

**Supplementary Table A.** Plant species having publicly available mutant resources conducive for cloning the gene of interest.

Sr. No.	Plant species	Name of mutant resource	Reference
1	Arabidopsis	T-DNA insertion mutants	(Belfield <i>et al.</i> , 2012; Enders <i>et al.</i> , 2015; Jia <i>et al.</i> , 2012; O'Malley <i>et al.</i> , 2015)
2	Soybean	<i>Tnt1</i> insertion mutants	(Cui <i>et al.</i> , 2013; Mathieu <i>et al.</i> , 2009)
3	Tomato	T-DNA activation tagged lines and Ds-tagged lines	(Matsukura <i>et al.</i> , 2008)
4	<i>Medicago truncatula</i>	<i>Tnt1</i> insertion mutants	(Tadege <i>et al.</i> , 2009; Tadege <i>et al.</i> , 2008)
5	<i>Lotus japonicus</i>	Transposon mutants	(Urbański <i>et al.</i> , 2012; Urbański <i>et al.</i> , 2013)
6	Rice	T-DNA or <i>Tos17</i> insertion mutants	(Krishnan <i>et al.</i> , 2009; Kuromori <i>et al.</i> , 2009)
7	<i>Brachypodium distachyon</i>	T-DNA insertion mutants	(Bragg <i>et al.</i> , 2012; Thole <i>et al.</i> , 2012)
8	Maize	Transposon tagged mutants	(Hanley <i>et al.</i> , 2000; Settles <i>et al.</i> , 2007)

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**Supplementary Table B.** Currently available predominantly used VIGS vectors and their target plant species\*

Sl. No.	VIGS vector	Examples for target plant species or family or common name	Reference
1.	<i>Apple latent spherical virus (ALSV)</i>	<i>Nicotiana benthamiana</i> , <i>Arabidopsis thaliana</i> , apple, cucurbits and pears	(Igarashi <i>et al.</i> , 2009)
2.	<i>Alternanthera mosaic virus (AltMV)</i>	<i>N. benthamiana</i> and <i>A. thaliana</i>	(Lim <i>et al.</i> , 2010)
3.	<i>Brome mosaic virus (BMV)</i>	Rice, maize, sorghum and foxtail millet	(Ding <i>et al.</i> , 2006)
4.	<i>Barley stripe mosaic virus (BSMV)</i>	Wheat and barley	(Panwar <i>et al.</i> , 2013; Scofield <i>et al.</i> , 2005)
5.	<i>Bamboo mosaic virus</i> and its satellite RNA (SatBaMV)	<i>N. benthamiana</i> and <i>Brachypodium distachyon</i>	(Liou <i>et al.</i> , 2014)
6.	<i>Bean pod mottle virus (BPMV)</i>	Common bean, soybean	(Díaz-Camino <i>et al.</i> , 2011; Zhang <i>et al.</i> , 2010)
7.	<i>Cucumber mosaic virus (CMV)</i>	<i>N. benthamiana</i> and soybean	(Matsuo and Matsumura, 2011; Nagamatsu <i>et al.</i> , 2007)
8.	<i>Cymbidium mosaic virus (CymMV)</i>	Phalaenopsis orchid	(Hsieh <i>et al.</i> , 2013a; Hsieh <i>et al.</i> , 2013b)
9.	<i>Potato virus X (PVX)</i>	<i>N. benthamiana</i> and potato	(Faivre-Rampant <i>et al.</i> , 2004)
10.	<i>Pea early browning virus (PEBV)</i>	<i>Pea</i> , <i>barrel medic</i> and <i>lotus</i>	(Constantin <i>et al.</i> , 2004; Grönlund <i>et al.</i> , 2008)
11.	<i>Tobacco rattle virus (TRV)</i>	<i>This vector has wide host range (a few examples include many members of Solanaceae family, Arabidopsis, California poppy and cotton.</i>	(Burch-Smith <i>et al.</i> , 2006; Gould and Kramer, 2007; Liu <i>et al.</i> , 2002; Xiquan <i>et al.</i> , 2011)
12.	<i>Turnip yellow mosaic virus (TYMV)</i>	<i>N. benthamiana</i> and <i>A. thaliana</i>	(Pflieger <i>et al.</i> , 2008)
13.	<i>African cassava mosaic virus (ACMV)</i>	<i>N. benthamiana</i> and <i>cassava</i>	(Fofana <i>et al.</i> , 2004)
14.	<i>Beet curly top virus (BCTV)</i>	<i>Spinach</i> and <i>tomato</i>	(Golenberg <i>et al.</i> , 2009)
15.	<i>Cabbage leaf curl virus (CaLCuV)</i>	<i>N. benthamiana</i> and <i>A. thaliana</i>	(Muangsan <i>et al.</i> , 2004)
16.	<i>Cotton leaf crumple virus (CLCV)</i>	<i>N. benthamiana</i> and <i>cotton</i>	(Tuttle <i>et al.</i> , 2012; Tuttle <i>et al.</i> , 2008)
17.	<i>Grapevine virus A (GVA)</i>	<i>N. benthamiana</i> and <i>grapevine</i>	(Muruganatham <i>et al.</i> , 2009)
18.	<i>Grapevine leaf roll-associated virus-2 (GLRaV2)</i>	<i>N. benthamiana</i> and <i>grapevine</i>	(Kurth <i>et al.</i> , 2012)
19.	<i>Pepper huasteco yellow vein virus (PHYVV)</i>	<i>Capsicum sp.</i>	(del Rosario Abraham-Juárez <i>et al.</i> , 2008)
20.	<i>Rice tungro bacilliform virus (RTBV)</i>	<i>Rice (Indica and Japonica)</i>	(Purkayastha <i>et al.</i> , 2010)
21.	<i>Tobacco curly shoot virus (TCSV)</i>	<i>Tobacco</i> and <i>tomato</i>	(Huang <i>et al.</i> , 2009)
22.	<i>Tomato yellow leaf curl China virus (TYLCCNV)</i>	<i>Tobacco</i> and <i>tomato</i>	(Cai <i>et al.</i> , 2007; Tao and Zhou, 2004)

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**Supplementary Table C.** Plant species suitable for TRV-VIGS for silencing target genes.

Sr. No.	Plant species	Suitable genotype or cultivar	Method of inoculation	Preferred age of plant	Environmental temperature (day)	Reference
1	<i>Nicotiana benthamiana</i>	-	Syringe-mediated inoculation; Agrodrench	3 week old plants	21±2 °C	(Ryu <i>et al.</i> , 2004; Senthil-Kumar <i>et al.</i> , 2013; Senthil-Kumar and Mysore, 2011)
2	Tomato ( <i>Solanum lycopersicum</i> )	Glamour & micro-tom	Syringe-mediated inoculation at cotyledonary leaves; vacuum infiltration	10 day old plants	21±2 °C	(Burch-Smith <i>et al.</i> , 2006; Liu <i>et al.</i> , 2002; Senthil-Kumar and Mysore, 2011)
3	<i>Arabidopsis thaliana</i>	Columbia	- Multiply the virus constructs in <i>N. benthamiana</i> and inoculate virus containing sap* - TRV1 and TRV2 mixed culture is delivered into apoplast of leaf cells by using needleless syringe	Two week old plants	23±2 °C	(Burch-Smith <i>et al.</i> , 2006; Lu <i>et al.</i> , 2003; Rojas <i>et al.</i> , 2012)
4	Cotton ( <i>Gossypium hirsutum</i> )	Coker 312	Agroinoculation by vacuum infiltration, infiltration by needleless syringe	Two week old plants	23-25 °C	(Qu <i>et al.</i> , 2012; Xiquan <i>et al.</i> , 2011)
5	<i>Aquilegia vulgaris</i> (also applicable to opium or California poppy)	-	Agroinoculation by vacuum infiltration	Varies (about 12-15 weeks)	Growth condition varies (20 °C suitable for VIGS)	(Gould and Kramer, 2007; Hileman <i>et al.</i> , 2005; Wege <i>et al.</i> , 2007)
6	<i>Jatropha curcas</i>	-	Agroinoculation by needle-less syringe or vacuum infiltration	Varies (2-3 leaf stage)	25 °C	(Ye <i>et al.</i> , 2009)
7	Tobacco ( <i>Nicotiana tabacum</i> )	Xanthi nc	Agrodrench	2-3 week old plants	21±2 °C	(Ryu <i>et al.</i> , 2004)
8	Petunia ( <i>Petunia hybrida</i> )	-	Agroinoculation by needle-less syringe	-	25 °C	(Chen <i>et al.</i> , 2004)
9	Pepper ( <i>Capsicum spp.</i> )	-	Agroinoculation by needle-less syringe; Agrodrench	2 week old plants	25 °C	(Chung <i>et al.</i> , 2006; Ryu <i>et al.</i> , 2004)
10	Several other plants of Solanaceae	-	Agroinoculation by needle-less syringe	-	-	(Senthil-Kumar <i>et al.</i> , 2007)
11	<i>N. benthamiana</i> , tomato and several other Solanaceae plants	-	Sprout vacuum inoculation	Immediately after germination	-	(Yan <i>et al.</i> , 2012)

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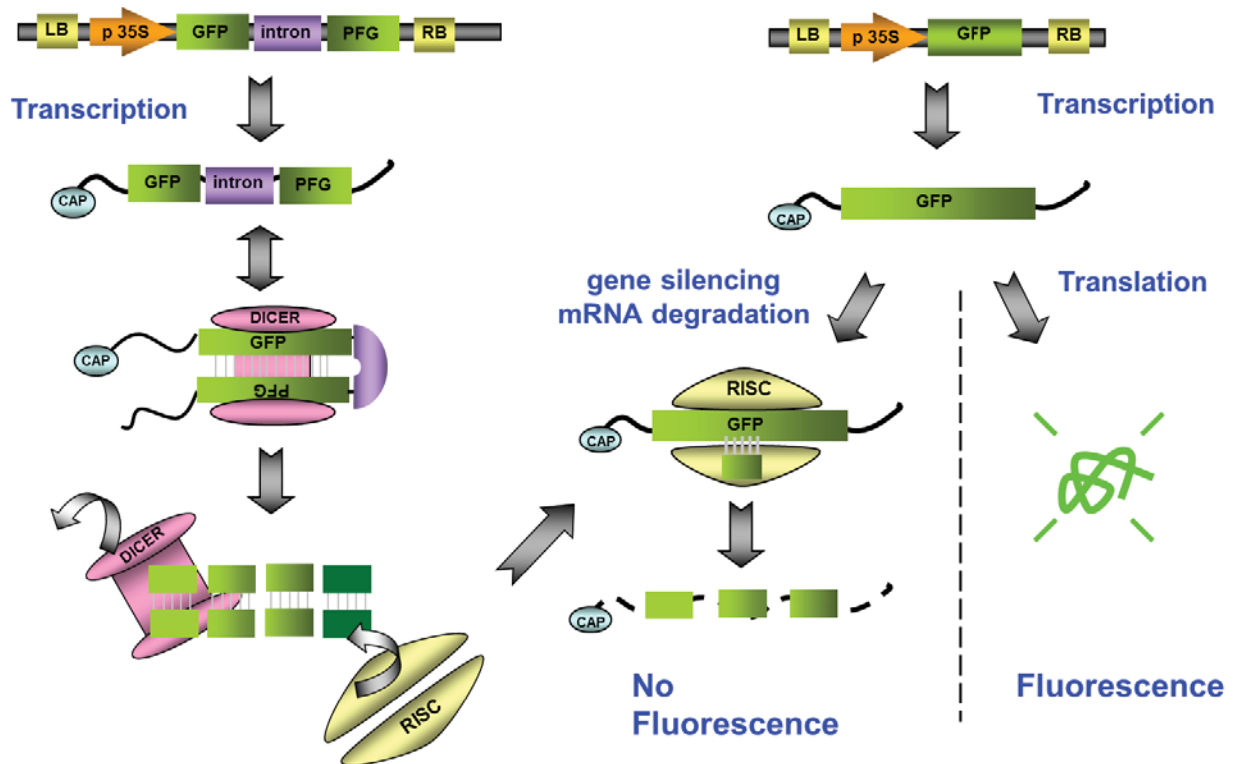
**Supplementary Table D.** Different methods of TRV inoculation for achieving VIGS.

Sr. No.	Name of the method	Brief protocol	Age of the plant used	Application	Reference
1	Syringe inoculation	TRV1 and TRV2 containing mixed <i>Agrobacterium</i> culture is delivered into apoplast of leaf cells by using needleless syringe	3 weeks old	For silencing one or few genes by a reverse genetics approach	(Velásquez <i>et al.</i> , 2009)
2	Agrodrench	Drench TRV1 and TRV2 containing mixed <i>Agrobacterium</i> culture into the plant growth medium near crown region	1-2 weeks old	For silencing large number of genes, especially for gene silencing in roots	(Ryu <i>et al.</i> , 2004)
3	Pricking leaves using toothpick	TRV1 <i>Agrobacterium</i> culture is inoculated using needleless syringe and at this inoculated site TRV2 colonies are pricked using toothpick	3 weeks old	For large scale gene silencing of genes using cDNA library for a forward genetics screening	(Senthil-Kumar <i>et al.</i> , 2013)
4	Spray inoculation	Leaf surface is dusted with carborundum. TRV1 and TRV2 containing mixed <i>Agrobacterium</i> culture is sprayed using a sprayer.	3 weeks old	Silencing a few genes independently in a large number of plants	(Padmanabhan and Dinesh-Kumar, 2009)
5	Vacuum infiltration of plant	Plant is immersed in TRV1 and TRV2 containing mixed <i>Agrobacterium</i> culture and vacuum is applied	All ages	Silencing genes in young seedlings	(Padmanabhan and Dinesh-Kumar, 2009)
6	Vacuum or syringe inoculation of fruit (examples, tomato & strawberry)	Inoculation of TRV1 and TRV2 containing mixed <i>Agrobacterium</i> cultures into detached fruit by vacuum suction or needle-less syringe	Detached fruit	Understanding fruit development (example, ripening) related genes	(Fu <i>et al.</i> , 2005)
7	Sprout vacuum inoculation	Inoculation of TRV1 and TRV2 containing mixed <i>Agrobacterium</i> in small seedlings by vacuum	Small seedlings immediately after germination	Gene function in the early stages of plant growth	(Yan <i>et al.</i> , 2012)
8	Rub inoculation of virus containing sap or virions on to the target plant leaf	Inoculation of TRV1 and TRV2 in <i>N. benthamiana</i> leaves and then virus particles or sap from the inoculated leaf is collected and inoculated on to the target plant species.	About 3 week old plants	This is an alternate method for Agroinoculation of TRV vectors. This method is efficient in some plant species (for example, Arabidopsis) where the Agroinoculation is not preferred.	(Lu <i>et al.</i> , 2003)

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**Supplementary Figure 1.** The virus induced gene silencing mechanism within plant body. VIGS is a PTGS method used by plants as a defense mechanism against invading viruses. During viral replication double-stranded RNA (dsRNA) is produced by an RNA dependent RNA polymerase and this triggers PTGS. One of the major aspects of this process involves Dicer-like enzyme-mediated cleavage of the dsRNA to produce siRNA. This siRNA binds to and activates the RNA-induced silencing complex (RISC) which cleaves the viral RNA in a homology-dependent manner.