【Short Report】

Breakdown of Cell Wall Polysaccharides in Rice Culms at the Early Ripening Stage

Keisuke Nemoto, Satoshi Ando, Eiichi Tanimoto[1], Nobuyuki Kabaki[2], Hiroshi Fujimoto[2] and Shigemi Akita*

(The University of Tokyo, Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan; [1] Nagoya City University, Mizuho, Nagoya 467-8501, Japan; [2]National Agriculture Research Center, Kannondai, Tsukuba, Ibaraki 305-8666, Japan)

The senescence process of a tissue often includes the degradation of cell wall polysaccharides. A well-known example is ripening fruit, where the degradation of pectic polysaccharides causes tissue softening (Huber, 1983a, b). In rice, the senescence of culms is relevant to such important phenomena as carbohydrate turnover and lodging resistance. However, little is known about the degradation process of cell walls during culm senescence. In this study, we examined the degradation of cell walls in ripening rice culms and found that (1) the cell wall thickness decreases, and (2) hemicellulose is remobilized as the grain filling proceeds in the culm.

Materials and Methods

Two field-grown japonica rice cultivars, M-401 and Tsukinohikari were grown at the National Agriculture Research Center, Tsukuba, under conventional growing conditions in 1994 (direct seeded) and 1996 (transplanted). In both years, the fourth internodes of the main culms below the peduncle, which was considered to have already reached its final size were taken at heading and two weeks after heading. For the measurement of cell wall thickness, small culm segments were fixed in FAA (5% formalin, 5% glacial acetic acid and 45% ethanol), dehydrated with graded ethanol and embedded in a glycol methacrylate resin (Historesin embedding kit, Leica, Germany). Cross sections ($3\,\mu$m thick) were cut using a retraction rotary microtome, mounted on a slide glass and stained with toluidine blue O to visualize cell walls or periodic acid. The Schiff reaction was also used to visualize total polysaccharides (Berlyn and Miksche, 1976). Cell wall thickness (means±standard deviations, n=60) was measured on parenchyma cells under an optical microscope with an eyepiece micrometer.

The amount of cell wall polysaccharides was measured using a modified method of Tanimoto and Huber (1997). Two-centimeter long segments were excised from the fourth internode of the main culm and stored at -60 oC. The segments were then homogenized in a mixture of phenol and Tris-HCl buffer, washed with Tris-HCl buffer and defatted with methanol-chloroform to obtain purified cell walls. Pectic polysaccharides were extracted with CDTA (trans-1,2-cyclohexanediamine-N,N,N',N'-tetraacetic acid) and ammonium oxalate after pronase treatment. Hemicellulosic polysaccharides were then extracted with KOH, and the alkaline-insoluble residue was designated as the cellulose fraction. The amounts of uronic acids and neutral sugars in the polysaccharide fractions were measured using the m-phenylphenol (Blumenkrantz and Asboe-Hansen, 1973) and phenol-sulfuric acid methods (Dubois et al., 1956), respectively.

Results and Discussion

In 1994, we made a preliminary study on culms at the heading and ripening stages in the M-401 and Tsukinohikari rice varieties. Our light-microscopical observations revealed that cell thickness markedly decreased after heading in M-401, while there was little change in Tsukinohikari (Fig.1). To verify these findings, we carried out a more detailed research in 1996. In contrast to the 1994 experiment, cell wall thickness decreased similarly in the two cultivars; the cell wall thickness at the ripening stage (average $0.49\pm0.06\,\mu$m and $0.46\pm0.04\,\mu$m for M-401 and Tsukinohikari, respectively) was about 70% of that at the heading stage (average $0.72\pm0.03\,\mu$m and $0.67\pm0.07\,\mu$m for M-401 and Tsukinohikari, respectively) in both cultivars. Thus the cell wall thickness decreased in 1996, but not in 1994, in Tsukinohikari. The amount of polysaccharides in the culm would be determined by the balance between sink and source sizes, which in turn is strongly affected by solar radiation and
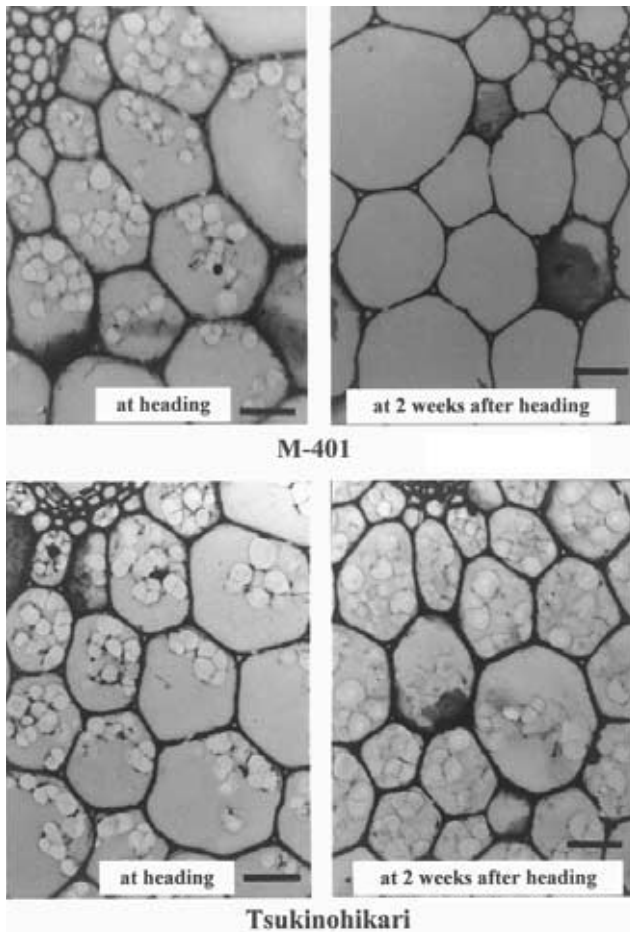
Fig. 1. Cross sections of the culms at heading and two weeks after heading in the M-401 and Tsukinohikari rice cultivars. Bars indicate 30 μm.
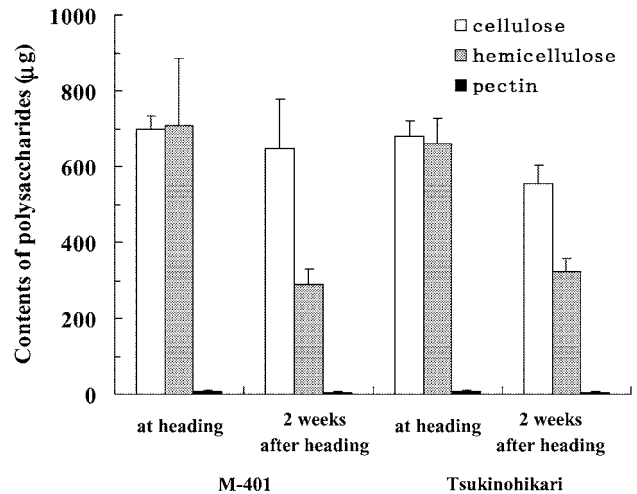

Fig. 2. Cell wall polysaccharides of the culms (2cm long) at heading and two weeks after heading in the M-401 and Tsukinohikari rice cultivars. Bars indicate standard deviations (n=8).

other environmental factors. Solar radiation was much higher in 1994 than in 1996.

In the 1996 experiment, we quantified the cell wall polysaccharides in the culms to understand the mechanisms involved in the decrease in cell wall thickness (Fig. 2). At the ripening stage, the content of total cell wall polysaccharides in the culms was about 70% of that at the heading stage in both M-401 and Tsukinohikari. This was consistent with the decrease in the cell wall thickness mentioned above. Thus, the decrease in cell wall polysaccharides could be responsible for the decrease in cell wall thickness. The cell wall at the heading stage was composed of nearly equal amounts of cellulose and hemicellulose. At the ripening stage, the content of hemicellulose was about 50% of that at the heading stage, whereas the content of cellulose at both the heading and ripening stages were not significantly different. Pectin occupied only a negligible part (about 0.5% and 0.3%, respectively) of the total polysaccharides at both the heading and ripening stages, as expected in grass cell walls. Thus, the decrease in cell wall polysaccharides was attributed mainly to the decrease in hemicellulose (Fig. 2).

These results agree well with previous reports that hemicellulose undergoes a turnover in the cell walls of the oat leaf (Loescher and Nevins, 1972), rice leaf (Zarra and Masuda, 1979a, b), maize leaf (Huber and Nevins, 1979) and wheat stem (van Herwaarden et al., 1998), while the turnover rate of the pectic component was not high compared with that of hemicellulosic polysaccharides in the oat leaf (Wada et al., 1968).

Components of hemicellulose vary across plant taxa and the primary component of hemicellulose in grasses is 1,3;1,4-β-glucan (Nevins et al., 1978; Sakurai, 1991). A decrease in the hemicellulose content in rice culms may result from a higher rate of β-glucan degradation, from a lower rate of β-glucan synthesis, or both. Previously, we found that the *Gns1* gene, a putative 1,3;1,4-β-glucanase gene of rice (Simmons et al., 1992), is highly expressed in ripening rice culms (Nemoto et al., 1999; Baba et al., 2001), which suggests that the higher rate of β-glucan degradation might be the main cause of the decrease in the hemicellulose content in ripening culms. The rate of β-glucan degradation might be very high in ripening culms, presumably because of its translocation to the panicle.

Acknowledgments

References

Baba, Y. et al. 2001. Plant Prod. Sci. 4 : 230-234.

Berlyn, G.P. and Miksche, J.P. 1976. Botanical Microtechnique and Cytochemistry, Iowa State University Press.

Blumenkrantz, N. and Asboe-Hansen, G. 1973. Anal. Biochem. 54 : 484-489.

Dubois, M. et al. 1956. Anal. Chem. 28 : 350-356.

Huber, D.J. and Nevins, D.J. 1979. Plant Cell Physiol. 20 : 201-212.

Huber, D.J. 1983a. Hortic. Rev. 5 : 169-219.

Huber, D.J. 1983b. Hort. Sci. 25 : 781-783.

Loescher, W. and Nevins, D.J. 1972. 50 : 556-563.

Nemoto, K. et al. 1999. Jpn. J. Crop Sci. 68 (Extra issue 1) : 32-33.

Nevins, D.J. et al. 1978. Phytochem. 17 : 1503-1505.

Sakurai, N. 1991. Bot. Mag. Tokyo. 104 : 235-251.

Simmons, C.R. et al. 1992. Plant Mol. Biol. 18 : 33-45.

Tanimoto, E. and Huber, D.J. 1997. Plant Cell Physiol. 38 : 25-35.

van Herwaarden, A.F. et al. 1998. Aust. J. Agric. Res. 49 : 1083-1093.

Wada, S. et al. 1968. Plant Cell Physiol. 9 : 369-376.

Zarra, I. and Masuda, Y. 1979a. Plant Cell Physiol. 20 : 1117-1124.

Zarra, I. and Masuda, Y. 1979b. Plant Cell Physiol. 20 : 1125-1133.