Sensory Test and Molecular Marker Based-Selection for Aromatic Rice in the F_3 Progenies

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ABSTRAK

Uji Sensori dan Seleksi Berbasis Marka Molekuler untuk Karakter Aroma Pada Padi Keturunan Generasi F3

Padi aromatik merupakan padi tipe khusus yang sangat disukai oleh masyarakat di Asia karena adanya aroma. Aroma di padi ditentukan oleh senyawa *2-acetyl-1-pyrroline* (2AP) yang dikendalikan oleh gen resesif *fgr*. Persilangan antara kultivar Sintanur (padi aromatik) dan PTB33 (non-aromatik, tahan wereng coklat) telah dilakukan, guna mengembangkan galur padi aromatic yang tahan terhadap wereng coklat. Pada F_2 hasil persilangan ini telah dilakukan seleksi berbasis marka molekuler dan bioassay untuk wereng coklat, akan tetapi seleksi untuk karakter aroma belum dilakukan khususnya di generasi F3 ini. Tujuan riset ini adalah untuk memperoleh individu padi galur F3 yang memiliki karakter aroma. Pengujian sensori dilakukan dengan menggunakan larutan KOH 1%, untuk seleksi berbasis marka molekuler digunakan empat marka terkait, yaitu ESP (*External Antisense Primer*), IFAP (*Internal Fragrant Antisense Primer*), INSP (*Internal Non fragrant Sense Primer*) and EAP (*External Antisense Primer*). Delapan puluh delapan tanaman padi dari dua galur F3 (SP#31 and SP#224) hasil persilangan cv. Sintanur dan PTB33 telah diteliti. Deteksi dengan menggunakan marka molekuler diperoleh 75 genotipe (85,23%) yang homosigot resesif aromatic dan satu heterosigot (non-aromatik). Delapan puluh lima (96,59%) genotipe termasuk aromatik berdasarkan hasil uji sensori. Tujuh puluh dua (81,82%) genotype termasuk aromatik berdasarkan uji sensory dan marka molekuler. Adanya inkonsistensi hasil dari kedua metode uji ini, maka disarankan untuk melakukan pengujian dengan menggunakan dua metode tersebut untuk menjamin kehandalan dan keakurasian karena aroma dipengaruhi oleh komposisi genetik dan kondisi lingkungan. Genotipe-genotipe yang terseleksi akan dilanjutkan untuk program pemuliaan guna mengembangkan padi aromatik dengan karakter agronomis yang lebih baik.

Keywords: EAP (External Antisense Primer), ESP (External Antisense Primer), IFAP (Internal Fragrant Antisense Primer), INSP (Internal Non Fragrant Sense Primer), Padi.

ABSTRACT

Aromatic rice is a special type of rice that highly preferred by people in Asia due to the presence of aroma. Aroma in rice is determined by *2-acetyl-1-pyrroline* (2AP) compound which is controlled by a recessive *fgr* gene. A hybridization between cv. Sintanur (aromatic rice) and PTB33 (non-aromatic, resistant to brown planthopper/BPH) has been done in order to develop aromatic rice lines that resistant to BPH. In the F_2 progeny, molecular marker-based selection and bioassay for the brown planthopper resistant lines have been carried out; however selection for the aromatic trait has not been performed yet. The objective of this study was to obtain the F3 progeny's individual with aromatic trait. Sensory test was conducted by KOH 1.7% solution, meanwhile molecular markers applied were ESP (External Antisense Primer), IFAP (Internal Fragrant Antisense Primer), INSP (Internal Non fragrant Sense Primer) and EAP (External Antisense Primer). Eighty-eight plants from two selected (SP#31 and SP#224) F3 lines progenies derived from cv. Sintanur and PTB33 have been

evaluated in this study. Detection by molecular markers found seventy-five genotypes (85.23%) were homozygous recessive (aromatic rice) and one was heterozygous (non-aromatic). Eighty-five (96.59%) genotypes were aromatic as detected by sensory test alone. Seventy-two (81.82%) genotypes were categorized as aromatic rice based on sensory test and molecular markers. Due to inconsistency results from each method alone, it is advised both methods to be applied to ensure the reliability and the accuracy since aroma in rice is affected by genetic composition and environment conditions. Selected genotypes will be continued for breeding program in developing aromatic rice with improved agronomic traits.

Keywords: EAP (External Antisense Primer), ESP (External Antisense Primer), IFAP (Internal Fragrant Antisense Primer), INSP (Internal Non Fragrant Sense Primer), Rice

INTRODUCTION

Aromatic rice is more preferred by farmers because it has a higher selling price in local as well as international markets as compared to non-aromatic one (Giraud, 2013). Therefore, it is necessary to develop the suitable breeding methods for aromatic rice in order to fulfill the consumer's demand and increase the farmer's income due to the increase demand for local and international markets. According to Buttery *et al.* (1983), *2-acetyl-1-pyrroline* (2AP) is the key for the aromatic compound in rice. This compound could be found in all plant parts except roots (Lorieux *et al.*, 1996; Yoshihashi *et al.*, 2002). Expression of this compound can be observed through sensory test (Yoshihashi *et al.*, 2002) and detection it with molecular markers (Bradbury *et al.*, 2005).

The sensory test of aromatic in rice can be performed by using solution of KOH 1.7% (Sood & Siddiq, 1978), meanwhile the molecular markers using a specific marker (Bradbury *et al.*, 2005) which is closely linked to the *fgr* gene that controlled the expression of aromatic trait. The *fgr* gene in rice is located on chromosome 8 (Jin *et al.*, 2010). The previous study was carried out using F$_3$ progeny from a cross of Sintanur x PTB-33 (SP). Sintanur is original variety from Indonesia with many valuable traits including aromatic trait (Balitpa, 2001), meanwhile PTB33 is originally from India and have a high resistance to brown planthopper/BPH (Rongbei *et al.*, 2001). The objective of this hybridization was to create new genotypes having both aromatic and resistant to BPH traits in one single genotype. In the F$_2$ progeny, molecular marker-based selection for the BPH resistant had been carried out and obtained some promising lines (Carsono *et al.*, 2016), however selection for aromatic trait has not been performed yet. There is a limited report regarding the effort in integrating aromatic and BPH resistance traits into one single genotype. Jairin *et al.* (2009) has reported in obtaining cv. Khao Dawk Mali 105 (KDML105), Thai aromatic rice cultivar with improved BPH resistance trait, which focused on the improvement of BPH resistance, not aromatic trait. Therefore, evaluation of F$_3$ progenies for aromatic trait is important to be conducted in order to obtain rice genotypes having both aromatic and brown planthopper resistance traits.

MATERIALS AND METHODS

Plant materials

Eighty eight genotypes of F$_3$ progeny derived from a crossing between Sintanur and PTB33 were evaluated, consisted of forty six genotypes derived from line SP#224 and forty two were from line SP#31. All genotypes have been identified as resistant to BPH based on SSR (Simple Sequence Repeats) molecular markers in the F$_2$ progeny (Carsono *et al.*, 2016). Seeds from these geneotypes were germinated and grown in the screen house of Experimental Station, Faculty of Agriculture, Universitas Padjadjaran, Jatinangor Campus. The leaves from every genotype were collected at the heading stage for the sensory test and DNA analysis.

Sensory test for aromatic detection

The aromatic test was conducted with 10 panelists using KOH 1.7% solutions. The test was done by inserting leaf pieces from plant (1.5 g) into the tube with 1.5 ml of KOH 1.7% and it was left there for 30 minutes. The aroma identification was performed by smelling the aroma in the tube then classified based on the presence or absence of aroma. All individual genotypes (88 plants) were scored for sensory test.

DNA extraction and PCR amplification

DNA extraction from young leaves was performed by CTAB method (Doyle & Doyle, 1987) with a slight modification in amount of sample and ethanol for DNA precipitation. DNA quality was analyzed using gel electrophoresis by using 2% agarose gel that dissolved in 100 ml of 0.5x TBE. The solution then was heated in a microwave for 2 minutes at the 150°C. Electrophoresis was done at 80 Volt for 45-60 min., and then it soaked in EtBr (ethidium bromide) solution for 30 min. and in distilled water for 10 minutes. DNA quantity measurement was done by measuring the concentration of DNA at a wavelength of 260 nm and 280 nm in spectrophotometer PCR was performed for molecular marker assessment for aromatic and non-aromatic rice in a total volume of 10 μl, consisted of 1μl of genomic DNA 20 ng/μl, 5 μl KAPA Taq DNA polymerase, and 1μl of each primer. Four specific markers were used according to Bradburry, 2005) i.e.: TTGTTTGGAGCTTGCTGATG; (ESP: external sense primer), CATAGGAGCAGCTGAAATATATACC (IFAP: internal fragrant antisense primer), CTGGTAAAAAGATTATGGCTTCA (INSP: internal non fragrant sense primer) and AGTGCTTTACAAAGTCCCGC (EAP: external antisense primer). Cycling conditions were an initial denaturation of 95°C for 5 min. followed by 35 cycles of 1 min. at 95°C, 1 min. at 58°C, 1 min. at 72°C; and a final extension of 72°C for 5 min. PCR products were analyzed using electrophoresis (2.0% agarose gel, and 1kb ladder) and ethidium bromide stained (0.5 μg/ml).

RESULTS AND DISCUSSION

Aromatic and non-aromatic rice lines derived from hybridization between Sintanur and PTB33 were studied in order to distinguish these lines.The results showed that aromatic and non-aromatic rice can be detected using1.7% KOH test (Sood &Siddiq, 1978) and specific primers (Bradbury et al., 2005). It was recorded as (+) for aromatic and (-) for non-aromatic (Table 1). Visualization of molecular marker for aromatic and non-aromatic detection is presented in Fig. 1.

In this study, 85 genotypes (96.59%) were selected as aromatic rice by sensory test, 75 genotypes (85.23%) by molecular markers (Bradburry's primer), and 72 genotypes (81.82%) by both methods (Table 1). Twelve genotypes (13.64%) have been detected as aromatic rice based on sensory test; however they were not confirmed by molecular markers (Table 1). It means that homozygous recessive lines (aa, aromatic) were not detected, only homozygous dominant (AA, non-aromatic) and heterozygous (Aa, non-aromatic) were found. In total 12 plants, out of 88, or around 13.64% (Table 2) which is much higher compared to the opposite condition (sensory negative, molecular markers positive) i.e., 3.41% or 3 rice plants. False positive plants identified by sensory test were higher than those confirmed by molecular markers (13.65% versus 3.41%).

This finding is in accordance data presented by Yeap et al. (2013) who found around 30-40% of F₁ rice population detected were non-fragrant rice but they expressed aroma in their leaves and grains. In addition Alrufaye et al. (2018) found the same thing, two genotypes, out of 16 genotypes were not detected by molecular markers but they expressing aroma. This condition may be due to the present other aromatic compound as revealed by Pachauri et al. (2010) stated that a significant variation in the type and intensity of aroma in the different groups of aromatic rice varieties, suggesting involvement of additional chemical compounds in varying proportions or Fitzgerald et al. (2008) argued that any other fgr gene controlling aroma in rice. Other explanation, this condition might be due to reduction of smell ability of panelists, so that they could not distinguish between scent of aromatic and non-aromatic compound. It could be also that there were allegations of minor genes that controlling rice aroma (Alrufaye et al., 2018).

Singh et al. (2007) reported that besides badh2 gene on chromosome 8, there is also badh1 gene on chromosome 4 as a candidate gene controlling the scent because it has the same function as badh2 gene on chromosome 8. The badh2 gene is also detected on chromosome 3, although badh2 on chromosome 8 had a major influence for phenotypic variation of aroma. So that, it is possible if the genotypes are detected by sensory test, but not by molecular marker. Other it might be that KOH solution may cause damage to the olfactory senses, so it can reduce the ability of panelists in analysis (Hien et al., 2006).

Table 1. Identification of aromatic and non-aromatic rice plants by both sensory and molecular markers.

No.	Sample	1.7% KOH[a]	Molecular Marker[a]	No.	Sample	1.7% KOH	Molecular Marker	No.	Sample	1.7% KOH	Molecular Marker
1	SP31-2	+	+	31	SP31-34	+	+	61	SP224-19	+	+
2	SP31-3	-	+	32	SP31-35	+	+	62	SP224-20	+	+
3	SP31-5	+	-	33	SP31-36	+	-	63	SP224-21	+	+
4	SP31-6	+	+	34	SP31-37	+	+	64	SP224-22	+	+
5	SP31-7	+	+	35	SP31-38	+	+	65	SP224-23	+	+
6	SP31-8	+	-	36	SP31-39	-	+	66	SP224-25	+	+
7	SP31-9	+	+	37	SP31-40	+	+	67	SP224-26	+	+
8	SP31-10	+	-	38	SP31-41	+	+	68	SP224-27	+	+
9	SP31-11	+	+	39	SP31-42	+	-	69	SP224-28	+	+
10	SP31-12	+	+	40	SP31-43	+	-	70	SP224-29	+	+
11	SP31-13	+	-	41	SP31-44	+	+	71	SP224-30	+	+
12	SP31-14	+	-	42	SP31-45	+	-	72	SP224-31	+	+
13	SP31-15	+	+	43	SP224-1	+	+	73	SP224-32	+	+
14	SP31-16	+	+	44	SP224-2	+	+	74	SP224-33	+	+
15	SP31-17	+	+	45	SP224-3	+	+	75	SP224-34	+	+
16	SP31-18	+	+	46	SP224-4	+	+	76	SP224-35	+	+
17	SP31-19	+	+	47	SP224-5	+	+	77	SP224-36	+	+
18	SP31-20	+	-	48	SP224-6	+	+	78	SP224-37	+	+
19	SP31-21	-	+	49	SP224-7	+	+	79	SP224-38	+	+
20	SP31-22	+	+	50	SP224-8	+	+	80	SP224-39	+	+
21	SP31-23	+	-	51	SP224-9	+	+	81	SP224-40	+	+
22	SP31-24	+	+	52	SP224-10	+	+	82	SP224-41	+	+
23	SP31-25	+	+	53	SP224-11	+	+	83	SP224-42	+	+
24	SP31-26	+	+	54	SP224-12	+	+	84	SP224-43	+	+
25	SP31-27	+	+	55	SP224-13	+	+	85	SP224-44	+	+
26	SP31-28	+	+	56	SP224-14	+	+	86	SP224-45	+	+
27	SP31-29	-	-	57	SP224-15	+	+	87	SP224-46	+	+
28	SP31-30	+	+	58	SP224-16	+	+	88	SP224-47	+	+
29	SP31-31	+	+	59	SP224-17	+	+				
30	SP31-33	+	-	60	SP224-18	+	+				

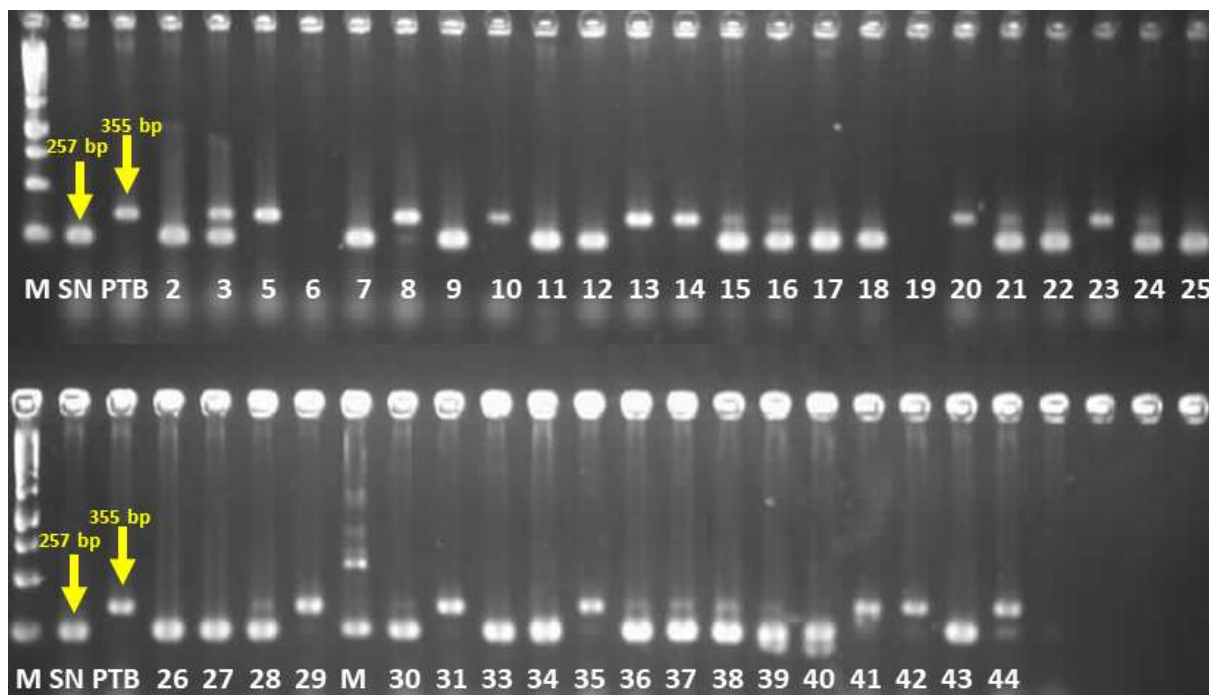Note: [a] (+): Aroma, (-): Non-aroma

Fig 1.   Visualization of molecular markers to distinguish aromatic (257bp) and non-aromatic rice (355bp). Remarks: M= ladder; SN: Cv. Sintanur; PTB= PTB33; Number represents genotype number of F3 progeny.

Table 2. Detection of aroma by sensory test and molecular markers in leaf tissues for 88 F3 progeny derived from the hybridization between Sintanur and PTB33.

Result	F3 segregants[a]		Number of F3 segregants	Percentage (%)
	Sensory test[a]	Molecular markers[b]		
Positive for both sensory and molecular markers	+	+	72	81.8
Recombination	+	-	12	13.6
Recombination	+	H	0	0.0
Recombination	-	+	2	2.3
Recombination	-	H	1	1.1
Negative for both sensory and molecular markers	-	-	1	1.1
Total number of plants			88	100

[a] For sensory test: (+): Aroma detected; (-): Non-aroma.
[b] For molecular markers: (+): homozygous recessive (aa); (-): homozygous dominant (AA); H: Heterozygous (Aa).

On the other hand, three genotypes were confirmed as aromatic rice based on molecular markers (aa, homozygous recessive) but not expressing aroma as detected by sensory test (Table 1 and 2). This might be due to the very low intensity of the *2-acetyl-1-pyrroline* (2AP) detected in the genotypes, so it cannot be captured by the panelist. Singh (2000) argued that the strength of aroma or the concentration of 2AP might be affected by environmental conditions. For example, rice cv. Basmati will be strong in aromatic scent if the temperature is relatively cool during growing period in the day (25-32°C) and night (20-25°C) with humidity of 70-80% during flowering and grain filling period.

Prodhan *et al.* (2017) mentioned that cultivation practice, genetic composition, environmental condition and its interaction affect the aroma quality in rice and they also confirmed that among environmental components, temperature is a vital component that can affect the aroma quality and chemical composition of aromatic rice.

From those information, it is clear that aroma in rice is affected by genetic composition and environmental condition. Thus, breeding for high quality aroma and cultivation methods that support aromatic expression is important to be done. However due to the effect of environment factors in evaluating aroma in segregating population (F_3), integration of sensory methods and molecular marker will provide more accurate and reliable result for determining some promising genotypes should be continued for the next generation.

CONCLUSION

Eighty eight plants from two selected (SP#31 and SP#224) F_3 lines progenies derived from cv. Sintanur and PTB33 have been evaluated in this study. Seventy two (81.82%) genotypes were categorized as aromatic rice based on sensory test and molecular markers. Seventy five (85.23%) genotypes carried homozygous recessive gene (aromatic rice) and one genotype was heterozygous detected by molecular markers. Eighty five (96.59%) genotypes were detected by sensory test alone. Due to inconsistency results from each method alone, it is advised both method to be applied to ensure the reliability and accuracy since aroma in rice is affected by genetic composition and environment conditions.

REFERENCES

Alrufaye, TAUH, BAHAL Khafaji, WMS Al-sheikh. 2018. Use molecular markers analysis and sensory methods in the revelation of fragrance in Iraqi rice. J. Pharm. Sci. and Res. 10 (10): 2531-2533.

Balitpa (Balai Penelitian Padi). 2001. Padi aromatic varietas Sintanur. Departemen Pertanian, Agdex 112/37. (in Bahasa Indonesia).

Bradburry, LMT, RJ Henry, Q Jin, RF Reinke, and DLE Waters. 2005. A perfect marker for fragrance genotyping in rice. Molecular Breeding, 16 (4):279-283. doi: 10.1007/s11032-005-0776-y.

Buttery, RG, LC Ling, OB Juliano, and JG Turnbaugh. 1983. Cooked rice aroma and 2-acetyl-1-pyrroline. Journal of Agricultural and Food Chemistry. 31:823-826.

Carsono, N, GI Prayoga, N Rostini, and D Dono. 2016. Molecular marker based selection on F_2 progeny for developing promising rice lines resistant to brown planthopper (in Bahasa Indonesia). Jurnal Agrikultura 27 (1): 9-15. Doi: 10.24198/agrikultura.v27i1.8471

Doyle, JF, and JL Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin. 19 (1): 11-15.

Giraud, G. 2013. The world market of fragrant rice, main issues and perspectives. International Food and Agribusiness Management Review. 16 (2): 1-20.

Fitzgerald, MA, NRS Hamilton, MN Calingacion, and VM Butardo. 2008. Is there a second fragrance gene in rice. Plant Biotechnology Journal. 6: 416–423.

Hien, NL, T Yoshihashi, WA Sahardi, VC Thanh, Y Oikawa, and Y Hirata. 2006. Evaluation of aroma in rice (*Oryza sativa* L.) using KOH method, molecular markers, and measurement of 2-Acetyl-1-Pyrroline concentration. Journal of Tropical Agriculture. 50(4):190-198, 2006.

Jairin, J, S Teangdeerith, P Leegalud, J Kothcharerk, K Sansen, M Yi, A Vanavichit, and T Toojinda. 2009. Development of rice introgression lines with brown plant hopper resistance and KDML105 grain quality characteristics through marker assisted selection. Field Crop Res. 110:263-271.

Jin L, Y Lu, Y Shao, G Zhang, P Xiao, S Shen, H Corke, and J Bao. 2010. Molecular marker assisted selection for improvement of the eating, cooking and sensory quality of rice (*Oryza sativa* L.). Journal of Cereal Science. 51:159-164.

Lorieux, M, M Perov, N Huang, E Guiderdoni, and A. Ghesquiere. 1996. Aroma in rice genetic analysis of a quantitative trait. Theoretical and Applied Genetics. 93:1145-1151.

Pachauri, V, MK Singh, AK Singh, S Singh, NA Shakeel, VP Singh, and Singh NK. 2010. Origin and genetic diversity of aromatic rice varieties, molecular breeding and chemical and genetic basis of rice aroma. Journal of

Plant Biochemistry & Biotechnology 19 (2):127-143.

Prodhan, ZH, G. Faruq, KA Rashid and RM Taha. 2017. Effects of temperature on volatile profile and aroma quality in rice. International Journal of Agriculture and Biology. 19: 1065-1072. DOI:10.17957/IJAB/15.0385.

Rongbei L, Q Xueyi, W Sumei, MP Pandey, and PK Pathak. 2001. Inheritance of resistance to brown planthopper in an *Oryza rufipogon* (Griff.)-derived line in rice. Current Science. 80:1421-1423.

Singh, R, AK Singh, TR Sharma, A Singh, and NK Singh. 2007. Fine mapping of aroma QTLs in Basmati rice (*Oryza sativa* L.) on chromosomes 3, 4 and 8. Journal of Plant Biochemistry and Biotechnology. 16 (2):75–82.

Singh, VP. The Basmati rice of India. 2000. p.135-153. In Sing, RK, US Singh and GS Kush (Eds). Aromatic Rices. Oxford & IBH Publ. Co. Pvt. Ltd. New Delhi, India.

Sood, BC, and EA Siddiq. 1998. A rapid technique for scent determination in rice. Indian Journal of Genetics and Plant Breeding. 38:268-271.

Yeap, HY, G Faruq, HP Zakaria, and JA Harikrishna. 2013. The efficacy of molecular markers analysis with integration of sensory methods in detection of aroma in rice. The Scientific World Journal: 1-6. http://dx.doi.org/10.1155/2013/569268.

Yoshihashi, T, NTT Huong, and N Kabaki. 2002. Quality evaluation of Khao Dawk Mali 105, an aromatic rice cultivar of Northeast Thailand. JIRCAS Working Report No. 30:150-160.