Maize streak virus-resistant transgenic maize: a first for Africa

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Received 27 March 2007; revised 28 June 2007; accepted 29 June 2007.
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Keywords: African biotechnology, geminivirus, maize streak virus, maize streak virus-resistant maize, maize yield improvement, pathogen-derived resistance, transgenic maize.

Summary

In this article, we report transgene-derived resistance in maize to the severe pathogen maize streak virus (MSV). The mutated MSV replication-associated protein gene that was used to transform maize showed stable expression to the fourth generation. Transgenic T1 and T2 plants displayed a significant delay in symptom development, a decrease in symptom severity and higher survival rates than non-transgenic plants after MSV challenge, as did a transgenic hybrid made by crossing T2 Hi-II with the widely grown, commercial, highly MSV-susceptible, white maize genotype WM3. To the best of our knowledge, this is the first maize to be developed with transgenic MSV resistance and the first all-African-produced genetically modified crop plant.

Introduction

Maize was first introduced to Africa by Portuguese traders in the 16th century, and has become the continent’s staple food crop, making up more than 50% of total caloric intake in local diets (Sinha, 2007). However, average maize yields of 1.2 tonnes/ha are just a quarter of global averages (http://faostat.fao.org), a disparity exacerbated by the susceptibility of maize to pathogen attack. Maize streak disease (MSD), caused by the geminivirus maize streak virus (MSV), is the major viral pathogenic constraint on maize production in Africa (Wambugu, 1999; Bosque-Pérez, 2000), making resistance to MSV a key target for crop improvement.

Biotechnological solutions to Africa’s food security problems (Motari et al., 2004) have been flagged as a vital component of the United Nation’s Millennium Development Goals (Acharya et al., 2003; Ayele et al., 2006). Yield increases and economic benefits to both commercial and resource-poor farmers of Bacillus thuringiensis (Bt) toxin transgenic cotton and maize have been well documented in South Africa and other developing countries (Pray et al., 2002; James, 2003; Qaim and Zilberman, 2003; Thirtle et al., 2003; Toenniessen et al., 2003; Gouse et al., 2004; Morse et al., 2004). Starting with the first accounts of pathogen-derived resistance (PDR) over 20 years ago (Sanford and Johnson, 1985; Powell-Abel et al., 1986), there have been numerous reports of genetically engineered virus resistance (mostly coat protein derived) in economically important food crops, including tomato (Nelson et al., 1988; Sanders et al., 1992; Kunik et al., 1994; Noris et al., 1996), papaya (Fitch et al., 1992; Lius et al., 1997) and potato (Kaniewski et al., 1990; Kawkuch et al., 1990; Lawson et al., 1990; van der Wilk et al., 1991). More recently, monocots have been engineered for virus resistance, including sugarcane (Ingelbrecht et al., 1999), ryegrass (Xu et al., 2001), barley (Wang et al., 2000), rice (Hayakawa et al., 1992; Pinto et al., 1999), wheat (Sivanani et al., 2000, 2002) and maize (to maize dwarf mosaic potyvirus and maize chlorotic mottle machlomovirus; Murry et al., 1993). Resistance to numerous geminiviruses has been achieved using a PDR approach (reviewed by Vanderschuren et al., 2007); however, none of these reports involved resistance in a monocot species. We have recently demonstrated the first successful PDR strategy for MSV resistance in a model...
monocot system (the MSV-susceptible grass *Digitaria sanguinalis*), using several dominant negative mutants of the MSV replication-associated protein gene (*rep*) (Shepherd et al., 2007). The multifunctional Rep protein is essential to viral replication, is required early in the viral lifecycle and functions as an oligomer, making it an ideal PDR target (Shepherd et al., 2007). In this article, we report the development of maize with transgenic resistance to MSV. This is a major advance in African agricultural biotechnology that should contribute to a substantial improvement in African maize yields.

**Results and discussion**

Although several mutant MSV *rep* constructs have previously been shown to inhibit MSV replication in transient and transgenic (model system) assays, only one (*prep*<sup>1-219Rb</sup>) resulted in phenotypically normal, fertile, MSV-resistant plants (Shepherd et al., 2007). In the present study, *prep*<sup>1-219Rb</sup>- was used to transform maize Hi-II, with bar (in pAHC25; Christensen and Quail, 1996) as the selectable marker, and T<sub>2</sub> seed was tested for MSV resistance in the laboratory. Generally, the younger the maize at the time of inoculation, the more susceptible the plant. There may be leaf necrosis, stunting and dieback if plants are infected before 6 weeks with severe MSV variants. In an initial screen for resistance, 110 T<sub>2</sub> plants and 50 non-transgenic controls were tested for MSV resistance by infection of 3-day-old seedlings via agroinoculation (method described in Martin and Rybicki, 1998). All trials were blind, with transgene presence/absence only determined by polymerase chain reaction (PCR) following symptom analysis [see ‘Experimental procedures’ and Figure S1 (Supplementary material)]. The percentages of chlorotic leaf areas were measured for leaves two and three [15 days post-inoculation (dpi)], four (22 dpi), five (29 dpi) and six (36 dpi) of each symptomatic plant using an image analysis technique (Martin and Rybicki, 1998), and the average percentages of chlorotic leaf areas were calculated for the transgenic and non-transgenic groups (Figure 1c). Significantly delayed symptom development was demonstrated by a decrease in chlorotic leaf area in leaf three of the transgenic group by a factor of 61 compared with non-transgenics (*P* < 0.0001; Mann–Whitney *U*-test). Transgenic plants were also significantly taller (30 ± 2.7 cm; 95% confidence interval) than non-transgensics (17 ± 2.4 cm) at 28 dpi (*P* < 0.0001; Mann–Whitney *U*-test), despite the death of the most severely infected plants (22% of non-transgensics, 2% of transgensics), effectively removing the most discriminatory plants from the analysis. Similarly, when the chlorotic areas of leaves five and six were analysed (29 and 36 dpi, respectively), only 2% and 6% of transgenic plants had died, compared with 22% and 33% of non-transgensics. Thus, the actual difference in resistance is probably greater as the most severely symptomatic (mostly non-transgenic) plants had been removed from the data set.

The decrease in resistance in the younger tissues of transgenic plants (leaves five and six, which only fully emerge after 24 dpi), together with eventual symptom development in initially asymptomatic transgenic plants, prompted us to investigate whether transgene expression was consistent over time. We therefore assayed transgene mRNA levels (see ‘Experimental procedures’). All transgenics tested at 7 dpi had detectable transgene transcript levels, whereas only 37% (17/46) of transgensics at 28 dpi had detectable transgene expression. This suggests that a decrease in transgene expression correlates with a decline in resistance, further indicating that our MSV resistance is caused by expression of the *prep*<sup>1-219Rb</sup>- transgene. Although it was not investigated, a most probable cause for the down-regulation of transgene expression is virus-induced gene silencing. This was shown previously for another geminivirus, Tomato yellow leaf curl Sardinia virus (TYLCSV), where expression of a resistance-conferring *rep* transgene (Rep-210) in tomato was shut down by a virus-induced homology-dependent mechanism of gene silencing (Lucioli et al., 2003; Noris et al., 2004). The authors suggested that the virus evaded silencing of its own Rep by evolving a silencing suppression mechanism to evade the plant’s natural defence system during a normal infection. We
are in the process of investigating whether a similar phenomenon is occurring in our system.

To test the efficacy of our transgenic resistance mechanism in a more agriculturally relevant genetic background, a highly MSV-susceptible elite white maize genotype, WM3, was crossed with MTA7E.2. Hybrid offspring, called WM3 × 7E, were challenged at 14 days old in a glasshouse using leafhopper transmission of an extremely severe MSV field isolate (described in Shepherd et al., 2007). In two initial challenges, only plants from hybrid populations (57/126, pooled results) were completely resistant, whereas all non-transgenic controls were sensitive.

To further investigate the association between resistance and our transgene in these hybrids, we challenged them a third time and extracted genomic DNA and RNA pre- and post-infection from 58 WM3 × 7E and 24 WM3 seedlings. We also recorded symptom severities for each plant at 28 dpi. Based on our genotyping, plants were split into three groups: group 1, WM3 controls (24 plants); group 2, WM3 × 7E non-transgenics (nt-WM3 × 7E; 19 plants); group 3, WM3 × 7E transgenics (39 plants). The transgenics had a significantly lower infection rate than groups 1 and 2 (Figure 2a). In addition, symptoms in infected transgensics were significantly milder than in control groups [Figures 2b, d and S3 (Supplementary material)], with chlorotic leaf areas being reduced by 12- and sixfold relative to WM3 and nt-WM3 × 7E plants, respectively. The resistance phenotypes observed in all three groups included no symptom development at all (10 transgenics, two plants in each non-transgenic group) and delayed symptom development (14 transgenics, 1/2 nt-WM3 × 7E, 0/2 WM3). In addition, for symptomatic plants without a delayed symptom phenotype, 11 of 15 transgenics, two of 20 WM3 and two of 15 nt-WM3 × 7E plants had mild symptoms (10% or lower chlorotic leaf areas recorded at 28 dpi). Combining the data for the three resistance phenotypes [examples of which are shown in Figure S4 (Supplementary Material)]

Figure 1 Maize streak virus (MSV) resistance in agroinoculated T3 transgenic Hi-II maize. (a) Transgenics (●) (n = 47) become symptomatic at a significantly lower rate than non-transgenics (■) (n = 18; P = 0.0047; Mann–Whitney U-test). (b) Transgenics (●) have a significantly higher survival rate than non-transgenics (■) between 1 and 50 days post-inoculation (dpi) (P < 0.0001; Kaplan–Meier log rank test). (c) Mean percentages of chlorotic areas on leaves two (n = 47 and 18), three (n = 47 and 18), four (n = 46 and 15), five (n = 44 and 13) and six (n = 37 and 11) of transgenics (●) and non-transgenics (■), respectively. Error bars represent 95% confidence intervals. Leaves two, three and four of transgenic plants showed a decrease in the percentage of chlorosis by factors of eight (P = 0.0456; Mann–Whitney U-test), 61.2 (P < 0.0001) and 2.5 (P < 0.0001), respectively, compared with non-transgenics. (d, e) Comparison of non-symptomatic (d) and symptomatic (e) transgenic plants with non-transgenics at 20 dpi.
material), 35 of 39 (90%) transgenic plants showed some resistance phenotype, compared with six of 24 (25%) WM3 and six of 19 (31.6%) nt-WM3 × 7E plants. The few asymptomatic controls were most probably escapes caused by reduced virus inoculum received by the plant, as found previously in our model system (Shepherd et al., 2007). Importantly, unlike the laboratory-challenged transgenic Hi-II, the transgene transcript was detected in all WM3 × 7E transgenics both pre- and post-infection. Accordingly, chlorotic areas on younger leaves analysed at 28 dpi were consistently significantly less than those in controls (Figure 2b).

The different responses of the Hi-II transgenics (Figure 1) and the Hi-IIWM3 transgenic hybrids (Figure 2) to virus infection are probably a result of two major differences between the two experiments.

1. Genotypes: the Hi-IIWM3 hybrid genetic background, even in the absence of the transgene, may provide more natural resistance than Hi-II or WM3 genotypes (this is indicated by the fact that even non-transgenic hybrid plants showed more resistance than non-transgenic WM3 in Figure 2b).

2. There are differences in the two experimental systems. Hi-II was agroinoculated with MSV at 3 days old, the most stringent challenge possible. In contrast, the Hi-IIWM3 hybrids were leafhopper inoculated at 14 days old (three- to five-leaf stage) which, although still young and therefore very sensitive to MSV, more closely reflects a natural field
infection. It is worth noting that even highly resistant conventionally bred maize genotypes (given a rating in the field of zero on a scale of 0–5, with zero being no symptom development when leafhopper inoculated with MSV) are susceptible to MSV when agroinoculated at 3 days old (Martin et al., 1999).

If there is indeed a balance between silencing, silencing suppression and virus replication (Lucioli et al., 2003; Noris et al., 2004) in our challenged transgenic plants, the slightly later inoculation period and possibly greater inherent resistance of the Hi-II/WM3 hybrids may tip the balance in favour of transgene expression. Expressed Rep\(^{1-219Rb}\) protein could then inhibit virus replication before the virus reaches the population density threshold required to induce silencing of the transgene or to suppress silencing of its own Rep. This suggests that introducing the transgene into breeding lines that already have some degree of tolerance (which includes most elite maize genotypes used in Africa) will significantly boost their observed resistance.

To gain an insight into the possible yield differences between MSV-infected transgenic and non-transgenic groups, challenged glasshouse-grown plants that had reached maturity were self-pollinated. Figure 2c shows the percentage of challenged glasshouse-grown plants that had reached maturity when leafhopper inoculated with MSV) are conventionally bred maize genotypes (given a rating in the field of zero on a scale of 0–5, with zero being no symptom development when leafhopper inoculated with MSV) are susceptible to MSV when agroinoculated at 3 days old (Martin et al., 1999).

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To gain an insight into the possible yield differences between MSV-infected transgenic and non-transgenic groups, challenged glasshouse-grown plants that had reached maturity were self-pollinated. Figure 2c shows the percentage of plants in each group that grew to maturity and yielded seed. Although 62% of challenged transgenics reached the flowering stage, only 16% and 13% of non-transgenic WM3 × 7E and WM3 plants, respectively, flowered. The rest of the plants either died before reaching maturity or never developed tassels. Interestingly, 48% of symptomatic transgenics grew to maturity and 21% yielded seed, compared with 6% of MSV-infected non-transgenic plants, which should greatly simplify strategies for dissemination of the trait.

Experimental procedures

Transformation of maize Hi-II

Embryogenic high type II (Hi-II) calli, derived from a cross between A188 and B73 genotypes and obtained from Dr W. Gordon-Kamm (Pioneer Hi-Bred International, Inc., Johnston, IA, USA), were maintained on N6 medium, pH 5.8, supplemented with 3% sucrose, 0.3% proline, 0.01% casamino acids and 2 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D), solidified with 2.4 g/L Gelrite.

Actively growing embryogenic calli were selected as target issue for gene delivery by particle bombardment using the Bio-Rad PDS1000-He system (Bio-Rad Inc., Hercules, CA, USA). The calli were placed on to target plates containing high osmoticum medium (N6 maintenance medium with 36.4 g/L mannitol, 36.4 g/L sorbitol and 10 mg/L silver nitrate) 4 h prior to bombardment. Plasmid DNA (2 μg) was precipitated on to gold particles as described in Shepherd et al. (2007). A mutated MSV rep plasmid rep\(_{2-199B}\) [which consists of the rep\(^{1-219Rb}\) gene inserted between the maize ubiquitin (Ubi) promoter and the Agrobacterium tumefaciens nopaline synthase (Nos) terminator in pAH17; Christensen and Quail, 1996] was co-bombarded with the bar-containing plasmid pAH25 (Christensen and Quail, 1996) at a pressure of 900 psi. The biolectic device settings were as follows: 8 mm between the rupture disc and macrocarrier, 10 mm between the macrocarrier and the stopping screen, and 7 cm between the stopping screen and the target. After the first delivery of DNA-coated gold, a recovery time of 4 h was allowed before the calli were bombarded a second time. Approximately 300 ng of each plasmid was delivered per shot. The calli were transferred from high osmoticum to maintenance N6 medium 24 h after bombardment. Non-bombarded calli and calli bombarded with pAH25 alone were used as controls in all experiments. Subsequent experiments have involved the bombardment of minimal transgene cassettes containing the Ubi promoter<rep\(^{1-219Rb}\><\text{Nos} terminator sequences and no plasmid backbone or ampicillin resistance gene, which are present in pAH25 and pAH17.

Selection and regeneration of transgenic calli

Calli were transferred to a gentle selection medium (N6 maintenance medium with 1 mg/L bialaphos) 4 days after bombardment, on which they were maintained for 2 weeks. All subsequent selection was more stringent at 3 mg/L bialaphos. Selection continued for 6-8 weeks in the dark, after which bialaphos-resistant calli were transferred to shoot initiation medium (Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) with 5 mg/L benzylaminopurine (BAP), 0.25 mg/L 2,4-D, 3% sucrose and 3 mg/L bialaphos). After 5 days in the dark, followed by 9 days under diffuse light, the calli were kept in the light for 16 h a day. Once shoots emerged, they were transferred to shoot elongation medium (shoot initiation medium without BAP or 2,4-D) and finally root elongation medium (MS supplemented with 1.0% sucrose and 3 mg/L bialaphos). Stringent selection was maintained during the whole regeneration process. Once roots had grown, plantlets were hardened off in a 1:1:1 mix of sand, compost and palm peat, and finally transferred to potting soil. Transgenic plants were maintained in a glasshouse in KwaZulu-Natal, South Africa. T\(_{1}\) generation offspring resulting from self-pollinations of transgenic lines were crossed with the elite white
maize genotype WM3 to give Hi-II/WM3 hybrids. T2 and T3 Hi-II plants and T4 Hi-II/WM3 hybrids were used in MSV resistance trials.

Determination of the presence/absence of transgene DNA and mRNA in challenged transgenic plants

As Hi-II transgenic seedlings were agroinoculated with MSV at 3 days old, it was impractical to determine transgene-containing and transgene transcript-expressing seedlings before inoculation with MSV. We therefore developed transgene- and transcript-specific PCR assays, which were carried out after symptom analysis. The same primers were used for molecular analysis of WM3 × 7E hybrid plants pre- and post-inoculation with MSV. The extraction of DNA from challenged plants for PCR purposes was performed using the Extract-n-Amp Plant PCR Kit (Sigma-Aldrich, St. Louis, MO, USA), and RNA was extracted using the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany).

Detection of transgene DNA

Referring to Figure S1 (Supplementary material), the vector sequence (grey blocks) contains the Ubi promoter, followed by an 83-bp untranslated first exon (dark grey block and uppercase lettering) and the intron of the maize Ubi-1 gene (broken line and lowercase lettering), a polylinker sequence (grey block; uppercase lettering), and the Nos terminator (3′ end). Including a polylinker sequence (grey block) and lettering), a polylinker sequence (grey block; uppercase lettering), and the Nos terminator 3′ (3′ end binds to the 5′ end of the primer sequence), whereas the 5′ end of the forward primer (UbiRep F; blue lettering) anneals to the 3′ end of the primer sequence containing transcription termination signals (grey block). PCR primers were designed that would specifically amplify the \( \text{rep}^{1-219\text{Rb}} \) transgene, but not MSV rep from replicating viral genomes in infected plants. The primer sequences were UbiRep F (5′-CAG-GTCACTCTAAGAGGATCCCTCAG-3′) and NosRep R (5′-GGGTCTACAGGAAATTCGATCATCAAGCTCG-3′). As can be seen in Figure S1 (Supplementary material), \( \text{rep}^{1-219\text{Rb}} \), which has a 3′ HindIII restriction site (AAGCCT), had been subcloned into pSK to create two BamHI sites, one already present 5′ of the replication initiation sequence and the other in the pSK multiple cloning site. Thus, there is a stretch of pSK sequence between the \( \text{rep}^{1-219\text{Rb}} \) and 3′ HindIII site and the pSK-derived BamHI site, which introduced a stop codon (TGA) directly after the \( \text{rep}^{1-219\text{Rb}} \) HindIII site. This stretch of pSK sequence is not present in pMSV or in MSV rep, allowing specific reverse primer design (NosRep R; blue arrow). The 5′ end of the NosRep R primer anneals to the pSK sequence, whereas the 3′ end of the primer anneals to the 3′ end of the \( \text{rep}^{1-219\text{Rb}} \). Similarly, the 5′ end of the forward primer (UbiRep F; blue arrow) anneals to the vector sequence (intronic and polylinker sequence) and the 3′ end binds to the 5′ end of \( \text{rep}^{1-219\text{Rb}} \).

To determine primer specificity, the following controls were used: control 1, plasmid \( \text{rep}^{1-219\text{Rb}} \) (negative transgene control); control 2, pAHC17 (negative vector control); control 3, pAHCC25 (co-bombarded plasmid containing the bar gene); control 4, MSV-infected non-transgenic maize RNA (negative MSV rep transcript control). Once cDNA was amplified from transgenic plants, the PCR product was inserted into pGEM-T Easy (Promega, Madison, WI, USA) and sequenced. Thereafter, this cDNA was used as a positive control for RT-PCR transgene expression assays.

RT-PCRs were carried out using the Access RT-PCR Kit (Promega).

Detection of transgene copy number and integration pattern

Southern analysis was carried out following standard protocols (Sambrook et al., 1989). Five micrograms of genomic DNA were digested with BglII, which cuts once in the expression cassette upstream of the transgene. The hybridization probe specific to the \( \text{rep} \) transgene was created using a PCR DIG Probe Synthesis Kit (Roche Diagnostics GmbH, Mannheim, Germany), according to the manufacturer’s instructions, using the rep-specific primers TrepF (5′-ATGGCAATCCTCCATCCAAAC-3′) and TrepR (5′-AAGCTTCG-GGACTAACCT-3′).

Virus challenges and symptom analysis

Challenges of Hi-II maize

Transgenic and non-transgenic 3-day-old seedlings were agroinoculated with MSV-Kom in the laboratory, following the method described by Martin et al. (1999). Chlorotic leaf areas (%) were measured for leaves two, three, four, five and six of each symptomatc plant using a microcomputer-based image analysis technique (Martin and Rybicki, 1998). Leaves two and three were assessed 15 dpi and leaves four, five and six were assessed 22, 29 and 36 dpi, respectively. The mean percentages of chlorotic areas on leaves two, three, four, five and six were used as a representative measure of chlorosis for each group of plants (transgenic and non-transgenic).

Challenges of WM3 × 7E maize hybrids

Transgenic Hi-II (MTA7E-2) T2 plants were crossed with an elite white maize genotype, WM3, at the South African seed company PANNAR (Pty) Ltd. (Greytown, Kwa-Zulu Natal, South Africa). WM3 is one of PANNAR’s main breeding lines and is extremely sensitive to MSV. Hybrid offspring (called WM3 × 7E) were challenged with MSV at 14 days old (three to five-leaf stage) in the glasshouse using viruliferous leafhoppers (Cicadulina mbila Naudé), as described for the challenge of Digitaria sanguinalis by Shepherd et al. (2007). The leafhoppers carried an extremely severe field isolate containing a mixed
viral population. Three leafhoppers were placed in each vial, which was clipped to the youngest leaf of each plant, and the leafhoppers were left to feed for 24 h. MSV only infects leaves that emerge from the whorl after viral inoculation; therefore, all leaves that emerged subsequent to the inoculated leaf were monitored daily for symptom development. Chlorotic areas on symptomatic leaves were quantified at 28 dpi using a symptom key (Shepherd et al., 2007).

Acknowledgements

This work was supported by grants from the South African National Research Foundation, PANNAR (Pty) Ltd. (Greytown, Kwa-Zulu Natal, South Africa) and fellowships from the Claude Leon Foundation (DNS and TM), Sydney Brenner Fellowship and Harry Oppenheimer Trust (DPM). The authors thank Eric van der Walt (University of Cape Town, Cape Town, South Africa) for helpful comments on the manuscript. The authors also thank Dr William Gordon-Kamm (Pioneer Hi-Bred International, Inc., Johnston, IA, USA) for the generous gift of Hi-II calli.

References


**Supplementary material**

The following supplementary material is available for this article:

**Figure S1** Schematic diagram of the rep$^{1-219Rb}$ transgene cloned in the monocotyledon expression vector pAHC17 (rep$^{1-219Rb}$), including relevant sequences from the ubiquitin (Ubi) promoter through to the nopaline synthase (Nos) terminator.

**Figure S2** Symptom severities in T$_3$ transgenic (MTA8D.3) and non-transgenic Hi-II maize agroinoculated at 3 days old. The percentages of leaf chlorosis are shown for leaves two to six of each individual plant analysed, to illustrate the range of symptom severities in each group. The horizontal bars show the mean percentage chlorosis for each group (see also Figure 1c). The percentages of chlorosis were measured at 15 days post-inoculation (dpi) in leaves two and three, at 25 dpi in leaf four, at 29 dpi in leaf five and at 36 dpi in leaf six. It can be seen that the emergence of symptoms only occurred in most plants in the transgenic group by 25 dpi, compared with 15 dpi in the non-transgenic group.

**Figure S3** Comparison of symptom severities in WM3 × 7E transgenic hybrids vs. WM3 × 7E non-transgenic hybrids (a), and in WM3 × 7E transgenic hybrids vs. WM3 non-transgenic plants (b). In (a), the percentages of leaf chlorosis are shown for leaves five to nine of each individual plant analysed, to illustrate the range of symptom severities in each group. All WM3 × 7E non-transgenics were leafhopper inoculated with maize streak virus (MSV) on leaf four or five, and thus there are no leaf four comparisons (maize streak disease symptoms only appear on leaves that emerge after virus inoculation). In (b), the percentages of leaf chlorosis are shown for leaves four to nine of each individual plant analysed in the WM3 × 7E transgenic and WM3 non-transgenic groups. The horizontal bars show the mean percentages of chlorosis for each group (see also Figure 2b), and the broken lines indicate a 10% chlorosis cut-off, on or below which plants were considered to have mild symptoms.

**Figure S4** Examples of maize streak virus (MSV) resistance phenotypes. (a) A non-symptomatic plant compared with severely infected plants. (b) Delayed symptom development, with chlorotic lesions only appearing on young leaves 36 days post-inoculation (indicated by arrow). (c) Reduced symptom severity manifested as speckling (enlarged inset), compared with (d) continuous streaks in susceptible plants. Overall, 90% (35/39) of WM3 × 7E transgenic plants exhibited some resistance characteristics, compared with 31.6% (6/19) of...
non-transgenic WM3 × 7E plants and 25% (6/24) of non-transgenic WM3 plants.

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