under room conditions, and then threshed and hulled before 2AP analysis. The brown rice was not sieved.

(3) Effect of temperature during ripening on 2AP concentration (Experiment 3)

In 1994, we transplanted two hills of rice plants (Hier and Sari Queen) from the rice field to 1/5000 a (16 cm in diameter, 19 cm in height) Wagner-pots. At 10 DAH, the plants were put in an artificial climate room, and exposed to either a high temperature (day : 35°C /night : 30°C) or a low temperature (25°C/20°C) until ripening. We conducted a similar experiment with the same two cultivars in 1996. We exposed them from 3 DAH to a high temperature (day : 35°C/night : 30°C), moderate temperature (30°C/25°C) or a low temperature (25°C/20v) until ripened. Before analyzing 2AP concentrations, the panicles harvested were dried in a laboratory, threshed and hulled. The brown rice was sieved through 1.9 mm (Hier) or 1.5mm sieves (Sari Queen).

2. Analysis of 2AP

2AP was directly extracted from a small amount of rice flour with hot ethanol and analyzed using a gas chromatograph mass spectrometry in the SIM mode. The analysis was conducted at Shikoku National Agricultural Experiment Station in Zentsuji between 1992 and 1994 (method 1), and at National Research Institute of Brewing in Higashihiroshima in 1996 (method 2).

In method 1, the brown rice sample was ground into a powder (less than 0.5 mm in diameter), and

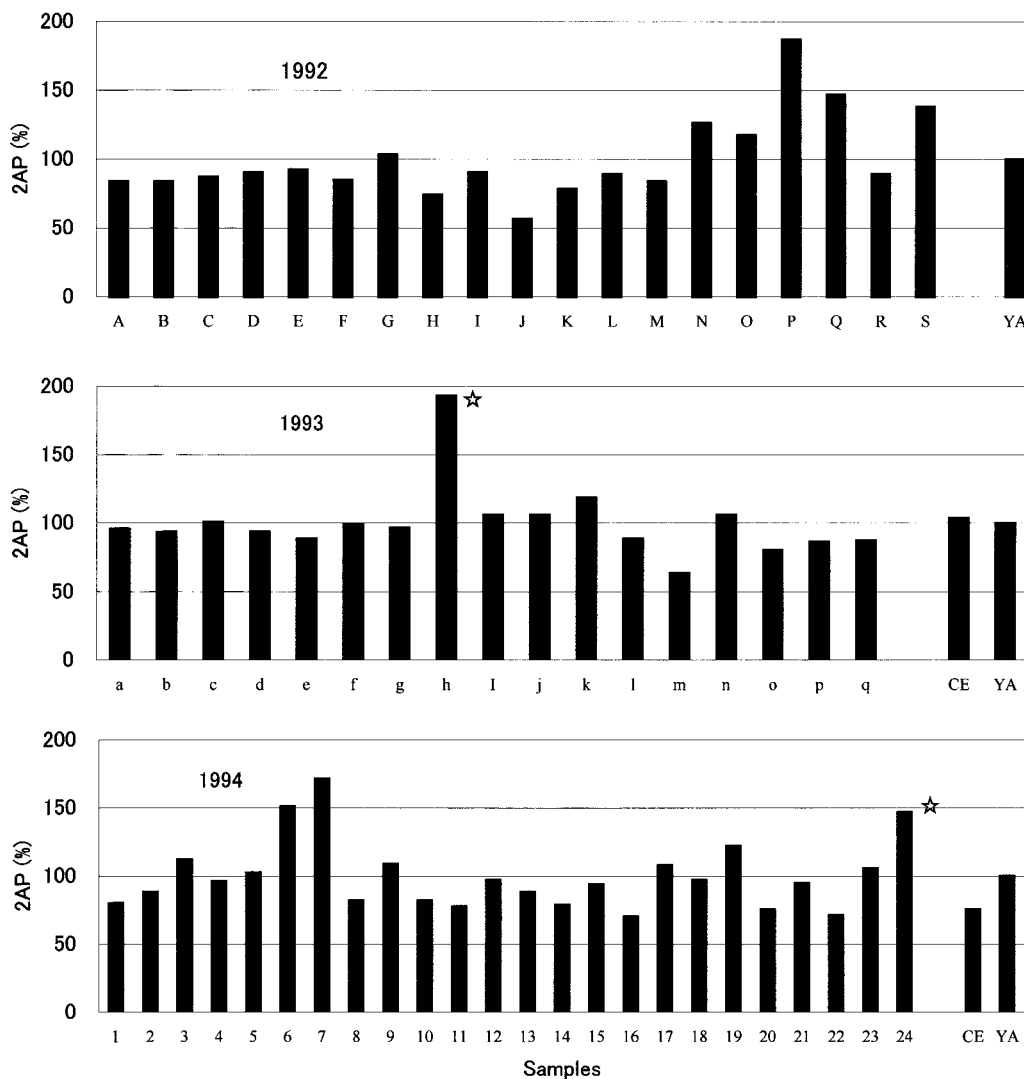


Fig. 1. Variation of 2-acetyl-1-pyrroline (2AP) concentrations in aromatic rice samples collected in the Kubokawa area, Kochi Prefecture in 1992, 1993 and 1994. The 2AP concentrations are shown as the relative value to each yearly average (YA). ☆ : sample cultivated after tobacco cultivation. CE : samples mixed and dried in the country-elevator.

0.08 g samples were placed into pressure-resistant microvials, and suspended in 0.8 ml 100% ethanol. 2AP was extracted in an 80°C water bath with shaking (160 cycles/min) for 60 minutes. After filtration through a membrane filter (pore size 0.45 μm), the filtrate was used for quantitative analysis of 2AP. We used the capillary GC/MS (GC : Shimadzu GC-14A, MS : Shimadzu QP-2000, capillary column : Shimadzu CBP1-S25-050). To adjust the sensitivity of the GC/MS, we measured an identical rice sample alternately with every sample (Fushimi, 1992; Fushimi and Ishitani, 1994).

In method 2, we extracted 0.5 g of rice powder with 2 ml ethanol. For the analysis, we used a Hewlett Packard 6890 gas chromatograph coupled to a Hewlett Packard 6890 MSD and 3-hexanol as an internal standard. The details of method 2 are described in a separate paper. The difference of method 2 to method

1 is the ratio of rice powder to ethanol and an internal standard.

Each sample was analyzed twice and three times in methods 1 and 2, respectively.

Results and Discussion

1. Variation of 2AP concentrations in the aromatic rice samples collected in Kubokawa (Experiment 1)

Fig. 1 shows the 2AP concentrations in the brown rice Hierri produced in 1992, 1993 and 1994 in the Kubokawa area. The values are shown as relative values of each yearly average (YA=100%). In 1992, 1993 and 1994, the 63% (12/19), 82% (14/17) and 58% (14/24) of the samples were within the range of $\text{YA} \pm 20\%$, and the standard deviations (%) were 30.6, 28.6 and 26.7, respectively. However, a few samples showed extremely high or low concentrations of 2AP. For example, the samples of P in 1992 and h in 1993 was almost 2-fold

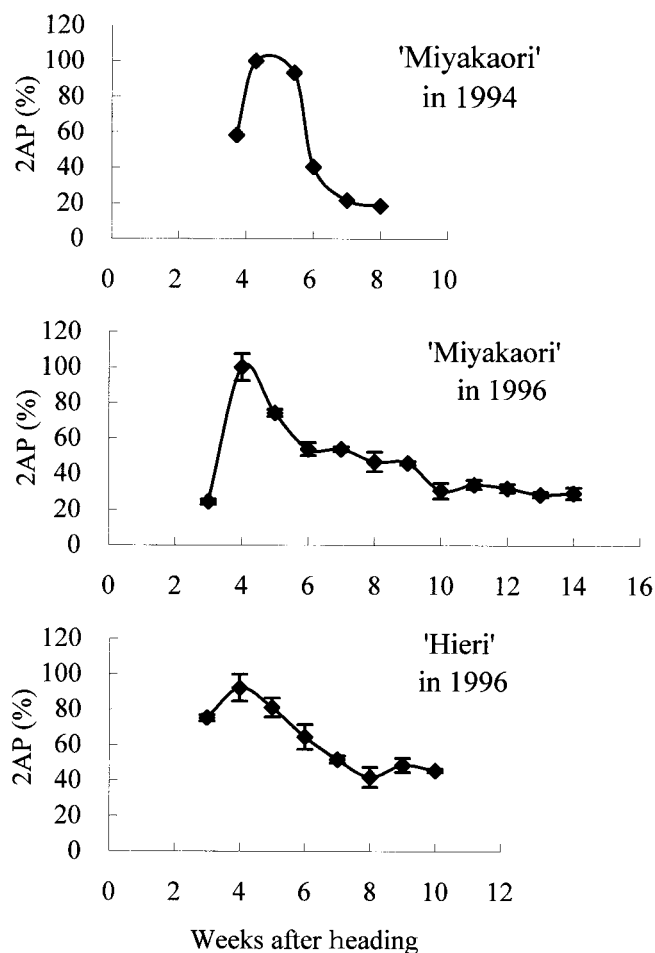


Fig. 2. Change in the 2-acetyl-1-pyrroline (2AP) concentrations in brown rice after heading. The maximum value of the relative 2AP concentration was plotted at 100%. The vertical bars in 1996 indicate standard deviations. Heading date : 25 July in Miyakaori in both years and 22 Aug. in Hieri.

of each YA and the samples of Q in 1992 and 6, 7, 24 in 1994 was 1.5-fold, while the samples in J in 1992 and m in 1993 were 60%. This suggests that the 2AP concentration in aromatic rice products of the same cultivar in the same area varied with the conditions of cultivation or soil.

We obtained an extremely high concentration of 2AP in the sample of h in 1993. This sample was cultivated as a succeeding crop after tobacco cultivation in that year. In the following year, sample 24 that was similarly cultivated also showed an extremely high 2AP concentration. According to the both farmers, aromatic rice cultivated as a succeeding crop after tobacco often had a strong aroma. This agrees with the findings in Experiment 3. The cultivation of aromatic rice as a succeeding crop after tobacco resulted in delayed rice planting, and therefore decrease in ripening temperature.

The sample from the country-elevator in 1993 showed almost the same concentration of 2AP to YA.

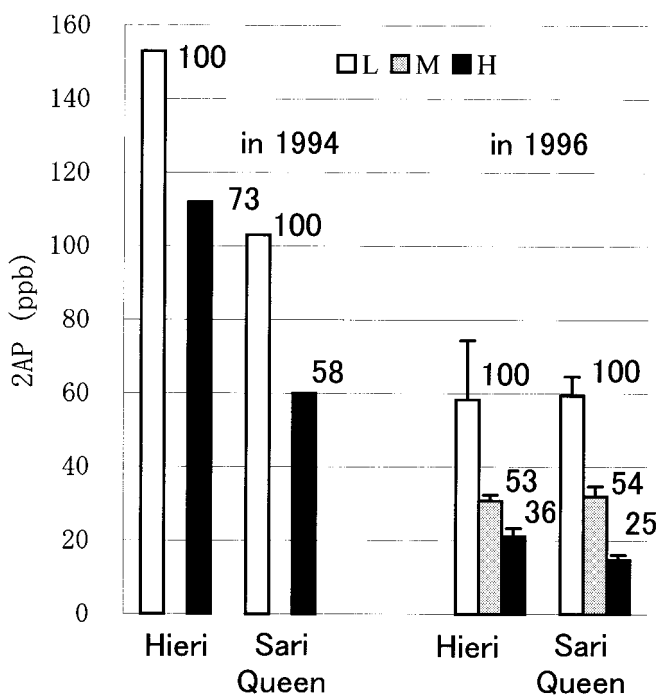


Fig. 3. 2-Acetyl-1-pyrroline (2AP) concentrations in brown rice under different temperature conditions during ripening : H : high (day : 35°C/night : 30°C), M : moderate (30°C/25°C) and L : low (25°C/20°C). In 1994, rice plants were exposed to H and L from 10 days after heading to ripening. In 1996, they were exposed to H, M and L from 2 days after heading to ripening. The number on the top of each column is the relative value to that at L in each year and cultivar. The vertical bars in 1996 show standard deviations.

This finding confirms that aromatic rice grains that were treated properly in the country-elevator did not lose as much 2AP as did the farmers' samples. However, the sample from the country-elevator in 1994 showed fairly low concentrations of 2AP in comparison with YA. Probably, the aromatic rice in that year was improperly treated during mixing and drying in the country-elevator. Unsuitable post-harvest conditions considerably reduce the concentrations of 2AP in aromatic rice. For example, the drying method of harvested raw grains is important for aroma. Drying at high temperatures lowers 2AP concentrations (Itani and Fushimi, 1996).

2. Change in the 2AP concentration after heading (Experiment 2)

As shown in Fig. 2, during grain development in 1994, the 2AP concentration in brown rice of Miyakaori showed a peak at approximately four or five WAH and then decreased rapidly. The concentration finally reached a level of 20% of the peak at 7 or 8 WAH. In 1996, the change in the 2AP concentration of Miyakaori showed a curve similar to that in 1994. The peak occurred at approximately 4 WAH and the 2AP concentration rapidly decreased thereafter. In

Hieri in 1996, the 2AP concentration peaked at four WAH and then gradually decreased to a level of 40% of the maximum at 8 WAH. The lower temperature to which Hieri was subjected during ripening might have caused the difference in the final concentration of 2AP between the two cultivars. The heading date of Hieri was 4 weeks later than that of Miyakaori.

3. Effect of temperature during ripening on 2AP concentration (Experiment 3)

The 2AP concentrations (on a dry basis) are shown in Fig. 3. The number on the top of each column is the relative value to that at L in each year and cultivar. The 2AP concentration was higher in brown rice ripened under a lower temperature in both Hieri and Sari Queen cultivars. The ratio of the 2AP concentration at a high or moderate temperature to that at the low temperature differed slightly with the cultivar or year. The relative concentration at a high temperature in 1996 was lower than that in 1994 in both cultivars.

The length of treatment may have affected the results because we started the experiment 8 days later in 1994. The results described above suggest that low temperature during ripening and early harvesting could increase 2AP concentration in brown rice.

The 2AP concentration in Hieri was higher than that in Sari Queen in 1994, but the two cultivars had similar values in 1996. The fertilizer level may be a possible effect of the difference. We observed recently that the level of N applied to the field changed the 2AP concentrations (data not shown). In both cultivars, all the rice grains ripened under a high temperature looked entirely chalky and those ripened under a low temperature a little chalky.

General Discussion

Aromatic rice has a distinct aroma in the field, and during harvest, storage, milling, and cooking. The strength of the aroma is considered to be very important, but there have been few studies on the aroma owing to the difficulty of sensory tests by panels and the easily volatilized aroma. In the present study, we focused on 2AP the key compound responsible for the aroma. In Experiment 1, extremely high or low 2AP concentrations were detected in aromatic rice samples produced by different farmers in the Kubokawa area in Kochi Prefecture. Both harvest time and temperature during ripening were suggested as possible causes. In Experiments 2 and 3, late harvest and high temperatures lowered the 2AP concentrations.

According to Lorieux et al. (1996), both Basmati and Thai rice are harvested at the beginning of winter in order to obtain a stronger aroma. Nakamura (1998) reported that aromatic rice cultivated at a high altitude or later than usual had a stronger aroma than that cultivated at a low altitude or according to the usual schedule. It is recently reported that 2AP is formed

from proline that is increased at dry conditions, therefore, 'Khao Dawk Mali 105', the most important aromatic rice cultivar in Thailand, have the strongest aroma and the best quality when grown at Tung Kula Rong Hai region, often suffered from drought (Yoshihashi et al., 2002; Yoshihashi, 2002). The region is located at a high altitude and often suffered from drought.

Concerning post-harvest conditions, the drying method of harvested raw grains is important for aroma. Drying rice at high temperature lowered 2AP concentrations (Itani and Fushimi, 1996). The 2AP concentration in an aged (shelf-stored) rice sample was about half that of a fresh rice sample of Thai aromatic rice (Khao Dawk Mali-105) (Laksanalamai and Ilangantileke, 1993).

There is a distinct difference in aroma strength among cultivars (Tsuzuki et al., 1977; Tripathi and Rao, 1979) and we observed a three-fold difference in 2AP of four cultivars cultivated under the same condition.

For excellent aromatic rice production, the Agricultural Cooperative Association of Kochi recommended the following: 1) proper selection of aromatic rice cultivars, 2) cultivation at a cool temperature and high altitude, 3) application of low levels of nitrogen, 4) harvesting earlier compared with ordinary cultivars, and 5) not drying at high air temperatures, and so on (Nomura, 1985; Itani, 2000). From the results of this study, early harvest and cool climate conditions are preferred for production of aromatic rice with a strong aroma.

Our findings agree with the above recommendations 2 and 4. Aroma strength varied with the genetic and environmental conditions (Pinson, 1994). Therefore, it is important to elucidate each factor that may influence aroma strength one by one.

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