

Virus vector for gene silencing in wheat

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Wheat, rice, and maize account for more than 85% of the world's cereal production (apps.fao.org/default.jsp), and the increasing global demand for wheat heightens the need for new varieties with improved disease resistance, tolerance to abiotic stresses, and grain quality. Incorporation of such desirable traits via biotechnological approaches first requires thorough characterization of genes and pathways, and high-throughput screening is an essential part of this process (1). Virus-induced gene silencing (VIGS) uses a viral-expressed transgene to trigger RNA silencing against an endogenous gene and can serve as a powerful tool for functional genomics by facilitating high-throughput screening processes (2). However, most VIGS vectors are only available for dicot plants. For example, potato virus X (PVX) and tobacco rattle virus (TRV) have been commonly used for solanaceous species (3,4) and pea early browning virus (PEBV) for legume species (5).

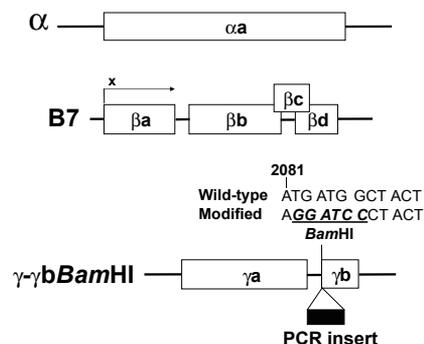


Figure 1. Genome of the barley stripe mosaic virus (BSMV) vector. The BSMV genome was modified to increase the efficiency of virus-induced gene silencing (VIGS). Expression of the coat protein (β a) from RNA β was prevented by introducing a point mutation into the β a initiation codon. To facilitate cloning of PCR products, a *Bam*HI site was introduced by modifying the γ b start codon (GenBank[®] accession no. U13917).

Currently, the only published example of the successful use of a VIGS vector in monocots is the use of barley stripe mosaic virus (BSMV) in barley (6,7).

The objective of the present study was to determine whether BSMV could serve as a useful VIGS vector in wheat (*Triticum aestivum*). Although predominantly a barley pathogen, BSMV occurs naturally in wheat as well. The genome of BSMV strain ND18 consists of three

RNAs, designated α , β , and γ , and has been described previously (8). We used modified β and γ components of the ND18 strain to facilitate insertion of a PCR product and to increase efficiency of VIGS (Figure 1). The BSMV RNA β mutant “B7” was derived from wild-type RNA β , in which the expression of coat protein was disrupted by a mutation in the start codon (9), thereby enhancing gene silencing (7). The start codon of the γ b open-reading frame was modified to create a *Bam*HI site for insertion of cloned DNA fragments (γ - γ b*Bam*HI in Figure 1, designated as “B2” in Reference 10), while also blocking expression of the VIGS suppressor γ b (8). This modification of γ b is the major difference between this vector and the version used previously in barley (6,7).

To verify its application, we tested VIGS with three marker genes

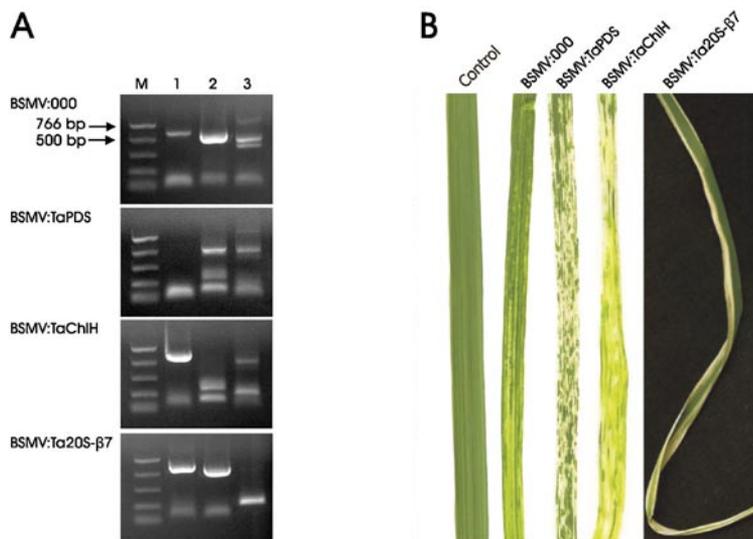


Figure 2. Barley stripe mosaic virus (BSMV)-based virus-induced gene silencing (VIGS) in wheat. (A) Verification of VIGS by reverse transcription PCR (RT-PCR). Wheat plants were inoculated with the empty vector (BSMV:000) or the constructs indicated at the left of each panel. Total RNA was isolated from leaves systemically infected with these constructs, and TaPDS-, TaChIH-, or Ta20S- β 7-specific RNA levels were analyzed by RT-PCR. Amplification products specific for phytoene desaturase (PDS), subunit H of the magnesium-protoporphyrin chelatase complex (ChIH), or the β 7 subunit of the 20S proteasome complex (20S- β 7) are in lanes 1, 2, and 3, respectively. PCR primers used were the same as those used for cloning [TaPDS, 889–1500; TaChIH, 534–1054; and Ta20S- β 7, 230–732 (A of the first ATG codon being +1)]. No RNA specific for the silencing target was detected in silenced plants, while RNAs specific for the nontargeted genes were detected. Major bands represent predicted PCR products (TaPDS, 624 bp; TaChIH, 533 bp; Ta20S- β 7, 515 bp). Bands appearing near the bottom of the gels are primer oligonucleotides. Other extraneous, nonspecific bands were not unexpected as a consequence of the very large, hexaploid wheat genome. (B) Visible phenotypes resulting from silencing three marker genes by BSMV-mediated VIGS (about 21 days post-inoculation). The wheat cultivar “Chinese Spring” was used. Plants were kept in a growth chamber at 25°C with a 9/15-h light/dark cycle. The leaf on the left is the healthy control, followed by leaves infected with empty vector (BSMV:000), BSMV:TaPDS, BSMV:TaChIH, and BSMV:Ta20S- β 7. Silencing of PDS, ChIH, and 20S- β 7 resulted in photobleaching, chlorosis, and necrosis, respectively. Four plants were used per construct, and the experiment was repeated twice.

Table 1. Primers Used to Amplify Wheat Gene Fragments

| Name | Sequence | PCR Product (bp) | TIGR Accession No. |
|-------------------------|---|------------------|--------------------|
| TaPDS_889FBH | 5'- GGATCC TTTCTCCAGGAGAAGCATGGCT-3' | 624 | TC150935 |
| TaPDS_1500RBH | 5'- GGATCC GGACCTCGGTGTCTTCACAA-3' | | |
| TaChlH_534FBH | 5'- GGATCC CGTCACCACCACCAAGATCA-3' | 533 | TC169257 |
| TaChlH_1054RBH | 5'- GGATCC GACACACCTGGGATATCTTAA-3' | | |
| Ta20S- β 7_230FBH | 5'- GGATCC AGGAGATTTGCGGTATCTGG-3' | 515 | TC169934 |
| Ta20S- β 7_732RBH | 5'- GGATCC TTACCATGTTCTACAGCAC-3' | | |

The bold sequences represent the *Bam*HI site used for cloning into the γ - γ b*Bam*H1 vector. Primers were designed based upon the sequences reported in the accession numbers listed.

as described by Ryu et al. (11): (i) phytoene desaturase (PDS); (ii) subunit H of the magnesium-protoporphyrin chelatase complex (ChlH); and (iii) the β 7 subunit of the 20S proteasome complex (20S- β 7). We cloned wheat homologs of these genes (referred to here as TaPDS, TaChlH, and Ta20S- β 7, respectively) based on sequences reported in The Institute for Genomic Research (TIGR; www.tigr.org) wheat gene index. DNA fragments of TaPDS (TC150935), TaChlH (TC169257), and Ta20S- β 7 (TC169934) were amplified using the primers listed in Table 1, *Bam*HI digested, and then inserted nondirectionally into the *Bam*HI site of BSMV γ - γ b*Bam*H I (Figure 1). These cloned fragments were 624, 533, and 515 bp long and contained sequences corresponding to the nucleotides 889–1500 of PDS, 534–1054 of ChlH, and 230–732 of 20S- β 7 (numbered with A of the first ATG codon being +1) as well as *Bam*HI-related sequence at both ends. TaPDS was incorporated in the (+) sense, while the other two were (-) sense constructs. Infectious BSMV RNAs were prepared by in vitro transcription using T7 RNA polymerase (New England Biolabs, Beverly, MA, USA) and inoculated as previously described (12). Although the modified BSMV itself caused a mosaic on leaves, the VIGS phenotypes were easily discernible and became prominent by 21 days post-inoculation (Figure 2). The decrease in PDS expression levels caused a deficiency in carotenoid biosynthesis, so that the

plants became sensitive to light and the leaves became photobleached (Figure 2, BSMV:TaPDS) (13). Silencing of ChlH blocked the biosynthesis of chlorophyll, resulting in chlorosis (Figure 2, BSMV:TaChlH) (14). Disruption of the 20S proteasome complex due to silencing of the β 7 subunit was lethal (Figure 2, BSMV:Ta20S- β 7) (11,15). Silencing phenotypes were relatively stable and lasted well beyond 21 days, but the stability over time was not specifically tested.

In addition to the visible symptoms of silencing in wheat plants, decreased RNA levels were evident following reverse transcription PCR (RT-PCR). Total RNA from systemically infected leaves was isolated at 21 days post-inoculation using the TRIzol[®] reagent (Invitrogen, Carlsbad, CA, USA). First-strand cDNA was synthesized from 1 μ g of total RNA using BD PowerScript[™] Reverse Transcriptase (SMART[™] II RACE kit; BD Biosciences Clontech, Palo Alto, CA, USA). Using the cDNA from the Ta20S- β 7-, TaChlH-, or TaPDS-silenced plants as templates, no PCR products were detected even after 45 amplification cycles (Figure 2). These results were confirmed with other primer sets and by evaluation with varying numbers of amplification cycles (data not shown). However, the cDNA generated from silenced plants was successfully used as a template for the PCR amplification of the other two genes tested, indicating that silencing one gene did not grossly interfere with the transcription levels of

other genes tested and supporting the conclusion that lack of amplification of silencing targets was likely due to silencing rather than experimental error or artifact.

To our knowledge, this is the first published report of the successful application of VIGS in wheat. BSMV-mediated VIGS has excellent potential for genome-wide application in wheat and provides a powerful tool for wheat functional genomics. We are currently using this tool for library screening to investigate wheat disease resistance against fungal pathogens.

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COMPETING INTERESTS STATEMENT

The authors declare no competing interests.

NOTE ADDED IN PROOF

Another report of the use of BSMV for silencing in wheat appeared (or will appear) in the August issue of *Plant Physiology*, although these authors used the original, unmodified vector reported by Holzberg et al. (7).

REFERENCES

1. Francki, M. and R. Appels. 2002. Wheat functional genomics and engineering crop improvement. *Genome Biol.* 3:1013.1-1013.5.
2. Baulcombe, D.C. 1999. Fast forward genetics based on virus-induced gene silencing. *Curr. Opin. Plant Biol.* 2:109-113.

3. **Burch-Smith, T.M., J.C. Anderson, G.B. Martin, and S.P. Dinesh-Kumar.** 2004. Applications and advantages of virus-induced gene silencing for gene function studies in plants. *Plant J.* 39:734-746.
4. **Robertson, D.** 2004. VIGS vectors for gene silencing: many targets, many tools. *Annu. Rev. Plant Biol.* 55:495-519.
5. **Constantin, G.D., B.N. Krath, S.A. MacFarlane, M. Nicolaisen, I.E. Johansen, and O.S. Lund.** 2004. Virus-induced gene silencing as a tool for functional genomics in a legume species. *Plant J.* 40:622-631.
6. **Fitzmaurice, W.P., S. Holzberg, J.A. Lindbo, H.S. Padgett, K.E. Palmer, G.M. Wolfe, and G.P. Pogue.** 2002. Epigenetic modification of plants with synthetic RNA viruses. *OMICS* 6:137-151.
7. **Holzberg, S., P. Brosio, C. Gross, and G.P. Pogue.** 2002. Barley stripe mosaic virus-induced gene silencing in a monocot plant. *Plant J.* 30:315-327.
8. **Bragg, J.N. and A.O. Jackson.** 2004. The C-terminal region of the Barley stripe mosaic virus γ b protein participates in homologous interactions and is required for suppression of RNA silencing. *Mol. Plant Pathol.* 5:465-481.
9. **Petty, I.T.D. and A.O. Jackson.** 1990. Mutational analysis of barley stripe mosaic virus RNA β . *Virology* 179:712-718.
10. **Petty, I.T.D., R. French, R.W. Jones, and A.O. Jackson.** 1990. Identification of barley stripe mosaic virus genes involved in viral RNA replication and movement. *EMBO J.* 9:3453-3457.
11. **Ryu, C.-M., A. Anand, L. Lang, and K.S. Mysore.** 2004. Agrodrench: a novel and effective agroinoculation method for virus-induced gene silencing in roots and diverse Solanaceous species. *Plant J.* 40:322-331.
12. **Petty, I.T.D., B.G. Hunter, N. Wei, and A.O. Jackson.** 1989. Infectious barley stripe mosaic virus RNA transcribed in vitro from full-length genomic cDNA clones. *Virology* 171:342-349.
13. **Demmig-Adams, B. and W.W. Adams.** 1992. Photoprotection and other responses of plants to high light stress. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 43:599-626.
14. **Hiriart, J.B., K. Lehto, E. Tyystjarvi, T. Junttila, and E.M. Aro.** 2002. Suppression of a key gene involved in chlorophyll biosynthesis by means of virus-inducing gene silencing. *Plant Mol. Biol.* 50:213-224.
15. **Dahan, J., P. Etienne, A.-S. Petitot, V. Houot, J.-P. Blein, and L. Suty.** 2001. Cryptogein affects expression of α 3, α 6 and β 1 20S proteasome subunits encoding genes in tobacco. *J. Exp. Bot.* 52:1947-1948.

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