In situ staining the activities of starch synthesis related enzymes of the rice grain

T.L. Jeng[1], C.S. Wang[1], **J.M. Sung[2]**

[1]Division of Agronomy, Agricultural Research Institute, Taichung 413, Taiwan, ROC
[2]Department of Food Science and Nutrition, Hung Kuang University, Sha Lu, Taichung 433, Taiwan, ROC. jmsung@nchu.edu.tw

Abstract

In the present study, the in situ grain enzyme activities expression by nitro-blue tetrazolium staining was compared between a low amylase rice mutant (SA419) and its wild type (Tainung 67). All enzymes including sucrose synthase, invertase, hexokinase, UDPglucose pyrophosphorylase, ADPglucose pyrophosphorylase, starch synthase could be visualized in the growing endosperm. The activities of enzymes for SA419 showed blue staining in all the endosperm cell at 7 days after anthesis (DAA), then de-colored from middle part at 11 DAA. The de-colored region increased progressively coinciding with grain development. Marked changes were observed in the growing grains of Tainung 67 at 18 DAA. The de-coloration patterns of NBT enzyme activity in situ staining for the starch synthesizing enzymes correlated with the shift in enzyme activities measured during grain development. It appears that the in situ enzyme activity staining could provide more information to support the biochemical analysis for physiological investigation.

Media summary

The activities of starch synthesizing enzyme were expressed in all the endosperm cells at early phase but progressively declined from central part of endosperm cells during rice grain development.

Keywords

Histochemistry, mutant, rice grain, starch, enzyme

Introduction

Starch is synthesized in the plastid of rice endosperm by ADPglucose pyrophosphorylase, starch synthase, starch branching and starch debranching enzyme. The conversion of sucrose to starch in endosperm is crucial in regulating the grain growth (Jeng et al., 2003). The ability of endosperm cells to synthesize starch changed during grain filling, starting from central part of endosperm at initial phase of grain-fill and progressively moving to outer part of endosperm adjacent to the aleurone at the end of grain fill. These phenomenons were undetectable through chemical determination using the conventional whole grain extraction. However, the histochemical staining may help to differentiate these differences. A potent method which coupling of NAD reduction to the reduction of nitroblue tetrazolium (NBT) and leading to the formation of a blue precipitate has been developed recently. This technique has been applied to some grain enzymes related to starch accumulation (Miller and Chourey, 1992; Wittich and Vreugdenhil, 1998; Sergeeva and Vreugdenhil, 2002).

The activities of sucrose synthase, invertase, hexokinase, ADPglucose pyrophosphorylase, UDPglucose pyrophosphorylase and starch synthase are related to NAD reduction. The activities of these enzymes are also associated to rapid grain-fill of rice grain （Jeng et al., 2003）. In the present study, the in situ NBT staining was use to examine the enzyme responses for two rice grains differing in starch accumulation rate during grain fill.

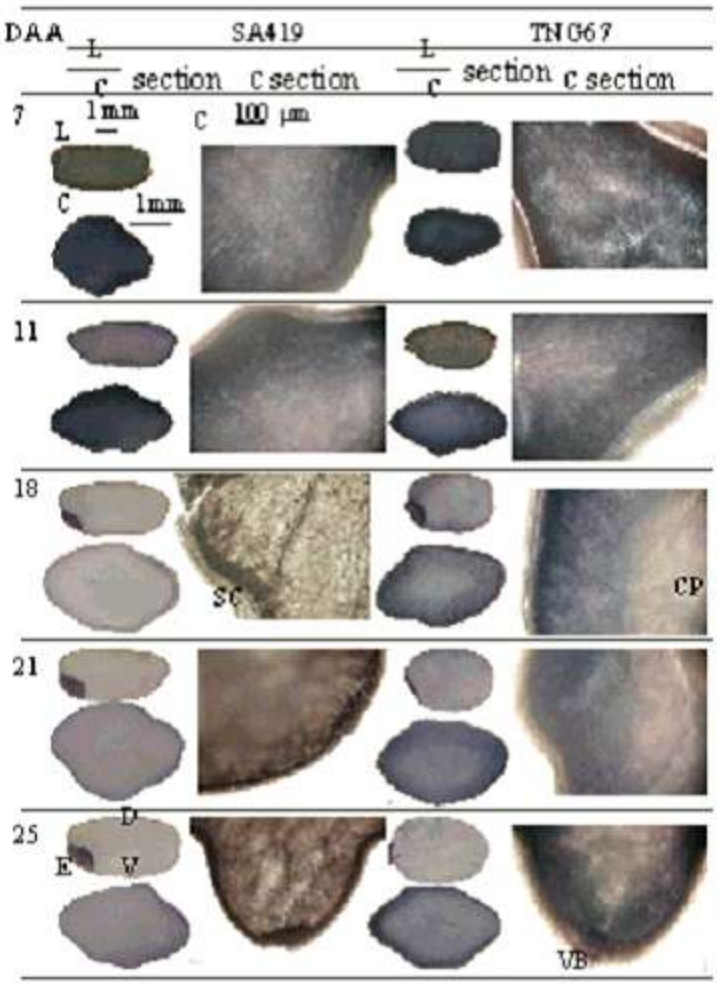

Fig. 1. Staining of activity of sucrose synthase, observed by cross-section (C) and longitudinal-section (L) of developing rice grain, of rice cultivar Tainung 67 and mutant SA419. (E) embryo (SC) seed coat (VB) vascular bundle (CP) central part (D) dorsal (V)ventral.

Methods

Rice (*O. sativa* L.) cultivar Tainung 67 and its NaN_3-induced mutant SA419 were grown at the experiment farm of Taiwan Agricultural Research Institute. The hand-harvested panicles were frozen in liquid nitrogen, then dried in freeze-dryer and stored at -20? for in situ staining study. The longitudinal-section and cross-section of rice grains were stained using the methods detailed by Sergeeva and Vreugdenhil (2002). The enzymes stained in this experiment were SUS, invertase, hexokinase, ADPglucose pyrophosphorylase, UDPglucose pyrophosphorylase and starch synthase.

Results and conclusion

All the tested enzymes were visible in the growing endosperm. The longitudinal-section (Fig. 1 and 2) of SA419 showed blue staining in all the endosperm cells sampled at 7 DAA, then started to de-colorize central part at 11 DAA. The de-colored region increased progressively following the grain development, with the rate of de-colorization for ventral part faster than dorsal part. The staining was disappeared after 18 DAA. Marked changes in staining pattern were observed in Tainung 67, in that the rapid de-colorization started on 18 DAA. Similar staining patterns were also visible on the cross-section of

developing rice grains differing in starch accumulation rate. In developing corn kernels, starch start to accumulate from the apical part of the endosperm to the basal endosperm during kernel development (Heinlein and Starlinger, 1989), and the expression of sucrose synthase activity coincided with the order of starch accumulation (Wittich and Vreugdenhil, 1998). Using the method of Histomorphology or ^{14}C isotope detection, it has been shown that, in rice grains, starch accumulated from the central part to the outer part of endosperm during grain development (Hoshikawa, 1968; He et al., 1989). Our data indicated that the NBT staining for all the tested enzymes also correlated well with the changes in the activities of starch synthesizing enzymes during grain development.

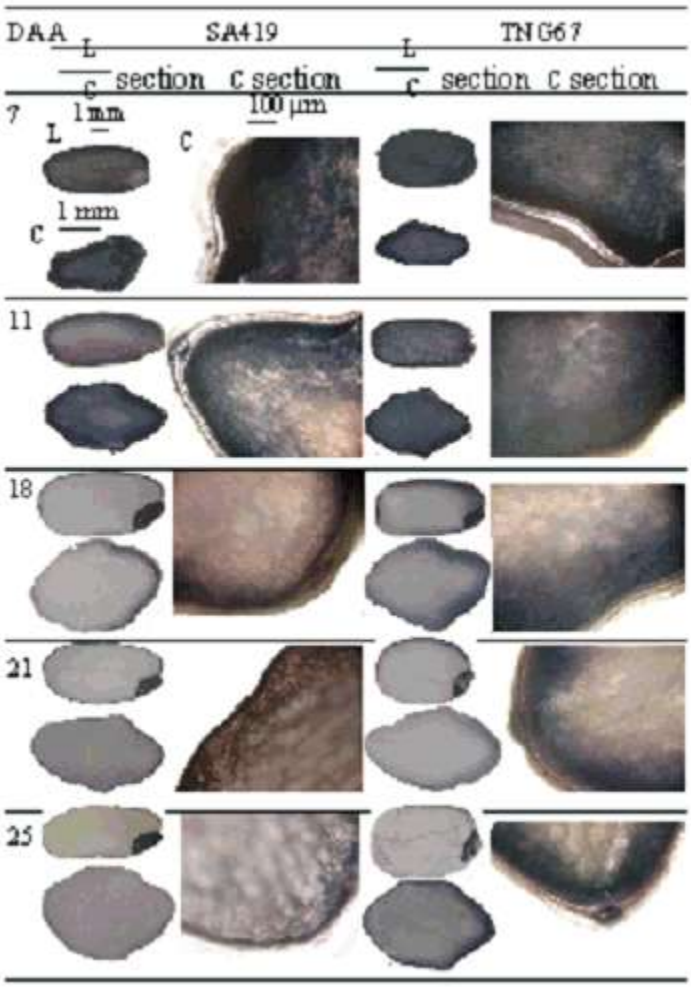

Fig.2. Staining of activity of starch synthase, observed by cross-section (C) and longitudinal-section (L) of developing rice grain, of rice cultivar Tainung 67 and mutant SA419.

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