

Identification of red rice (*Oryza sativa* 'Akamai') cultivated in paddy fields in Tsushima, Kyusyu, Japan

— Plant opal analysis of tubercle forms developed from upper epidermal cells of red rice hulls —

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Red rice, called “Akamai” in Japanese, has been studied for many years in the field of natural science and humanities in Japan. In the present study, red rice (*Oryza sativa* 'Akamai') was identified by analyzing plant opals through the investigation of tubercle forms in the upper epidermal cells of red rice hulls. There have been no reports of the identification of red rice hulls by plant opal analysis. Plant opals of hulls were extracted from soil samples obtained from a paddy field where only red rice was cultivated as dedication to a shrine whose name can be found in a Japanese document dating from the 10th century AD. The result of the plant opal analysis suggests that red rice is a temperate *japonica* rice variety and not a tropical *japonica* or *indica* rice variety. Plant opal analysis of hulls of *Oryza sativa* L. is thus helpful for identifying species of rice from the soil samples at archeological sites. This study defined a morphological character of tubercles of red rice hulls, which will be a useful marker for distinguishing *japonica* type red rice from other rice varieties of *O. sativa* L. by plant opal analysis.

Keywords: plant opal analysis; red rice; Tsushima; *japonica*.

1. Introduction

One of the methods for discriminating red rice between the *japonica* and *indica* type is the measurement of the grain size (Arashi, 1974; Hamada, 1956). Recently, DNA analysis has been used for identifying red rice (*Oryza sativa* 'Akamai') (Ishikawa et al., 1992; Okoshi et al., 2004). The purpose of the study is to determine whether plant opal analysis can be used for the classification of red rice. The festival of red rice cultivation on these fields is designated as National Important Intangible Folk-Cultural Properties. Tsushima is an island that lies between Kyusyu and Korea; it is approximately 138 km from Hakata port in Fukuoka prefecture and 49.5 km from Pusan port in Korea. The island measures 82 km from the north to the south and 18 km from the east to the west, with an area of approximately 708 km². Seven paddy fields of red rice are located at 34°7' N and 129°11' E in Tsutsu town, southern Tsushima, approximately 40 m above the sea level (Figs. 1 and 2). The harvested red rice is dedicated to a shrine called “Tagutsutama jinjya” in Japanese. This shrine has been identified as one of the shrines described in a Japanese document, Engishiki, dating to 927 AD. However, the original age of these paddy fields is unknown. Seven soil samples for the plant opal analysis were collected from the seven paddy fields, and one sample gained from the oldest paddy field, was used for the analysis (Fig. 3). In general, the older the paddy field, the more the plant opals of red rice will increase quantitatively.

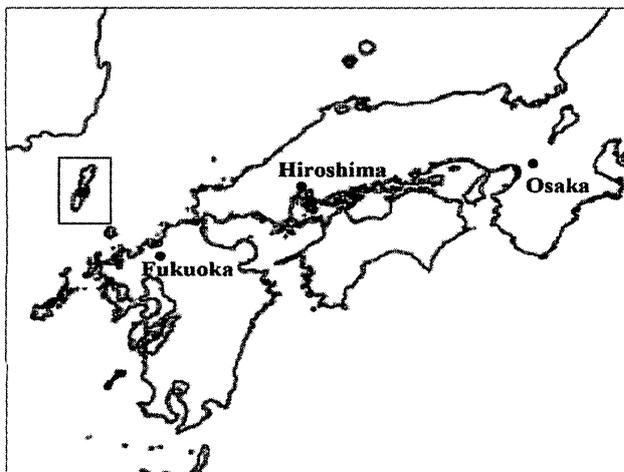


Fig. 1 The rectangle indicates the location of Tsushima in western Japan.

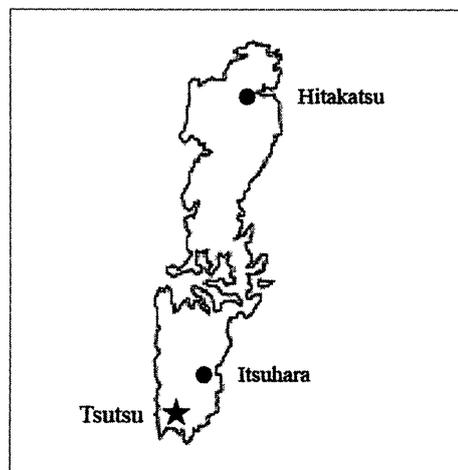


Fig. 2 The star mark indicates the location of red rice paddy fields at Tsutsu town.



Fig. 3 The oldest paddy field of red rice viewed from the southeast.

2. Plant opal extraction

- (1) Soil samples were dried in an electric furnace at 110 °C for 72 h.
- (2) On an electronic precision balance with a precision of 0.001 g, 1 g of soil sample was weighed.
- (3) The weighed sample was treated with 30 ml of 30 % H₂O₂ solution for rinsing, and it was heated until the liquid reduced to 10 ml.
- (4) Deionized water (50 ml) was added to this residue and according to Stokes' law, other impurities were repeatedly removed from the residue.
- (5) To separate any remaining mineral component, the plant opal component of the resulting residue was extracted by gravimetric separation (3000 rpm/s for 13 min) using a sodium polytungstate solution of density 2.30 g/ml.
- (6) After extraction, 20 μl aliquots of liquid containing plant opal components were placed on slides using a micropipette and dried in a desiccator for 3–4 h.
- (7) Cover glasses were placed over dried samples with a mounting reagent, and they were observed under an optical microscope at 200× and 400× magnifications. Hulls of *Oryza sativa* L. were counted and microphotographs were obtained with a single-lens reflex digital camera mounted on the microscope.

3. Results and Discussion

Tubercles and their morphological characters have been classified by Takahashi et al. (2005) and reviewed by Kobayashi (2013). The classification of tubercles in this study is based on these reports. Five hundred samples were microscopically examined. This examination required 6 g of dried soil sample and 66 slides, and 131 frames of each were examined. The examination revealed that all 500 tubercle samples were of type S. A marked struma was observed at the lateral branch of the long cell and the papilla was observed to exit from this struma. In many cases, strumae on adjacent long cells formed an intricate pattern (Fig. 4. 1–8). Types C and P were not found during the examination. According to Takahashi (2010), the latter two types belong to tropical *japonica* or *indica* rice varieties, whereas type S is observed only in temperate *japonica* varieties (Takahashi et al., 2005; Kobayashi 2008). Therefore, it was concluded that the red rice grown here was the temperate *japonica* type. This result of the plant opal analysis agreed with that of the DNA analysis of isozyme genes (Ishikawa et al., 1992). Recent studies (Takahashi 2008, 2010)

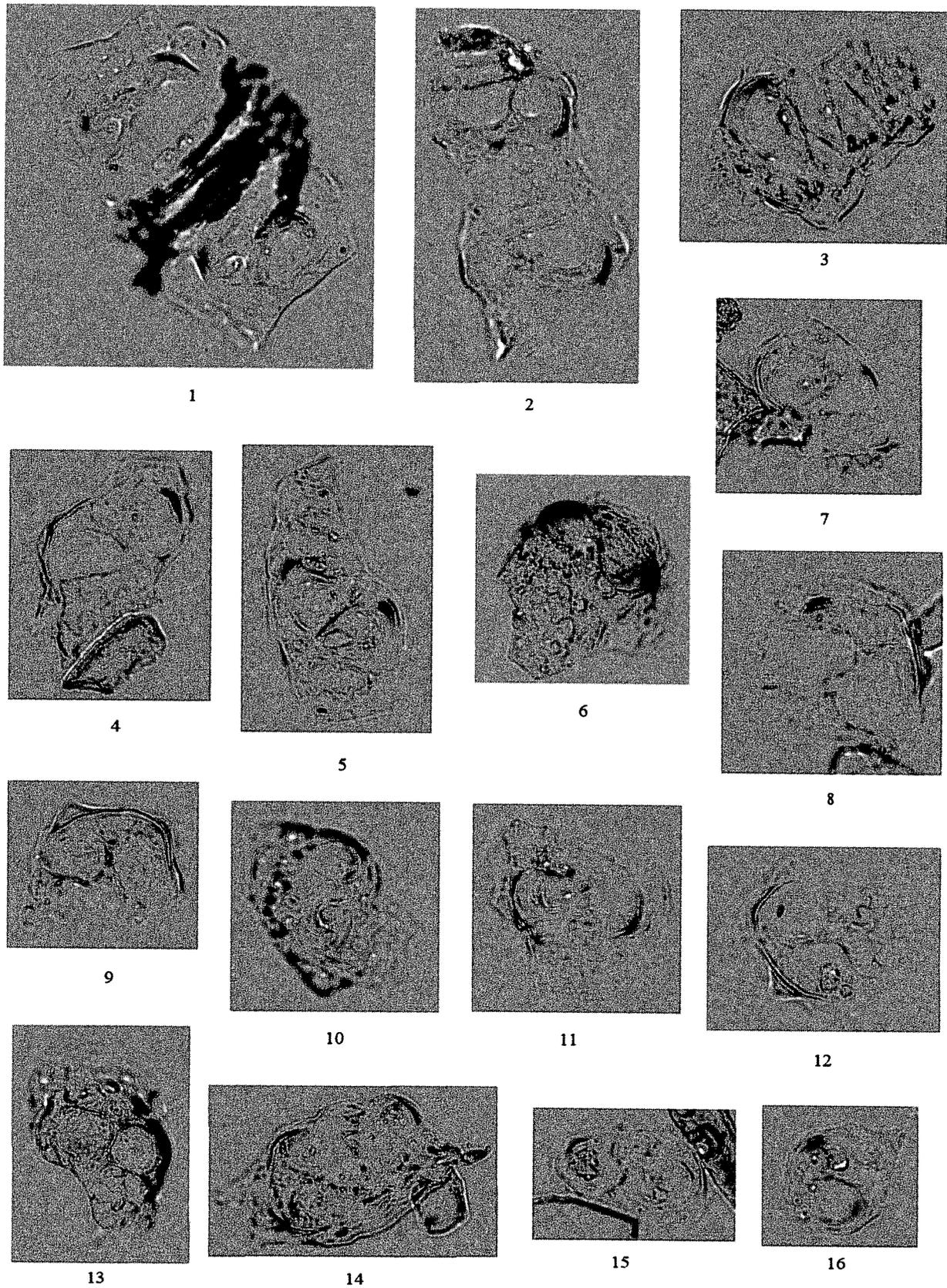


Fig. 4 Microphotographs showing plant opals of the tubercles of upper epidermal cells of red rice hulls.

(approximately 500×)

have shown that these three types already existed in the early Jomon period in Japan. A distinctive form of the tubercle was observed in 28 samples (5.6% of the 500 samples). The strumae face each other and do not form an intricate pattern in this tubercle. Moreover, each struma is slightly larger and appears round (Fig. 4. 9–16). Although its frequency is not large, this shape can be used to identify red rice among rice varieties of type S by plant opal analysis. It may be advisable to define a new type for this tubercle type.

5. Conclusion

The red rice grown in paddy fields in Tsushima is the temperate *japonica* type. Round strumae at the tubercles developed from the upper epidermal cells of hulls, and they may contribute toward the identification of *japonica* type red rice in *O. sativa* L. plant opals extracted from soil samples at archeological sites.

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References

- Arashi, K., 1974. Study of red rice in Japan. Yusankaku Press Co., Ltd., Tokyo. (in Japanese)
- Hamada, H., 1956. The distribution of Japanese red rice and its characters. The Crop Science Society of Japan, vol. 24, no. 3, pp. 147-148
- Ishikawa, R., Maeda, K., Harada, T., Niizeki, M., Saito, K., 1992. Genotypic variation for 17 isozyme genes among Japanese upland varieties in rice. *Ikushugaku zasshi*, vol. 42, no. 4, pp. 737-746
- Kobayashi, H., 2008. The study of *Oryza sativa* L. found at sites in Japan: Plant opal analysis of tubercles at epidermal cells of the glumes of *Oryza sativa* L. Memorial collected papers for Prof. Chousuke Serizawa "Archaeology, Ethnology and History," Rokuichi Syobou Co., Ltd., Tokyo, pp. 595-606 (in Japanese)
- Kobayashi, H., 2013. Species of *Oryza sativa* L. stored in Aoyama castle destroyed in the late 16th century AD, Okayama, western Japan—Plant opal analysis of tubercles developed from the epidermal cells of the glumes of *Oryza sativa* L. — The bulletin of Okayama University of Science, no.49, A, pp.1-4
- Okoshi, M., Jingjie, H., Ishikawa, R., Fujimura, T., 2004. Polymorphic analysis of landraces of Japanese rice using microsatellite markers. *Japanese Soc. of Breeding* 6, pp. 125-133 (Summary in English)
- Takahashi, M., Tajima, M., Kobayashi, H., 2005. The excavation of Hikozaiki shell-mound, Okayama prefecture. *Koukougaku Journal* no. 527, pp. 28-31 (in Japanese)
- Takahashi, M., 2008. Epidemic tissues at hulls of Asian rice and its classification. Memorial collected papers for Prof. Chousuke Serizawa "Archaeology, Ethnology and History," Rokuichi Syobou Co., Ltd., Tokyo, pp.57-66 (in Japanese)
- Takahashi, M., 2010. Plant culture at Hikozaiki shell-mound. *Bull. Center for Okayama city Archaeological operations*, no. 2, pp. 19-46 (in Japanese)