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VIGS (Virus Induced Gene Silencing)

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Abstract

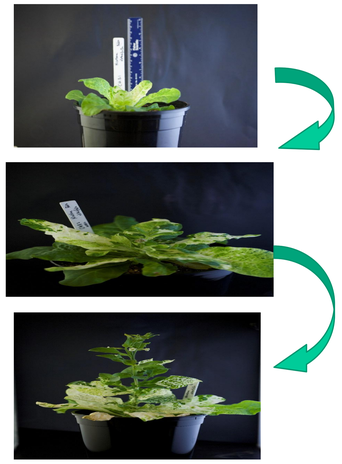
VIGS (virus induced gene silencing) is a relatively efficient new technique for insertion of plasmid vectors into plants through administration of agrobacterium via inoculation. Through study and experimentation with VIGS various methods of transmission for gene silencing have been found. The method commonly used and also the one used in our laboratory exercise was the leaf inoculation method by syringe. This method is direct in its approach where the agrobacterium with the plasmid vector holding the gene of interest is inserted into a leaf of the plant in multiple areas. The method of inoculation of leaf through syringe is used to administer agrobacterium with PDS gene vector of Ti plasmid to manipulate the coloration of the leaves of the plant species N. Obtusifolia. This method is one of many methods used to study VIGS. Vacuum infiltration provides a more efficient way of administering agrobacterium by soaking the whole plant in bacterium and then filtering out unwanted moisture through a filtration system. Agrodench method introduces agrobacterium through the root and soil. The roots from the soil absorb the agrobacterium overtime traveling from the bottom roots to the stem. Also DNA abrasion method is useful in direct contact through the tissues of the plant to also yield relatively quick results.
VIGS, PDS, plasmid, agrobacterium, inoculation, vector, transmission

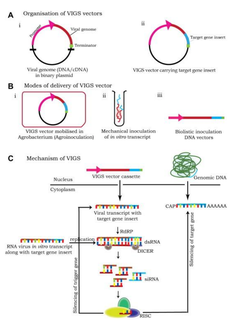
Introduction

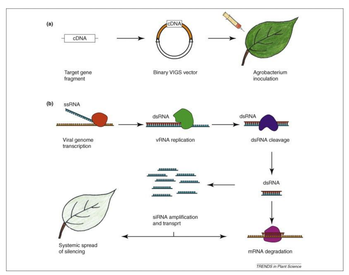
VIGS is a relatively new and unique technique used to induce gene silencing in plants. This VIGS method is a technology that exploits RNA-mediated anti-viral defense mechanism. VIGS utilizes the mechanism(s) of RNA interference (RNAi) which deals with post transcriptional gene silencing and transcriptional gene silencing that silences the gene of interest. The silencing of a gene is a consequence of degradation of RNA into short RNAs that activate ribonucleases to target homologous mRNA. This mechanism is used to bypass degradation of siRNA and successfully insert a PDS gene insert into a vector to be silenced in expression. There are various methods that each follows this same method; however the administration of the virus with the plasmid vectors into the plant is performed in many ways. The syringe method (which is the mostly used) involves the agro bacterium containing the plasmid vectors being inserted into a syringe where after the leaf is lacerated typically using a razor blade, inoculation occurs through various points at the cut of the leaf. The spray method involves administration of the virus through an airbrush device aimed at the ventral side of the leaves at a distance of about nine inches for about five seconds. This method insures more exposure to the plant but may not be as efficient depending on area of inoculation and distance. The agrodench method is concentrated in the soil. The soil adjacent to the plant root is drenched with an Agrobacterium suspension carrying the TRV-derived VIGS vectors. This method relies on the root absorption of the agrobacterium as a means of affecting the plant as a whole. This tool is a good method in which you can work on a large scale functional analysis of cDNA libraries and is rapid fast just as the vacuum infiltration method is because of the way the agrobacterium is administered and the amount absorbed per unit time. Vacuum filtration is a method that is found to transcend other methods previously used such as: leaf inoculation, and spray inoculation. The method is simple, lost cost, and rapid compared to other techniques such as leaf infiltration or agorodench. SVI (sprout vacuum infiltration) will be an effective tool to overcome the limitations of current inoculation methods such as agrobacterium exposure, time for spreading of agrobacterium from plant to plant, and effectiveness of inoculation overall. This method in turn will help to facilitate large-scale VIGS analysis.

Results/Discussion

Result were that the PDS gene was successfully silenced through syringe inoculation method. Administration of TRV (tobacco rattle virus) with plasmid vector through agrobacterium occurred by inoculation of the leaves of a collection of adult *N. Obtusifolia* plants. Over a course of 2 weeks the leaves slowly lost their pigment. However the end result was not as powerful as expected and even though the leaves did lose coloration a portion of the various sample plants were either lightly effected or not effected at all in terms of coloration of the leaves. The reasoning behind this phenomenon was the stage of development of the plants themselves and also the time of incubation for the agrobacterium. Using younger plant samples and giving adequate time incubating for incubation would have a much stronger overpowering effect on the gene silencing process. Also the method of inoculation is decent but maybe not as efficient as some of the other newer methods mentioned.







Conclusion

Virus Induced Gene silencing is very useful in studying genes function and also analysis of gene relation in conjunction with other genes. This concept is very sound in its approach and especially for using plants; this method of study can be used to explain different morphological characteristics that plants carry such as leaf coloration, symmetry, and also various parts that make up the plant such as stamen and pistil formation. This is a relatively new technique and new methods of introducing the virus with plasmid vector into the plant are being found with more efficient distribution for better results. This method is very promising for future studies and can be implemented in both small scale and large scale analysis.

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