17. Screening of pullulanase deficient mutant of rice

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The starch in endosperm is synthesized by various kinds of enzymes, ADP-Glc pyrosphorylase, soluble starch synthase, starch-branching enzyme and starch-debranching enzyme (DBE) (Smith et al., 1997 Myers et al., 2000 Nakamura, 2002). Two types of DBE, isoamylase and pullulanase have been reported in rice endosperm and the function of isoamylase is well characterized (Kubo et al., 1999). However, the function of pullanase is not clear. In order to clearly define the role of pullulanase in starch synthesis in plant seeds, a pullulanase deficient mutant was found by screening and the genetic analysis of this mutant was performed.

We have selected for 100kD pullulanase deficient mutants by SDS-PAGE of endosperm protein from more than 1,200 endosperm mutant lines which were induced from fertilized egg cells of the *japonica* rice, cv Taichung 65 treated with N-methyl-N-nitrosourea. A mutant line, EM1003, which lacked the 100kD protein was obtained (Fig. 1A). Western blot analysis with antiserum raised against pullulanase showed that the mature endosperm of EM1003 was absent from pullulanase protein (Fig. 1B). Pullulanase activity was not detected in developing endosperm of EM1003 (Fig. 1C). These results show that EM1003 lacks pullulanase protein.

To elucidate the inheritance of the deletion of pullulanase in EM1003, the proteins of F_2 seeds of the cross between EM1003 and the original cultivar were analyzed by SDS-PAGE. The segregation mode in the F_2 seeds is shown in Table 1. The segregation ratio fitted to the expected 3:1 ratio, suggesting that pullulanase deficiency in EM1003 is controlled by a single recessive gene.

This pullulanase deficient mutant is useful for the resolution of pullulanase function in the system of starch biosynthesis in plant seed.

Fig. 1

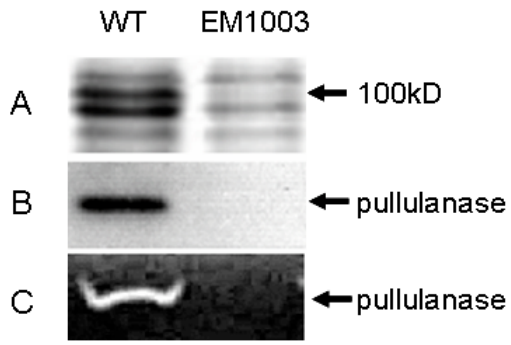

Fig. 1 Western blot analysis of a pullulanase deficient mutant, EM1003.
A: CBB staining; B: Western blot analysis with antiserum raised against pullulanase purified from rice endosperm; C: Native/PAGE pullulanase activity staining.

Table 1 Segregation of pullulanase present(+) and absent(-) phenotypes in F_2 seeds from the cross between EM1003 and its parent cv Taichung 65

Cross combination	Segregation in F_2seeds		total	χ^2 (3:1)
	+	-		
TC65×EM1003	90	28	118	0.05

References

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