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Insights from controlled, comparative experiments highlight the limitations of using BSMV and FoMV for virus-enabled reverse genetics in rice

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Authors

Guilherme M. Turra

ORCiD
https://orcid.org/0000-0002-4806-7613

Affiliations
- Crop Science Department, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil

Aldo Merotto Junior

Affiliations
- Crop Science Department, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil

Dana R. MacGregor
Corresponding Author
Submitting Author

ORCiD
https://orcid.org/0000-0003-0543-0408

Affiliations
- Translating Biotic Interactions, Rothamsted Research, Harpenden, Hertfordshire, UK

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Running head: BSMV and FoMV in rice
Title: Insights from controlled, comparative experiments highlight the limitations of using BSMV and FoMV for virus-enabled reverse genetics in rice

Guilherme M. Turra[1], Aldo Merotto Junior[1], Dana R. MacGregor[2]*

[1]Crop Science Department, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil
[2]Translating Biotic Interactions, Rothamsted Research, Harpenden, Hertfordshire, UK
Corresponding author: dana.macgregor@rothamsted.ac.uk

Summary

Virus-enabled reverse genetics (VERG) is a powerful tool for transient gene expression modulation in plants, particularly where stable transformation is challenging. However, the efficacy of VERG varies across species. In this study, we tested two commonly used viral vectors, barley stripe mosaic virus (BSMV) and foxtail mosaic virus (FoMV), for their ability to induce virus-induced gene silencing (VIGS) and virus-mediated overexpression (VOX) in rice (*Oryza sativa*). While both vectors successfully altered gene expression in wheat (*Triticum aestivum*), they failed to do so in six rice cultivars from different subspecies, despite rigorous optimization of inoculation methods and environmental conditions. The BSMV vector carrying a portion of *PHYTOENE DESATURASE* (*PDS*) in antisense did not induce the expected photobleaching phenotype, and FoMV-driven GFP expression was absent in rice. These findings contrast with previous reports of successful VERG in other monocots and suggest that intrinsic resistance mechanisms exist in rice which may inhibit or reduce viral vector efficacy. Our results highlight the species-specific limitations of VERG and underscore the need for alternative viral systems or novel vector designs for functional genomics research in rice. By sharing our unsuccessful attempts, we aim to prevent redundant efforts and encourage further exploration of VERG in *Oryza* species.

Keywords**:** VIGS, VOX, VERG, *Oryza sativa*, gene silencing, overexpression

1 Introduction

Virus-enabled reverse genetics (VERG) tools are useful for rapidly testing genotype-to-phenotype hypotheses as they transiently change gene expression *in planta*. VERG either induces or reduces gene expression, known as virus-mediated overexpression (VOX) or virus-induced gene silencing (VIGS), respectively. These techniques are particularly useful in plants where stable transformation protocols are either undeveloped or unsuitable such as for allogamous plants with long generation times and under-developed genomic resources (MacGregor, 2020). Accordingly, VERG have successfully been used in i.e. *Alopecurus myosuroides* Huds. (Mellado-Sánchez et al., 2020; Patterson et al., 2025), *Setaria italica* (L.) Beauv., *Solanum* spp., *Thalictrum thalictroides* (L.) Eames & Boivin, and *Zingiber officinale* Roscoe (reviewed in Dommes et al., 2019). VERG are also well-established in a wide range of agronomically-important species and model plants (Dommes et al., 2019). However, not all VERG protocols are successful or repeatable, even within the

same laboratory; for instance, there are conflicting reports on the efficacy of VIGS in *Pennisetum glaucum* (L.) R. Br. (Van Nugteren et al., 2007; Schoeman, 2011). Its effectiveness can vary depending on host compatibility and experimental conditions (Dommes et al., 2019)

Among the most commonly used viral vectors for VERG studies, barley stripe mosaic virus (BSMV) and foxtail mosaic virus (FoMV) stand out due to their efficacy, flexibility and broad host ranges (Dommes et al., 2019; Rössner et al., 2022). BSMV, a tripartite, positive-sense RNA virus from the genus *Hordeivirus*, has been extensively utilized for VIGS applications in monocots, particularly in cereal crops (Lee et al., 2015a). On the other hand, FoMV, a *Potexvirus*, has demonstrated robust systemic infection in multiple monocots and dicots, making it a promising alternative to BSMV. FoMV's relatively simple genome organization and high replication efficiency alongside a capacity to hold and retain longer inserts than BSMV contribute to its effectiveness as a VERG tool (Beernink & Whitham, 2023) and a well-characterized vector for overexpression of the GREEN FLUORECEST PROTEIN (GFP) is available (Bouton et al., 2018).

Despite the evidenced usefulness of VERG for functional genomics research, there is a distinct lack of data reporting VERG based on BSMV or FoMV in *Oryza* species, even though rice is both experimentally and agronomically important. There is evidence for rice coding sequences having been used for silencing targets in wheat driven by a BSMV-based system (Holzberg et al., 2002), for the heterologous expression of rice genes (e.g. *Hd3a*) in *Panicum miliaceum* in a FoMV-based system (Yuan et al., 2020), and more recently the use of a modified FoMV vector for silencing in japonica rice (Huang et al., 2020). Additionally, a few studies (Ding et al., 2006a; Kant et al., 2015; Kant & Dasgupta, 2017; Ding et al., 2006b; Purkayastha et al., 2010) have reported the use of other viral vectors in rice. Brome mosaic virus (Ding et al., 2006b) and Rice tungro bacilliform virus (Purkayastha et al., 2010) were both validated for VIGS in Asian rice (*Oryza sativa* L.) but the literature building on or effectively using their vectors is scarce. Although *O. sativa* ssp. *japonica* can be reliably transformed, efficient and effective stable transformation of *O. sativa* ssp. *indica* remains challenging (Mohammed et al., 2019) and we found no data about stable or transient transformation of weedy rice, *Oryza sativa* f. *spontanea*. Therefore, the aim of this study was to test if well-established visual reporters of VERG could induce transient gain or loss of function in *O. sativa* subspecies to gain insights in the gene regulation of this complex species.

2 Materials and Methods

2.1 Plant material and growth conditions

Seeds from *Nicotiana benthamiana*, wheat (*Triticum aestivum* L. cv. Riband), rice (*O. sativa* ssp. *indica* cv. IR 64, *O. sativa* ssp. *indica* cv. Kasalath, *O. sativa* ssp. *indica* cv. CO-39, *O. sativa* ssp. *japonica* cv. Kitaake*, O. sativa* ssp. *japonica* cv. Koshihikari, *O. sativa* ssp. *japonica* cv. Balilla), and green foxtail (*Setaria viridis*) were used. All seeds except for the *S. viridis*, which were acquired from Kew's Millenium Seed Bank (Serial Number 31491), were available from Rothamsted Research Seed Stocks. *N. benthamiana* plants were grown according (Lee et al., 2015b). All plants were grown in a high-end DEFRA/HSE-registered controlled environment room set for 26.7/21.1 ºC in a 16/8 h of light/dark regime at 200 µmol m^{-2} s^{-1}. Seeds from wheat, green foxtail, and rice genotypes were surface sterilized with bleach solution (50%) for 5 minutes and rinsed 3 times with distilled water. Seeds were surfaced sowed in 20x15 cm pots containing wet Rothamsted Standard Compost Mix (75% Medium grade (L&P) peat, 12% Screened sterilised loam, 3% Medium grade vermiculite, 10% Grit (5 mm screened, lime free), 3.5 kg per m3 Osmocote® Exact Standard 3-4 M, 0.5 kg per m3 PG mix, 3kg lime pH 5.5-6.0 and 200 ml per m3 Vitax Ultrawet) and covered with perlite. The pots were covered with lids to maintain high humidity. After five to seven days, seedlings (~2 cm) were chosen to be transplanted to square 4 cm pots filled with Rothamsted Standard Compost Mix. Propagator lids covered transplanted seedlings for 3 days following transplant. Plants were kept in the same growth room and watered every two days.

2.2 Preparation and cloning of viral vectors

The creation of BSMV:asOsPDS1 and of BSMV:asOsPDS2 followed published procedures (Lee et al., 2015b) with minor changes detailed below. The *O. sativa PHYTOENE DESATURASE* (*OsPDS*, LOC_Os03g08570) was aligned with *Triticum aestivum PDS* fragments previously used (Lee et al., 2012; Mellado-Sánchez et al., 2020). Two fragments of the *OsPDS* coding sequence were synthetized in the antisense (as) direction. These covered 185 nucleotides targeting 430 to 614bp as *asOsPDS1* or 200 nucleotides targeting 900 to 1100bp as *asOsPDS2* (Figure 1). Synthetized dsDNA fragments were cloned into BSMV:RNAγ vector pCa-γbLIC (Yuan et al., 2011) and transformed into *Escherichia coli* JM109. Cloning was confirmed by colony PCR using primers in the viral backbone (2235.F - GATCAACTGCCAATCGTGAGTA and 2615.R - CCAATTCAGGCATCGTTTTC). Purified plasmid DNA from verified colonies were sequenced using the same primers to confirm cloning orientation and sequence accuracy. A selected positive transformant was transferred to *Agrobacterium tumefaciens* GV3101

following published procedures (Lee et al., 2015b). Recombinants were selected based on survival of dual selection with kanamycin and gentamycin. Individual colonies were selected, multiplied, and verified by colony PCR with the same primers described above. A selected confirmed transformant colony for each BSMV:asOsPDS1 and BSMV:asOsPDS2 was stocked in glycerol (15%) and stored at -80 ºC. BSMV:asTaPDS, BSMV:MCS, BSMV:RNAα (pCaBS-α) and BSMV:RNAβ (pCaBS-β) VIGS constructs (Lee et al., 2012; Yuan et al., 2011) and FoMV:MCS, FoMV:GFP (PV101-GFP), and P19 (pBIN61-P19) VOX constructs (Bouton et al., 2018) were used without further modifications from transformed *Agrobacterium tumefaciens* strain GV3101 stocked in 15% glycerol at -80ºC (Mellado-Sánchez et al., 2020).

2.3 Preparation of the virus inoculum from *Nicotiana benthamiana* for rub inoculation

Detailed protocols using BSMV or FoMV were followed (Mellado-Sanchez et al., 2024). Briefly, glycerol stocks (15% glycerol with 85% bacterial culture at logarithmic growth stage) of *A. tumefaciens* containing the recombinant vectors were grown in LB broth supplemented with kanamycin (50 µg/ml) and gentamycin (25 µg/ml). *A. tumefaciens* cultures were pelleted and resuspended in infiltration buffer [10 mM MgCl2, 10 mM 2-(N-morpholino)ethanesulfonic acid (MES) pH 5.6, and 150 µM acetosyringone] to a final OD600 of 1.5-1.55 for BSMV constructs, 0.6 for FoMV constructs, and 0.3 for P19. BSMV:RNAγ (MCS, asTaPDS, asOsPDS1, and asOsPDS2), BSMV:RNAα, and BSMV:RNAβ were mixed in equal proportions, hereafter BSMV inoculum. FoMV:GFP and FoMV:MCS were equally mixed with P19, hereafter FoMV inoculum. BSMV and FoMV inoculums were propagated via agroinfiltration in *N. benthamiana*. Infiltrated leaves were harvested 3-5 days for BSMV and 5-7 days for FoMV after infiltration. Three leaves from different *N. benthamiana* plants were weighed into foil packets and instantly frozen in liquid nitrogen before being stored at -80°C.

2.4 Preparation of the BSMV inoculum from *Agrobacterium tumefaciens* for injection inoculation and dipping

A. tumefaciens glycerol stocks were grown and pelleted as described above. For dipping inoculation, pelleted cultures containing BSMV constructs were resuspended in a modified dipping buffer (10 mM MgCl2, 10 mM MES pH 5.6, 200 µM acetosyringone, 1% sucrose, and 0.01% Silwet L-77) to a final OD600 of 0.6 (Andrieu et al., 2012). For injection inoculation, *A. tumefaciens* cultures were resuspended in a published injection buffer (Kant

& Dasgupta, 2017) (10 mM MgCl2, 10 mM MES pH 5.6, 500 µM acetosyringone) and adjusted to a final OD600 of 0.8.

2.5 Inoculation of plants from BSMV or FoMV infiltrated *Nicotiana benthamiana*

Rub-inoculation using the abrasive carborundum [Technical, SLR, Extra Fine Powder, ~ 36 µm (300 Grit), Fisher Chemical cat 10345170] was performed as published (Lee et al., 2015b) with minor adjustments. Frozen *N. benthamiana* leaves that had been infiltrated as described above were ground in a 2:1 (w/v) ratio in 10 mM potassium phosphate buffer pH 7. The sap was used for inoculation by rubbing a leaf 6 to 10 times. Inoculation by microneedling was performed using a DermaRoller device to wound the leaf once prior the sap application. The third leaf of 14-day old wheat plants and 21-day old green foxtail and rice plants was inoculated.

2.6 Inoculation of rice from *Agrobacterium tumefaciens*

For dipping inoculation, the third leaf of 21 days old rice was wounded by microneedling and dipped in the BSMV:MCS and BSMV:asOsPDS2 *A. tumefaciens* resuspensions for 30 minutes. For injection inoculation, approximately 0.2 mL of the resuspended BSMV:MCS and BSMV:asOsPDS2 inoculums were injected into the meristematic region located at the crown of 14 days old rice plants using a 1 mL syringe and a 26G needle. After inoculation, rice plants were treated in the same way as those inoculated by rubbing. Only rice cultivars IR64 (*indica*) and Kasalath (*aus*) were used for this experiment.

2.7 Visual assessment of VIGS and VOX

Whole plants or leaf segments were photographed using a 48 megapixels camera from iPhone 15 Pro (Apple, Cupertino, CA, USA) and Velour Vinyl black backdrop (Superior Seamless 234312). For GFP fluorescence, a yellow long-pass (510 nm) filter (Midwest Optical Systems, Palatine, IL, USA) was used in front of the camera and plants were illuminated with blue light from a Dual Fluorescent Protein flashlight (Nightsea, Lexington, MA, USA).

3 Results

In biological and technical replicates, individual plants were inoculated and evaluated and for each experiment, Riband plants were inoculated in parallel as positive control for the inoculum and the rubbing process. A total of 286 rice plants from subgroups *indica*, *aus*,

and *japonica* were inoculated alongside 105 wheat plants (Table 1). As expected, the efficacy in wheat was high, with an overall efficiency of 77% for FoMV:GFP, whereas the different BSMV:asPDS constructs induced photobleaching in over 91% of the treated plants (Table 1). Unexpected, none of the rice plants showed the expected gain or loss of function phenotypes (Table 1). These results are explained further below.

3.1 Virus-induced gene silencing (VIGS) in wheat and rice

BSMV vectors driving an antisense portion of *PHYTOENE DESATURASE* are a well-accepted standard for VIGS and have been validated in several different monocot and dicot species under a range of different growth, inoculation, and propagation conditions (Mellado-Sánchez et al., 2020; Patterson et al., 2025; Lee et al., 2015b, 2012; Yuan et al., 2011). Like these previous results, Figure 2A shows that inoculating Riband with either of the BSMV:asOsPDS1 or BSMV:asOsPDS2 constructs created herein induced photobleaching to a similar degree as inoculation with BSMV:asTaPDS from Lee et al. (2015b). Further details about the asOsPDS1 and asOsPDS2 sequences and their cloning can be found in the Methods. Also as expected, Riband plants inoculated with empty vector control (BSMV:MCS) remained green (Figure 2A). Systemic bleaching was seen in BSMV:asOsPDS treated Riband plants from 12 days after inoculation and were observed up to 35 days after inoculation when the experiment was terminated (Figure 2C). However, parallel inoculation of BSMV:MCS, BSMV:asOsPDS1, BSMV:asOsPDS2 or BSMV:asTaPDS into indica rice (IR64) led to a different phenotype (Figure 2B). In IR64, inoculation with any variant of the BSMV vectors led to development of white spots on the top of the leaf (Figures 2B and 2D). Importantly, not only was this non-bleaching phenotype similar regardless of which vector was introduced (Figure 2B), similar white spots were also observed in uninoculated rice plants grown in the same room (Figure 2E).

Many different standard “transient expression” protocols that used rice as the target species are available in the literature (Andrieu et al., 2012; Ding et al., 2006a, 2006b; Huang et al., 2020; Kant et al., 2015; Kant and Dasgupta, 2017; Purkayastha et al., 2010). To determine whether the lack of VIGS phenotype resulted from insufficient introduction of virus by the rub inoculation protocol, we tried a variety of inoculation methods (e.g. using microneedles, dipping, or injection) and resuspension buffers (see Material and Methods for details). None of the alternative inoculation methods induced photobleaching in rice cultivars from the *indica* or *aus* subgroups (Figure 2F). In addition, injection-based inoculation led to mortality in approximately 20% of the treated plants.

3.1 Virus-mediated overexpression (VOX) in wheat, *Setaria viridis,* and rice

FoMV driving expression of GFP (PV101-GFP) (Bouton et al., 2018) is a well-established visual reporter which has been proven to be effective in several species, including several different types of monocots (Yuan et al., 2020; Bouton et al., 2018; Torti et al., 2021). As expected, the FoMV:GFP inoculum induced characteristic GFP fluorescence in wheat (Figure 3A). In Riband, fluorescence was visible 7 to 10 days post rub-inoculation and spread systemically (Figure 3B). Rub inoculation with FoMV:GFP also successfully led to GFP fluorescence in *Setaria viridis,* while those treated with FoMV:MCS showed no observable fluorescence or viral infection phenotypes (Figure 3C). Rice plants inoculated in parallel with either FoMV:MCS or FoMV:GFP showed no observable symptoms of viral infection or GFP fluorescence (Figure 3D). This lack of observable phenotype was similar across all the different rice genotypes treated. Plants from all the species were monitored regularly for five weeks after inoculation.

4 Discussion

Data from our well-replicated and highly-controlled experiments do not support the hypothesis that our protocols (fully detailed at Mellado-Sanchez et al., 2024) for BSMV-driven VIGS or FoMV-driven VOX are effective in the different cultivars and subspecies of *O. sativa* we tested. Although the efficacy of VERG systems is affected by myriad factors such as plant growth temperature, insert orientation and length (Bouton et al., 2018; Lee et al., 2015b), and there are different variations on the BSMV and FoMV vectors used by the community (described in e.g. Kant and Dasgupta, 2019) ultimately the success of VERG is dependent on the appropriate and balanced interaction between the viral vector and the host.

In accordance with our data, previous work showed FoMV was symptomless after mechanical inoculation in rice (Paulsen, 1977). Although we did see an unexpected, non-green phenotype in the BSMV experiments in rice (Figures 2B, 2D-F), the lack of a clear correlation between this phenotype and the presence of *PDS* sequence, or even BSMV inoculum (Figures 2B, 2E, and 2F), suggests that this phenotype is not caused by BSMV infection or target-induced VIGS. One possible reason that we are not seeing VERG-induced phenotypes in rice could be that neither BSMV nor FoMV are able to mount a successful infection due to rice's multiple defence pathways against viruses (Qin et al., 2019); further work would be required to prove this hypothesis but if true, scientists wanting to do VERG would need to use alternative viruses as the VERG chassis. Alternative viral systems are available (reviewed in Kant and Dasgupta, 2019) and novel vectors specific to

rice could be created. As we recognise the potential usefulness of VERG tools for research in *Oryza* species, we hope that by clearly outlining our failed experimental protocols and combinations, we can help others take to alternative routes to deploying VERG protocols for *O. sativa* functional genomics research.

As stated above, successful VERG depends on a balance between the virus and the host that supports successful infection and either gene silencing or heterologous expression of the gene of interest. The first step in this process is infection of the host by the virus. Although rub inoculation is sufficient in wheat and other monocots (Bouton et al., 2018; Ellison et al., 2021; Lee et al., 2015a; Mei et al., 2019; Mellado-Sánchez et al., 2020), we recognise that there are multiple inoculation techniques and buffer formulations commonly used for transient expression in rice (Andrieu et al., 2012; Ding et al., 2006a, 2006b; Huang et al., 2020; Kant et al., 2015; Kant and Dasgupta, 2017; Purkayastha et al., 2010). We tested many of these and despite our efforts, none of these protocols induced the expected photobleaching phenotype in either *indica* or *aus* rice genotypes or were more effective than the standard rub inoculation described by (Mellado-Sanchez et al., 2024). Although further work is required to determine which of the many steps of the infection process is not successful in rice, these results suggest BSMV cannot mount a sufficient infection in rice to lead to VERG. We recognise there are places in the literature that report BSMV is able to infect rice (Benedito et al., 2004), but our data show that even if BSMV is successfully infecting rice, the asOsPDS it carries is not leading to a VIGS response. A similar statement applies to FoMV, where even if we are successfully infecting rice with the FoMV:GFP, no functional GFP is being produced under our conditions in these rice cultivars.

Working in wheat or in blackgrass Bouton et al. (2018) and Mellado-Sanchez et al. (2020) respectively demonstrated that not all biotypes have equivalent VERG responses, even if treated with the same vector under equivalent environmental conditions and protocols. We had hoped that by trialling different rice cultivars, we would find an equivalent cultivar-specific response in rice. However, Table 1 shows that none of the plants of O*. sativa* ssp. *indica* cv. IR 64, *O. sativa* ssp. *indica* cv. Kasalath, *O. sativa* ssp. *indica* cv. CO-39, *O. sativa* ssp. *japonica* cv. Kitaake*, O. sativa* ssp. *japonica* cv. Koshihikari, or *O. sativa* ssp. *japonica* cv. Balilla showed the expected VERG phenotypes. These varieties and subspecies are both experimentally and agronomically important. We therefore believe that our lack of VERG is not due to either cultivar- or subspecies-specific issues.

In contrast to our data, successful VIGS was reported in rice using vectors based on brome mosaic virus (BMV) (Ding et al., 2006b) or rice tungro bacilliform virus (RTBV) (Purkayastha et al., 2010). There, two strains of BMV were used to generate a modified

BMV vector harbouring an 86 bp fragment of the *OsPDS* and this vector was used to inoculate *Oryza sativa* ssp. *indica* cv. IR64 (Ding et al., 2006b). However, as no untreated controls were shown, it is not entirely clear whether the reported phenotype is a function of *PDS* silencing or viral symptoms similar to what we observed. Similarly, modified RTBV was tested for its ability to silence *PDS* where inoculation by injection into two cultivars of *indica* rice resulted in a weak streaking phenotype (Purkayastha et al., 2010). Our data also contradict the report of successful VIGS in *O. sativa* ssp. *japonica* cultivars Tatsuen No.2, Taikeng No.9, and Taoyuan No.3 using a 441 bp sequence of *PDS* from Barley that was cloned into a modified FoMV vector (Huang et al., 2020). While their FoMV vector differed from the one we tested, they employed a similar approach by infiltrating *Chenopodium quinoa* and rub-inoculating rice seedlings at the 2-leaf stage. However, we found no literature repeating or building on their successful FoMV-enabled VIGS in rice.

As temperature plays a crucial role in the effectiveness of either VERG method, all our experiments were conducted in high-end, well-monitored controlled environment rooms using conditions previously demonstrated to be ideal for BSMV and FoMV infection and VIGS and VOX efficacy (Bouton et al., 2018; Fei et al., 2021; Lee et al., 2015b; Mellado-Sanchex et al., 2020; Mellado-Sanchez et al., 2024). The studies reporting successful VIGS in rice utilized standard growth conditions, including constant temperatures ranging from 25 to 28ºC or a 25/20ºC day/night regime, a 16/8-hour light/dark photoperiod, and relative humidity levels exceeding 80% (Ding et al., 2006a; Huang et al., 2020; Kant and Dasgupta, 2017). These conditions align with those employed in our study, suggesting that the observed ineffectiveness of the VERG protocols we tested is unlikely due to environmental factors.

In addition to environmental conditions, it is well known that efficacy of VIGS depends on the accuracy of the match between target and endogenous RNA (reviewed in MacGregor, 2020). That said, systematic VIGS was successful in *N. benthamiana* using a wide range of heterologous inserts indicating that a perfect match to the endogenous copy is not required providing the viral vector can successfully infect and spread (Senthil-Kumar et al., 2007). Nevertheless, we used two rice-specific BSMV:asOsPDS vectors that targeted two different regions of the *PDS* cDNA. These regions were chosen as they target the same *PDS* regions previously shown to be highly efficacious in wheat (Lee et al., 2015b) or blackgrass (Mellado-Sánchez et al., 2020). They were also cloned in antisense, as the antisense orientation was shown to be more effective than those cloned in a sense direction (Zhang et al., 2010). Despite the controlled and optimized conditions, and although these newly created vectors efficiently induced photobleaching in wheat (Figure 2A), neither

vector caused a characteristic photobleaching phenotype in rice (Figure 2B). We therefore do not believe that lack of sequence specificity was the cause for our unsuccessful VIGS.

We did not find any reports that viral vectors could induced heterologous expression in rice in the literature. While ineffective for VOX in rice, our methods successfully replicated previous publications where GFP was induced in wheat (Bouton et al., 2018; Mei et al., 2016) and *Setaria viridis* (Ellison et al., 2021). These FoMV:GFP vectors also successfully induced fluorescence in blackgrass (Mellado-Sanchez et al., 2020), and *Nicotiana benthamiana* (Torti et al., 2021) demonstrating they can be applied to a variety of species.

5 Conclusion

This study demonstrates that barley stripe mosaic virus (BSMV) and foxtail mosaic virus (FoMV) vectors which have been optimised for virus-enabled reverse genetics (VERG) were unable to induce or reduce gene expression in rice (*O. sativa*). Although the vectors we trialled successfully altered gene expression in wheat in our experiments, and have been effective in a number of well-developed model and non-model species monocots and dicots, these vectors and their associated protocols failed to induce virus-induced gene silencing (VIGS) or virus-mediated overexpression (VOX) in any of six different cultivars and subspecies of rice. Our well-controlled experiments did not generate the expected VERG phenotypes despite using conditions previously optimised for viral efficacy and a variety of inoculation methods and rice genotypes. Herein, we aim to make these data public to prevent other researchers from embarking on similar attempts and to emphasize that VERG techniques, which rely on a precise balance of viral infection with plant response, are not always easily transferred between species.

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8 Data Accessibility

All data supporting the findings of this study, as well as all references to cited material, are provided within the main article. No additional datasets were generated or analysed during the current study.

9 Conflict of Interests

We have no conflict of interests.

10 Authors' Contributions

All authors conceived the original idea and formulated the research plan; GMT designed the experiments with input from DRM; GMT performed the experiments; GMT and DRM developed the rice specific VIGS constructs; GMT wrote the manuscript with contributions from DRM and AMJ; DRM agrees to serve as the author responsible for contact and ensures communication.

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12 Tables

Table 1. Description of plant materials and number of plants inoculated with each vector and different inoculation methods. The number in parenthesis represents plants that showed positive signs of inoculation: bleaching for BSMV and green fluorescence for FoMV:GFP

Species	Subgroup	Total number of plants	FoMV: GFP rubbing	FoMV: MCS rubbing	BSMV: asOsPDS1 rubbing	BSMV: asOsPDS2 rubbing	BSMV: asOsPDS2 microneedling	BSMV: asOsPDS2 dipping	BSMV: asOsPDS2 injection	BSMV: MCS rubbing
Triticum aestivum cv Riband	-	105	48 (37)	4 (0)	15 (12)	22 (19)				4 (0)
Oryza sativa ssp. *indica* cv. IR 64	*Indica*	182	30 (0)	24 (0)	24 (0)	40 (0)	10 (0)	10 (0)	10 (0)	34 (0)
Oryza sativa ssp. *indica* cv. Kasalath	*Aus*	56	6 (0)			10 (0)	10 (0)	10 (0)	10 (0)	10 (0)
Oryza sativa ssp. *indica* cv. CO-39	*Indica*	12	6 (0)			6 (0)				
Oryza sativa ssp. *japonica* cv. Kitaake	*Japonica*	12	6 (0)			6 (0)				
Oryza sativa ssp. *japonica* cv. Koshihikari	*Japonica*	12	6 (0)			6 (0)				
Oryza sativa ssp. *japonica* cv. Balilla	*Japonica*	12	6 (0)			6 (0)				

13 Figure Legends

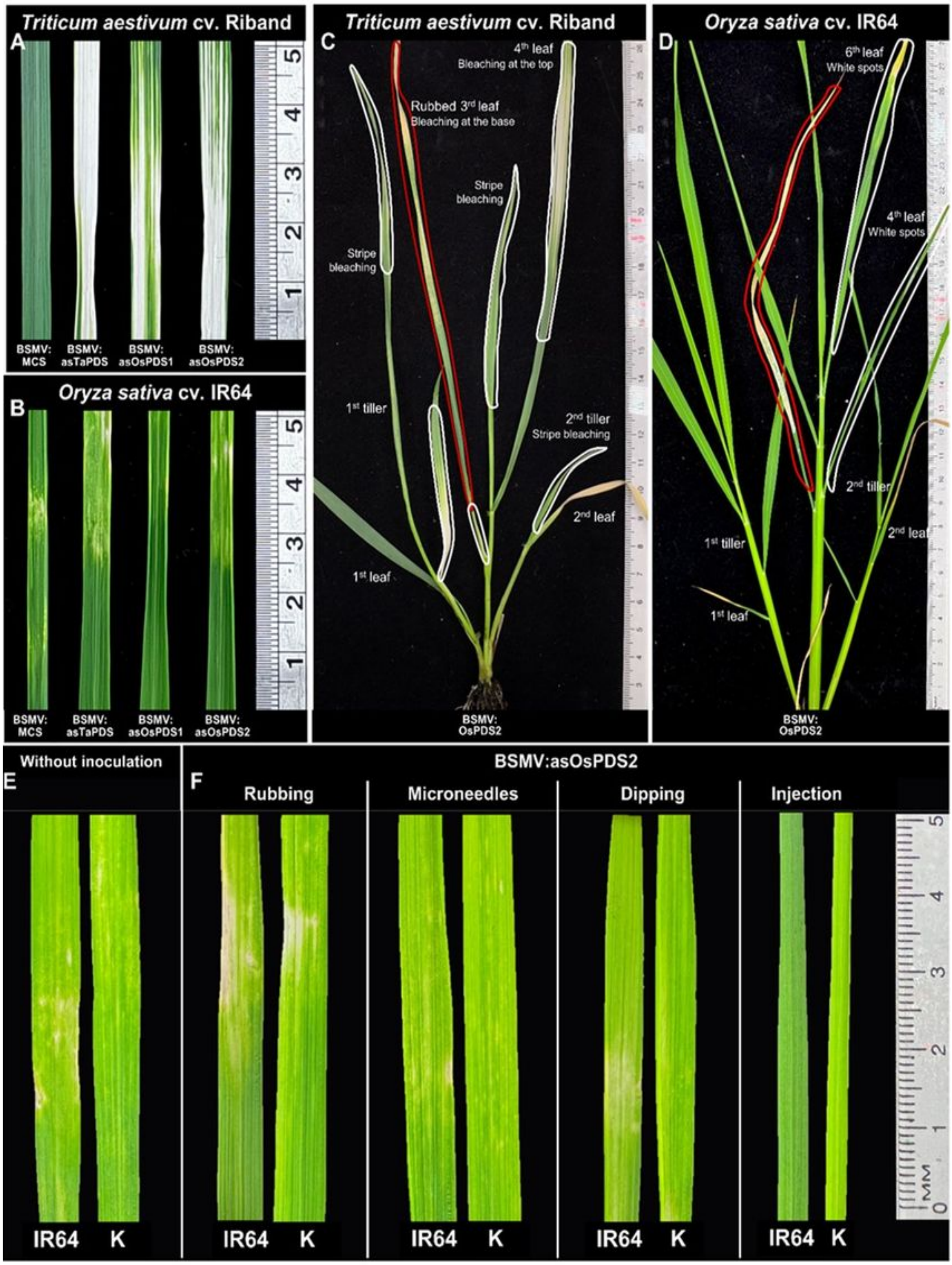
Figure 1. Antisense (as) Oryza sativa PDS fragments synthetised for cloning in BSMV vectors. Bold regions are ligation independent cloning adaptors.

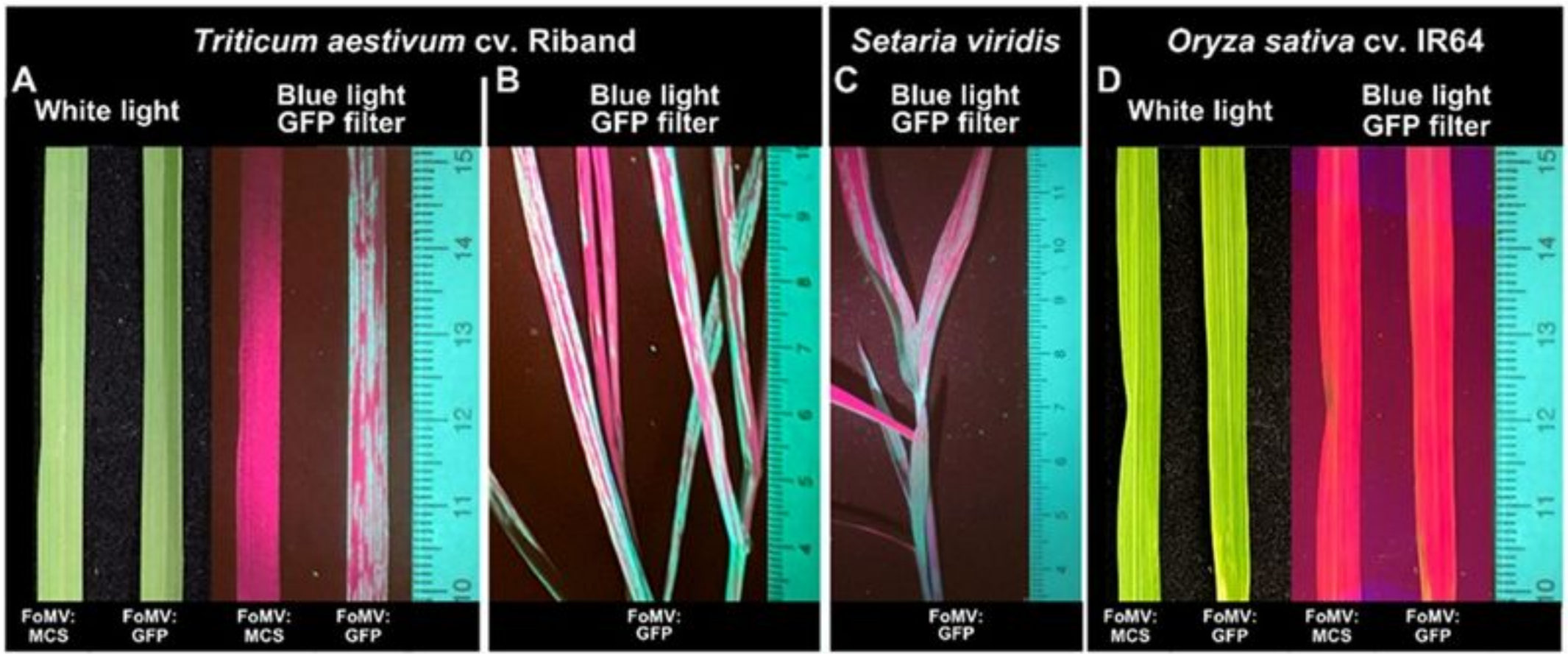
Figure 2. Virus-induced gene silence (VIGS) using barley stripe mosaic virus (BSMV) vector. Phenotypes of wheat (A) and rice (B) leaves that have been inoculated with BSMV vector carrying multi cloning site (MCS) or partial sequences of *phytoene desaturase* (*PDS*) from wheat (asTaPDS) or rice (asOsPDS1 and asOsPDS2). Photos of the fourth leaf (up next the rub-inoculate leaf). Whole-plant phenotypes from wheat (C) and rice (D) that have been inoculated with BSMV:asOsPDS2. Use of different methods of inoculation were not sufficient to improve the efficacy of VIGS in *Oryza sativa* ssp. *indica* cv. IR64 from indica subgroup and *Oryza sativa* ssp. *indica* cv. Kasalath (represented by K) from aus subgroup (F). The named BSMV vector was introduced by rub-inoculation of the third leaf of 21 days old plants 6-10 times with ground leaves of *Nicotiana benthamiana* agroinfiltrated with BSMV:asOsPDS2 and the abrasive carborundum. Instead of carborundum, a device containing small metallic needles was used to wound the third leaf before the rubbing of BSMV:asOsPDS2 sap. Inoculation by dipping and injection followed published procedures with adaptations. Photos of the fourth leaf (up next the inoculated leaf) for rubbing, microneedles, and dipping and of the second leaf for injection.

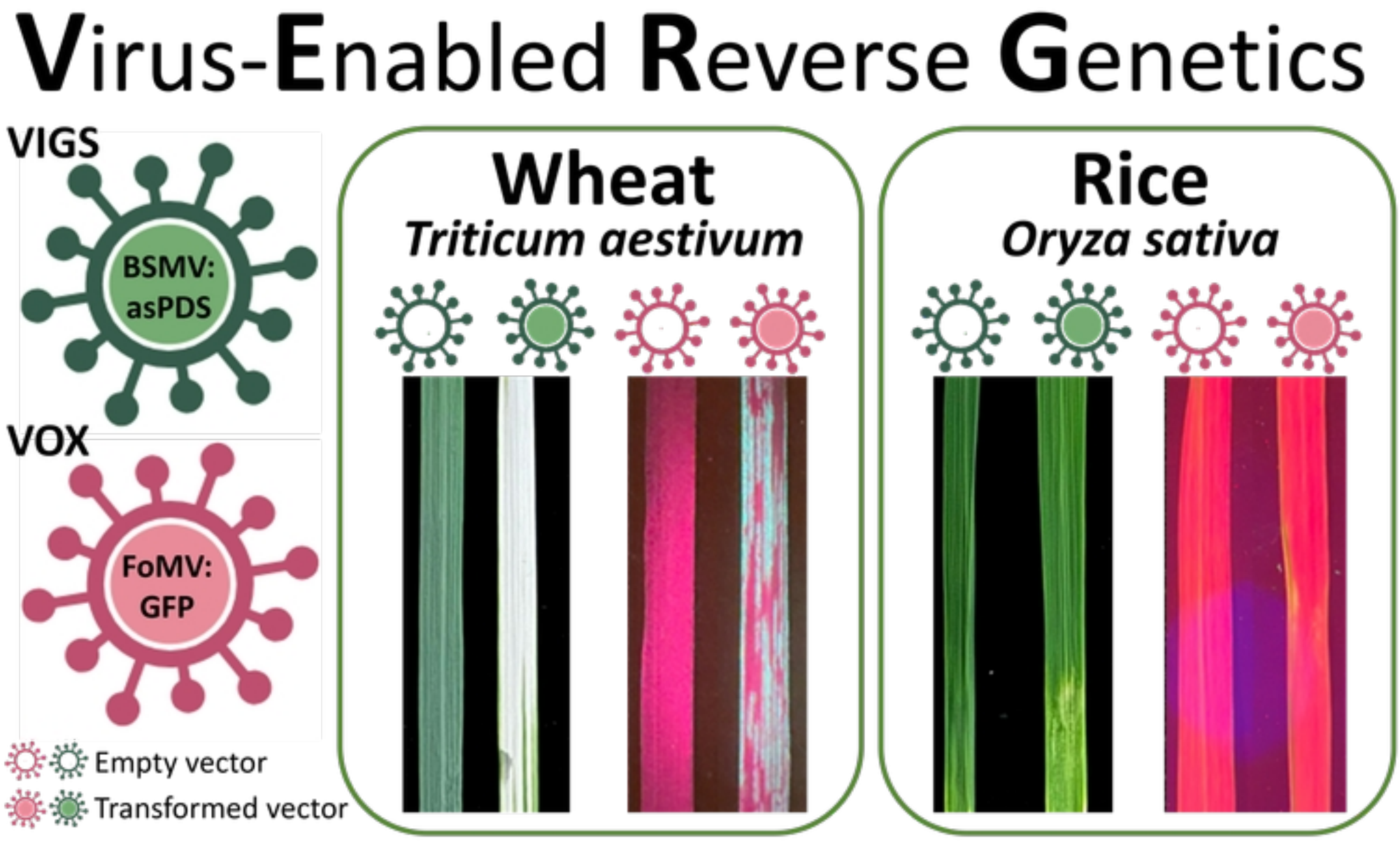
Figure 3. Virus-mediated protein overexpression (VOX) using foxtail mosaic virus (FoMV) vector. Phenotypes of wheat (A) and rice (D) leaves that have been inoculated with FoMV carrying a multi cloning site (MCS) or GFP gene under white light or using blue light with a GFP filter set. Photos of the fourth leaf, up next the rub-inoculate leaf with a sap containing grinded leaves of *Nicotiana benthamiana* agroinfiltrated with FoMV:GFP and the abrasive carborundum. Phenotype of whole wheat (B) and *Setaria viridis* (C) plants that have been inoculated with FoMV:GFP showing systemic infection.

>asOsPDS_VIGS1
AAGGAAGTTTAAGGAAAATCAAACCGGCTGAATTCTCCTGGCTTGTTTGGCATGGCAAATATCATGGAGTGTTCCT
TCCATTGCAACCGATCATTAATACCAAGCTCGCCAAACAAGTTCTGTATGTTGGGATAAGCTCCAAAAAAGATATG
AAGCCCAGTTTCATACCAATCTCCATCTTCATCCTTCCAAGCAG**CACGGTGGTGGTGGTT**

>asOsPDS_VIGS2
AAGGAAGTTTAATCTCCAGTTATTTGAGTTCCATCAGTAAGTGCAAAGTGTTTCACTGTTCCATCAGGATTAAGTT
CTATTTTCTGAATACGAGAATTCAGCCGAACCTCACCACCCAAAGAGCGAACATGGTCAACAATAGGCATGCATAA
CCTTTCAGGAGGATTACCATCCAAGAATGCCATCTTAGAACCATGCTTCTCCTGAAGAAA**ACGGTGGTGGTGGTT**


Triticum aestivum cv. Riband
A
BSMV: MCS
BSMV: asTaPDS
BSMV: asOsPDS1
BSMV: asOsPDS2
Oryza sativa cv. IR64
B
BSMV: MCS
BSMV: asTaPDS
BSMV: asOsPDS1
BSMV: asOsPDS2
Triticum aestivum cv. Riband
C
4th leaf
Bleaching at the top
Rubbed 3rd leaf
Bleaching at the base
Stripe bleaching
Stripe bleaching
1st tiller
2nd tiller
Stripe bleaching
2nd leaf
1st leaf
BSMV: OsPDS2
Oryza sativa cv. IR64
D
6th leaf
White spots
4th leaf
White spots
2nd tiller
2nd leaf
1st tiller
1st leaf
BSMV: OsPDS2
Without inoculation
E
BSMV:asOsPDS2
F
Rubbing
Microneedles
Dipping
Injection
IR64
K
IR64
K
IR64
K
IR64
K
IR64
K
0 mm


Triticum aestivum cv. Riband
A
White light
Blue light
GFP filter
B
Blue light
GFP filter
Setaria viridis
C
Blue light
GFP filter
Oryza sativa cv. IR64
D
White light
Blue light
GFP filter
FoMV:
MCS
FoMV:
GFP
FoMV:
MCS
FoMV:
GFP
FoMV:
GFP
FoMV:
GFP
FoMV:
MCS
FoMV:
GFP
FoMV:
MCS
FoMV:
GFP


Virus-Enabled Reverse Genetics
VIGS
BSMV: asPDS
VOX
FoMV: GFP
Empty vector
Transformed vector
Wheat
Triticum aestivum
Rice
Oryza sativa