**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 *et al*., 2012; Enders *et al*., 2015; Jia *et al*., 2012; O’Malley *et al*., 2015) |
| 2 | Soybean | *Tnt1* insertion mutants | (Cui *et al*., 2013; Mathieu *et al*., 2009) |
| 3 | Tomato | T-DNA activation tagged lines and Ds-tagged lines | (Matsukura *et al*., 2008) |
| 4 | *Medicago truncatula* | *Tnt1* insertion mutants | (Tadege *et al*., 2009; Tadege *et al*., 2008) |
| 5 | *Lotus japonicus* | Transposon mutants | (Urbański *et al*., 2012; Urbański *et al*., 2013) |
| 6 | Rice | T-DNA or *Tos17* insertion mutants | (Krishnan *et al*., 2009; Kuromori *et al*., 2009) |
| 7 | *Brachypodium distachyon* | T-DNA insertion mutants | (Bragg *et al*., 2012; Thole *et al*., 2012) |
| 8 | Maize | Transposon tagged mutants | (Hanley *et al*., 2000; Settles *et al*., 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. | *Apple latent spherical virus* (*ALSV*) | *Nicotiana benthamiana*, *Arabidopsis thaliana*, apple, cucurbits and pears | (Igarashi *et al*., 2009) |
| 2. | *Alternanthera mosaic virus* (*AltMV*) | *N. benthamiana* and *A. thaliana* | (Lim *et al*., 2010) |
| 3. | *Brome mosaic virus* (*BMV*) | Rice, maize, sorghum and foxtail millet | (Ding *et al*., 2006) |
| 4. | *Barley stripe mosaic virus* (*BSMV*) | Wheat and barley | (Panwar *et al*., 2013; Scofield *et al*., 2005) |
| 5. | *Bamboo mosaic virus* and its satellite RNA (SatBaMV) | *N. benthamiana* and *Brachypodium distachyon* | (Liou *et al*., 2014) |
| 6. | *Bean pod mottle virus* (*BPMV*) | Common bean, soybean | (Díaz-Camino *et al*., 2011; Zhang *et al*., 2010) |
| 7. | *Cucumber mosaic virus* (*CMV*) | *N. benthamiana* and soybean | (Matsuo and Matsumura, 2011; Nagamatsu *et al*., 2007) |
| 8. | *Cymbidium mosaic virus* (*CymMV*) | Phalaenopsis orchid | (Hsieh *et al*., 2013a; Hsieh *et al*., 2013b) |
| 9. | *Potato virus X* (*PVX*) | *N. benthamiana* and potato | (Faivre-Rampant *et al*., 2004) |
| 10. | *Pea early browning virus (PEBV)* | *Pea, barrel medic and lotus* | (Constantin *et al*., 2004; Grønlund *et al*., 2008) |
| 11. | *Tobacco rattle virus (TRV)* | *This vector has wide host range (a few examples include many members of Solanaceae family, Arabidopsis, California poppy and cotton.* | (Burch-Smith *et al*., 2006; Gould and Kramer, 2007; Liu et al., 2002; Xiquan et al., 2011) |
| 12. | *Turnip yellow mosaic virus (TYMV)* | *N. benthamiana and A. thaliana* | (Pflieger *et al*., 2008) |
| 13. | *African cassava mosaic virus (ACMV)* | *N. benthamiana and cassava* | (Fofana *et al*., 2004) |
| 14. | *Beet curly top virus (BCTV)* | *Spinach and tomato* | (Golenberg et al., 2009) |
| 15. | *Cabbage leaf curl virus (CaLCuV)* | *N. benthamiana and A. thaliana* | (Muangsan *et al*., 2004) |
| 16. | *Cotton leaf crumple virus (CLCV)* | *N. benthamiana and cotton* | (Tuttle *et al*., 2012; Tuttle *et al*., 2008) |
| 17. | *Grapevine virus A (GVA)* | *N. benthamiana and grapevine* | (Muruganantham *et al*., 2009) |
| 18. | *Grapevine leaf roll-associated virus-2 (GLRaV2)* | *N. benthamiana and grapevine* | (Kurth *et al*., 2012) |
| 19. | *Pepper huasteco yellow vein virus (PHYVV)* | *Capsicum sp.* | (del Rosario Abraham-Juárez *et al*., 2008) |
| 20. | *Rice tungro bacilliform virus (RTBV)* | *Rice (Indica and Japonica)* | (Purkayastha *et al.,* 2010) |
| 21. | *Tobacco curly shoot virus (TCSV)* | *Tobacco and tomato* | (Huang *et al*., 2009) |
| 22. | *Tomato yellow leaf curl China virus (TYLCCNV)* | *Tobacco and tomato* | (Cai *et al*., 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 | *Nicotiana benthamiana* | - | Syringe-mediated inoculation; Agrodrench | 3 week old plants | 21±2 °C | (Ryu *et al*., 2004; Senthil-Kumar *et al*., 2013; Senthil-Kumar and Mysore, 2011) |
| 2 | Tomato (*Solanum lycopersicum*) | Glamour & micro-tom | Syringe-mediated inoculation at cotyledonary leaves; vacuum infiltration | 10 day old plants | 21±2 °C | (Burch-Smith *et al*., 2006; Liu *et al*., 2002; Senthil-Kumar and Mysore, 2011) |
| 3 | *Arabidopsis thaliana* | Columbia | - Multiply the virus constructs in *N. benthamiana* 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 *et al.,* 2006; Lu *et al*., 2003; Rojas *et al*., 2012) |
| 4 | Cotton (*Gossypium hirsutum*) | Coker 312 | Agroinoculation by vacuum infiltration, infiltration by needleless syringe | Two week old  plants | 23-25 °C | (Qu *et al*., 2012; Xiquan *et al*., 2011) |
| 5 | *Aquilegia vulgaris* (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 *et al*., 2005; Wege *et al*., 2007) |
| 6 | *Jatropha curcas* | - | Agroinoculation by needle-less syringe or vacuum infiltration | Varies (2-3 leaf stage) | 25 °C | (Ye *et al*., 2009) |
| 7 | Tobacco (*Nicotiana tabacum*) | Xanthi nc | Agrodrench | 2-3 week old plants | 21±2 °C | (Ryu *et al*., 2004) |
| 8 | Petunia (*Petunia hybrida*) | - | Agroinoculation by needle-less syringe | - | 25 °C | (Chen *et al*., 2004) |
| 9 | Pepper (*Capsicum spp*.) | - | Agroinoculation by needle-less syringe; Agrodrench | 2 week old plants | 25 °C | (Chung *et al*., 2006; Ryu *et al*., 2004) |
| 10 | Several other plants of Solanaceae | - | Agroinoculation by needle-less syringe | - | - | (Senthil-Kumar *et al*., 2007) |
| 11 | *N. benthamiana*, tomato and several other Solanaceae plants | - | Sprout vacuum inoculation | Immediately after germination | - | (Yan *et al*., 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 *Agrobacterium* 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 *et al*., 2009) |
| 2 | Agrodrench | Drench TRV1 and TRV2 containing mixed *Agrobacterium* 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 *et al*., 2004) |
| 3 | Pricking leaves using toothpick | TRV1 *Agrobacterium* 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 *et al*., 2013) |
| 4 | Spray inoculation | Leaf surface is dusted with carborundum. TRV1 and TRV2 containing mixed *Agrobacterium* 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 *Agrobacterium* 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 Agrobacterium cultures into detached fruit by vacuum suction or needle-less syringe | Detached fruit | Understanding fruit development (example, ripening) related genes | (Fu *et al*., 2005) |
| 7 | Sprout vacuum inoculation | Inoculation of TRV1 and TRV2 containing mixed Agrobacterium in small seedlings by vacuum | Small seedlings immediately after germination | Gene function in the early stages of plant growth | (Yan *et al*., 2012) |
| 8 | Rub inoculation of virus containing sap or virions on to the target plant leaf | Inoculation of TRV1 and TRV2 in N. benthamiana 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 *et al*., 2003) |

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