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SHORT COMMUNICATION

Establishment of an efficient screening system to isolate rice mutants deficient in green leaf volatile formation

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Green leaf volatiles (GLVs) are involved in defense responses of plants against wide range of stresses. In order to investigate the ecological significances of GLVs in rice plants, we started screening of rice mutants that showed abnormalities in GLV formation. For this, we established an efficient system to quantify the amounts of GLVs. With the system their amounts can be analyzed within 20 min.

Keywords: rice; headspace GC; green leaf volatile

Introduction

Green leaf volatiles (GLVs) are carbon six compounds produced from the hydroperoxide lyase pathway of oxylipin metabolism (Matsui 2006). GLV emissions increase when plant tissues are damaged or after suffering biotic/abiotic stresses. It is postulated that GLVs act as semiochemicals, such as repellents for herbivorous pests, or attractants for organisms antagonistic to these pests, and that they may also induce a defense response in neighboring plants (Paré and Tumlinson 1999; Matsui 2006). Identification of a rice mutant deficient in GLV formation can help elucidate the roles of GLVs in the plant. In this study we established an efficient screening system for the rice mutants by analyzing amounts of (*E*)-2-hexenal and (*Z*)-3-hexen-1-ol in a short time.

Materials and methods

Rice mutant lines mutagenized with *N*-methyl-*N*-nitrosourea were collected in Kyushu University by National Bioresource Project (http://www.nbrp.jp/). The rice seeds were soaked for two weeks in tap water at 25°C under 14/10 h light/dark. The seedlings were then sown on soil and grown under the same conditions for additional two weeks. The rice leaves (0.2 g fr wt) were collected and ground in liquid N_2, then, put into a headspace vial (22 ml, Perkin Elmer, Waltham, MA) and frozen at −80°C overnight. Then they were thawed at 25°C, and then, incubated for 30 min at 25°C in order to facilitate formation of volatiles. The enzymes were killed by addition of 5 ml saturated $CaCl_2$ solution. After sealed with a butyl stopper and crimp top seal, the headspace volatiles were analyzed with a headspace sampler (HS 40XL, Perkin Elmer) equipped with a GC (GC2014, Shimadzu, Kyoto). A Stabiliwax column (30 m × 0.25 mm i.d., Restek, Bellefonte, PA) was used with 40°C (5 min) to 180°C (1 min) at 15°C min^{-1}. Detection was with a flame ionization detector.

Results and discussion

The screening system relies on the formation of GLVs after disrupting the rice leaf tissues with freeze-thaw treatment. We confirmed that GLVs were rapidly formed after the treatment and subsequent incubation at 25°C for 30 min. With the automated headspace sampler coupled with GC, the three major volatile compounds, i.e. 1-penten-3-ol, (*E*)-2-hexenal, and (*Z*)-3-hexen-1-ol, were detected within 12 min (Figure 1). The system enabled us to determine the amounts of damage-induced GLVs with as many as 70 mutant lines of rice within one day. After analyzing more than 1000 lines with this system, we found that four potential mutant candidates to be used for future experiments. This system is promising and we found this can be used for screening with the other plant tissues (Kakumyan et al. 2009).

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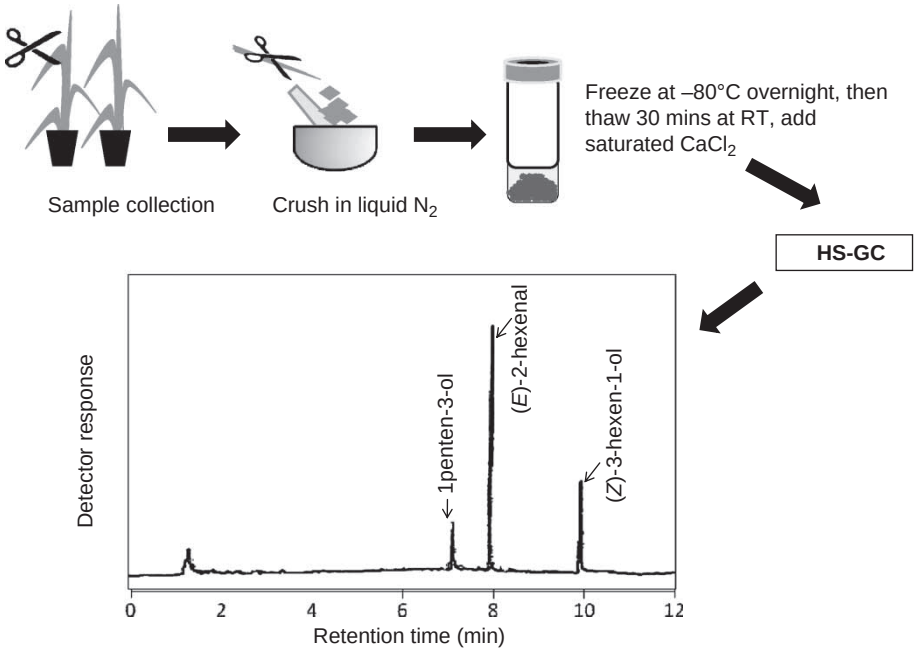

Figure 1. A schematic outline of headspace GC screening. A representative chromatogram obtained with rice seedlings is also shown.

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