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A study of the relationship between wild rice (*Oryza rufipogon*) and *japonica* rice varieties (*Oryza sativa* L.)

—Using phytoliths to investigate tubercle forms developed from the upper epidermal cells of *Oryza rufipogon* and *japonica* rice varieties hulls—

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Focus has been recently placed on the relationship of between wild rice and *Oryza sativa* L., with some studies, using DNA analyses, suggesting that *japonica* was originated from *Oryza rufipogon*. This study aimed to examine variations in tubercle forms developed from the upper epidermal cells of *Oryza rufipogon* collected from different areas of the world, using phytoliths extracted from their hulls. There have been very few reports on this topic. Moreover, a comparison with tubercle forms of *japonica* varieties was attempted to investigate the relationship between cultivated *japonica* and *Oryza rufipogon*. The result suggests that the tubercle form of *Oryza rufipogon* is much the same as that of tropical *japonica*; however, it is quite different to that of temperate *japonica*. This indicates that *Oryza rufipogon* and temperate *japonica* cultivated in paddy fields have no direct relation. On the other hand, the *Oryza rufipogon* type of tubercle form currently occurs in upland rice in Japan.

Keywords: phytolith; *Oryza rufipogon*; *japonica*; hull.

1. Introduction

As is the case for *Oryza sativa* L., it has been considered that *japonica* and *indica* have a common origin (Oka, 1988) or *japonica* was originated from *indica* (Ting, 1957). However, according to recent DNA analyses (Sato, 1996, 2008, 2013), it is suggested that the ancestor is not common, with *japonica* originating from *Oryza rufipogon*, and the emergence of *indica* is the result of hybridization between *japonica* and an unknown wild *Oryza*. To examine whether differences between tubercle forms of *Oryza rufipogon* collected from different areas of the world exist or not, phytoliths originating from their hulls were examined. A phytolith is mostly consisted of silica and is therefore called a "silica body." This study examines siliceous phytoliths of tubercle forms developed from the upper epidermal cells of *Oryza rufipogon* hulls and examines its relationship with that of *japonica* varieties.

2. Materials and Methods

Four kinds of *Oryza rufipogon* sample accessions analyzed in this study originated from the National Bioresource Project, MEXT, Japan. These samples were identified as follows (Fig. 1 and Table 1): W0593, W1236, W1294, and W1954 (the code listed here are the sample codes used in the source institution). These four samples were collected from four areas in Asia and Oceania, namely China, the Philippines, Malaysia, and Papua New Guinea. Each sample was separately ashed for extracting siliceous phytoliths of tubercle forms from their hulls.

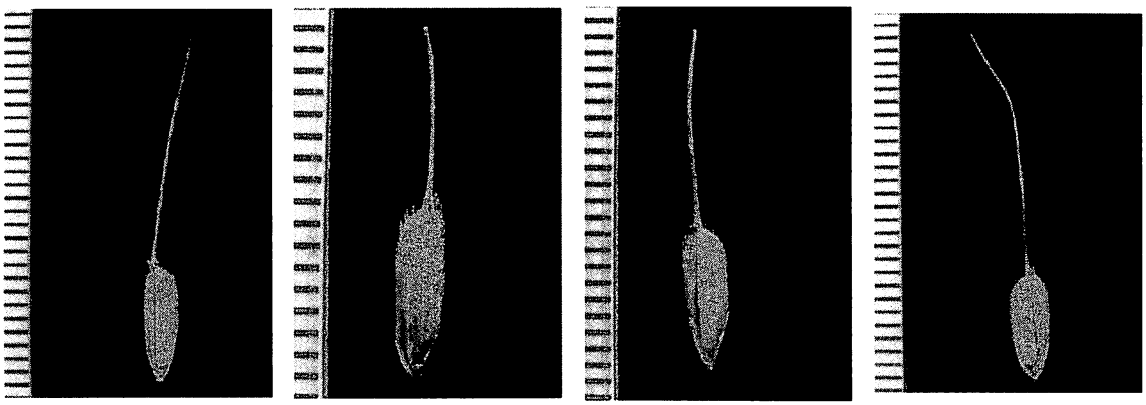

Fig. 1 Four samples of *Oryza rufipogon* used for extracting phytoliths: 1-W0593; 2-W1236; 3-W1294; 4-W1954. (Scale: mm)

Table 1 Places of origin of *Oryza rufipogon* samples used for extracting phytoliths.

No	Original place
W0593	Binjai Rendah, Malaya (Malaysia)
W1236	Madang, Australian New Guinea (Papua New Guinea)
W1294	Mususan, Mindanao, the Philippines
W1954	China

3. Phytolith extraction

(1) Each sample was cleaned in deionized water for 30 min using an ultrasonic cleaning machine. (2) Each sample was dried in an electric furnance at 85°C for 4 h. (3) Using a set of tweezers and a surgical knife, all segments of the hull were removed from each sample, and these segments were burnt in an electric furnace at 315°C for 9–19 h until the color changed to white or gray. The time required depended on the thickness or hardness of the hull. (4) The hulls were treated with 30 ml of 30% H_2O_2 solution to remove their carbons. The hull segments were then heated until they became almost white in color. (5) After removing carbons, deionized water (50 ml) was added to this residue, and 20 µl aliquots of liquid containing phytolith components were placed on slides using a micropipette or standard pipette, and dried in a desiccator for 3–4 h. (6) Cover glasses were placed over dried samples with a mounting reagent and were observed under an optical microscope at 200 × and 400 × magnifications. Microphotographs were obtained with a SLR digital camera mounted on the microscope.

4. 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. A total of 12–24 slides were made per sample, with 70 slides and 140 frames examined overall. All tubercle forms of *Oryza rufipogon* samples from different areas were of typical type C (Fig. 2). Considerably larger papillae developed with forms similar to strumae. Papillae on adjacent lateral branches of long cells did not form intricate patterns, but faced each other and formed circular cones. These remarkable circular cones were concentrated at one spot and were similar in appearance to closely packed mountains with sharp peeks. Detailed differences were not observed in type C of the four samples. Type C is typical in tropical *japonica* (Takahashi et al., op. cit.). In Japan, this type can be observed in upland rice; these rice varieties have descended from tropical *japonica* (Takahashi 2008, 2011). The ratio of upland rice is currently extremely small in Japan. In contrast, most rice varieties comprise the rice cultivated in paddy fields, with the dominant species being temperate *japonica*. The tubercle form of temperate *japonica* is type S (Takahashi et al. op. cit.; Kobayashi 2008). A marked struma can be observed at the lateral branch of the long cell and the papilla exits from this struma. Strumae on adjacent long cells form an intricate pattern. This type does not occur in tropical *japonica* and *indica* varieties. Therefore, the result of investigating

tubercle forms suggests that tropical *japonica* has a direct relation to *Oryza rufipogon,* and it is suggested that the latter is the ancestor of the former and that the latter has no relation to temperate *japonica*. Recent studies (Takahashi 2008, 2010) have shown that type C and S already existed in the early Jomon period dated 7,600–5,500 years ago in Japan. The appearance of both types seems to be ancient. As for type S, other varieties of *Oryza rufipogon* may have this type. Otherwise, type S is possibly the result of hybridization of *Oryza rufipogon* with type C and other unknown *Oryza* species.

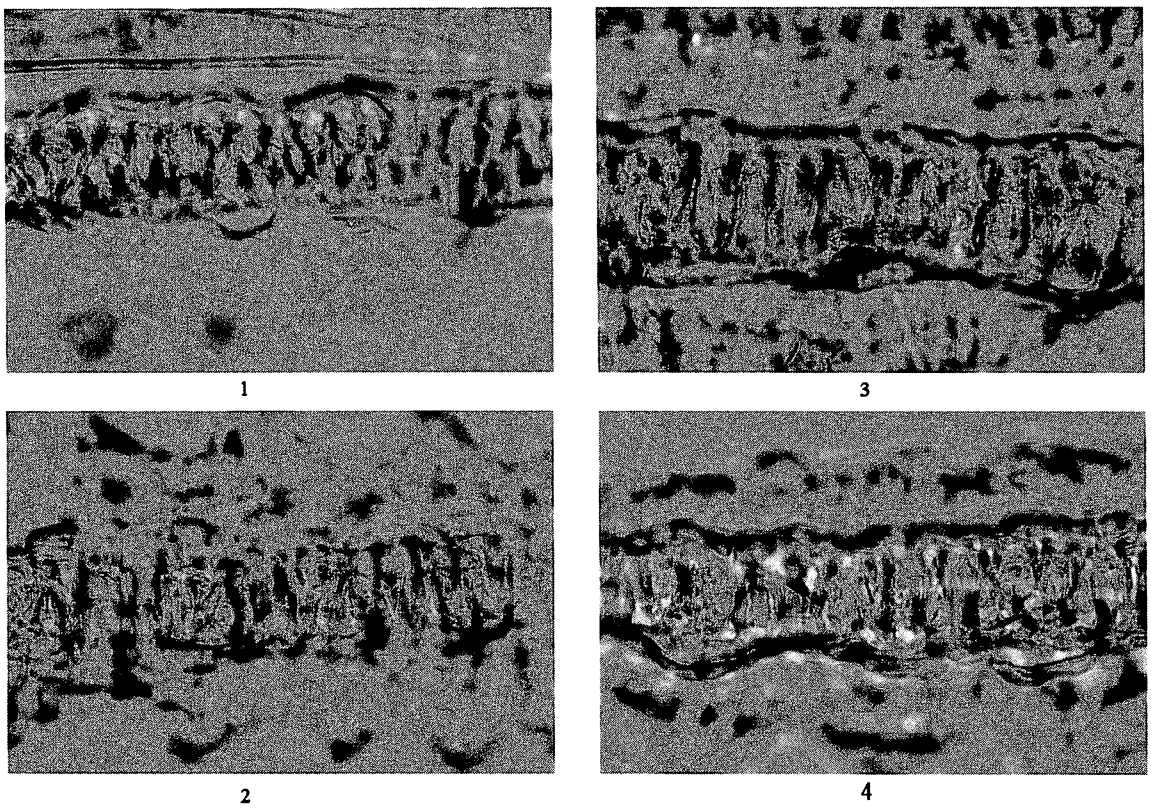

Fig. 2 Microphotograph shows phytoliths of tubercles of upper epidermal cells of *Oryza rufipogon* hulls: 1-W593; 2-W1236; 3-W1294; 4-W1954. (Magnification approximately 400 ×)

5. Conclusion

The tubercle form of *Oryza rufipogon* was of type C. Therefore, it is suggested that *Oryza rufipogon* is the ancestor of tropical japonica and this type occurs in upland rice in Japan. Further investigation is required to clarify the relevance of *Oryza rufipogon* and temperate *japonica* cultivated in paddy fields.

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