In germinating cereal seeds, the expression of hydrolytic enzymes is generally enhanced by gibberellin. Examples of this phenomenon include the gibberellin acid-induced gene expression of α-amylase in wheat and cysteine proteinases in barley. We have found a rice cysteine proteinase that can efficiently degrade the major rice storage protein, rice glutelin, in vitro, and also observed that the cysteine proteinase activity of germinating rice seeds is greatly enhanced by added GA₃. We have recently isolated three cDNA clones encoding cysteine proteinases from a rice seed cDNA library and have named them oryzain α, β and γ. Northern blot analyses showed that the expression of the three respective oryzain mRNAs is enhanced by GA₃. Although the GA₃-induced expression of oryzain β mRNA is transient, that of oryzain α and γ mRNAs continues over a period of five days after the start of germination. In this study, we investigated the regulation of the GA₃-induced expression of oryzain α and γ mRNAs in a dwarf rice cultivar and a normal cultivar in the presence of an inhibitor of gibberellin biosynthesis (uniconazole). In addition, we examined the expression of these mRNAs in the GA₃-treated endosperm.

We examined the expression of oryzain α and γ mRNAs in whole rice seeds of a dwarf cultivar (Tan-ginbozou) and a uniconazole-treated normal cultivar (Nipponbare). It has been reported that Tan-ginbozou contains extremely low levels of the active form of gibberellin. In addition,
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of GA₃, the oryzain z mRNA level upon germination was as high as in Nipponbare seeds germinating in the absence of added GA₃. The expression of oryzain z mRNA in germinating seeds of Nipponbare was almost completely repressed by the addition of uniconazole. Similar results were obtained for the expression of oryzain γ mRNA.

These data indicate that the mRNAs for oryzains z and γ exist at low levels in both Tan-ginbozu and uniconazole-treated Nipponbare and also that their levels increase when Tan-ginbozu seeds germinate in the presence of added GA₃. These results strongly suggest that the expression of these two oryzain mRNAs is dependent on GA₃.

We next examined the site at which the oryzain z and γ mRNAs are detected in seeds of the Nipponbare cultivar. Seeds were dehulled, polished, and soaked in water in the presence or absence of 1 μM GA₃, and incubated at 37°C for 24 hr. Northern blot analysis was done as described above. In dehulled Nipponbare seeds, the expression of oryzain z and γ mRNAs was not detected even in the presence of added GA₃ (Fig. 2). This indicates that oryzain z and γ mRNAs are not expressed in the endosperm. However, we previously isolated oryzain z from the endosperm at an early stage of germination. Therefore, although speculative, oryzain z and γ mRNAs are probably expressed in the aleurone layer or in the germ, and rice seeds possibly have a system to transport oryzains from the site(s) of synthesis similar to the transport system for α-amylase.

To our knowledge, this study is the first to deal with GA₃-dependent gene expression in a gibberellin-deficient dwarf cultivar of rice and a normal rice cultivar after treatment with a gibberellin biosynthesis inhibitor. The results contribute to our basic information about the function of gibberellin in germinating rice seeds.

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References