**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) |

**References**

Belfield, E.J., Gan, X., Mithani, A., Brown, C., Jiang, C., Franklin, K., Alvey, E., Wibowo, A., Jung, M. and Bailey, K. (2012) Genome-wide analysis of mutations in mutant lineages selected following fast-neutron irradiation mutagenesis of *Arabidopsis thaliana*. *Genome Research* 22, 1306-1315.

Bragg, J.N., Wu, J., Gordon, S.P., Guttman, M.E., Thilmony, R., Lazo, G.R., Gu, Y.Q. and Vogel, J.P. (2012) Generation and characterization of the Western Regional Research Center Brachypodium T-DNA insertional mutant collection. *PLoS One* 7, e41916.

Cui, Y., Barampuram, S., Stacey, M.G., Hancock, C.N., Findley, S., Mathieu, M., Zhang, Z., Parrott, W.A. and Stacey, G. (2013) Tnt1 retrotransposon mutagenesis: a tool for soybean functional genomics. *Plant physiology* 161, 36-47.

Enders, T.A., Oh, S., Yang, Z., Montgomery, B.L. and Strader, L.C. (2015) Genome Sequencing of *Arabidopsis* abp1-5 Reveals Second-Site Mutations That May Affect Phenotypes. *The Plant Cell* 27, 1820-1826.

Hanley, S., Edwards, D., Stevenson, D., Haines, S., Hegarty, M., Schuch, W. and Edwards, K.J. (2000) Identification of transposon tagged genes by the random sequencing of Mutator tagged DNA fragments from *Zea mays*. *The Plant Journal* 23, 557-566.

Jia, Q., Bundock, P., Hooykaas, P.J. and De Pater, S. (2012) Agrobacterium tumefaciens T-DNA integration and gene targeting in *Arabidopsis thaliana* non-homologous end-joining mutants. *Journal of Botany* 2012.

Krishnan, A., Guiderdoni, E., An, G., Yue-ie, C.H., Han, C.-d., Lee, M.C., Yu, S.-M., Upadhyaya, N., Ramachandran, S. and Zhang, Q. (2009) Mutant resources in rice for functional genomics of the grasses. *Plant physiology* 149, 165-170.

Kuromori, T., Takahashi, S., Kondou, Y., Shinozaki, K. and Matsui, M. (2009) Phenome analysis in plant species using loss-of-function and gain-of-function mutants. *Plant and Cell Physiology* 50, 1215-1231.

Mathieu, M., Winters, E.K., Kong, F., Wan, J., Wang, S., Eckert, H., Luth, D., Paz, M., Donovan, C. and Zhang, Z. (2009) Establishment of a soybean (*Glycine max Merr*. L) transposon-based mutagenesis repository. *Planta* 229, 279-289.

Matsukura, C., Aoki, K., Fukuda, N., Mizoguchi, T., Asamizu, E., Saito, T., Shibata, D. and Ezura, H. (2008) Comprehensive resources for tomato functional genomics based on the miniature model tomato micro-tom. *Current genomics* 9, 436.

O’Malley, R.C., Barragan, C.C. and Ecker, J.R. (2015) A User’s Guide to the *Arabidopsis* T-DNA Insertion Mutant Collections. *Plant Functional Genomics: Methods and Protocols*, 323-342.

Settles, A.M., Holding, D.R., Tan, B.C., Latshaw, S.P., Liu, J., Suzuki, M., Li, L., O'Brien, B.A., Fajardo, D.S. and Wroclawska, E. (2007) Sequence-indexed mutations in maize using the UniformMu transposon-tagging population. *BMC genomics* 8, 116.

Tadege, M., Wang, T.L., Wen, J., Ratet, P. and Mysore, K.S. (2009) Mutagenesis and beyond! Tools for understanding legume biology. *Plant physiology* 151, 978-984.

Tadege, M., Wen, J., He, J., Tu, H., Kwak, Y., Eschstruth, A., Cayrel, A., Endre, G., Zhao, P.X. and Chabaud, M. (2008) Large scale insertional mutagenesis using the Tnt1 retrotransposon in the model legume *Medicago truncatula*. *The Plant Journal* 54, 335-347.

Thole, V., Peraldi, A., Worland, B., Nicholson, P., Doonan, J.H. and Vain, P. (2012) T-DNA mutagenesis in *Brachypodium distachyon*. *Journal of experimental botany* 63, 567-576.

Urbański, D.F., Małolepszy, A., Stougaard, J. and Andersen, S.U. (2012) Genome wide LORE1 retrotransposon mutagenesis and high throughput insertion detection in *Lotus japonicus*. *The Plant Journal* 69, 731-741.

Urbański, D.F., Małolepszy, A., Stougaard, J. and Andersen, S.U. (2013) High-throughput and targeted genotyping of *Lotus japonicus* LORE1 insertion mutants. In: *Legume Genomics* (Rose, R.J. ed) pp. 119-146. Springer.

**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) |

**References**

Burch-Smith, T.M., Schiff, M., Liu, Y. and Dinesh-Kumar, S.P. (2006) Efficient virus-induced gene silencing in Arabidopsis. *Plant physiology* 142, 21-27.

Cai, X., Wang, C., Xu, Y., Xu, Q., Zheng, Z. and Zhou, X. (2007) Efficient gene silencing induction in tomato by a viral satellite DNA vector. *Virus research* 125, 169-175.

Constantin, G.D., Krath, B.N., MacFarlane, S.A., Nicolaisen, M., Elisabeth Johansen, I. and Lund, O.S. (2004) Virus‐induced gene silencing as a tool for functional genomics in a legume species. *The Plant Journal* 40, 622-631.

del Rosario Abraham-Juárez, M., del Carmen Rocha-Granados, M., López, M.G., Rivera-Bustamante, R.F. and Ochoa-Alejo, N. (2008) Virus-induced silencing of Comt, pAmt and Kas genes results in a reduction of capsaicinoid accumulation in chili pepper fruits. *Planta* 227, 681-695.

Díaz-Camino, C., Annamalai, P., Sanchez, F., Kachroo, A. and Ghabrial, S.A. (2011) An effective virus-based gene silencing method for functional genomics studies in common bean. *Plant methods* 7, 16.

Ding, X.S., Schneider, W.L., Chaluvadi, S.R., Mian, M.R. and Nelson, R.S. (2006) Characterization of a Brome mosaic virus strain and its use as a vector for gene silencing in monocotyledonous hosts. *Molecular Plant-Microbe Interactions* 19, 1229-1239.

Faivre-Rampant, O., Gilroy, E.M., Hrubikova, K., Hein, I., Millam, S., Loake, G.J., Birch, P., Taylor, M. and Lacomme, C. (2004) Potato virus X-induced gene silencing in leaves and tubers of potato. *Plant physiology* 134, 1308-1316.

Fofana, I.B., Sangaré, A., Collier, R., Taylor, C. and Fauquet, C.M. (2004) A geminivirus-induced gene silencing system for gene function validation in cassava. *Plant molecular biology* 56, 613-624.

Golenberg, E.M., Sather, D.N., Hancock, L.C., Buckley, K.J., Villafranco, N.M. and Bisaro, D.M. (2009) Development of a gene silencing DNA vector derived from a broad host range geminivirus. *Plant methods* 5, 9.

Gould, B. and Kramer, E.M. (2007) Virus-induced gene silencing as a tool for functional analyses in the emerging model plant Aquilegia (columbine, Ranunculaceae). *Plant methods* 3, 6.

Grønlund, M., Constantin, G., Piednoir, E., Kovacev, J., Johansen, I.E. and Lund, O.S. (2008) Virus-induced gene silencing in *Medicago truncatula* and *Lathyrus odorata*. *Virus research* 135, 345-349.

Hsieh, M.-H., Lu, H.-C., Pan, Z.-J., Yeh, H.-H., Wang, S.-S., Chen, W.-H. and Chen, H.-H. (2013a) Optimizing virus-induced gene silencing efficiency with Cymbidium mosaic virus in Phalaenopsis flower. *Plant Science* 201, 25-41.

Hsieh, M.-H., Pan, Z.-J., Lai, P.-H., Lu, H.-C., Yeh, H.-H., Hsu, C.-C., Wu, W.-L., Chung, M.-C., Wang, S.-S. and Chen, W.-H. (2013b) Virus-induced gene silencing unravels multiple transcription factors involved in floral growth and development in Phalaenopsis orchids. *Journal of experimental botany* 64, 3869-3884.

Huang, C., Xie, Y. and Zhou, X. (2009) Efficient virus induced gene silencing in plants using a modified geminivirus DNA1 component. *Plant biotechnology journal* 7, 254-265.

Igarashi, A., Yamagata, K., Sugai, T., Takahashi, Y., Sugawara, E., Tamura, A., Yaegashi, H., Yamagishi, N., Takahashi, T. and Isogai, M. (2009) Apple latent spherical virus vectors for reliable and effective virus-induced gene silencing among a broad range of plants including tobacco, tomato, *Arabidopsis thaliana*, cucurbits, and legumes. *Virology* 386, 407-416.

Kurth, E.G., Peremyslov, V.V., Prokhnevsky, A.I., Kasschau, K.D., Miller, M., Carrington, J.C. and Dolja, V.V. (2012) Virus-derived gene expression and RNA interference vector for grapevine. *Journal of Virology* 86, 6002-6009.

Lim, H.-S., Vaira, A.M., Domier, L.L., Lee, S.C., Kim, H.G. and Hammond, J. (2010) Efficiency of VIGS and gene expression in a novel bipartite potexvirus vector delivery system as a function of strength of TGB1 silencing suppression. *Virology* 402, 149-163.

Liou, M.R., Huang, Y.W., Hu, C.C., Lin, N.S. and Hsu, Y.H. (2014) A dual gene silencing vector system for monocot and dicot plants. *Plant biotechnology journal* 12, 330-343.

Liu, Y., Schiff, M. and Dinesh Kumar, S. (2002) Virus induced gene silencing in tomato. *The Plant Journal* 31, 777-786.

Matsuo, K. and Matsumura, T. (2011) Deletion of fucose residues in plant N-glycans by repression of the GDP-mannose 4, 6-dehydratase gene using virus induced gene silencing and RNA interference. *Plant biotechnology journal* 9, 264-281.

Muangsan, N., Beclin, C., Vaucheret, H. and Robertson, D. (2004) Geminivirus VIGS of endogenous genes requires SGS2/SDE1 and SGS3 and defines a new branch in the genetic pathway for silencing in plants. *The Plant Journal* 38, 1004-1014.

Muruganantham, M., Moskovitz, Y., Haviv, S., Horesh, T., Fenigstein, A., du Preez, J., Stephan, D., Burger, J.T. and Mawassi, M. (2009) Grapevine virusA-mediated gene silencing in *Nicotiana benthamiana* and *Vitis vinifera*. *Journal of virological methods* 155, 167-174.

Nagamatsu, A., Masuta, C., Senda, M., Matsuura, H., Kasai, A., Hong, J.S., Kitamura, K., Abe, J. and Kanazawa, A. (2007) Functional analysis of soybean genes involved in flavonoid biosynthesis by virus induced gene silencing. *Plant biotechnology journal* 5, 778-790.

Panwar, V., McCallum, B. and Bakkeren, G. (2013) Host-induced gene silencing of wheat leaf rust fungus *Puccinia triticina* pathogenicity genes mediated by the Barley stripe mosaic virus. *Plant molecular biology* 81, 595-608.

Pflieger, S., Blanchet, S., Camborde, L., Drugeon, G., Rousseau, A., Noizet, M., Planchais, S. and Jupin, I. (2008) Efficient virus induced gene silencing in Arabidopsis using a ‘one step’ TYMV derived vector. *The Plant Journal* 56, 678-690.

Purkayastha, A., Mathur, S., Verma, V., Sharma, S. and Dasgupta, I. (2010) Virus-induced gene silencing in rice using a vector derived from a DNA virus. *Planta* 232, 1531-1540.

Scofield, S.R., Huang, L., Brandt, A.S. and Gill, B.S. (2005) Development of a virus-induced gene-silencing system for hexaploid wheat and its use in functional analysis of the Lr21-mediated leaf rust resistance pathway. *Plant physiology* 138, 2165-2173.

Tao, X. and Zhou, X. (2004) A modified viral satellite DNA that suppresses gene expression in plants. *The Plant Journal* 38, 850-860.

Tuttle, J.R., Haigler, C.H. and Robertson, D. (2012) Method: low-cost delivery of the cotton leaf crumple virus-induced gene silencing system. *Plant methods* 8, 1-8.

Tuttle, J.R., Idris, A., Brown, J.K., Haigler, C.H. and Robertson, D. (2008) Geminivirus-mediated gene silencing from Cotton leaf crumple virus is enhanced by low temperature in cotton. *Plant physiology* 148, 41-50.

Xiquan, G., Robert C Jr, B., Libo, S. and Ping, H. (2011) Agrobacterium-mediated virus-induced gene silencing assay in cotton. *Journal of Visualized Experiments*.

Zhang, C., Bradshaw, J.D., Whitham, S.A. and Hill, J.H. (2010) The development of an efficient multipurpose bean pod mottle virus viral vector set for foreign gene expression and RNA silencing. *Plant physiology* 153, 52-65.

**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) |

**References**

Burch-Smith, T.M., Schiff, M., Liu, Y. and Dinesh-Kumar, S.P. (2006) Efficient virus-induced gene silencing in Arabidopsis. *Plant physiology* 142, 21-27.

Chen, J.-C., Jiang, C.-Z., Gookin, T., Hunter, D., Clark, D. and Reid, M. (2004) Chalcone synthase as a reporter in virus-induced gene silencing studies of flower senescence. *Plant molecular biology* 55, 521-530.

Chung, E., Ryu, C.M., Oh, S.K., Kim, R.N., Park, J.M., Cho, H.S., Lee, S., Moon, J.S., Park, S.H. and Choi, D. (2006) Suppression of pepper SGT1 and SKP1 causes severe retardation of plant growth and compromises basal resistance. *Physiologia plantarum* 126, 605-617.

Gould, B. and Kramer, E.M. (2007) Virus-induced gene silencing as a tool for functional analyses in the emerging model plant Aquilegia (columbine, Ranunculaceae). *Plant methods* 3, 6.

Hileman, L.C., Drea, S., Martino, G., Litt, A. and Irish, V.F. (2005) Virus induced gene silencing is an effective tool for assaying gene function in the basal eudicot species *Papaver somniferum* (opium poppy). *The Plant Journal* 44, 334-341.

Liu, Y., Schiff, M. and Dinesh Kumar, S. (2002) Virus induced gene silencing in tomato. *The Plant Journal* 31, 777-786.

Lu, R., Martin-Hernandez, A.M., Peart, J.R., Malcuit, I. and Baulcombe, D.C. (2003) Virus-induced gene silencing in plants. *Methods* 30, 296-303.

Qu, J., Ye, J., Geng, Y.-F., Sun, Y.-W., Gao, S.-Q., Zhang, B.-P., Chen, W. and Chua, N.-H. (2012) Dissecting functions of KATANIN and WRINKLED1 in cotton fiber development by virus-induced gene silencing. *Plant physiology* 160, 738-748.

Rojas, C., Senthil-Kumar, M., Wang, K., Ryu, C., Kaundal, A. and Mysore, K. (2012) Glycolate oxidase plays a major role during nonhost resistance responses by modulating reactive oxygen species mediated signal transduction pathways. *Plant Cell* 24, 336-352.

Ryu, C.M., Anand, A., Kang, L. and Mysore, K.S. (2004) Agrodrench: a novel and effective agroinoculation method for virus-induced gene silencing in roots and diverse Solanaceous species. *The Plant Journal* 40, 322-331.

Senthil-Kumar, M., Hema, R., Anand, A., Kang, L., Udayakumar, M. and Mysore, K.S. (2007) A systematic study to determine the extent of gene silencing in *Nicotiana benthamiana* and other Solanaceae species when heterologous gene sequences are used for virus-induced gene silencing. *New Phytologist* 176, 782-791.

Senthil-Kumar, M., Lee, H.-K. and Mysore, K.S. (2013) VIGS-mediated forward genetics screening for identification of genes involved in nonhost resistance. *JoVE (Journal of Visualized Experiments)*, e51033-e51033.

Senthil-Kumar, M. and Mysore, K.S. (2011) Virus-induced gene silencing can persist for more than 2 years and also be transmitted to progeny seedlings in *Nicotiana benthamiana* and tomato. *Plant biotechnology journal* 9, 797-806.

Wege, S., Scholz, A., Gleissberg, S. and Becker, A. (2007) Highly efficient virus-induced gene silencing (VIGS) in California poppy (*Eschscholzia californica*): an evaluation of VIGS as a strategy to obtain functional data from non-model plants. *Annals of Botany* 100, 641-649.

Xiquan, G., Robert C Jr, B., Libo, S. and Ping, H. (2011) Agrobacterium-mediated virus-induced gene silencing assay in cotton. *Journal of Visualized Experiments*.

Yan, H.-x., Fu, D.-q., Zhu, B.-z., Liu, H.-p., Shen, X.-y. and Luo, Y.-b. (2012) Sprout vacuum-infiltration: a simple and efficient agroinoculation method for virus-induced gene silencing in diverse solanaceous species. *Plant cell reports* 31, 1713-1722.

Ye, J., Qu, J., Bui, H.T.N. and Chua, N.H. (2009) Rapid analysis of *Jatropha curcas* gene functions by virus induced gene silencing. *Plant biotechnology journal* 7, 964-976.

 **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) |

**References**

Fu, D.-Q., Zhu, B.-Z., Zhu, H.-L., Jiang, W.-B. and Luo, Y.-B. (2005) Virus-induced gene silencing in tomato fruit. *The Plant Journal* 43, 299-308.

Lu, R., Martin-Hernandez, A.M., Peart, J.R., Malcuit, I. and Baulcombe, D.C. (2003) Virus-induced gene silencing in plants. *Methods* 30, 296-303.

Padmanabhan, M. and Dinesh-Kumar, S.P. (2009) Virus-induced gene silencing as a tool for delivery of dsRNA into plants. *Cold Spring Harbor Protocols* 2009, pdb. prot5139.

Ryu, C.M., Anand, A., Kang, L. and Mysore, K.S. (2004) Agrodrench: a novel and effective agroinoculation method for virus-induced gene silencing in roots and diverse Solanaceous species. *The Plant Journal* 40, 322-331.

Senthil-Kumar, M., Lee, H.-K. and Mysore, K.S. (2013) VIGS-mediated forward genetics screening for identification of genes involved in nonhost resistance. *JoVE (Journal of Visualized Experiments)*, e51033-e51033.

Velásquez, A.C., Chakravarthy, S. and Martin, G.B. (2009) Virus-induced gene silencing (VIGS) in *Nicotiana benthamiana* and tomato. *Journal of visualized experiments: JoVE*.

Yan, H.-x., Fu, D.-q., Zhu, B.-z., Liu, H.-p., Shen, X.-y. and Luo, Y.-b. (2012) Sprout vacuum-infiltration: a simple and efficient agroinoculation method for virus-induced gene silencing in diverse solanaceous species. *Plant cell reports* 31, 1713-1722.