JARQ 38 (2), 105 – 109 (2004) http://www.jircas.affrc.go.jp

Area Dependency of 2-Acetyl-1-Pyrroline Content in an Aromatic Rice Variety, Khao Dawk Mali 105

Tadashi YOSHIHASHI[1]*, Thi Thu Huong NGUYEN[2] and Nobuyuki KABAKI[3]

Crop Production and Postharvest Technology Division, Japan International Research Center for Agricultural Sciences (JIRCAS) (Tsukuba, Ibaraki 305–8686, Japan)

Abstract

It has been stated that the quality of the aromatic rice variety, Khao Dawk Mali 105 is influenced by the environment of the production area or cultivation method. In this study, 2-acetyl-1-pyrroline content of various rice samples in Thailand was investigated. The results showed that the samples from the Northeastern region, where Khao Dawk Mali 105 was cropped in rain-fed paddy fields, were higher in 2-acetyl-1-pyrroline content. The “Tungkularonghai” area, stated as being the highest quality rice production area, had the highest content among the areas in Northeastern Thailand. Samples collected from non-drought condition areas showed lower content even in the Tungkularonghai area. However, differences in seeding method did not affect 2-acetyl-1-pyrroline content in the Tungkularonghai area. These results showed that dry climate might be a factor for 2-acetyl-1-pyrroline content in Khao Dawk Mali 105. Since Khao Dawk Mali 105 is a photosensitive variety, it was expected that the ripening stage becomes uniform at a certain period which should be suitable for 2-acetyl-1-pyrroline formation in paddy fields.

Discipline: Food
Additional key words: Thailand, drought stress, Tungkularonghai

Introduction

Consumers have become more quality conscious about the rice they consume. Therefore, it is needed to focus on the quality of rice, which should be considered as the key to linking farmers to markets. When farmers become conscious of their rice quality, they are driven to produce better quality rice. Aromatic rice varieties are very popular in South and South East Asia and have recently gained wider acceptance in the USA, Europe and East Asia, especially in China[5,6]. Due to their characteristics such as aroma and flavor, they are highly favored, and command higher prices in markets. An aromatic variety, Khao Dawk Mali 105 (KDML105), is mainly produced in Northeastern Thailand. Demand for this variety is increasing in both domestic and international markets, due to the recognition of its good quality. Although an increase in production is urgently needed, the cultivation is limited due to infertile and drought-stricken sandy soil. Also it has been stated that the quality of the aromatic rice variety, Khao Dawk Mali 105 might be influenced by the environment of the production area, especially the difference in water supply and salinity of soil[7].

2-acetyl-1-pyrroline, a “popcorn”-like flavor compound was reported as the main flavor component of aromatic rice[1,2]. Also this compound contributed to the “roasted aroma” of cooked beef, crusts of wheat and rye breads[10], popcorn[9], and wetted ground pearl millets[8]. The

This paper reports the results obtained in the joint project on “Comprehensive Studies on Sustainable Agricultural Systems in Northeast Thailand” sponsored by Japan International Research Center for Agricultural Sciences (JIRCAS).

Present address:
[1] Food Science and Technology Division, Japan International Research Center for Agricultural Sciences (JIRCAS) (Tsukuba, Ibaraki 305–8686, Japan)
[2] College of Bioresource Sciences, Nihon University (Fujisawa, Kanagawa 252–8510, Japan)
[3] National Institute of Crop Science, National Agricultural Research Organization (Tsukuba, Ibaraki 305–8517, Japan)
*Corresponding author: fax +81–29–838–6358; e-mail tadashi@jircas.affrc.go.jp

Received 9 December 2003; accepted 21 January 2004.

"aroma" quality of aromatic rice in sensory evaluation showed strong correlation to its content[7]. We previously reported that 2-acetyl-1-pyrroline did not form during cooking or postharvest processing of aromatic rice varieties[12]. Instead, it is formed in aerial parts of plants during growth in paddy fields. We also investigated some amino acids as precursor molecules of 2-acetyl-1-pyrroline, and assumed that L-proline is related to the formation of 2-acetyl-1-pyrroline in aromatic rice[13]. It is well established that L-proline accumulation is a common metabolic response to water deficits and salinity stresses, and has been the subject of numerous reviews[4,11].

The results of storage tests indicated that this compound in aromatic rice kernels can exist as a complex with the hydrophobic region of crystalline starch. Only during the ripening stage, this compound could stack into non-crystalline formed starch in the kernel. Hence, it was assumed that the amount of L-proline in rice plants was related to water availability in soil, especially during the ripening stage.

The ecological conditions of an area such as weather and soil conditions are highly correlated with the quality of rice. However, the correlation factors for the production of high quality rice of an aromatic variety have not been confirmed with scientific data. The aim of this study is to elucidate the factors influencing the final aromatic quality of Khao Dawk Mali 105 during its cultivation, especially the area dependency of 2-acetyl-1-pyrroline content among areas in Thailand.

Materials and Methods

1. Rice samples and preparations

Rough rice samples of the Khao Dawk Mali 105 rice grown in 2000 and 2001 at various production areas of Thailand, summarized in Table 1, were collected and stored at 5ºC in sealed polypropylene bags prior to the experiment. Samples were cropped following conventional methods in the areas. Samples from Suwannaphum experimental field were cropped with direct seeding or transplanting which is conventional in the area. They were hulled using a rice huller (TR-200; Kett, Tokyo, Japan). Broken and colored kernels were removed by an automated rice analyzer (RN-500; Kett, Tokyo, Japan).

Brown rice samples were ground with a 0.5-mm-mesh screen. (ZM-100; Retsch, Haan, Germany) Moisture content of samples was measured using oven drying at 105ºC for 3 h. All experiments were performed within 6 months from the harvest.

2. Chemicals

Iodomethane-^{13}C was purchased from CDN isotopes (Quebec, Canada). Ethanol (residual pesticide analysis grade) was purchased from Wako Chemicals (Osaka, Japan). Other reagents used were of analytical grades.

3. Synthesis of 2-acetyl-1-pyrroline and 2-acetyl-(methyl-^{13}C)-1-pyrroline

2-acetyl-1-pyrroline was synthesized as described by De Kimpe et al[3]. 2-acetyl-(methy-^{13}C)-1-pyrroline was also synthesized using methyl-^{13}C-magnesium iodide

Table 1. Khao Dawk Mali 105 samples collected in various areas of Thailand

Area collected	Year collected	Remarks
Chiang Mai (North)	2000	Irrigated, usually didn't crop this variety Farm field
Kalasin (Northeastern)	2000	Rain-fed, stated as being of middle quality Farm field
Khao Suan Kwang (Northeastern)	2000, 2001	Rain-fed Experimental field
Khon Kaen (Northeastern)	2000	Rain-fed Farm field
Loei (Northeastern)	2000	Rain-fed, mountainous area Farm field
Suwannaphum (Northeastern)	2000, 2001	Rain-fed, stated as being of highest quality Experimental field, seed production area
Pathumthani (Central)	2000	Irrigated, usually didn't crop this variety Experimental field

from iodomethane-^{13}C. Purity of each compound was confirmed by GC-MS and ^{1}H & ^{13}C-NMR; yields were 35 and 34%, respectively. The yield of the last reaction for labeling the acetyl group was 99%. Actual quantities were confirmed by ^{1}H -NMR using ethanol as an internal standard. Stock solutions (2%) were prepared with dichloromethane and stored at –80ºC until use.

4. Extraction of 2-acetyl-1-pyrroline from rice samples

The extraction vials used were 12 × 32 mm and closed with a PTFE septa and screw caps. 2-acetyl-1-pyrroline was extracted from 200 mg samples using 0.75 mL ethanol containing 200 ppb 2-acetyl-(methy-^{13}C)-1-pyrroline as an internal standard at 75°C. After centrifugation, the supernatant was subjected to GC-MS-SIM analysis.

5. Gas chromatography - mass spectrometry - selected ion monitoring (GC-MS-SIM)

The extract (2 μL) was injected into a fused silica capillary column (DB-WAX; 60 m × 0.25 mm I.D. × 0.25 μm film thickness; J & W Scientific, Folsom, CA) installed in a Hewlett-Packard (HP) 5980 series 2 gas chromatograph (Palo Alto, CA). Helium gas (purity 99.9999%, passed through a molecular sieve and an oxygen trap) with a head pressure set at 40 p.s.i was used as the GC carrier gas. The injector and the GC-MS interface temperatures were set at 150ºC and 250°C, respectively. The column temperature was maintained isothermally at 40ºC for 2 min, then temperature was increased at a rate of 10ºC/min to 100ºC and was increased further to 140ºC at 5ºC/min. After this program, the column temperature was maintained isothermally at 250ºC for 10 min. An HP 5989A mass spectrometer was used in the electron ionization mode with the ion source temperature set at 250°C, the analyzer temperature was set at 100°C, and ionization energy at 70 eV. SIM was set up to monitor m/z 111 for 2-acetyl-1-pyrroline and m/z 112 for 2-acetyl-(methy-^{13}C)-1-pyrroline. MS detection dwell time was 100 ms for each ion. Under these conditions, the retention times of these compounds were found to be 12.47 and 12.46 min, respectively. Quantification was performed by measuring the area ratios between ions at m/z 111 and 112, corresponding to 2-acetyl-1-pyrroline and 2-acetyl-(methy-^{13}C)-1-pyrroline, respectively. Each extract was analyzed 3 times to obtain an average peak area. The amount in samples was calculated from a calibration curve of various concentrations for synthetic 2-acetyl-1-pyrroline against an internal standard.

Results and Discussion

1. Comparison of 2-acetyl-1-pyrroline content among areas in Thailand

Fig. 1 shows the results from 7 samples to compare the differences in Thailand, in the year 2000. Content of

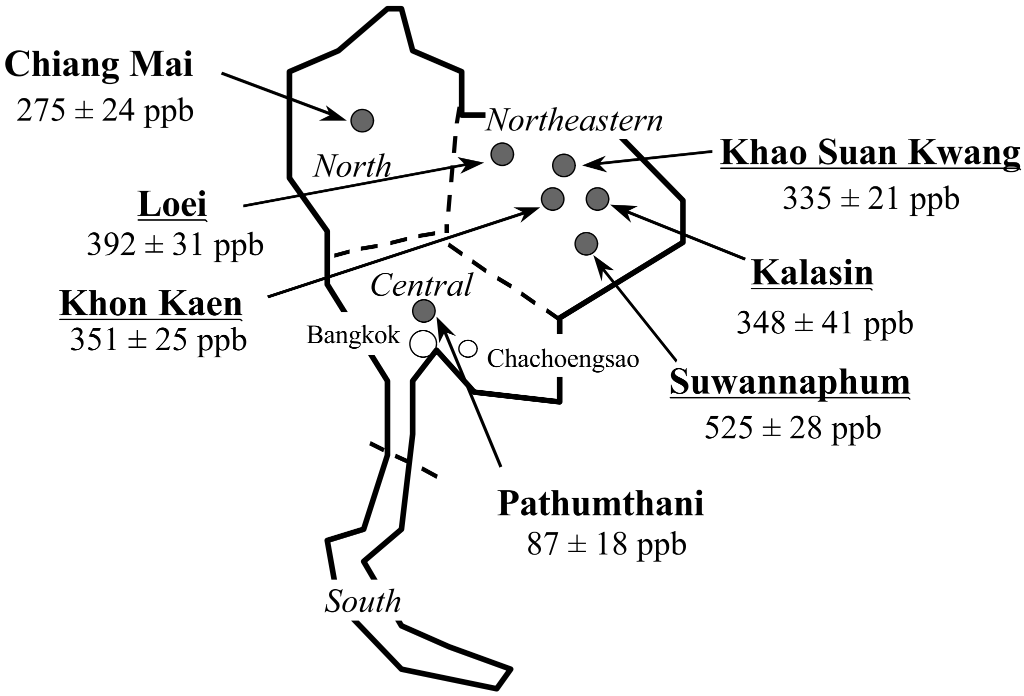

Fig. 1. Content of 2-acetyl-1-pyrroline in Khao Dawk Mali 105 from various areas in Thailand in the year 2000
Samples from Northeastern Thailand are underlined.

2-acetyl-1-pyrroline in rice samples was different among production areas. Since ecological conditions among the areas and cultivation methods, like irrigation or fertilizer conditions, are different in Thailand, content of 2-acetyl-1-pyrroline in Khao Dawk Mali 105 might be controlled not only by genetic background, but also other factors, such as ecological or cultivation factors. Thailand can be divided into 4 regions, Northern, Northeastern, Central and South. There are quite clear ecological characteristics for each region. Khao Dawk Mali 105 is usually cultivated in Northeastern Thailand, though this variety was collected in Chachoengsao, Central Thailand. Historically, Khao Dawk Mali 105 was collected and selected by Suphanburi Rice Experimental Station in Central Thailand. During selection, it was accidentally exposed to severe drought in the area, and Khao Dawk Mali 105 showed tolerance for drought. Therefore it was selected as a drought tolerant variety for Northeastern Thailand. The climate of the South region is rather different from other regions. In this study, we omitted the samples from the South region, where the rice crop pattern is completely different. Contents of 2-acetyl-1-pyrroline in rice samples from Northeastern Thailand were higher than other regions in Thailand. Especially the samples from irrigated areas were lower than that of rain-fed areas. Among rain-fed areas, Northeastern Thailand is characterized as infertile, sandy with salinity affected soil, and dry weather severely affected by monsoon activity. Especially during the ripening season of Khao Dawk Mali 105, the region usually does not have rain. Therefore, dry climate was expected to be a factor for 2-acetyl-1-pyrroline content, basically. On the other hand, other important aromatic rice varieties in the world, Basmati lines, are also cultivated in dry and highland areas, near the border of India and Pakistan. Therefore, areas with high quality aromatic rice are suspected of being dry and sandy.

2. Comparison of 2-acetyl-1-pyrroline content among the samples from "Tungkularonghai" area

Fig. 2 shows the difference in the content among the samples from "Tungkularonghai". The area "Tungkularonghai" is stated as being a high quality Khao Dawk Mali 105 production area[7], and also characterized as drought-stricken with sandy soil. The samples from typical fields in the area showed higher 2-acetyl-1-pyrroline content than other areas in Thailand. However, the sample in an area not considered to be a drought situation (flooded area) showed rather lower 2-acetyl-1-pyrroline content. Also, the sample from a clay soil field that could maintain soil moisture until harvest, even in the "Tungkularonghai" area, showed lower content of 2-acetyl-1-pyrroline. Therefore drought conditions during cultivation are expected to be the most important factor affecting the

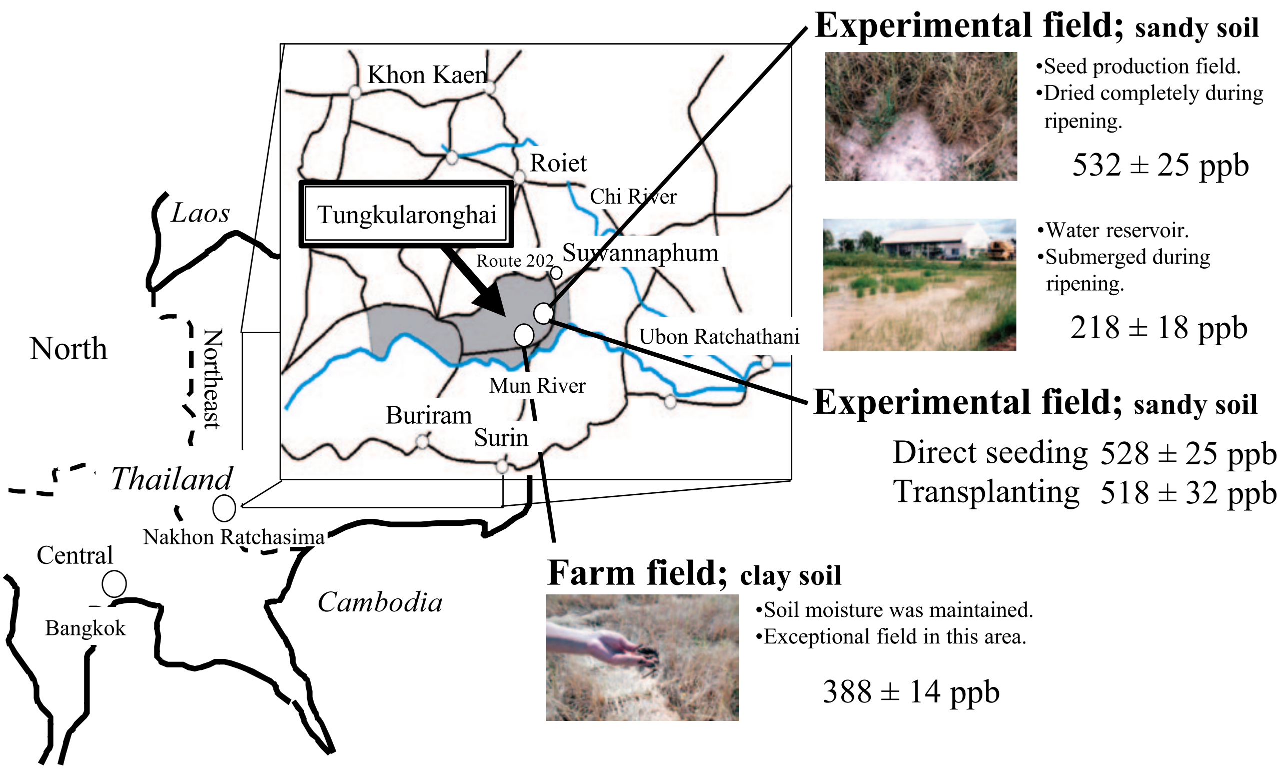

Fig. 2. Content of 2-acetyl-1-pyrroline in Khao Dawk Mali 105 samples from the area of "Tungkularonghai" in 2001

quality of Khao Dawk Mali 105. The sample from a flooded area showed the typical effect, because the sample from a field with similar ecological conditions (neighbor) but only different in drought conditions showed different contents of 2-acetyl-1-pyrroline. Since drought conditions are difficult to control in rain-fed paddy fields, it is suspected that photosensitivity of Khao Dawk Mali 105 can be controlled to set the ripening stage uniformly to a certain period which is suitable for 2-acetyl-1-pyrroline formation in paddy fields.

The sample which was cultivated with a direct seeding method did not show any significant difference from traditional transplanting cultivation in 2-acetyl-1-pyrroline content. The results showed that application of direct seeding, which deals with the labor shortage in this area, did not affect the aromatic quality of Khao Dawk Mali 105.

Further studies are needed to investigate the effect of drought conditions during cultivation on 2-acetyl-1-pyrroline content, especially during the ripening stage when the compound is considered to stack into non-crystalline formed starch in the rice kernel.

References

1. Buttery, R. G. & Ling, L. C. (1982) 2-acetyl-1-pyrroline: An important aroma component of cooked rice. *Chem. Ind. (Lond.),* **1982**, 958–959.
2. Buttery, R. G., Ling, L. C. & Mon, T. R. (1986) Quantitative analysis of 2-acetyl-1-pyrroline in rice. *J. Agric. Food Chem.,* **34**(1), 112–114.
3. De Kimpe, N. G., Stevens, C. V. & Keppens, M.A. (1993) Synthesis of 2-acetyl-1-pyrroline, the principal rice flavor component. *J. Agric. Food Chem.*, **41**(9), 1458–1461.
4. Delauney, A. J., & Verma, D. P. S. (1993) Proline biosynthesis and osmoregulation in plants. *Plant J.,* **4**, 215–223.
5. Hori, K. et al. (1992) Knowledge and preference of aromatic rice by consumers in East and South-east Asia. *J. Consum. Stud. Home Econ.*, **16**, 199–206.
6. Hori, K. et al. (1994) Comparison of sensory evaluation of aromatic rice by consumers in East and South-east Asia. *J. Consum. Stud. Home Econ.*, **18**, 135–139.
7. Ishitani, K. & Fushimi, C. (1994) Influence of pre-and post-harvest conditions on 2-acetyl-1-pyrroline concentration in aromatic rice. *Koryo,* **183**, 73–80.
8. Seitz, L. M. et al. (1993) Contribution of 2-acetyl-1-pyrroline to odors from wetted ground pearl millet. *J. Agric. Food Chem.,* **41**, 955–958.
9. Schieberle, P. (1991) Primary odorants in popcorn. *J. Agric. Food Chem.,* **39**, 1141–1144.
10. Schieberle, P. & Grosch, W. (1985) Identification of the volatile flavour compounds of wheat bread crust. Comparison with rye bread crust. *Z. Lebensm. Unters. Forsch.,* **185**, 111–113.
11. Taylor, C. B. (1996) Proline and water deficit: Ups and downs. *Plant Cell,* **8**, 1221–1224.
12. Yoshihashi, T. (2002) Quantitative analysis on 2-acetyl-1-pyrroline of an aromatic rice by stable isotope dilution method and model studies on its formation during cooking. *J. Food Sci.,* **67**(2), 619–622.
13. Yoshihashi, T., Nguyen, T. T. H. & Inatomi, H. (2002) Precursors of 2-acetyl-1-pyrroline, a potent flavor compound of an aromatic rice variety. *J. Agric. Food Chem.,* **50**(7), 2001–2004.