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Grant Title:

Glycotechnology to regulate plant development through glycogene control

1. Objectives

It has been reported that free *N*-glycans (FNGs), which are produced from misfolded glycoproteins in the ERAD system or glycopeptides in the degradation system of senescent glycoproteins, ubiquitously occur in developing plants. More than two decades ago, it was hypothetically reported that FNGs, Man3Xyl1Fuc1GlcNAc2 or Man5GlcNAc, stimulates the ethylene production [1], but the biological significance(s) of these FNGs involved in plant development or differentiation remain to be elucidated. On the other hand, we have found that the amount of high-mannose type FNGs increased with the tomato fruit maturation and the gene-expression levels of ENGase responsible for the release of high-mannose type *N*-glycans remained unchanged [2]. As a part of the study to clarify the biological significances of plant FNGs and acidic PNGase responsible for the release of N-glycans from N-glycopeptides, we constructed a transgenic tomato plant (T-2 and T-3 generations), in which one of acidic PNGase genes has been overexpressed, and we have analyzed gene-expression levels of some glycosidases involved in the cell wall degradation or carotene synthetic enzymes in the transgenic tomato plant.

2. Methods

aPNGase Le gene [3] was transformed into a model tomato plant, Micro-Tom, using the method of agrobacterium-mediated transformation, and the transformed aPNGase gene was expressed under the control of CAMV 35S promoter. After redifferentiating the transformed callus tissue, tomato seeds (T-0 generation) were harvested from the full-grown transgenic tomato fruits. By passage breeding, T-2 and T-3 lines were obtained, and the expression levels of transformed aPNGase Le gene were confirmed by PCR. The amount of FNGs produced in the control fruits and the transgenic fruits were analyzed by the method described in the previous paper [4], and the aPNGase activity was assayed using the *N*-glycopeptide as a substrate prepared form egg yolks. The expression-levels of some glycosidases involved in the degradation of cell walls and ethylene-response related enzymes were analyzed by the real-time PCR method.

3. Results

The amount of plant complex type FNGs produced in the transgenic tomato fruits increased more than twice in comparison with that in the control fruits, and the aPNGase activity in the transgenic fruits increased nearly fourfold. Promotion of fruit ripening or increase in the rate of fruit coloring (nearly a week) was observed in the aPNGase-Le overexpressed tomato plants, indicating that the plant complex type FNGs produced by aPNGase or the amidase activity itself might play an important role in the fruit ripening process. Furthermore, we found that the gene-expressions of polygalacturonase and β-galactosidase significantly increased in the aPNGase-Le overexpressed fruits, while significant changes in the expression levels of ethylene-response related enzymes were not observed.

[1] Priem, B. and Gross, K.C. *Plant Physiol.* **98**, 399-401 (1992). [2] Nakamura, K. *et al., Biosci. Biotechnol. Biochem.*, **72**, 2936-2945 (2008). [3] Hossain, A. *et al., J. Biochem.*, **147**, 157-165 (2010). [4] Maeda, M. *et al.*, *Carbohydr. Res.* **435**, 50-57 (2016).