PRODUCTION OF TRANSGENIC PLANTS EXPRESSING *DIOSCOREA BATATAS* TUBER LECTIN 1 TO CONFER RESISTANCE AGAINST SUP-SUCKING PESTS

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ABSTRACT

Dioscorea batatas tuber lectin 1 (DB1) is a storage protein isolated from yam tuber, and is shown to be a mannosebinding lectin. It shows 58% amino-acid identity to insecticidal snowdrop bulb lectin GNA, and 55% identity to Garlic leaf lectin ASAL. We demonstrated that ≥1 mg/ml DB1 in an artificial diet decreased the survival and fecundity of green peach aphid, *Myzus persicae*. The number of survival aphids was reduced to 60% in transgenic tobacco expressing cDNA of DB1 under the control of Cauliflower mosaic virus 35S promoter (35S-DB1) or phloem-specific promoter of rice sucrose synthase-1 gene (RSs1-DB1). We also produced transgenic rice (cv. Taichung 65) with 35S-DB1 and RSs1-DB1, which accumulated DB1 at a level of 2.2% and 0.33%, respectively, of total soluble protein. In our preliminary small-scale test, these transgenic rice seedlings showed resistance against brown planthopper (*Nilaparvata lugens*), white back planthopper (*Sogatella fucifera*) and small brown planthopper (*Nilaparvata lugens*). Our results indicate that DB1 can be used to enhance resistance to sap-sucking pests in transgenic crops.

Keywords *: Dioscorea batatas tuber lectin 1, insect resistance, planthopper, transgenic rice*

INTRODUCTION

Dioscorea batatas tuber lectin 1, DB1, has been isolated from yam tuber, *Dioscorea batatas* Decne., as a storage protein. DB1 is a mannose-binding lectin (23 kDa) consisting of identical 12-kDa subunits. It has 58% amino-acid identity to snowdrop lectin GNA and is classified into the GNA-related lectin family (Gaidamashvili et al. 2004). The insecticidal properties of DB1 have been reported against moth larvae (*Helicoverpa armigera*). The rate of adults emerging from pupae has been reduced to 33%, when fed on 0.01% (w/v) DB1 in an artificial diet (Ohizumi et al. 2009). We also demonstrated that ≥1 mg/ml DB1 in an artificial diet significantly decreased the survival and fecundity of green peach aphid, *Myzus persicae* (Kato et al. 2010). The number of survival aphids was reduced to 60% in transgenic tobacco expressing cDNA of DB1 under the control of Cauliflower mosaic virus 35S promoter (35S-DB1) or phloem-specific promoter of rice sucrose synthase-1 gene (RSs1-DB1). Our results indicate that *DB1* can be used to enhance resistance to sap-sucking insects in transgenic crops. Here we report the production of transgenic rice expressing DB1.

MATERIALS AND METHODS

The cDNA covering full-length ORF (accession no. AB513659) was PCR cloned into pGEM T-vectors (Promega, Madison, USA) using KOD+ polymerase (Toyobo, Osaka, Japan) and primers XB/NDB1 F 5'-TCTAGAGGATCCATGGCTAACCCAGGAGCA-3' (*Xba* I and *Bam* HI sites are underlined) and S/DB1 R 5'-GAGCTCTCACTTGTTGACGACC-3' (*Sac* I site is underlined). For phloem-specific expression of DB1, the rice sucrose synthase-1 (RSs1) promoter (accession no. AJ401233) was employed as described (Shi et al. 1994). The promoter sequence (3 kb) was amplified from rice cv. Taichung 65 using KOD+ polymerase and specific primers Sal/RSs1 F 5'-GTCGACCTTTCGTGACTTGTTTTCGC-3' (Sal I site is underlined) and Bam/RSs1_R 5'-GGATCCTAGCTTGGCAGCCAT-3' (*Bam* HI site is underlined), and was subcloned into pGEM-T vector. Then RSs1 promoter was inserted into *Sal* I/*Bam* H I sites of

pBI101H, which contained the hygromycin resistance cassette. The resulting construct was named RSs1-DB1 (Kato et al. 2010). The construct was transfered into *Agrobacterium tumefaciens* strain EHA105.

Transformation of rice (*Oryza sativa* L. cv. Taichung 65) was carried out by the method of *Agrobacterium*-mediated transformation. Taichung 65 is a japonica rice cultivar that originated in Taipei, Taiwan. The transformants were selected on a medium containing 30 mg/l hygromycin and 40 mg/l meropen (Dainippon Sumitomo Pharma Co., Ltd., Osaka, Japan). The introduced DB1 was detected by PCR analysis using the following primers; DB1-F1 primer 5'-CAGAATGACTGCAACCTGGT-3', and DB1-R2 primer 5'-ACCAAAGATGGTGGCCTTAC-3' with annealing temperature at 57^0C. For southern blot analysis, genomic DNA was digested with *BamH*I, which cut once within the T-DNA, and probed with DIG-labeled DB1 cDNA. The DB1 concentration in the total soluble protein in leaves was determined by comparing the intensity of bands reacted with anti-DB1 polyclonal antiserum with those of series of known amounts of purified DB1, as described previously (Kato et al. 2010).

Evaluation of planthopper resistance was carried out at Life Science Research Institute, Kumiai Chemical Industry Co., Ltd. (Shizuoka, Japan). T1 seedlings at 5th leaf stage were infested separately with 5 female adults of small brown planthopper (*Nilaparvata lugens*), brown planthopper (*Nilaparvata lugens*), or white back planthopper (*Sogatella fucifera*). All the female adults were removed after 5 days. For brown planthopper, additional 5 females were supplemented at the 20^{th} day. The numbers of nymphs and adults were counted daily. A transgenic plant with GUS driven by RSs1 promoter (RSs1-GUS) was used as a negative control.

RESULTS

We obtained three transgenic lines for 35S-DB1 and two lines for RSs1. The accumulated DB1 in leaves was detected using western blot analysis. An approximately 12-kd band corresponding to mature DB1 monomer was detected at the same position as that of standard DB1 purified form yam tuber, indicating the proper processing of 16-kd DB1 premature protein in transgenic rice. In the case of transgenic lines with 35S-DB1, DB1 accumulated at a level of 2.2% of total soluble protein in plant no. 35S-2, 1.3% in no. 35S-6 and 0.4% in no. 35S-9. In the case of transgenic lines with

RSs1-DB1, DB1 accumulated at a level of 0.36% in plant no. RSs1-3 and 0.33% in no. RSs1-2.

A plant carrying a single copy of the integrated DB1 was selected based on the detection of a single band on Southern blot analysis. Consequently the plant nos. 35S-2 and RSs1-2 were selected. The segregation ratio of PCR positive plants and negative plants in the selfed progeny was 39:10 for 35S-2 and 5:3 for RSs1-2, which fitted to a theoretical ratio of 3:1 expected from a single copy integration.

The PCR positive plants were used for the evaluation of resistance against small brown planthopper, brown planthopper, or white back planthopper. We have tested only two plants for each, and present here the champion data in Table 1. The numbers of nymphs and adults were greatly reduced when fed on the transgenic plants with 35S-DB1 and RSs1-DB1, but not on transgenic plant with RSs1-GUS (negative control).

Table 1 Number of nymphs and adults after fed on transgenic T_1 plants. Five female adults were released at day 0 and the numbers counted on the day in parenthesis are shown.

	Number of nymphs and adults		
Line	35S-DB1	RSs1-DB1	RSs1-GUS
Amount of DB1*	2.2%	0.33%	0%
Small brown planthopper (28th day)	12	8	38
Brown planthopper (34th day)**	1	4	14
White back planthopper (34the day)	1	0	40

* Amount of DB1 protein per total soluble protein in leaves of T_0 generation.

** Five female adults were supplemented at the 20th day.

Discussion

DB1 was accumulated in 35S-DB1 and RSs1-DB1 lines at almost identical levels to those of previously reported for garlic leaf lectin ASAL accumulation in transgenic rice (Saha et al. 2006; Yarashi et 2008). Accumulation ASAL has been shown to confer substantial resistance to brown planthopper, green rice planthopper, and whitebacked planthopper, in terms of increased insect mortality, retarded development and decreasing fecundity. Recently, virus resistance has been also reported in transgenic rice expressing ASAL under the control of RSs1 promoter (Saha et al. 2006). Acquisition of resistance against planthoppers and rice tungro virus is expected in the transgenic rice plants expressing DB1. Our preliminary small scale test indicated that both 35S-DB1 and RSs1-DB1 lines exhibited high-level resistance against small brown planthopper, brown planthopper and white back planthopper, although we need to repeat the experiment in a large scale to have definite conclusion.

RSs1 promoter has also been shown to direct phloem-specific expression of beta-glucuronidase and GNA in transgenic tobacco (Shi et al. 1994). RSs1 promoter has also been successfully used to drive garlic lectin, ASAL, in transgenic rice (Saha et al. 2006). Our current study indicated that the level of planthopper resistance is the same between the RSs1-DB1 and 35S-DB1 lines, although the amount of DB1 per soluble protein extracted from leaves of the RSs1-DB1 line was approximately one-seventh of that in the 35S-DB1 line, suggesting that the amount of DB1 in phloem might be almost identical. RSs1 promoter has the advantage of maximizing expression of the insecticidal protein at the site of attack by sap-sucking insects, while minimizing it elsewhere in plants.

The plant no. RSs1-2 homozygous for the introduced RSs1-DB1 was propagated. Evaluation of planthopper resistance in a large scale is now in progress using the T3 generation of the RSs1-DB1 line (RSs1-2) in collaboration with Dr. Nono Carsono, Padjadjaran University, and Dr. M. Herman, Indonesia Centre for Agriculture Biotechnology and Genetic Resources (BB Biogen, Bogor).

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