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Production of transgenic sweetpotato plants resistant to stem nematodes using *oryzacystatin*-I gene

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ABSTRACT

Stem nematode (*Ditylenchus destructor* Thorne) is one of most serious diseases limiting sweetpotato (*Ipomoea batatas* (L.) Lam.) production, and it is urgent to develop sweetpotato varieties resistant to this disease. In present study, we have developed transgenic sweetpotato (cv. Xushu 18) plants resistant to stem nematodes using *oryzacystatin*-I (*OC*I) gene with *Agrobacterium tumefaciens*-mediated transformation. *A. tumefaciens* strain EHA105 harbors a binary vector pCAMBIA1301 with *OC*I gene, *uidA* gene and *hpt*II gene. Selection culture was conducted using 7 mg/l hygromycin. A total of 2119 plants were produced from the inoculated 1710 cell aggregates of Xushu 18 via somatic embryogenesis. GUS assay and PCR analysis of the regenerated plants randomly sampled showed that 92.8% of the regenerated plants were transgenic plants. Transgenic plants exhibited enhanced resistance to stem nematodes compared to the untransformed control plants by the field evaluation and the inoculation test with stem nematodes and stem nematode-resistant plants were selected from the transgenic plants. Stable integration of the *OC*I gene into the genome of resistant transgenic plants was confirmed by Southern blot analysis, and the copy number of integrated *OC*I gene ranged from 1 to 3. High level of *OC*I gene expression in the resistant transgenic plants was demonstrated by real-time quantitative PCR analysis. This study provides a way for improving stem nematode resistance of sweetpotato.

1. Introduction

Sweetpotato, *Ipomoea batatas* (L.) Lam., is an important food and industrial material crop in the world. It is also an alternative source of bio-energy as a raw material for fuel production (Zang et al., 2009). The improvement of this crop by conventional hybridization is limited because of its high male sterility, incompatibility and the hexaploid nature (Dhir et al., 1998). Gene engineering offers great potential for the improvement of sweetpotato. There have been several reports on this subject in the literature. Transgenic plants expressing cowpea trypsin inhibitor (*CpTI*), snowdrop lectin, delta-endotoxin, soybean kunitz trypsin inhibitor (*SKTI*-4), sweetpotato feathery mottle virus (SPFMV-S) coat protein, granule-bound starch synthaseI (*GBSS*I), tobacco microsomal ω-3 fatty acid desaturase (*NtFAD*3), starch branching enzyme II (*IbSBE*II) or *bar* gene have been produced (Newell et al., 1995; Morán et al., 1998; Cipriani et al., 1999; Okada et al., 2001; Kimura et al., 2001; Wakita et al., 2001; Shimada et al., 2006; Otani et al., 2003; Yi et al., 2007; Choi et al., 2007; Zang et al., 2009). But, in most cases only a low transformation efficiency was obtained, which limits the successful application of genetic engineering in sweetpotato improvement.

Stem nematode (*Ditylenchus destructor* Thorne) is one of most serious diseases limiting sweetpotato production. This disease usually decreases sweetpotato yield by 20–50%, and even no yield in the field if seriously infected by stem nematodes in China (Xie et al., 2004). Thus, the breeding of sweetpotato varieties resistant to stem nematodes has become especially important. A significant negative correlation between stem nematode resistance and important quality traits (starch content, soluble sugar content, etc.) was observed, which limits the improvement of these important traits for sweetpotato by conventional hybridization (Ma et al., 1997). The use of transgenic plants expressing stem nematode resistance gene will be an alternative approach to improve the nematode resistance of sweetpotato.

Transformation of plants with proteinase inhibitor genes has great potential to enhance resistance against pathogens and insects (Masoud et al., 1993). Protein inhibitors mainly exist in storage organs of plants. The content of protein inhibitors can be as much

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as 1–10% of total proteins in seeds and bulbs of plants (S.Y. Lu et al., 1998; X.F. Lu et al., 1998). Proteinase inhibitors accumulate when the leaves of plants suffer from mechanical injury or chemical treatment. Up to date, more than 90 kinds of plants have been found to produce protease inhibitors.

Oryzacystatin-I (OCI) protein is one member of proteinase inhibitors, which can inhibit the proteinase activity in insects' intestinal canal, finally prevent the assimilation of proteins (Murdock et al., 1988). *OC*I gene with a length of 1.4 kb, cloned from rice cDNA library, is composed of three exons and two introns. The gene encodes 102 amino acid residues, and has a typical conserved sequence as Gln-Val-Val-Ala-Gly (Abe et al., 1987), which is necessary for its inhibiting effect (Meng et al., 2000). Oryzacystatin plays a role in inhibiting cysteine proteinase and may play an important role in biodefense in rice seed (Abe et al., 1987, 1988; Kondo et al., 1989).

It has been found that the *OC*I gene confers the improved resistance to plant nematodes such as *Meloidogyne incognita* and *Globodera pallida*, and nematode-resistant transgenic rice and tomato plants have been produced (Vain et al., 1998; Atkinson et al., 1996). Cipriani et al. (2001) and Jiang et al. (2004) reported the regeneration of transgenic sweetpotato plants with *OC*I gene at a low frequency, and the nematode resistance of transgenic plants was not evaluated.

We succeeded in developing an efficient system of embryogenic suspension cultures for a wide range of sweetpotato genotypes especially for commercial cultivars (Liu et al., 2001). Using embryogenic suspension cultures of sweetpotato, we have also established an efficient *Agrobacterium tumefaciens*-mediated transformation system (Yu et al., 2007) and obtained transgenic plants exhibiting complete herbicide resistance with the *bar* gene (Zang et al., 2009). In this study, we have developed transgenic sweetpotato plants resistant to stem nematodes using the *OC*I gene.

2. Materials and methods

2.1. Plant material

Sweetpotato cv. Xushu18 used in this study is one of most important commercial cultivars widely planted in China, but it is susceptible to sweetpotato stem nematodes. Embryogenic suspension cultures of Xushu 18 were prepared according to the method of Liu et al. (2001). Sixteen weeks after initiation, cell aggregates 0.7–1.3 mm in size from embryogenic suspension cultures of 3 days after subculture were employed for the transformation.

2.2. Bacterial strain and plasmid

The *A. tumefaciens* strain EHA 105 harboring a binary vector, plasmid pCAMBIA1301 was employed in this study. This binary vector contains the *OC*I, *uidA* and hygromycin phosphotransferase II (*hpt*II) genes driven by a CaMV 35S promoter, respectively (Fig. 1). *Eco*RI has a unique cleavage site in the T-DNA region in the vector.

2.3. Sensitivity of cell aggregates to hygromycin

The sensitivity of uninoculated cell aggregates to hygromycin (Hyg) added to the medium was tested according to the method of Yu et al. (2007) using 0, 3, 5, 7, 9, 12, 15, 20, 25, and 30 mg/l Hyg in order to determine the optimal concentration of this antibiotic in the selective medium. The experiments were repeated three times with 50 cell aggregates per treatment. The data were analyzed using SAS V8.02 (SAS Institute Inc., Caly, NC, USA) and the differences between the means were compared by Duncan's (1955) multiple range tests at the 0.05 level.

2.4. Transformation, selection and plant regeneration

Transformation and selection of the cell aggregates were conducted as described by Zang et al. (2009). The *Agrobacterium* single colony was cultured in 25 ml Luria–Bertani (LB) liquid medium containing 50 mg/l kanamycin and 50 mg/l rifampycin on a reciprocal shaker (200 rpm) at 28 °C for 16–18 h until $OD_{600\,nm} = 0.5$ was reached. The bacteria were collected by centrifugation at 5000 rpm for 5 min, washed with LB liquid medium and further with Murashige and Skoog (1962) (MS) medium containing 2.0 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D), and then were resuspended in 25 ml MS medium containing 2.0 mg/l 2,4-D for the inoculation. Cell aggregates were infected for 5 min in the bacteria suspension at room temperature. Following inoculation, the cell aggregates were blotted on sterile filter paper and placed on filter paper in a Petri dish with 25 ml MS solid medium with 2.0 mg/l 2,4-D and 30 mg/l acetosyringone (AS) for the cocultivation. The cocultivation was conducted for 3 days in the dark at 27 ± 1 °C. After cocultivation, the cell aggregates were washed twice with MS liquid medium containing 2.0 mg/l 2,4-D and 500 mg/l carbenicillin (Carb) and maintained in MS liquid medium with 2.0 mg/l 2,4-D and 100 mg/l Carb on a reciprocal shaker (100 rpm) at 27 ± 1 °C under 13 h of cool-white fluorescent light at 10 μmol/(m^2 s) for 1 week, and then were cultured at 2-week interval on MS solid medium supplemented with 2.0 mg/l 2,4-D, 100 mg/l Carb and 7 mg/l Hyg for the selection culture in the dark at 27 ± 1 °C.

Eight weeks after selection, the obtained Hyg-resistant embryogenic calluses were transferred to MS solid medium supplemented with 1.0 mg/l abscisic acid (ABA), 100 mg/l Carb and 7 mg/l Hyg to induce formation of somatic embryos and regeneration of plantlets at 27 ± 1 °C under 13 h of cool-white fluorescent light at 54 μmol/(m^2 s). The regenerated plantlets were further transferred to the basal medium for the development of whole plants under 13 h of cool-white fluorescent light at 54 μmol/(m^2 s).

2.5. GUS assay

The Hyg-resistant calluses and leaves, stems and roots of transgenic plants were tested for GUS expression using histochemical GUS assay according to the method of Jefferson et al. (1987). These tissues were incubated in GUS assay buffer at 37 °C for 12 h. Blue staining of the cells or tissues denoted positive reaction.

2.6. PCR analysis

Genomic DNA was extracted from fresh leaf tissues of in vitro-grown GUS-positive/-negative transgenic plants and untransformed control plants by the cetyltrimethylammonium bromide (CTAB) method (Saghai-Maroof et al., 1984). Equal amounts of 200 ng of total DNA were amplified in 50 μl reactions using specific primers for the *OC*I gene: 5′-ATG TCG AGC GAC GGA GGG-3′ and 5′-TTA GGC ATT TGC CGA GGC ATC-3′. These primers were expected to give products of 311 bp. PCR amplifications were performed with an initial denaturation at 95 °C for 4 min, followed by 35 cycles at 94 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s and final extension at 72 °C for 10 min. PCR products were separated by electrophoresis on a 1% (w/v) agarose gel.

2.7. Assay for stem nematode resistance

The transgenic plants and the untransformed control plants were transplanted to pots with a mixture of soil and vermiculite (1:1) in a greenhouse for the domestication. These plants were then planted in a field infected by stem nematodes at a density of 300 nematodes per 100 g soil and their stem nematode resistance was evaluated for 2 years according to the method of S.Y. Lu et al. (1998)

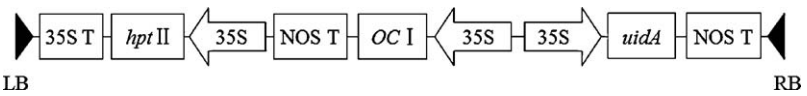

Fig. 1. Schematic diagram of the T-DNA region of binary plasmid pCAMBIA1301-*OC*I. LB: left border; RB: right border; *hpt*II: hygromycin phosphotransferase II gene; *OC*I: *oryzacystatin*-I gene; *uidA*: β-glucuronidase gene; 35S: cauliflower mosaic virus (CaMV) 35S promoter; 35S T: CaMV 35S terminator; NOS T: nopaline synthase terminator.

and X.F. Lu et al. (1998). Transgenic plants resistant to stem nematodes selected through the field evaluation were further identified for their stem nematode resistance by nematode inoculation test. After surface-sterilized with 70% ethanol, the storage root was perforated on it in a depth of its semidiameter with a stopper borer, 500 sweetpotato stem nematodes were inoculated in the hole, then one half of the root column was inserted back into the hole, and finally the hole was sealed with paraffin. Stem nematodes were isolated from storage roots of the control plants. The inoculated storage roots were maintained in an incubator in the dark at 25 °C for 4 weeks to investigate the symptom development. Three storage roots were used per treatment. The data were analyzed as described above.

2.8. Southern blot analysis

Southern blot analysis was conducted as described by Zang et al. (2009). Coding sequence of the *OC*I gene was used as probe. The labeling of probe, prehybridization, hybridization and detection were performed by the protocol of DIG High Prime DNA Labeling and Detection Starter Kit II (Roche Diagnostics GmbH, Germany).

2.9. Real-time quantitative PCR analysis

Storage roots of transgenic plants were used for real-time quantitative PCR analysis because stem nematodes mainly damage the storage roots in sweetpotato. Total RNA was extracted from storage roots of transgenic plants and untransformed control plants using the RNAprep Pure Plant Kit (Tiangen Biotech, China). RNA samples were reverse-transcribed using Quantscript Reverse Transcriptase Kit (Tiangen Biotech, China). The cDNA solution was used as templates for PCR amplification with a pair of gene-specific primers of the *OC*I gene: 5′ AGC TCC TTG AAG TCC ATC CA 3′ and 5′ CAA GAA GGC CAA TTC TCT GC 3′, which are expected to give the product of 164 bp. A 169 bp fragment of *β-actin* gene, used as an internal control, was amplified by the specific primers: 5′ AGC AGC ATG AAG ATT AAG GTT GTA GCA C 3′ and 5′ TGG AAA ATT AGA AGC ACT TCC TGT GAA C 3′. Amplification was conducted with ABI PRISM 7500 (Software for 7500 and 7500 Fast Real-Time PCR Systems, V2.0.1, USA) using Real Master Mix (Tiangen Biotech, China). The amplifications were performed with an initial denaturation at 95 °C for 5 min, followed by cycling stage with 40 cycles of 95 °C for 15 s, 60 °C for 20 s, and 68 °C for 30 s, then followed by melt curve stage for 95 °C for 15 s, 60 °C for 60 s, 95 °C for 30 s, and 60 °C for 15 s. Quantification of the gene expression was done with comparative C_T method (Schmittgen and Livak, 2008). The experiments were repeated three times with three storage roots per treatment. Each data represents the average of three experiments.

2.10. Western blot analysis

Storage roots of transgenic plants were further used for Western blot analysis. Protein was extracted from storage roots of transgenic plants and the untransformed control plant by the protocol of plant total protein extraction Kit (Applygen Technologies Inc., China). The extracted total protein was determined by bicinchoninic acid (BCA) method. Fifty micrograms of protein was separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) (15% separation gel/5.4% stacking gel) for Western blot analysis. After electrophoresis, the protein on the gel was transferred to a nitrocellulose transfer membrane (Whatman Inc., Germany) by semi-dry electrophoretic transfer cell for immunostaining. Immunostaining was performed as described by Wang and Fang (2002). The preparation of primary antibody (OCI antiserum) was completed in rabbit in accordance to the standard procedures using the purified protein (Wang and Fang, 2002).

3. Results

3.1. Sensitivity of cell aggregates to hygromycin

The sensitivity of cell aggregates from embryogenic suspension cultures of sweetpotato cv. Xushu 18 to Hyg was tested to establish an efficient selection system. The results showed that Hyg concentrations significantly influenced the growth and survival of Xushu 18 cell aggregates, and a 7 mg/l concentration of Hyg was enough to inhibit the growth of Xushu 18 cell aggregates. This concentration of Hyg was less than that reported by Yu et al. (2007) who suggested that 25 mg/l Hyg completely inhibited the growth of sweetpotato cv. Lizixiang cell aggregates. Thus, the sensitivity of cell aggregates against Hyg was different among different sweetpotato varieties. This allowed us to confirm the selective medium used in the present study.

3.2. Transformation, selection and plant regeneration

A total of 1710 cell aggregates of Xushu18 (Fig. 2A) cocultivated with the *A. tumefaciens* strain EHA 105 were cultured on the selective medium with 2.0 mg/l 2,4-D, 100 mg/l Carb and 7 mg/l Hyg. Eight weeks after selection, 488 Hyg-resistant embryogenic calluses were produced from them (Fig. 2B). Pieces of 25 Hyg-resistant calluses were randomly sampled for GUS assay. The results showed that all of the tested calluses expressed the *uidA* gene (Fig. 2C).

The 488 Hyg-resistant embryogenic calluses were transferred to MS medium supplemented with 1.0 mg/l ABA, 100 mg/l Carb, and 7 mg/l Hyg, after 5–6 weeks 419 of them formed somatic embryos which further germinated into plantlets on the same medium (Fig. 2D and E). These plantlets developed into whole plants on the basal medium (Fig. 2F). A total of 2119 putatively transgenic plants were obtained in the present study.

3.3. GUS assay of transgenic plants

Of the 2119 putatively transgenic plants, 194 were randomly sampled for detecting GUS expression in leaf, stem and root tissues; 180 (92.8%) had visible GUS activity in these tissues (Fig. 2G–I), indicating stable *uidA* gene integration into the genome of the plants. The remaining 14 plants and untransformed control plants showed no GUS expression (Fig. 2G–I). These 14 plants showing no GUS expression probably came from non-transgenic cells of the transgenic calluses which possibly contained a few non-transgenic cells as reported by Yu et al. (2007) and Zang et al. (2009).

3.4. PCR analysis of transgenic plants

The 194 GUS-positive/-negative plants and untransformed control plant were analyzed by PCR amplification. All of the 180 GUS-positive plants had a specific 311 bp band of the *OC*I gene,

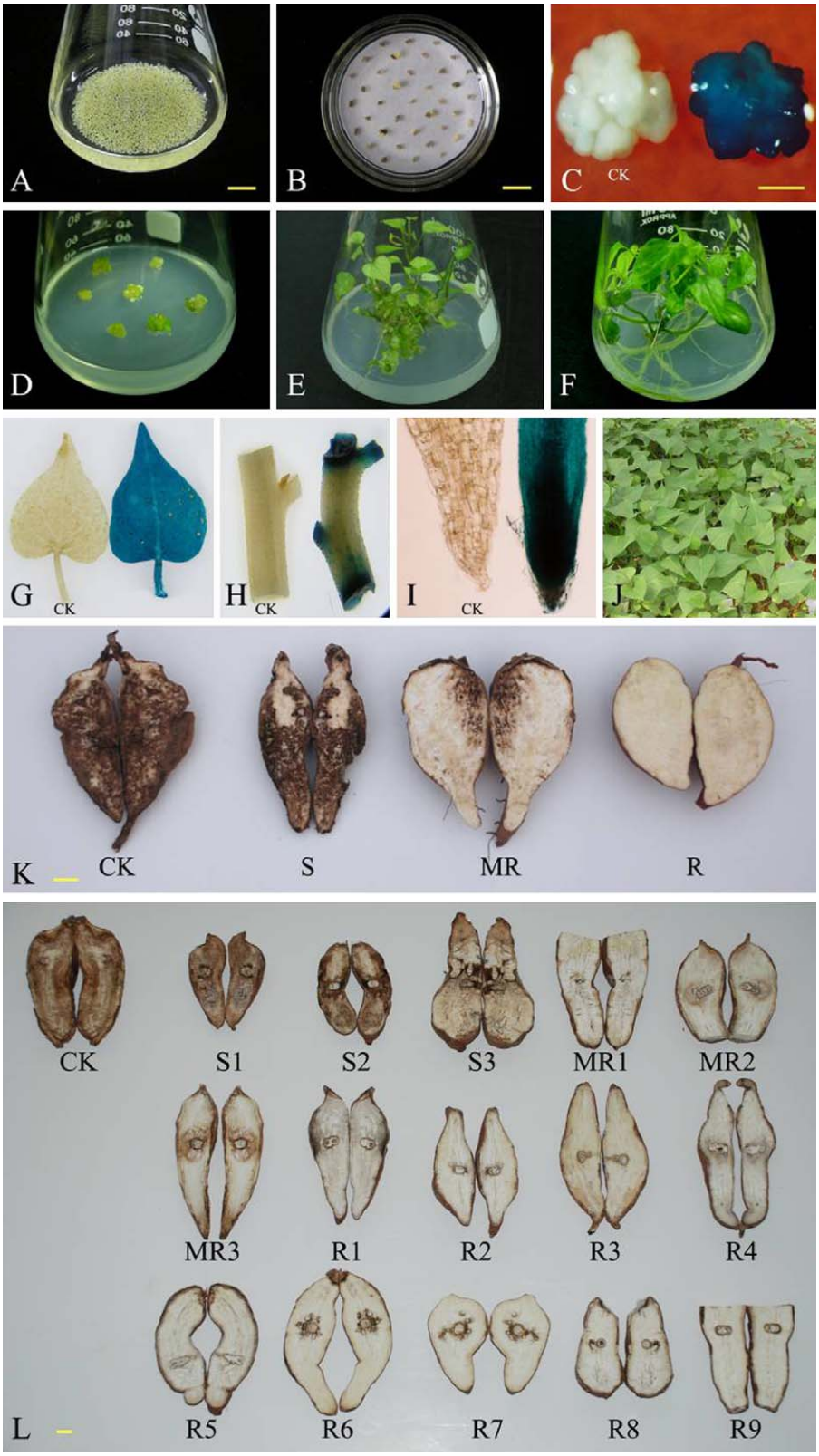

Fig. 2. Production of transgenic plants from sweetpotato cv. Xushu18 cell aggregates and evaluation of stem nematode resistance. (A) Embryogenic suspension cultures proliferating in MS medium containing 2.0 mg/l 2,4-D (bar = 10 mm). (B) Hyg-resistant calluses formed on MS medium with 2.0 mg/l 2,4-D, 100 mg/l Carb and 7 mg /l Hyg after 8 weeks of selection (bar = 10 mm). (C) GUS expression in a piece of Hyg-resistant calluses and no GUS expression in untransformed control callus (CK) (bar = 1 mm). (D and E) Formation and germination of somatic embryos from Hyg-resistant embryogenic calluses on MS medium with 1.0 mg/l ABA, 100 mg/l Carb and 7 mg/l Hyg. (F) Whole regenerated plants formed on the basal medium. (G, H and I) GUS expression in leaf, stem and root of a transgenic plant and no GUS expression in the control (CK). (J) Transgenic plants grown in a greenhouse. (K) Storage roots of transgenic plants and untransformed control plant displaying different reaction to stem nematodes in the field infected by stem nematodes. CK: untransformed control plant; S: susceptible transgenic plant; MR: middle resistant transgenic plant; R: resistant transgenic plant (bar = 10 mm). (L) Storage roots of transgenic plants and untransformed control plant displaying different reaction to stem nematodes after 4 weeks of inoculation. CK: untransformed control plant; S1–S3: susceptible transgenic plants; MR1–MR3: middle resistant transgenic plants; R1–R9: resistant transgenic plants (bar = 10 mm).

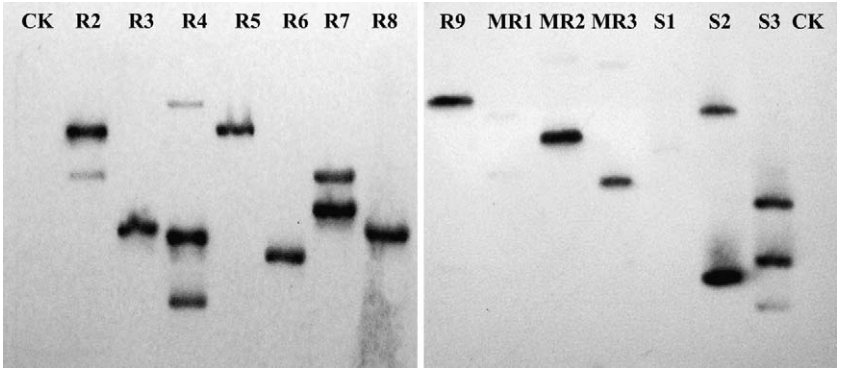

Fig. 3. Southern blot analysis of transgenic plants to detect the copy number of integrated *OC*I gene. DNA was digested with *Eco*RI and hybridized with the DIG-labeled *OC*I gene probe. Lane CK: untransformed control plant. Lanes R2–R9: resistant transgenic plants. Lanes MR1–MR3: middle resistant transgenic plants. Lanes S1–S3: susceptible transgenic plants.

while no specific band was observed in the 14 GUS-negative plants and untransformed control plant, indicating that the 180 plants were transgenic.

3.5. Assay for stem nematode resistance of transgenic plants

The 2119 regenerated plants showed 100% survival when transferred to the soil (Fig. 2J). No obvious variations in morphology were observed. These plants were planted in a field infected by stem nematodes and their stem nematode resistance was evaluated for 2 years. Of the 2119 plants, 9 were resistant, 167 middle resistant, and 1943 susceptible to stem nematodes (Fig. 2K). The untransformed control plants were susceptible to stem nematodes (Fig. 2K).

The nine resistant plants, three middle resistant and three susceptible plants randomly sampled, and untransformed control plant were further identified for their stem nematode resistance by nematode inoculation test and showed the similar resistance reaction in the field evaluation (Table 1). Storage roots of the untransformed control and three susceptible plants were rotten completely; storage roots of one middle resistant plant (MR1) were rotten completely and the rotten areas of storage roots from the remaining two middle resistant plants (MR2 and MR3) reached 21 mm and 18 mm, respectively, from the hole; the inoculated nematodes did not almost spread in storage roots of eight of the nine resistant plants except for the nematodes spread to 2 mm from the role in the remaining one resistant plant (R1) (Fig. 2L). The results indicated that the resistant plants had significantly higher stem nematode resistance compared to the susceptible plants and untransformed control plant, R2–R9 plants had the best stem nematode resistance, and R1 showed a little weaker resistance than R2–R9.

Table 1
Resistance reaction of the transgenic plants after 4 weeks of inoculation with stem nematodes.

Plant lines	Rotten semidiameter of storage roots (mm)
R1	2 ± 2a*
R2	0a
R3	0a
R4	0a
R5	0a
R6	0a
R7	0a
R8	0a
R9	0a
MR1	Rotten completely
MR2	21 ± 6b
MR3	18 ± 7b
S1	Rotten completely
S2	Rotten completely
S3	Rotten completely
CK	Rotten completely

* Values presented as mean ± SE, with different letters within a column indicating significant differences at the 0.05 level.

3.6. Southern blot analysis of transgenic plants

Transgene integration patterns of R2–R9, MR1–MR3, S1–S3 plants were analyzed by Southern blot. The DNA of the transgenic plants and untransformed plant was digested with *Eco*RI, which has a unique cleavage site in the T-DNA region in the vector and hybridized with the *OC*I gene probe. The resistant, middle resistant and susceptible transgenic plants displayed different integration patterns and the copy number of integrated *OC*I gene varied from 1 to 3, but there was no clear relationship between stem nematode resistance and copy number of integrated *OC*I gene (Fig. 3). No hybridizing band was observed in the untransformed control plant as expected (Fig. 3).

3.7. Real-time quantitative PCR analysis of transgenic plants

R2–R9, MR1–MR3, S1–S3 plants were further analyzed by real-time quantitative PCR. The results showed that the level of *OC*I gene expression varied among the transgenic plants, the highest level of transgene expression was found in the resistant plants, the susceptible plants exhibited the lowest expression level, and the middle resistant plants shown medium level (Fig. 4). Especially, the expression level of *OC*I gene was significantly higher in the resistant plants than in the susceptible plants and some of the middle resistant plants, even though the expression level of R3 was also higher by 5.9% than that of MR3 (Fig. 4).

3.8. Western blot analysis of transgenic plants

R2–R9, MR1–MR3, S1–S3 plants were also further analyzed by Western blot. All of these eight resistant plants had the strong specific OCI protein band with a molecular weight of 11.4 kDa; the three middle resistant plants only showed the weak specific band; no obvious specific band was observed in the three susceptible plants (Fig. 5). The results verified that the expression level of *OC*I gene was significantly related to the resistance of transgenic plants.

4. Discussion

Stem nematode is one of most serious diseases limiting sweetpotato production. In China, almost all of the commercial

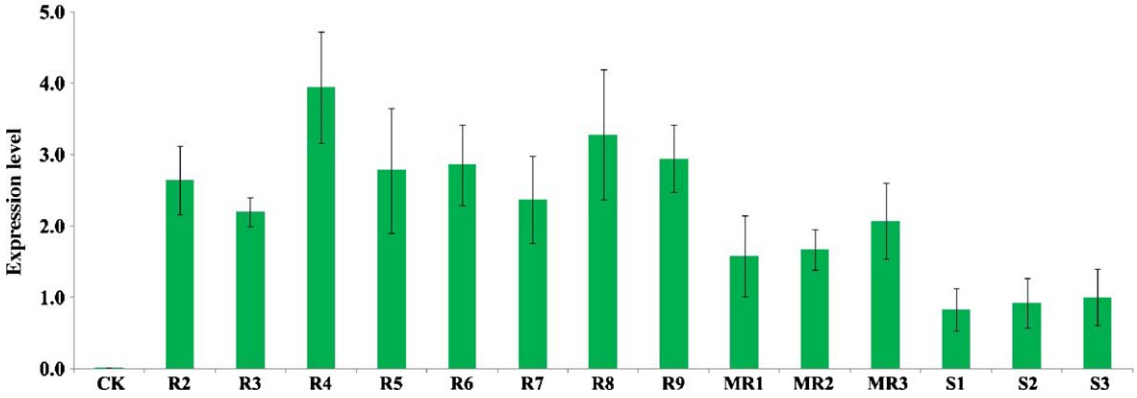

Fig. 4. Real-time quantitative PCR analysis of transgenic plants. CK: untransformed control plant. R2–R9: resistant transgenic plants. MR1–MR3: middle resistant transgenic plants. S1–S3: susceptible transgenic plants.

sweetpotato cultivars are not resistant to stem nematodes. The objective of the present study is to develop sweetpotato resistant to this disease by gene engineering.

It has been found that the *OC*I gene confers the improved resistance to some plant nematodes such as *M. incognita* and *G. pallida* (Vain et al., 1998; Atkinson et al., 1996). But, there is no report on producing transgenic plants resistant to stem nematode (*D. destructor* Thorne) using this gene. Cipriani et al. (2001) and Jiang et al. (2004) obtained only a few transgenic sweetpotato plants with the *OC*I gene and the nematode resistance of transgenic plants was not evaluated.

The present study is the first report to for production of transgenic sweetpotato plants resistant to stem nematodes using the *OC*I gene. Eight transgenic plants resistant to stem nematodes were obtained from the 2119 transgenic plants by the field evaluation and the artificial inoculation test. Our results verified that the *OC*I gene can enhance the resistance of sweetpotato to stem nematodes.

The frequency of resistant transgenic plants was low (0.42%) in the present study. Real-time quantitative PCR and Western blot analyses showed that the expression level of *OC*I gene was significantly higher in the resistant plants than in the susceptible plants and some of the middle resistant plants (Figs. 4 and 5). Thus, it is thought that relatively high level of expression of *OC*I gene can confer the resistance of sweetpotato to stem nematodes, while low level of *OC*I gene expression in the middle resistant and susceptible plants is not enough to provide the resistance to stem nematodes. The similar results were also observed in transgenic tobacco plants with *OC*I gene (Masoud et al., 1993). The present results demonstrated that no obvious OCI protein expression was observed in the three susceptible plants, suggesting the silence of the transgene may occur at the translation level in the susceptible transgenic plants. Vain et al. (1998) reported at least one of the transgenes was totally silenced in the progeny of approximately 50% lines in transgenic rice plants with *OC*IΔD86. In addition, MR2 and R7 exhibited the similar protein expression level and it is thought that the relationship between OCI expression and stem nematode resistance is probably complicated in some transgenic plants. The detailed analysis will be conducted in the future study.

Masoud et al. (1993) suggested that high level of *OC*I expression might be due to multiple copies of the T-DNA segment. But, the present results indicated that there was no clear relationship between expression level of *OC*I gene and copy number of integrated *OC*I gene (Figs. 3–5), similar to the results reported by Zang et al. (2009), in which the copy number of integrated *bar* gene also ranged from 1 to 3 in transgenic plants exhibiting functional resistance to herbicide.

Though the present study gave a low frequency of resistant transgenic plants, truly resistant plants can be selected from a large population of the transgenic plants with the *OC*I gene. Sweetpotato is a clonally propagated crop, the obtained resistant transgenic plants can be clonally propagated rapidly for the application.

In conclusion, we have succeeded in the development of transgenic sweetpotato resistant to stem nematodes by the transfer of *OC*I gene to cell aggregates from embryogenic suspension cultures of Xushu 18 using *A. tumefaciens*-mediated transformation system. Overexpression of *OC*I gene in storage roots of transgenic plants can enhance stem nematode resistance of sweetpotato. The present study provides an effective way for improving stem nematode resistance of this crop.

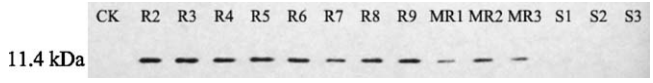

Fig. 5. Western blot analysis of transgenic plants. CK: untransformed control plant. R2–R9: resistant transgenic plants. MR1–MR3: middle resistant transgenic plants. S1–S3: susceptible transgenic plants.

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