[Short Report]

**Gas Exchange through the Slit between the Lemma and the Palea in the Rice (Oryza sativa L.) Floret before Anthesis**

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A rice floret emerges from the sheath of the flag leaf just before anthesis and opens for about one hour in the morning. Before flower-opening, stamen and pistil are enclosed in a hull, probably to avoid direct contact with water (Corbet, 1990; Fagri et al., 1979) and protect them from damage by insects and diseases. Since the stomata on the outer surface of rice hull are vestigial (Hoshikawa, 1993), the slit between the lemma and palea is considered to be the primary pathway for passage of gas between the inside and outside of floret, although the edge of the lemma is hooked to the edge of the palea (Hoshikawa, 1993) and the slit looks tightly closed. The radical preparatory process such as decomposition of starch in the pollen grains occurs in the rice floret just before the anthesis (Koike et al., 1987). Such a radical preparatory process may require oxygen for respiration. If so, where does the oxygen for respiration during this process come from? The purpose of this report is to clarify whether the preparatory process requires supply of oxygen from outside of the floret and the route of the oxygen supply. For this purpose, we examined the effect of sealing of the slit between lemma and palea on the preparatory processes for the anthesis.

**Materials and Methods**

1. **Plant materials**

   A japonica rice cultivar, Akitakomachi, was used. Seedlings at the 5.8 leaf stage were transplanted into four 1-liter pots in a circular pattern, 20 seedlings per pot, early in June, and grown outdoors (Osaka, Japan) under submerged soil conditions. The heading date was early in August. Each pot was provided with 0.6 g of P<sub>2</sub>O<sub>5</sub> as basal-dressing and with 0.3 g each of N, and K<sub>2</sub>O as top-dressing at about 40 days before heading. Tillers were removed during the experiment when they appeared. The natural opening of florets started at 10:30 to 11:00 on the days of the experiments.

2. **Experiment 1**

   At the mid-flowering stage, the florets expected to open on the next day were marked at 17:00, divided into three groups and treated as follows within one hour (17:00-18:00). In the first group, the slits between the lemma and the palea of florets were sealed with petroleum jelly (Nacalai tesque, Vaseline (white)) using a tooth-pick (Fig. 1). In the second group, the slits were sealed with petroleum jelly, and a pin-hole was made on every lemma with a sewing needle. The third group was the control.

   On the next day, at the beginning of the natural opening of florets (10:00), 16 florets per group were sampled. Ten florets from each group were used to examine the ability of anther to dehisce. Immediately after sampling, the lemmata were removed from these florets, which were then kept at 28°C and 60% R.H. for 30 minutes (artificial floret opening), and the percentage of dehisced thecae was examined (Matsui et al., 1999). The remaining six florets were used to examine the decomposition of starch in pollen grains. The anthers were detached from the florets and split using tweezers, and the pollen grains were stained with iodine-potassium iodide solution. Pollen grains were classified into 3

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Before decomposition Partly decomposed Fairly decomposed
(Unstained area = 0) (0 < Unstained area < 20) (20 ≤ Unstained area)

Score 0 0.5 1.0

Table 1. The effects of sealing of slits between paleae and lemmata and pin-hole made on the lemmas on the decomposition of starch in pollen grains, the dehiscence of anthers in florets and the opening of florets. The florets were sealed and pin-holes made from 17:00 to 18:00 on the day before the natural anthesis of florets.

| Treatment          | Degree of starch decomposition* | Percentage of dehisced thecae* | Percentage of opened florets |
|--------------------|---------------------------------|---------------------------------|-------------------------------|
| Control            | 0.643±0.023a                    | 96.7±2.2a                      | 100.0                         |
| Sealing            | 0.017±0.017b                    | 5.0±2.5b                       | 8.3                           |
| Sealing + pin-hole | 0.673±0.047a                    | 93.3±3.7a                      | 100.0                         |

*Values are mean±S.E.
Within columns, values followed by different letters are significantly different (p<0.001).

Fig. 2. Schematic diagram of the decomposition process of starch in pollen grains and score of the degree of starch decomposition. White: decomposed region.

Fig. 3. Change in CO₂ concentration in the sealed (●) and unsealed (□) florets. The florets were sealed at 17:00 to 18:00 on the day before their natural anthesis (17.5 to 16.5 hrs before anthesis). Vertical bars indicate±S.E. (n=2).

Fig. 4. Change in the degree of decomposition of starch in pollen grains in the sealed (●) and unsealed (□) florets. The florets were sealed at 17:00 to 18:00 on the day before natural anthesis (17.5 to 16.5 hrs before anthesis). Vertical bars indicate±S.E. (n=6).

3. Experiment 2
At the mid-flowering stage, the florets expected to open on the next day were marked at 17:00. Within one hour (17:00–18:00), the slits between the lemmata and the paleae were sealed with petroleum jelly in one group, but not sealed in the other group (control). The marked florets were sampled eight times from 18:00 on the day before anthesis to 10:00 on the day of anthesis (30 minutes before the natural floret opening). The three florets in each group at each time were used to measure the CO₂ concentration inside the florets. The degree of the decomposition was measured and calculated in the same way as in Experiment 1.

Results and Discussion
The sealing of the slits between paleae and lemmata suppressed the decomposition of starch in the pollen grains, the dehiscence of the anthers in response to the artificial floret opening and the spontaneous opening of florets (Table 1). The florets were released from the suppression by making pin-holes in the lemma. Although we did not measure the gas exchange rate in all treatments, these findings suggest that the sealing
disturbed the gas exchange though the slit between the lemma and the palea, and that the piercing of lemma broke the effect of sealing. The results also suggest that these processes required the gas exchange through the slit.

Measurement of CO₂ concentration inside the sealed florets revealed that CO₂ concentration in the sealed florets increased up to 0.15 mol mol⁻¹ before sunrise (Fig. 3). The drastic increase of CO₂ concentration shows the effect of the sealing on the gas exchange between inside and outside of the floret. Most of the O₂ in the sealed florets might be consumed during this period by respiration and the concentration of O₂ might decrease to below 0.1 mol mol⁻¹. A high CO₂ concentration (Chaves and Tomas, 1984; Li and Kader, 1989; Grodzinski et al., 1982; Kao and Yang, 1982; Gepstein and Thimann, 1982) and low O₂ concentration (Burg and Thimann, 1959; Kader, 1980) affect the biosynthesis of ethylene. Such a gaseous condition also inhibits receptivity to hormones (Burg and Burg, 1967; Kader, 1986). On the other hand, a low O₂ concentration condition of 0.045 to 0.09 mol mol⁻¹ itself limits the respiration rate in plants (Kader, 1980; Weichmann, 1987). Although any role of hormones such as ethylene can not be excluded, a high CO₂ and low O₂ concentration in the florets at night may be the primary cause of the disturbance of starch decomposition, anther dehiscence and floret opening.

The concentration of CO₂ in the unsealed floret was 0.008–0.025 mol mol⁻¹, which is rather higher than that of ambient air (Fig. 3). The high concentration in the unsealed floret suggests the resistance to gas exchange between lemma and palea. The increase in CO₂ concentration preceding the sunrise was observed also in the unsealed floret although the change in the concentration was within 0.016 mol mol⁻¹ (Fig. 3). The increase in CO₂ concentration in the sealed and unsealed florets would result from the increase in respiratory rate in the stamen as has been suggested by Keijzer (1999). Since starch did not decompose in the sealed florets in which CO₂ concentration drastically increased before dawn (Fig. 4), the increase of CO₂ concentration may be attributed to some O₂ requiring process just before the beginning of the decomposition of starch. Keijzer (1999) claimed that the endogenous CO₂ concentration level in the flower of maize determines the time of anthesis.

After dawn, the CO₂ concentration decreased in the sealed and unsealed florets. The rice glume has stomata on its inner surface. The decrease of CO₂ concentration suggests the consumption of CO₂ in the florets by assimilation in the glume. Wakasugi et al. (2000) reported that the assimilation rate in the glume amounts to 18 nmol O₂ gFW⁻¹ s⁻¹, which is about 530 nmol O₂ h⁻¹ per floret. Thus, 0.15 mol mol⁻¹ of the CO₂ in the sealed floret can be consumed within a few hours by the assimilation.

During the daytime, CO₂ in florets is consumed by the assimilation of florets. The gas exchange through slits is, therefore, important for anthesis particularly at night, because O₂ is mainly supplied from slits and CO₂ is not consumed in florets at night.

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