Internal Microstructures of Wheat Grains as Affected by Post-anthesis Waterlogging: Observations with a Scanning Electron Microscope

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開花後に過湿土壌ストレスを受けたコムギ子実の内部微細構造：走査顕微鏡による観察

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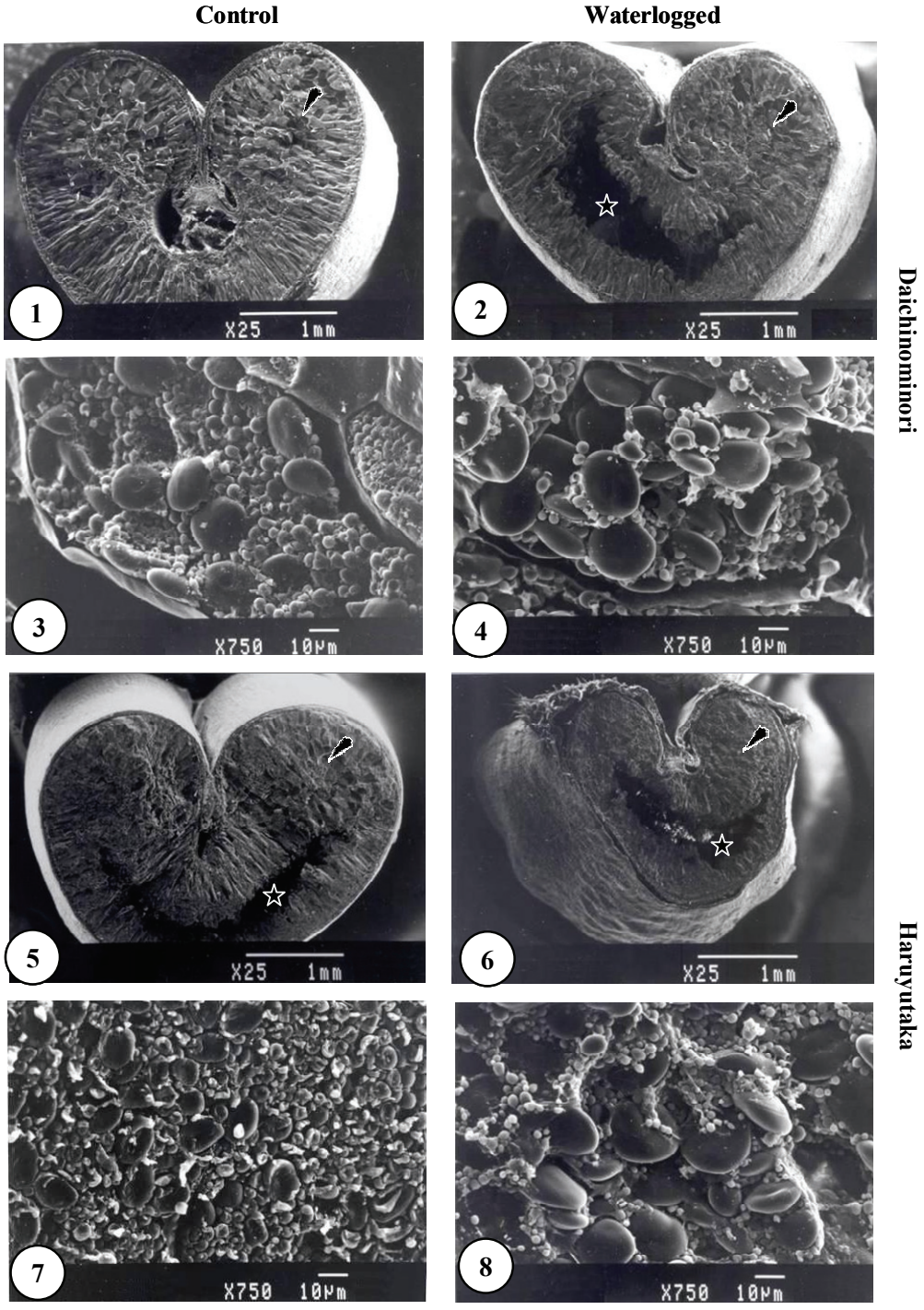
In our recent article, we reported poor grain growth at the later part of grain filling, which led to a lighter grain in two Japanese wheat cultivars, Daichinominori and Haruyutaka, under post-anthesis waterlogging (Hossain et al., 2011, Field Crops Research 123, 100-108). Endosperm, the main part of wheat grain occupying about 90% of the grain, is made mainly of starch in the two forms of starch granules, larger A (10-50μm in diameter) and smaller B (1-10μm diameter) granules. Hence, the grain weight of wheat largely depends on the numbers and arrangements of the starch granules in the endosperm. Therefore, we conducted scanning electron microscopic study on the grains affected by post-anthesis waterlogging—especially the starch granules in endosperm.

Materials and Methods: The plants of Daichinominori (bred in Western Japan) and Haruyutaka (bred in Hokkaido) were grown in field of Experimental farm, Yamaguchi University in 2009–2010 (November–June). The plants of the both cultivars were waterlogged at surface during grain-filling period (anthesis to maturity). Haruyutaka experienced the stress from 2 weeks before anthesis due to its delayed anthesis. Five spikes were sampled every week starting from 2 weeks after anthesis until maturity. The waterlogged and control plants exhibited maturity 28 and 38 days after anthesis (DAA) in Haruyutaka, and 35 and 38 DAA in Daichinominori, respectively. The grains developed in first and second floret of the spikes were sampled from the middle part of the spike (5th-8th spikelets), and were stored in a fixative containing 70% alcohol. For specimen preparation, three grains were dehydrated with a 50-100% ascending ethanol series. The completely dehydrated grains were frozen in liquid nitrogen, and were transversely cracked using a hand-made device (two copper plates with small holes, scalpel, and a hammer). The fractured pieces of grains were infiltrated with 100% t-butyl alcohol, frozen in a refrigerator, and then were dried using a freeze-drying device (JFD-300, JEOL). Fractured surfaces of the dried grains were coated with 300Å gold using an ion-sputtering device (JFC-1500, JEOL), and were observed with a scanning electron microscope (JSM-6100, JEOL).

Results and Discussion: Post anthesis waterlogging had a greater negative impact on internal structures of matured wheat grains in both cultivars, although the impact was not pronounced in young grains. The effect was severer in Haruyutaka than in Daichinominori. The stress induced to form an endosperm with loosely arranged starch granules and with a cavity (Figs. 1−8). However, Haruyutaka in control condition also exhibited a cavity in endosperm (Fig. 5). The stress drastically reduced the formation of B granules, which

might contribute to poorly developed endosperm, and finally lighter grains. The lighter grain in Haruyutaka, a common phenomenon under western Japan environment, might be related to poorer development in endosperm due to over wetting soils in the area.



Figs. 1–8: SEM micrographs of the fractured surface of matured grains in control (left side) and waterlogged (right side) plants of the two Japanese wheat cultivars, Daichinominori (1–4) and Haruyutaka (5–8). Figs. 1–2, 5–6: transversally cleaved grains; arrows indicate the endosperm and asterisks indicate the damaged portion (cavity) in endosperm. Figs. 3–4, 7–8: Enlarged endosperm with two types of starch granules, larger A type and smaller B type granules.