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ORIGINAL RESEARCH

RNA Interference-Based Transgenic Maize Resistant to Maize Dwarf Mosaic Virus

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Abstract Maize dwarf mosaic virus (MDMV) is a widespread pathogenic virus that causes serious loss of yield in maize (*Zea mays*). RNA interference (RNAi) triggered by hairpin RNA (hpRNA) transcribed from a transgenic inverted-repeat sequence is an effective way to defend against viruses in plants. In this study, an hpRNA expression vector containing a sense arm and an antisense arm of 150 bp separated by an intron of the maize actin gene was constructed to target the P1 protein (protease) gene of MDMV and used to transform *Agrobacterium tumefaciens* strain EHA105. The transformed *Agrobacterium* strain was used to transform maize embryonic calli isolated from immature embryos by an improved culture technique. In all, 46 plants were regenerated after stringent hygromycin B selection, and 18 of them were certified to be positive by PCR amplification. Of these positive plants, 13 were grown to produce offspring, and nine were identified by Southern blotting to have the transgene integrated with one or two copies. The resistance of three T_2 lines was evaluated in a field trial of dual MDMV inoculation in two environments and was found to be improved compared with the non-transformed control. The disease indexes of the transgenic plant lines h2, 13, and h1 were not significantly different from the highly resistant control line H9-21. The viral titers of the inoculated plants were detected by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA), and the result was in accord with the resistance evaluated in the field trial. The addition of uniconazole S3307 (0.25 mgl^{-1}) and ABT root-promoting powder (0.5 mgl^{-1}) showed a significant improvement of hardening in regenerated plantlets, which were stronger and generated a better fibrous root system than the control. This improvement could facilitate the transgenic operation of maize.

Keywords *Agrobacterium*-mediated transformation · Embryonic callus · Hairpin RNA · Maize dwarf mosaic virus · Resistance · RNA interference

Abbreviations

IgG	Immunoglobulin G
T-DNA	Transfer DNA

Introduction

Maize dwarf mosaic virus (MDMV) is a worldwide pathogen that causes chlorosis, stunting, and significant loss of yield in maize (Redinbaugh et al. 2004; Uzarowska et al. 2009). In China, MDMV causes a yield loss of about 10% in a year, and this loss has been increasing steadily in recent years (Jiang and Zhou 2002; Wu et al. 2007). Strategies for the management of virus diseases normally include control of the vector population using insecticides, adjusting seedtime, and the use of virus-free propagating material and appropriate cultural practices (Dasgupta et al. 2003). However, these methods can become ineffective because of the non-persistent model of virus transmission by aphids (Wu et al. 2007). The use of resistant germplasm

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is an environmentally sustainable and effective way for controlling virus diseases of maize (Redinbaugh et al. 2004), but it is time consuming because identification and development of resistant inbred lines or hybrids needs to respond to year-to-year inconsistency of viral disease pressure. The biological control of MDMV remains a challenge.

In many crops, cross-protection resistance to virus infection has been developed by introducing a sequence of the viral genome into the target crop by genetic transformation (Baulcombe 1996; Lu et al. 1998; Sun et al. 2001; Liu et al. 2009). The specific capsid protein (CP) and P1 protein (protease) encoded by a single sense RNA strand of the MDMV genome are crucial to viral particle coating and cell-to-cell transmission (Cronin et al. 1995). Transgenic resistant lines have been obtained by introduction of the sense (Murry et al. 1993; Liu et al. 2009) or the antisense (Bai et al. 2006) sequence of the MDMV CP gene. Waterhouse et al. (1998) considered this kind of cross protection as viral gene silencing triggered by RNA interference (RNAi) of double-stranded RNA, which is characterized as stable, sustainable, and safe (Waterhouse et al. 1998, 2001; Prins and Goldbach 1996; Wang and Metzlaff 2005). Among those, however, the range of resistances obtained varied from extreme resistance to susceptibility, even in lines obtained with the same transgene (Morroni et al. 2008), and the resistance is not complete (Dasgupta et al. 2003).

RNAi triggered by hairpin RNA (hpRNA) transcribed from the transgenic inverted-repeat sequence provides a straight-forward natural defense mechanism against invasive viruses in plants and has been proved to be more efficient against viruses than the cross protection triggered by the introduction of a sense or an antisense sequence (Chuang and Meyerowitz 2000; Kusaba 2004; Ding and Voinnet 2007; Prins et al. 2007; Rahman et al. 2008; Aliyari and Ding 2009; Obbard et al. 2009). The defense effect can be enhanced by the inclusion of an intron in the hpRNA construct (Wesley et al. 2001). This technique has been used to engineer virus resistance in rice (Tyagi et al. 2008), tomato (Abhary et al. 2006; Ramesh et al. 2007), potato (Vargas et al. 2008), common bean (Bonfim et al. 2007), cucumber (Chen et al. 2004; Morroni et al. 2008), tobacco (Waterhouse et al. 1998; Qu et al. 2007), poinsettia (Clarke et al. 2008), pepper (Negrete et al. 2009), and *Arabidopsis* (Fusaro et al. 2006).

In maize, the only attempt to induce RNAi-mediated transgenic virus resistance was reported by Bai et al. (2008), who transformed maize with an hpRNA expression vector containing the inverted-repeat sequence of the MDMV replicase gene, and obtained transgenic resistant lines. In this study, an hpRNA expression vector containing reverted-repeat sense and antisense arms was constructed to target the MDMV gene encoding the P1 protein (protease) and used to transform maize embryonic calli. Transgenic lines were regenerated after selection with hygromycin B, identified by PCR amplification and Southern blotting, and evaluated for their virus resistance in inoculated field trials. Viral titers were detected by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA).

Materials and Methods

Construction of hpRNA Expression Vector

A 150-bp specific fragment of the P1 protein (protease) gene was selected from the genomic sequence of MDMV (GenBank accession number NC003377.1) and synthesized at Sangon Co., Shanghai, China. This fragment was amplified using PCR primers (Table 1) containing the restriction sites necessary for sense (*Xho*I/*Apa*I) and antisense (*Bam*HI/*Pst*I) orientation. The amplified fragments were inserted into the pSK vector (Stratagene, USA) in sense and antisense orientations, separated by an intron of the maize actin gene, to construct an hpRNA expression vector. The hpRNA expression construct was cloned into the plant expression vector pCAMBIA1300 (Stratagene, USA), under the control of the ubiquitin promoter and the *nos* terminator, generating the hpRNA expression vector pASP150 (Fig. 1). For selection, the hygromycin phosphotransferase gene conferring hygromycin B resistance was used under the control of the cauliflower mosaic virus 35S promoter (P-35S) and 35S terminator (T-35S). Sequence analysis was used to verify the junctions and orientations in the hpRNA expression vector pASP150. This vector was introduced by electroporation into the disarmed *Agrobacterium tumefaciens* strain EHA105.

Table 1 PCR primers used to construct hpRNA expression vector

Primer	Restriction site	Sequence
F1	*Xho*I	5′-CCCCTCGAGAATCATGGAGCTGTTCGCTG-3′
R1	*Apa*I	5′-TTTGGGCCCTCTTTCCATATCTGTGCACACTTC-3′
F2	*Pst*I	5′-AAACTGCAGAATCATGGAGCTGTTCGCTG-3′
R2	*Bam*HI	5′-CAAGGATCCTCTTTCCATATCTGTGCACACTTC-3′

Fig. 1 The T-DNA regions of hpRNA expression vector pASP50. *LB* left border, *RB* right border, *Hpt* hygromycin phosphotransferase gene, *P-35S* cauliflower mosaic virus *35S* promoter, *T-35S* cauliflower mosaic virus *35S* terminator, *P-Ubi* ubiquitin promoter, *T-nos* terminator of nopaline synthase, *intron* intron of maize actin gene, *P150* 150-bp fragment of MDMV P1 protein (protease) gene

Agrobacterium-Mediated Transformation and Plant Regeneration

Maize immature embryos of inbred line 18-599 were inoculated onto N6 inducement medium that had been modified by increasing the Ca^{2+} content from 1.13 to 5 mmol l^{-1} and the addition of 0.5 mg l^{-1} uniconazole S3307 (Fu et al. 2005), and cultured in darkness for callus isolation. Embryonic calli were screened, subcultured, and transformed by co-cultivation with the transformed *Agrobacterium* strain as described by Frame et al. (2002) and Ishida et al. (2007). After cultivation for 7 days, the calli were transferred to selection medium containing hygromycin B at a concentration of 5, 10, or 15 mg l^{-1} and cultured for 20 days. Then, the screened resistant calli were transferred to regeneration medium modified by the addition of 0.25 mg l^{-1} uniconazole S3307 (Fu et al. 2005) and 0.5 mg l^{-1} ABT root-promoting powder (Tang et al. 2004), and cultured for plantlet regeneration at 27°C with a photoperiod of 12 h dark/12 h light (1,000 μmol/m^2s). Plantlets with fully grown shoots and roots were transplanted into plastic pots containing vermiculite and margarite (3:1, *w*/*w*), allowed to acclimatize for 2–3 weeks in greenhouse, and then transplanted into the field for self-pollination to produce T_0 seeds.

PCR Detection of Transformed Plants

After planting in the field, a leaf blade was collected from each regenerated plant and used for DNA extraction. For screening the putative transgenic plants, the 150-bp fragment of the P1 gene was amplified using forward (5′-AATCATGGAGCTGTTCGCTG-3′) and reverse (5′-TCTTTCCATATCTGTGCACACTTC-3′) primers, which were the sequences used to construct the hpRNA expression vector without the restriction sites. A 20-μl sample of reaction mixture containing 10× PCR buffer, 200 μmol l^{-1} dNTPs, 1.5 mmol l^{-1} Mg^{2+}, 0.5 μmol l^{-1} of each primer, and 0.1 μg of template DNA was subjected to PCR amplification under the following conditions: 5 min at 95°C, then 35 cycles of 30 s at 95°C, 30 s at 58°C, 30 s at 72°C, and a final extension step of 8 min at 72°C. PCR products were analyzed by electrophoresis in 2.0% (*w*/*v*) agarose gels.

Southern Blotting Identification of Transformed Plants

Southern blotting with the genomic DNA extracted from the leaf samples of 13 T_1 lines derived from the fertile T_0 plants positive in PCR was used to identify the stable integration of the transgene into the maize genome and to evaluate the transgene copy number. A 20-μg sample of genomic DNA was digested with restriction enzyme *Bam*HI for 4 h and separated by electrophoresis in 0.8% (*w*/*v*) TBE agarose gel overnight at 1 V cm^{-1} followed by transfer onto Gene Screen Transfer membrane (Millipore Corporation, Bedford, MA, USA). Membranes were hybridized overnight with a digoxigenin-labeled (Roche Diagnostics, Germany) probe of the sense fragment of the hpRNA construct.

Field Inoculated Evaluation

The transgenic T_2 plant lines derived from the T_1 lines positive in Southern blotting, together with non-transformed controls of a highly resistant line (H9-21, Xi et al. 2008), a highly susceptible line (Mo17, Li et al. 2007), and the non-transformed control line 18-599 were grown in a randomized block design with three replications at Xinzhou in Shanxi province and at Ya'an in Sichuan province, China, which have the highest frequency of MDMV in China. On the basis of reports by Louie (1986) and Kuntze et al. (1995), mechanical inoculation was done twice within 1 week at the three- to four-leaf stage, using inoculum prepared from

Table 2 Promotion effect of uniconazole S3307 and ABT root-promoting powder addition to plant regeneration

Addition of uniconazole S3307 and ABT root-promoting powder	Piece of resistant calli	Number of regenerated plantlets	Number of survived plantlets
0.25 mg l^{-1} uniconazole S3307 and 0.5 mg l^{-1} ABT root-promoting powder	250	69	33
0.5 mg l^{-1} uniconazole S3307 and 0.5 mg l^{-1} ABT root-promoting powder	200	37	12
Negative control	400	27	1

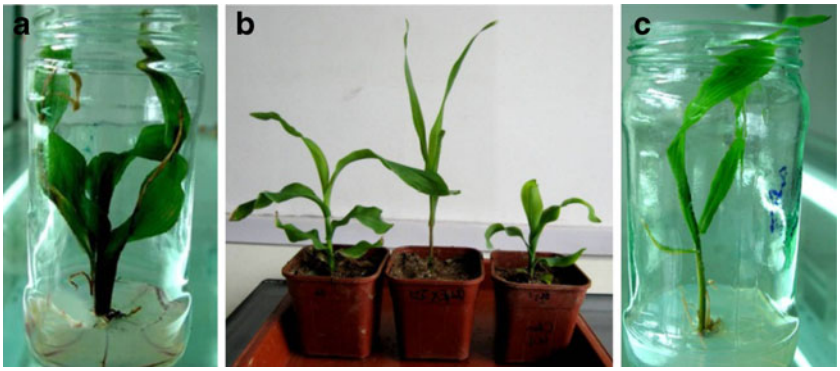

Fig. 2 Effects of uniconazole S3307 and ABT root-promoting powder to the growth of regenerated plants. **a** A regenerated plant on the regeneration medium with 0.25 mg l^{-1} uniconazole S3307 and 0.5 mg l^{-1} ABT root-promoting powder. **b** A regenerated plant transferred from the regeneration medium with 0.25 mg l^{-1} uniconazole S3307 and 0.5 mg l^{-1} ABT root-promoting powder (*left*), a regenerated plant transferred from the regeneration medium without uniconazole S3307 and ABT root-promoting powder (*middle*), and a regenerated plant transferred from the regeneration medium with 0.5 mg l^{-1} uniconazole S3307 and 0.5 mg l^{-1} ABT root-promoting powder (*right*). **c** A regenerated plant on the regeneration medium without uniconazole (S3307) and ABT root-promoting powder

the leaf sap of maize plants systemically infected with MDMV. The disease incidence and symptom scale were investigated at the adult stage according to the standard proposed by Lin (1989). The disease index was calculated as:

$$\text{Disease index}(\%) = \frac{\sum(\text{Number of infected plants} \times \text{symptom scale}) \times 100\%}{\text{Number of total plants} \times \text{maximum symptom scale}}$$

Resistance was classified into five grades: highly resistant (HR, disease index 0–10%), resistant (R, disease index 10.1–25.0%), intermediate (I, disease index 25.1–40.0%), susceptible (S, disease index 40.1–60.0%), and highly susceptible (HS, disease index >60.1%). One-way analysis of variance test was done with SPSS 18.0 software (http://www.spss.com/). The level of significance was set at $p<0.05$.

DAS-ELISA

The virus titer of the transformed lines was quantified by DAS-ELISA using an MDMV DAS ELISA kit (AC Diagnostics Inc., USA) according to the manufacturer' instructions. At the adult stage, the third leaf from the top was sampled from each plant of the same lines in the three replications in the field inoculated trial at Xinzhou, cut into 5 mm×5 mm pieces after removing the midribs, weighed as a mixed sample of 0.2 g, and homogenized in 2 ml of 0.01 mol l^{-1} phosphate buffer (pH 7.0). The homogenate was centrifuged for 1 min at 10,000×*g*. Microtiter plates (Nunc from Inter Med., Denmark) were coated with 2 μg ml^{-1} IgG freshly prepared from antiserum to MDMV and incubated overnight at 4°C. After a thorough wash in PBS-T buffer [50 mmol l^{-1} Tris–HCl, pH 7.4, 150 mmol l^{-1} NaCl, 0.1% (*v*/*v*) Tween 20], 100 μl of the centrifuged homogenate was dispensed into each triplicate sample well. The microtiter plates were incubated in a humid chamber for 2 h at room temperature and then washed again as described above. A 100-μl sample of freshly prepared enzyme-IgG conjugate was placed into each sample well, and the plates were washed three times with PBS-T buffer. A 100-μl sample of PNP solution [0.6 mmol l^{-1} $MgCl_2 \cdot 6H_2O$, 3.8 mmol l^{-1} NaN_3, 12% (*v*/*v*) diethanolamine, pH 9.8] was added to each well. After reaction in a humid chamber at room temperature for 30–60 min until clear color developed in the positive control wells, absorbance at 405 nm (A_{405}) was measured with an ELISA microtiter plate reader (BioTech, model ELX-800, USA).

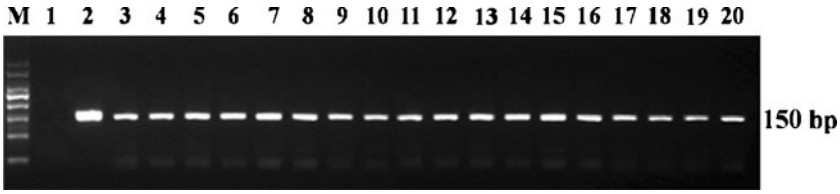

Fig. 3 PCR detection of the regenerated plants. *Lane M* 50-bp DNA ladder marker, *lane 1* non-transformed control 18-599, *lane 2* positive control of expression vector pASP150, *lanes 3–20* transformed plants 3, 5, 9, 10h, 10L, h1, h2, 12, 13, 14, m-1, 15, 16, 18, 20, 21, 24, and 25, respectively

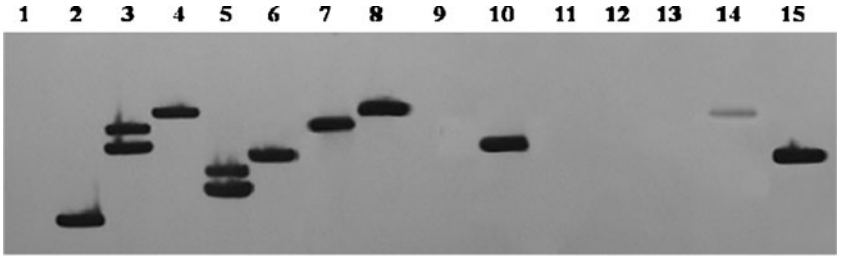

Fig. 4 Southern blotting of putative transgenic T_1 plant lines positive in PCR detection. *Lane 1* non-transformed control 18-599; *lanes 2–14* transformed plant lines 3, 5, 9, 10h, 10L, h1, h2, 12, 13, 14, 15, 20, and 21; *lane 15* plasmid control of hpRNA expression vector pASP150

Results

Transformation Rate and Effect on Regeneration of Uniconazole S3307 and ABT Root-Promoting Powder

From the 800 pieces of embryonic calli transformed by co-cultivation, 98 (12.3%) pieces of resistant embryonic calli were obtained after hygromicin B selection. A total of 46 (46.9%) plantlets were regenerated after the recovering subculture and the multiplication subculture (Table 2).

The addition of 0.25 mg l^{-1} uniconazole S3307 and 0.5 mg l^{-1} ABT root-promoting powder induced a significant increase of plant regeneration (Table 2). The regenerated plantlets were stronger and generated a more developed fibrous root system than the negative control. The growth of the regenerated plantlets was inhibited when the concentration of uniconazole added was increased to 0.5 mg l^{-1} (Fig. 2); the dwarfed regenerated plants became sterile when transferred into the field.

Integration of the Transgene into the Maize Genome

Of the 46 regenerated plants, 18 (39.1%) were detected as positive by specific PCR amplification and certified as putative transgenic plants (Fig. 3). Out of these 18 plants, 13 (72.2%) grew to reproduce seeds of the T_1 generation. Out of the 13 T_1 lines, nine (69.2%) were shown by Southern blotting to have stable transgene integration, and seven (77.8%) of them showed single-copy integration, and double-copy integration was found in the other two (22.2%) lines. The other four (30.8%) lines were not identified to be transformants (Fig. 4). On the basis of embryonic callus pieces used in the co-cultivation, the transformation rate was 1.13%.

Virus Resistance of T_2 Plant Lines

Differences among MDMV symptoms were observed in the field trials of the T_2 plant lines, the non-transformed control, the susceptible control, and the resistant control (Fig. 5). After the pollination stage, systemic infection of MDMV was observed in the non-transformed control (18-599), the susceptible control (Mo17), and in some T_2 plant lines. The disease indexes of the different T_2 plant lines and the controls matched each other in the two environments (Table 3). The non-transformed control (18-599) was evaluated as susceptible (S) to MDMV with a disease index between 40.1% and 60.0%, the susceptible control (Mo17) was evaluated as highly susceptible (HS) with a disease index >60.1%, while the resistant control (H9-21) was evaluated as resistant with a disease index between 10.1% and 25.0%. Of the nine transgenic T_2 plant lines derived from the T_1 lines positive in Southern blotting, lines h2, 13, and h1 were judged to have intermediate resistance to MDMV with a disease index between 25.1% and 40.0%, showing no systemic infection. This resistance is increased significantly when compared with the non-transformed control line 18-599, but was not significantly different from the highly resistant control line H9-21. The

Fig. 5 A different performance of MDMV symptom in T_2 plants and controls at the shooting stage. **a** The resistant control (H9-21), **b** the susceptible control (Mo17), **c** the non-transformed control (18-599), **d** the T_2 plant line h1

Table 3 MDMV resistance of T_2 transformed plant lines

T_2 plant line and control	Disease incidence (%)			Disease index (%)			Resistance grade
	Xinzhou	Ya'an	Average	Xinzhou	Ya'an	Average	
H 9-21 (resistant control)	56.2	48.1	52.2	22.9	17.9	20.4 a	R
h2	26.0	29.5	27.8	25.4	27.5	26.5 a	I
13	45.3	38.2	41.8	33.3	27.1	30.2 ab	I
h1	52.2	38.6	45.4	34.1	28.8	31.5 ab	I
9	61.5	65.2	63.4	42.7	46.0	44.4 bc	S
5	72.0	65.6	68.8	47.8	49.3	48.6 c	S
3	82.9	78.4	80.7	55.1	46.7	50.9 c	S
18-599 (non-transformed control)	93.7	100.0	96.9	48.6	55.8	52.2 c	S
21	88.7	83.5	86.1	60.4	57.4	58.9 c	S
10L	100.0	95.7	97.9	85.4	72.7	79.1 d	HS
10h	100.0	100.0	100.0	86.8	81.2	84.0 d	HS
Mo17 (susceptible control)	100.0	100.0	100.0	85.5	83.5	84.5 d	HS

In the column of average disease index, the same lowercase letters indicate non-significance, and the different lowercase letters indicate significance at possibility level of 0.05

R resistance, *I* intermediate, *S* susceptible, *HS* highly susceptible to MDMV

resistant grades of the other six transgenic T_2 plant lines were evaluated as susceptible (lines 9, 5, 3, and 21) and highly susceptible (lines 10L and 10h).

Virus Titer Detected by DAS-ELISA

The virus titers of the inoculated transgenic T_2 plant lines were obtained by measurement of A_{405} in DAS-ELISA (Table 4). The highly susceptible and susceptible lines (3, 5, 9, 10h, 10L, 21, and Mo17) evaluated in the field inoculated trial had higher virus titers than other lines. The virus titers of transgenic plant lines h2, 13, and h1 were lower than that of the non-transformed control 18-599 and as low as the highly resistant control line H9-21.

Table 4 Absorbance value at 405 nm in DAS-ELISA of the T_2 plant lines and controls

T_2 plant line and control	Absorbance at 405nm
H 9-21 (resistant control)	0.201
h2	0.207
13	0.285
h1	0.311
9	0.503
5	0.692
3	0.778
21	0.896
18-599 (non-transformed control)	0.904
10L	1.002
10h	1.323
Mo17 (susceptible control)	1.580

Each absorbance value is the average of three wells

Discussion

MDMV Resistance of the RNAi-Based Transgenic Lines

The integration of the hpRNA expression construct was certified for nine transgenic lines by Southern blotting. The MDMV resistance of lines h2, 13, and h1 was increased significantly compared with that of the non-transformed control line 18-599, although their resistance grade was evaluated as intermediate. The disease indexes of these three lines were not significantly different from that of the highly resistant control line H9-21, which was regarded as MDMV-free under ordinary non-inoculated conditions and was used as a source of highly resistant germplasm (Xi et al. 2008). The virus-free resistance was not obtained in the inoculated field evaluation of RNAi-based transgenic plant lines developed by Bai et al. (2008) to introduce an inverted-repeat sequence of the MDMV replicase gene into the maize genome. The disease incidence was ~15%.

Four of the RNAi-based transgenic lines (9, 5, 3, and 12) were susceptible to MDMV, and two of them (10L and 10h) were highly susceptible, including lines 5 and 10h with double-copy integration. Their susceptibility to MDMV was not significantly different from that of the non-transformed 18-599. This result could be due to three

possible reasons: (1) T-DNA rearrangement is well documented in some transgenic attempts (Deroles and Gardner 1988; Azhakanandam et al. 2000; Yin and Wang 2000; Rai et al. 2007). Almost 50% of the 27 transgenic rice lines studied showed rearrangement of T-DNA inserts according to Rai et al. (2007). Such T-DNA rearrangements could occur in our study and truncate the integrated hpRNA expression construct. (2) Numerous studies have shown that many plant viruses encode proteins that are able to suppress RNAi (Mallory et al. 2001; Vance and Vaucheret 2001; Hannon 2002; Savenkov and Valkonen 2002; Kubota et al. 2003; Moissiard and Voinnet 2004; Love et al. 2007). Further study is necessary to explore if MDMV encodes any RNAi suppressor. (3) As reported by Wesley et al. (2001), Chen et al. (2004), Missiou et al. (2004), Di Nicola-Negri et al. (2005), and Ritzenthaler (2005), the effective length of the expressed hpRNA constructs to trigger RNAi in transgenic plants is 300–800 bp, and the short limit is ~98 bp. The 150-bp hpRNA expression construct we introduced into the maize genome might be a little too short to trigger efficient RNAi. The underlying reason for the lack of resistance in some of the transgenic lines remains to be clarified.

Effect of Uniconazole S-3307 and ABT Root-Promoting Powder to Plant Regeneration

The inducement, subculture, and regeneration of embryonic calli are a bottleneck in maize transgenic operation (Sidorov and Duncan 2009). Fu et al. (2005) improved the inducement and subculture of maize embryonic calli by increasing the Ca^{2+} content from 1.13 to 5 mmol l^{-1} and addition of 0.5 mg l^{-1} uniconazole S3307 to N6. In this study, we added 0.25 mg l^{-1} uniconazole S3307 and 0.5 mg l^{-1} ABT root-promoting powder to the N6 regeneration medium. The regenerated plantlets became stronger, and their fibrous root system was more developed than that of the negative control (Fig. 2). Uniconazole S3307 is a category of triazole that regulates the growth of plants and protects them from abiotic stresses by inhibiting the synthesis of gibberellin (Fletcher and Hofstra 1990; Fletcher et al. 2001; Mizutani 2006; Saito et al. 2006). One of the conspicuous responses of plants to treatment with triazoles is that they are darker green with more chlorophyll than that in the controls (Davis et al. 1988; Fletcher and Hofstra 1990; Khalil and Rahman 1995). The regulation and protection effects of uniconazole could induce tolerance to water deficiency (Zhang et al. 2007). The active components of ABT root-promoting powder include indole-3-butytric acid and indole-3-acetic acid, which are regulators of plant growth. ABT root-promoting powder has been certified to have the ability to promote rooting and to increase the survival rate of plants under abiotic stress (Zhang et al. 1994; Tang et al. 2004). Therefore, the mechanism underlying the success of the addition of uniconazole and ABT root-promoting powder to promote plantlet regeneration in our study could be attributable to the ability to increase chloroplast formation and tolerance to water deficiency, and the increased root generation of the regenerated plants upon transfer from regeneration medium to nutrient soil.

In summary, we have transformed maize with an hpRNA expression construct of the MDMV P1 protein (protease) gene by *Agrobacterium*-mediated transformation and obtained transgenic lines resistant to MDMV. The disease index of the transgenic plant line h2 had no significant difference from the highly resistant control line H9-21. The viral titers of the inoculated plants were detected by DAS-ELISA, and the results were in accord with the resistance investigated in the field evaluation. This kind of effective resistance conferred by a transgene has significant potential because no chemical is used to control virus diseases. The improvement of the N6 regeneration medium by the appropriate addition of uniconazole and ABT root-promoting powder could facilitate the transgenic operation of maize.

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