

# 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.—

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I studied tubercles developed from the epidermal cells of the glumes of *Oryza sativa* L. and species of this plant in soil samples from the remains of an archeological site using plant opal analysis. Excavation of the remains of the Aoyama castle was conducted in July 2010. During the excavation, soil samples were collected from the ditch-like remains attached to the embedded-pillar building of the castle. In the process of analysis, tubercles developed from the epidermal cells of the glumes were extracted from the samples. The species of *O. sativa* L. consisted of temperate and tropical japonica, paddy rice and upland rice varieties. These japonica rice varieties were stored in the ditch-like remains, attached through their blades and shafts without being threshed.

**Keywords:** plant opal analysis; *Oryza sativa* L.; epidermal cell; archeology.

## 1. Introduction

The remains of a castle are located at Wake-cho, Wake-gun in Okayama prefecture, approximately 200 km west of Osaka (Fig.1, 2), atop a mountain approximately 220 m above sea level. These remains have been designated as a historic site. According to the private educational bureau of Wake-cho (1909), this castle has been called as the Aoyama castle. The age of foundation of the castle is unknown, but it was attacked and destroyed by a feudal lord named Naoie Ukita in 1577. As per the report of Yoneda (2011), excavation was initiated in the castle by the educational bureau of Okayama prefecture and Wake-cho for the construction of a broadcast station in the end of July 2010. During the excavation, large amounts of carbides were found in the ditch-like remains attached to an embedded-pillar building number 3. Soil samples for plant opal analysis were acquired from these remains during the excavation.

## 2. Procedures of plant opal extraction

- (1) Soil samples were dried in an electric furnace maintained at 110 °C for 72 h.
- (2) Using an electronic precision balance with a precision of 0.001 g, 1 g of soil sample was weighed.
- (3) The weighed aliquot was treated with 30 ml of a solution of 30 % H<sub>2</sub>O<sub>2</sub> for rinsing and heated until the liquid was reduced to 10 ml.
- (4) Deionized water (50 ml) was added to this residue, and other impurities were repeatedly removed from

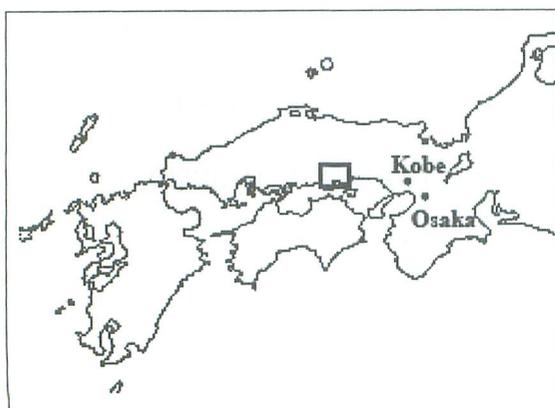


Fig. 1 The Rectangle shows the location of the remains of the Aoyama castle in western Japan.

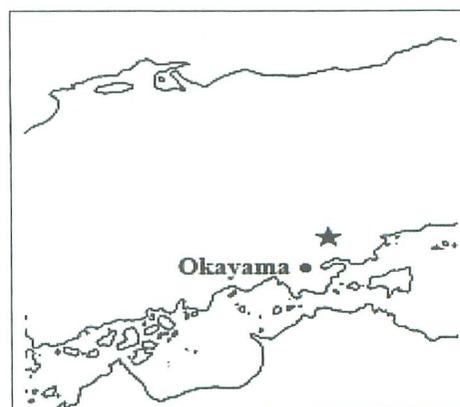


Fig. 2 The star mark shows the location of the remains of the Aoyama castle.

the residue on the basis of Stokes' law.

- (5) 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 a density of 2.30 g/ml to separate any remaining mineral component.
- (6) After extraction, each 20  $\mu$ l of liquid containing plant opal component was stored on a stageglass with a micropipette. These samples were dried in a desiccator for 3–4 h.
- (7) One of dried samples was covered with a coverglass with a mounting reagent and scanned using an optical microscope at 200X and 400X magnification. The glumes of *Oryza sativa* L. were counted and microphotographs were taken with a single-lens reflex digital camera mounted on the microscope.

### 3. Classification of tubercles developed from the epidermal cells of the glumes of *O. sativa* L.

Classification of tubercles followed the definition of Takahashi et al. (2005):

Type S: 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 (Fig. 3. 1–3).

Type C: Strumae on adjacent lateral branches of long cells do not form intricate patterns. Papillae face each other and strumae form circular cones (Fig. 3. 4–9).

Type P: Intricate patterns and circular cones cannot be observed on adjacent lateral branches of long cells.

Type T: This type is included in type S in a broad sense. Strumae adjacent lateral branches are oblong compared with those of type C and do not form intricate patterns (Fig. 3. 13).

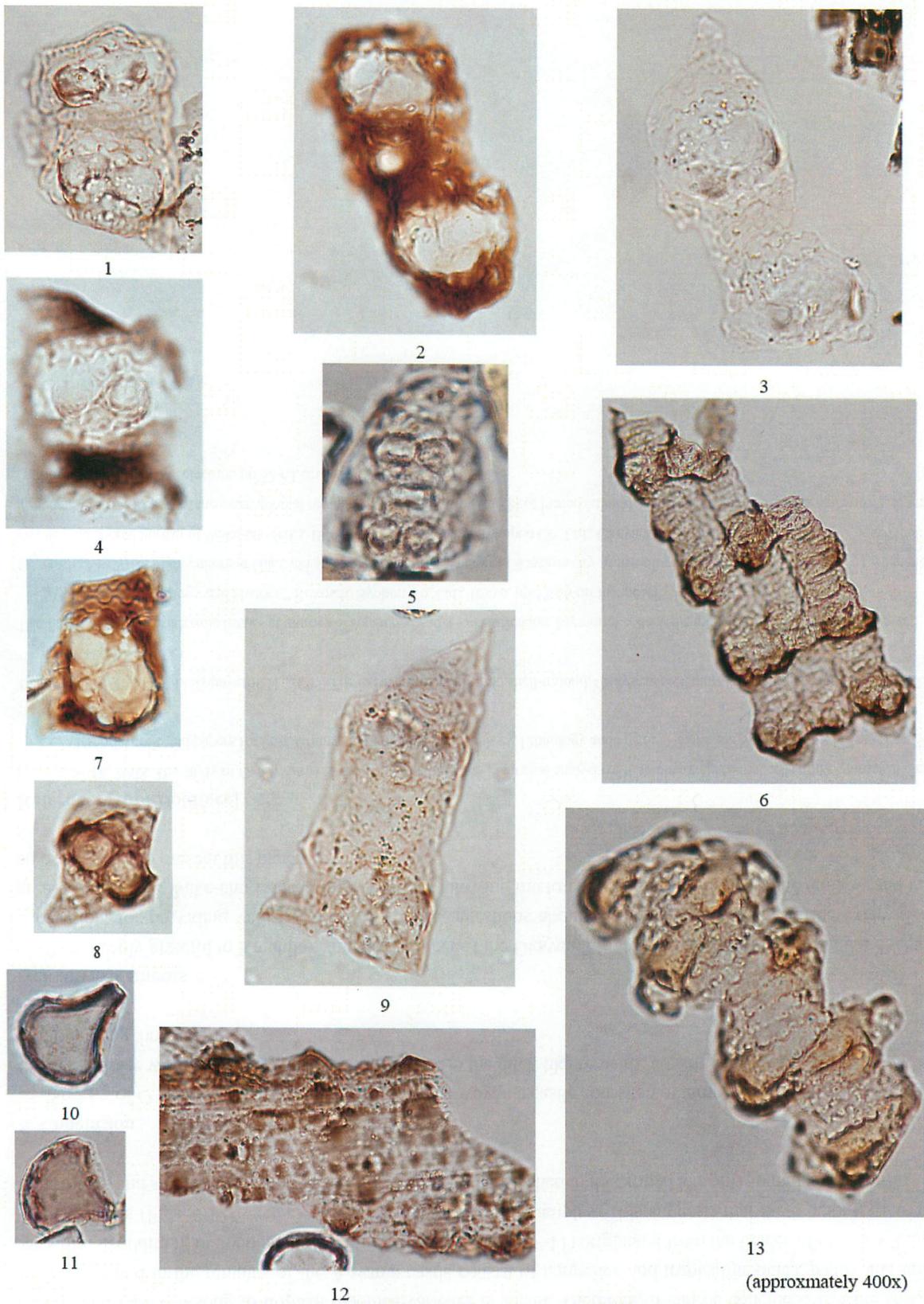
Microscopic examination was performed on 500 samples. This examination required 5 g of dried soil sample and 102 pieces of stageglass, and 204 frames on them were examined.

Table 1. The ratio of types of tubercles developed from the epidermal cells of glumes of *O. Sativa* L.

Type	C	S	P	T	total
Number	84	367	7	42	500
Percentage	16.8	73.4	1.4	8.4	100.0

### 4. Results and discussion

Type S accounted for 367 pieces or 73.4% of the 500 pieces (Table 1). The next most numerous type was C and the rarest was type P. Recent studies have shown that these three types already existed in the early Jomon period (from 7600 to 5500 years ago) and both type C and P are typical in tropical japonica and indica rice varieties (Takahashi et al., op. cit.). Type C can be observed in upland rice in Japan in the present day; these rice



**Fig. 3** Microphotographs showing plant opals of *O. sativa* L. extracted from soil samples from the ditch-like remains of the Aoyama castle. 1-9, 13: Tubercles developed from the epidermal cells of the glumes; 8-11: Motor cells; and 12: Cells from the shaft.

varieties have descended from tropical japonica (Takahashi 2008, 2011). In contrast, type B occurs only in temperate japonica varieties (Takahashi et al. op. cit.; Kobayashi 2008). Analysis of spodogram samples from the glumes of nine tropical japonica varieties showed that they belonged to type C (Kobayashi op. cit.). There are no reports on detection of indica varieties by DNA analysis from the prehistoric age to the recent times in Japan. These data provide evidence that type C and type P belong to tropical japonica varieties in Japan. Therefore, it can be concluded that the species of *O. sativa* L. found in the remains of the Aoyama castle consist of temperate and tropical japonica, paddy and upland rice varieties. In addition, many plant opals of motor cells (Fig. 3. 10–11) originated from the blades of *O. sativa* L., and cells of their shafts (Fig. 3. 12) were extracted from the same soil samples. This suggests that these japonica varieties with their blades and shafts were stored in the ditch like remains attached to the embedded-pillar building number 3.

## 5. Conclusion

Species of *O. sativa* L. found in the remains of the Aoyama castle consisted of temperate and tropical japonica, paddy and upland rice varieties. These varieties were stored in the ditch-like remains, attached through their blades and shafts without being threshed.

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