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Short Communication

Transformation of Soft Rot Resistant *aiiA* Gene into Chinese Cabbage (*Brassica rapa* ssp. *pekinensis*) by the Pollen-tube Pathway

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Abstract

Bacterial soft rot of Chinese cabbage is the most destructive disease caused by *Erwinia carotovora*. Until now reliable sources of resistance and effective control methods are unavailable, so development of highly resistant breeding lines is desirable. In the present study, we established an *aiiA* gene transformation system of soft rot resistant. The *aiiA* gene was introduced into Chinese cabbage through the pollen-tube pathway using the plasmid vector pBBBast-*aiiA* (7, 315 kb), which included *aiiA* gene, expression regulatory sequence (CaMV 35S promoter, OCS terminator), the herbicide resistant *Bar* gene and other elements. Five out of 20632 (0.02%) of T_0 plants was positive for *aiiA* gene through PCR. Further PCR Southern blot analysis displayed that the gene *aiiA* segments were transformed into T_0 plants. These transgenic plants could be the potential cultigens against the pathogens of soft rot for Chinese cabbage.

Keywords: Chinese cabbage; Soft rot; *aiiA*; Coat protein-mediated resistance; Pollen-tube pathway

Introduction

Chinese cabbage is one of the main leafy vegetables that is widely grown in Asia and exhibits the most genetic diversity with distinct morphological characteristics and the largest planting area and yield (Cao *et al*., 2006; Ge *et al*., 2011a,b; 2012). However, outbreaks of soft rot can cause substantial losses in the yield and marketability of Chinese cabbage (Kikumoto, 2000; Park *et al*., 2012).

Bacterial soft rot caused by *Erwinia carotovora* is one of the most destructive diseases including carrots, potato, Chinese radish and Brassicas, especially Chinese cabbage in East Asian (Kikumoto, 2000). This soil borne, facultative anaerobic pathogen far and wide distributed in farmland as well as in uncultivated land usually produces abundant of pectic enzymes, which cause plant tissue maceration, eventually resulting in plant death (Collmer and Keen, 1986). Chemical medicines during the vegetation period have been apparent to protect the plants from fungal infection but recurrent chemical medicines applications are usually inappropriate because pathogens could quickly develop resistance and also because medicines could contribute to environmental pollution (Kikumoto, 2000).

The use of transgenic technology is considered as one of the most promising alternative for more rational and safe crop-management practices. The first disease-control application of *AiiA* protein was the introduction of the *aiiA* gene into transgenic potato and tobacco plants (Dong *et al*., 2001). From then, the *aiiA* has been successfully transformed into other species (Ban *et al*., 2009), which exhibited enhanced resistance to soft rot disease. The technique of the pollen-tube pathway transformation was first reported by Zhou *et al*. (1983). This approach has been successfully applied in wheat (Chong *et al*., 1998), maize (Yang *et al*., 2009), melon (Hao *et al*., 2011), soybean (Yang *et al*., 2011) and cotton (Huang *et al*., 1999). In the present, *aiiA* gene was inserted into Chinese cabbage through the pollen-tube pathway technology. The transformed plants were confirmed by PCR and PCR-Southern blotting analysis.

Materials and Methods

Transformation experiments were conducted with Chinese cabbage cultivar genotype Er Niu Xin. The plasmid vector was pBBBast-*aiiA* (7, 315 kb), which was kindly supplied by Beijing Agricultural Biotechnology Research Center. The plasmid vector contained the soft rot resistant *aiiA* gene

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fused between the CaMV 35S promoter and the OCS terminator, the herbicide resistant *Bar* gene and other elements (Fig. 1). Plasmid extraction was carried out according to the alkaline lysis method (Sambrook *et al*., 1989). Purified plasmid DNA of pBBBast-*aiiA* was used for transformation with the pollen-tube method. Basta herbicide is a sterilant herbicide and the actual site of its target is glutamine synthetase. The herbicide resistant *Bar* gene with functions of detoxification could remove Basta herbicide's damange.

After 24 h of artificial pollination, 2/5 of the stigmas were removed by knife, and 5 µL DNA solutions (1000 µg/mL) were dropped evenly onto the exposed surfaces of the stigmas by a pipette. Treated flowers were marked and untreated flowers and buds in the same node were simultaneously eliminated. Seed pods that derived from the treated flowers were harvested respectively. Seeds from wild-type plants were harvested to serve as untransformed controls.

T_0 seeds were screened to gain transgenic plants through spraying of Basta herbicide (0.15% and 0.2%) on the seedlings at the cotyledon period and the first euphylla period (transverse length of the first euphylla ≥ 5 cm), respectively and then, after the second euphylla period, total gDNA of Basta herbicide resistant plants were extracted from the fresh leaves of plants through the modified SDS method (Ge *et al*., 2011b). PCR amplifications for detection of the presence of CP sequences in the transgenic plants were achieved with the primer: the forward primer 5′-ctcgagatgacagtaaaaaag-3′ (underlined *Xho*I site added) and reverse primer 5′-tctagactatatatactctgggaac -3′ (underlined *Xba*I site added), which corresponded to *aiiA* core conserved sequence. PCR amplifications, PCR product separation and observation were implemented according to the PCR conditions (Ge *et al*., 2011b). The expected amplification fragments were 753 bp in length.

Purified PCR amplification product of *aiiA* gene served as a probe to be used in the Southern blot analyses. The hybridization method was implemented according to the manufacturer's instructions of the DIG High Prime Labeling and Detection Starter Kit (Roche).

Results

An exogenous *aiiA* gene was transformed into Chinese cabbage through the pollen-tube pathway method and finally integrated into genome sequence in progeny. As shown in the present study, 8 out of 20, 632 T_0 seedlings were identified as Basta herbicide resistant plants, and then, PCR identification was used in 8 T_0 seedlings for transgenic screening. 5 out of 8 T_0 plants contained *aiiA* gene detected by PCR amplifications of the 753 bp (Fig. 2). PCR-positive candidate plants were further identified through PCR-Southern (Fig. 3) and resulted in a transformation frequency of 0.02% (5/20, 632) of the germinated T_0 plants.

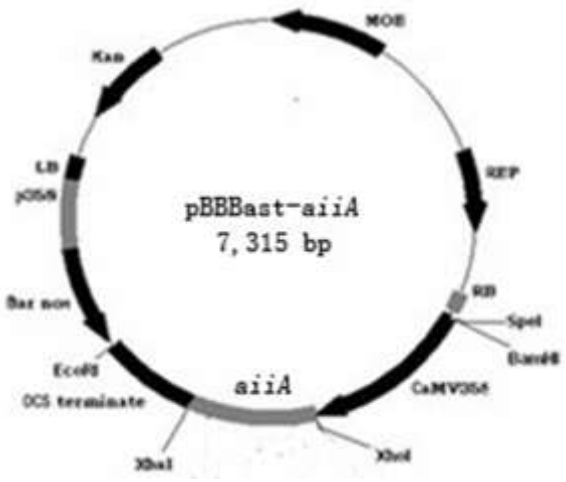

Fig. 1: Schematic maps of plasmid vector pBBBast-*aiiA*

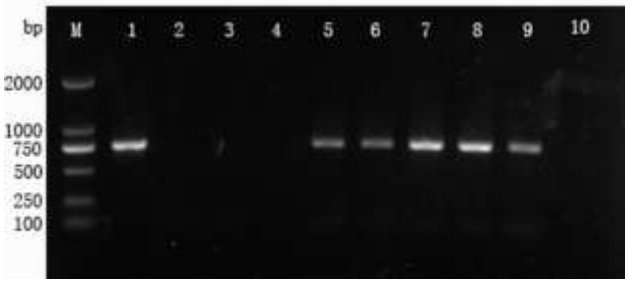

Fig. 2: PCR analysis of T_0 transgenic Chinese cabbage plants. Primer 1 was used for identification of the *aiiA* gene. PCR products were 753 bp in length. Lane M: DL2000 Marker; Lane 1: *aiiA*; Lane 5, 6, 7, 8, 9: PCR-positive result; Lane 2, 3, 4: PCR-negative result; Lane 10: untransformed controls

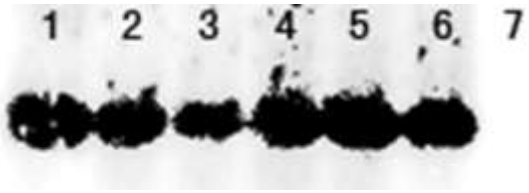

Fig. 3: PCR-southern blot analysis of T_0 transgenic Chinese cabbage plants. Lane 1: *aiiA*; Lane 2-6: transformed plant; Lane 7: untransformed controls

Discussion

The gene transformation efficiency of 0.02% by PCR analyses in this study was lower than this previously reported in the same Chinese cabbage line using the same pathway by our researchers, which was estimated as 0.15% for *TuMV-CP* gene (Han, 2011). Because the sequence length of *aiiA* gene (753bp) was longer than the sequences length of *TuMV-CP* gene (132bp), we suggested that the

difference of the transformation efficiency of these genes could be in accordance with the targets genes sequences length. Longer fragment exogenous DNA was more difficult in transformation in Chinese cabbage via the pollen-tube pathway.

Conclusion

Our result displayed that only 5 transgenic plants were obtained from 20, 632 T_0 seeds. The prior stages of the pollen-tube method are convenient, but amount of progeny were required to screen in order to obtain abundant available transformed plants. In the subsequent study, a large number of T_0 seeds should be needed to gain more transgenic plants to verify the effect of transgenic plants.

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