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Short Communication

Coat Protein-mediated Resistance to *Turnip mosaic virus* in Chinese Cabbage (*Brassica rapa* ssp. *pekinensis*) by the Pollen-tube Pathway

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Abstract

Turnip mosaic virus of Chinese cabbage is the most destructive disease. Until now reliable sources of resistance and effective control methods are unavailable, so development of highly resistant breeding lines is desirable. In this research, we established a plant transformation system of coat protein-mediated resistance to *Turnip mosaic virus*. The *TuMV-CP* gene was introduced into Chinese cabbage through the pollen-tube pathway using the plasmid vector pBBBast-*TuMV-CP* (9.79 kb), which included *TuMV-CP* gene, expression regulatory sequence (CaMV 35S promoter, OCS terminator), the herbicide resistant *Bar* gene and other elements. Totally, 0.15% of T_0 plants showed positive for the gene *TuMV-CP* through PCR. Further PCR Southern blot analysis displayed that the gene *TuMV-CP* segments were transformed into T_0 plants. © 2014 Friends Science Publishers

Keywords: Chinese cabbage (*B. rapa* ssp. *pekinensis*); *Turnip mosaic virus*; *TuMV-CP*; 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, 2012). However, outbreaks of *Turnip mosaic virus* (TuMV) can lead to abundant decreases in the yield of Chinese cabbage (Lehmann *et al*., 2003; Peng *et al*., 2012).

TuMV is the only *potyvirus* known to infect the genus *Brassica*, causing significant economic loss in *Brassica* crops (Shattuck, 1992). TuMV was first founded in *B. rapa* by Gardner and Kendrick (1921) and Schultz (1921) and then has been deeply investigated. Various symptoms were described such as systemic mosaic patterns, malformation of leaves, growth retardation, stunting, and even death after TuMV infection (Walsh and Tomlinson, 1985; Kim *et al*., 2008). Chemicals in growing season have become apparent to protect the plants from disease infection and repetitive chemicals applications also arouse concerns on environmental security, either for older copper-based or modern synthetic protectants (Sarfraz *et al*., 2005).

Genetically engineering resistance to viruses through using their genes (or fragments of the viral genome) in transgenic plants is an established means of generating resistance to viral infection in plants (Walsh, 2000; Simeon *et al*., 2005). This coat protein-mediated resistance has been widely applied to various species of viruses and plants (Lomonossoff, 1995; Malpica *et al*., 1998; Lehmann *et al*., 2003). In the present, *TuMV-CP* 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 vector used was pBBBast-*TuMV-CP* (9.79 kb), which was kindly supplied by Beijing Agricultural Biotechnology Research Center. The vector contained the TuMV resistant *TuMV-CP* gene fused between the CaMV 35S promoter and the OCS terminator, the herbicide resistant *Bar* gene and other elements (Fig. 1). Plasmid was extracted according to the method (Sambrook *et al*., 1989). Purified plasmid DNA of pBBBast-*TuMV-CP* was used for transformation with the pollen-tube technology.

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 using a pipette. Treated flowers were marked and untreated buds and flowers in the same node were simultaneously eliminated. Seed pods that derived from the treated flowers were harvested respectively. Seeds derived from wild-type plants were harvested to serve as untransformed controls.

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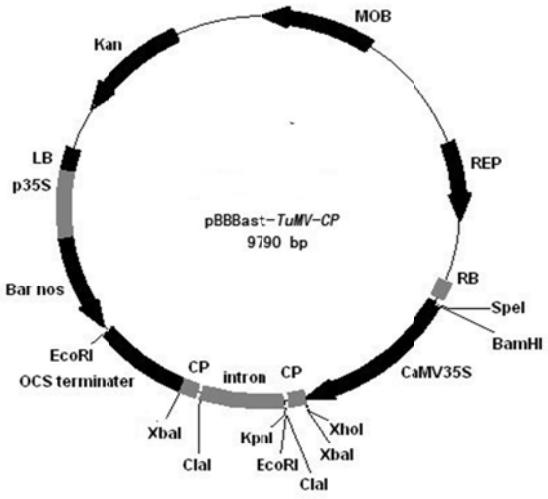

Fig. 1: Schematic maps of plasmid vector of pBBBast-*TuMV-CP*

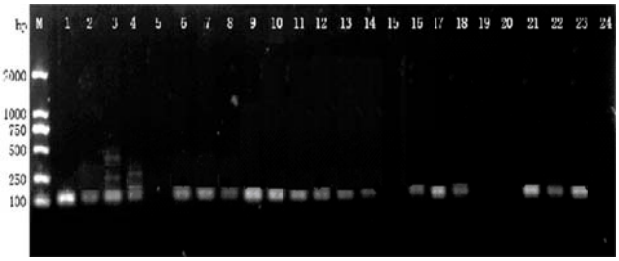

Fig. 2: PCR analysis of T_0 transgenic Chinese cabbage plants. Primer 1 was used for identification of the*TuMV-CP* gene. PCR products were 132 bp in length. Lane M: DL2000 Marker; Lane 1: *TuMV-CP*; Lane2-4, 6-14, 16-18, 21-23: PCR-positive result; Lane 5, 15, 19, 20: PCR-negative result; Lane 24: untransformed controls

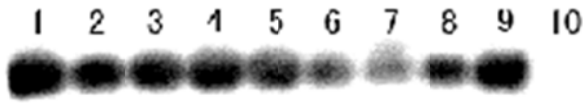

Fig. 3: PCR-southern blot analysis of T_0 transgenic Chinese cabbage plants. Lane 1: *TuMV-CP*; Lane 2-9: transformed plant; Lane 10: untransformed controls

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, respectively, and then, after the second period, total gDNA of Basta herbicide resistant plants were extracted from the fresh leaves of plants through the modified SDS procedure (Ge *et al*., 2011b). PCR amplifications for detection of CP sequences in the transgenic seedlings were achieved with the primer: the forward primer (5′-CACGCCAAACCCACATT-3′) and reverse primer (5′-GTTAAATTGCGCTGAAGACC-3′), which corresponded to *TuMV-CP* 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 132 bp in length.

Purified PCR amplification product of *TuMV-CP* 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.

Results

The time is the key factor for alien gene transformation through the pollen-tube pathway transgenic technology when the sperm and egg fused and zygote divided. In the present, the 24 h after pollination was set for foreign gene transformation via the pollen-tube pathway.

An exogenous *TuMV-CP* gene was transformed into Chinese cabbage through the pollen-tube pathway method and finally integrated into genome sequence in progeny. As shown in this report, 33 out of 18936 T_0 seedlings were identified as Basta herbicide resistant plants, and then, PCR identification was used in 33 T_0 seedlings for transgenic screening. 29 out of 33 T_0 plants contained *TuMV-CP* gene detected by PCR amplifications of the 132 bp (Fig. 2). PCR-positive candidate plants were further identified through PCR-Southern (Fig. 3) and resulted in a transformation frequency of 0.15% (29/18936) of the germinated T_0 plants.

Discussion

In Chinese cabbage, the mature pollen tube passed into the embryo sac 16 h after self-pollination which needed more times than that of soybean (Gao *et al*., 2007). At 32-34 h after self-pollination, the first mitotic division began in the zygote. Therefore, 16-34 h after self-pollination could be the prime time for foreign gene transformation into Chinese cabbage through the pollen-tube pathway (Peng and Shen, 2005). In the previous study, our researchers also produced a stable transgenic line Chinese cabbage Er Niu Xin containing the $hrpN_{Ecc}$ gene through 24 h after pollination for alien gene transformation time via the pollen-tube pathway (Gao, 2010), which was the same time as the present study.

The gene transformation efficiency of 0.15% by PCR analyses in this study was higher than these previously reported in the same Chinese cabbage line using the same pathway by our researchers, which was estimated as 0.02% for $hrpN_{Ecc}$ gene (Gao, 2010) and 0.0242% for *aiiA* gene (Han, 2011). In light of sequence length of *TuMV-CP* gene (132 bp) was shorter than sequences length of $hrpN_{Ecc}$ gene(1068 bp) and *aiiA* gene (753 bp), we suggested that the difference of the transformation efficiency of these genes couldbe accordance with the targets genes sequences length. Smaller fragment exogenous DNA was more favor

for transformation in Chinese cabbage via the pollen-tube pathway. Our result displayed that only 29 transgenic plants were obtained from 18936 T_0 seeds. Zhang *et al*. (2005) suggested that the prior steps of the pollen-tube transgenic technology are convenient, but amount populations of progeny was 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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