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Note

Maize plants mutated in NAD(P)H-dependent HC-toxin reductase gene (*Hm1*) is vulnerable to H_2O_2 stress

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Abstract Maize *Hm1* gene encodes a NAD(P)H-dependent HC-toxin reductase, which detoxify HC-toxin produced by fungus *Cochliobolus carbonum* (Meeley and Walton 1991 *Plant Phys* 97: 1080). Measurements of ion leakage indicated that H_2O_2 treatment of a recessive mutant (*hm1*) of maize resulted in accelerated death in excised leaves. Furthermore, an *hm1* maize showed quantitative decrease of NAD(H) level. Thus, the *Hm1* gene may confer other functions related to ROS stress tolerance

Key words: HC-toxin reductase, *Hm1*, maize, H_2O_2 stress.

The defense mechanism of plants against outside adversity involves one or more changes in plant cells. In maize, a NAD(P)H-dependent HC-toxin reductase (HCTR) is activated upon the invasion of fungus *Cochliobolus carbonum* to detoxify the HC-toxin produced by this fungus (Meeley and Walton 1991). The HCTR is encoded by maize *Hm1* gene (Johal and Briggs 1992). This disease resistance gene is conserved in all monocots examined, such as rice (Hihara et al. 1997) and barley (Han et al. 1997). Whether a molecular mechanism similar to the maize-*C. carbonum* interaction has evolved as a common defense system in the plant kingdom remains unknown (Briggs and Johal 1994). The fact that *C. carbonum* is specialized for growth on maize and HC-toxin is only biosynthesized in race 1 has cast a strong doubt on the hypothesis that the role of *Hm1* homologues in other monocots is to detoxify toxins (Walton 1996). Here we present the evidence that knock-out of *Hm1* in maize resulted in alteration of the level of nicotinamide dinucleotide contents, which may be related to reactive oxygen species (ROS)-mediated cell death.

Maize plants were cultivated in a green house at 25°C. We used maize mutants (*Hm1hm2* and *hm1hm2*) provided by Maize Genetics Cooperation Stock Center (Urbana, IL, USA). These mutants are recessive in *hm2* locus, whereas *Hm1* locus was either dominant (*Hm1hm2*) or recessive (*hm1hm2*). For the study of ion leakage measurement, leaf segments (about 5 mm in size) from 14-day-old maize plants were floated on distilled water with or without 10 mM H_2O_2. During 24 h treatment (0, 2, 4, 8, 24 h), electrolytes leaked from leaf tissues in the water were measured using electrical conductivity meter (Horiba, B-173, Japan) (Kawai-Yamada et al. 2004). The ion leakage assay using excised leaves showed that *hm1hm2* line leaked more ions than *Hm1hm2* (Figure 1). Thus, the *hm1hm2* line showed more accelerated death than the *Hm1hm2* line by H_2O_2.

Nicotinamide dinucleotides were measured according to Tezuka et al. (1994). Plant samples were homogenized with 0.1 N HCl (95°C) to extract oxidized coenzymes (NAD), or 0.1 N NaOH (95°C) to extract reduced

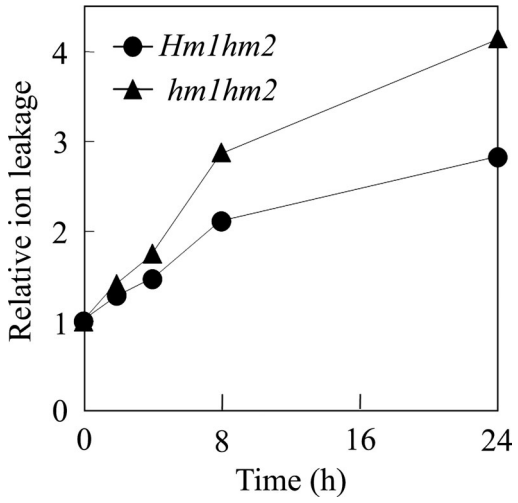

Figure 1. Comparison of ion leakage in maize leaves treated with H_2O_2. Ion leaking assay from maize leaf samples were used to monitor cell death. Relative values for 0 mM H_2O_2 were shown (n=3).

coenzymes (NADH). Each homogenate was then cooled down on ice bath and pH was adjusted to 6.5 with NaOH (NAD) or 7.5 with HCl (NADH), followed by the addition of 0.2 M glycylglycine (pH 7.5). After centrifugation (10,000×*g*, 20 min at 4°C), the supernatant was saved for further analyses. For NAD and NADH measurements, reaction mixture contained 50 mM glycylglycine (pH 7.4), 20 mM nicotinamide, 1 mM phenazine methosulfate (PMS), 1 mM thiazolyl blue (MTT), and alcohol dehydrogenase solution (final concentration: 40 μg ml^{-1}) and the supernatant fraction. The cuvette was placed in a UV/Visible spectrophotometer for measurement at 570 nm. Prior to the reaction, 8% ethanol was added. After the reaction (2 min at 25°C), 0.2 μM authentic NAD was added and the change in absorbance was followed for additional 2 min. Quantitative measurement of NAD(H) level in maize plants indicated that *hm1hm2* line showed reduced amounts of NAD(H) than the *hm1hm2* line (Figure 2).

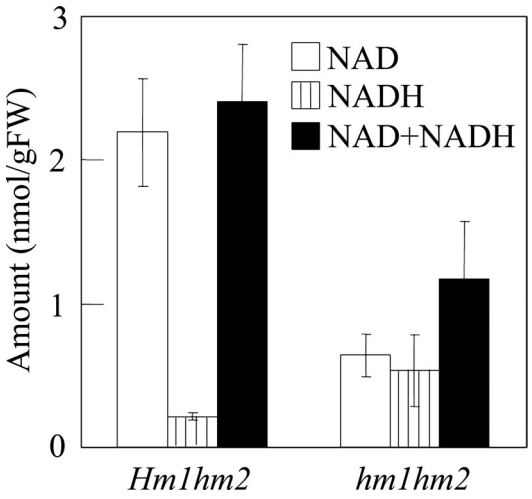

Figure 2. Quantitative measurements of NAD(H) levels in maize plants. NAD(H) level in 14-day-old plants. Data, mean±SD (n=9). gFW, gram fresh weight.

This study suggests that maize *Hm1* gene may confer H_2O_2 stress tolerance *in vivo*. Furthermore, alterations of nicotinamide dinucleotides imply that *Hm1* gene may regulate the pool of NAD level. We could not see any changes in NAD synthetase and ATP-NMN adenylyltransferase activities (unpublished results). Our findings indicate that the *Hm1* gene may confer other functions related to ROS stress tolerance.

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