

Epigenetics and Gene Silencing in Transgenic Plants

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Abstract: The introduction of transgenes in genetically modified plants can be silenced and also cause the silencing of endogenous plant genes. In this mechanism, silencing can occur transcriptional level and post transcriptional level but in case of silencing the endogenous genes seems predominantly post-transcriptionally. A major factor of this research to dissect the process of these sequence homology dependent gene silencing phenomena and different factors plays a role such as DNA methylation, transgene copy number as well as gene expression factors and possible modification of RNAs. Undesired transgene silencing is one of major concern in transgene technology used in crop improvements and this review gives the relationship between these factors and the bridge between transcriptional and post transcriptional gene silencing and discuss various observation correlated with silencing of gene.

Keywords: Epigenetics, gene silencing, transgenic plants, transgene.

1. INTRODUCTION

Gene silencing is defined as regulation of expression gene in a cell to prevent the exposed of a particular gene. Transgenes don't always same as expected and changing in the expression of transgenes in individual that is not because of difference in copy number. Nowadays, silencing of gene has been a confounding area of research and development course and the mechanism of gene silencing is not yet fully understood. In gene silencing process, a lot of mutations chemistry has been shown in various plant species like Para mutation. Recently the mechanism has become so much apparent that associated with the transgene-mediated silencing.

Epigenetic gene silencing is also a biological phenomenon in which the gene expression is mediated by DNA modification and RNA modification and its main mechanism is responsible for the defending the host genomes contrary to jumping genes elements and duplicated gene members as a silencer of transgenes. The epigenetic gene silencing mechanism is highly complex and not fully known yet. Transcription and post-transcriptional gene silencing mechanism mainly defined the degradation of cytosolic mRNA of endogenous genes. For plant expressing a transgene stability and segregation is main required factor (Shrawat et al., 2007; Weinhold et al., 2013).

2. GENE SILENCING AND EPIGENETICS

The main important factor associated with silencing of gene is based on number of transgene per integration site and transmission of T-DNA may enter two or more T-DNAs at the common chromosomal site (Prols and Meyer, 1992). The two main ways of gene silencing 1) transcriptional gene silencing that analyse from promoter inactivation and 2) post – transcriptional gene silencing in which promoter is active but mRNA can't accumulate. And mainly epigenetics deals with the correlation of genes and their results which gives the phenotype of a system (Waddington, 1942). In study of epigenetics, there are two main classes of transgene silencing in which first position effects related to expression of a foreign gene that is negatively regulated by flanking host DNA (Matzke et al., 2000). And the second main type of transgene silencing phenomena is related to epigenetic regulation and inactivation mechanism that can uptake multiple copies of same or homologous sequence inserted in a genome and it is also known as homology dependent gene silencing (Meyer and Saedler, 1996).

3. TRANSCRIPTIONAL LEVEL GENE SILENCING

Transcriptional level gene silencing states methylated patterns and transgenes are easily methylated to cytosine residue that is locus within CG and CHH sequences. For specific transgene, DNA methylation should be sequence specific (Matzke et al., 2007). At both transcriptional and post transcriptional levels, methylation in promoter regions, histones or any coding regions under influence the expressions of genes (Huettel et al., 2006) (Regulski et al., 2013; Tsuchiya and Eulgem, 2013). And another proper fact about transcriptional gene silencing in transgenic plants is correlated DNA methylation with structural changes and showing an increased level of chromatin condensation (Van Blokland et al., 1997). The transgenes response to environmental change was confirmed at transcriptional gene silencing (Meyer et al., 1992; Meyer, 2015).

According to transcriptional cis inactivation in plants, the transgenes penetrates into genome at random places by copy number, penetration site as well as local arrangements in every transformation process. It is stated that cis transcriptional gene silencing occurs as an outcome of combination between closely related species of transgenes that leads to the formation of secondary DNA structures which are the main sites for DNA methylation (Vaucheret and Fagard, 2001). 35 S promoter at the CpG and CpNpG sites of transgene for cytosine methylation were also showed in transgene grapevine transformed with Grapevine fan leaf virus coat protein gene (Gambino et al., 2010).

In transcriptional trans-inactivation process, the gene silencing process may predict from unidirectional effects of one particular transgene on another transgene. And silenced and methylated gene, when it is crossed with a plant in which the homologous gene in silenced position (Meyer et al., 1993). Vaucheret and Fagard (2001) and Yamasaki et al. (2011) reported that role of different genes indulged *ddm1* and *ddm2* in transcriptional gene silencing in Arabidopsis transgenic lines and also stated asymmetric cytosine methylation is enhancer region of 35 S promoter in transgenic procedure.

4. POST- TRANSCRIPTIONAL LEVEL GENE SILENCING

Under post- transcriptional level gene silencing, transcripts don't accumulate to continuous transcription (Vaucheret et al., 2001). If the genes are homologous, post transcriptional can silence both endogenous genes and transgenes and even endogenous genes can be switched off when a plant species is transformed with another copy of common gene. Transgene induced viral resistance which counteract post transcriptional gene silencing suggested it's a defense answer to check infection caused by virus (Brigneti et al., 1998; Kasschau and Carrington, 1998; Dalmay et al., 2000).

In post transcriptional gene silencing, there are cis and trans-inactivation in which cis inactivation is showed when any foreign genes like beta-Glucuronidase, neomycin etc. were regulate under strong 35S promoter (Dehio and Schell, 1994; Ingelbrecht et al., 1994; Elmayan and Vaucheret, 1996). When a 35S promoter used with double enhancer, showed post transcriptional gene silencing (Elmayan and Vaucheret, 1996; English et al., 1996). After some time, it was found that the transcriptional level was not always higher in silenced plants. The different models came for post transcriptional gene silencing were proposed RNA abundance and IRs (Baulcombe, 1996). And in cis inactivation process, DNA-DNA correlation can proceed to methylation process that can go ahead interfere with transcription and ultimately producing aberrant RNA (Wassenegger et al., 1994).

Both transgenes and endogenous genes reported in post transcriptional gene silencing termed as co-suppression (Napoli et al., 1990). Besides the efficacy of co – suppression is delayed due to not expressed of endogenous host genes (Smith et al., 1990; Vaucheret et al., 1997). Although, it can be calculated that co-suppression may not be reminded as the uni-directional silencing effect of transgenes instead of interaction of host genes and transgenes aids aberrant RNA leading to post transcriptional gene silencing.

5. METHYLATED TRANSGENES AND POST TRANSCRIPTIONAL GENE SILENCING

Methylated transgenes correlated with post transcriptional genes (Hobbs et al., 1990, 1993; Ingelbrecht et al., 1994; Smith et al., 1994; English et al., 1996). In case of sensitive plants, (Smith et al, 1994) reported that the viral transgene of the viral resistant plants was more involved in methylated process. Some examples like PTGSed *nptII* genes elaborated the positive and negative impact on methylation of the coding region (Ingelbrecht et al. (1994). Another one is PTGSed *uidA* genes in the tobacco plants were more methylated near the 3' end of the gene which included the poly-adenylation region Hobbs et al. (1990, 1993). English et al, 1996 explained more evidence that seems to be a bridge between DNA methylation and the RNA based mechanism.

6. MECHANISM CORRELATED WITH DELETION OF HOMOLOGOUS TRANSCRIPTS

According to RNA threshold model if the post transcriptional gene silencing induced, the cells those are capable to measure the levels of specific RNAs. And RNA controlled activity doesn't exist in normal cells or modified by the transgenes, may be as a sort of defense mechanism against the maximum harmful effects of these foreign DNAs. The complementary RNAs synthesized by the plant-encoded RNA dependent RNA polymerase in the cytoplasm (Lindbo et al., 1993; Dougherty and Parks, 1995). It is stated that these complementary mRNAs which are destructed by ds-RNA specific RNases. The explanation of the RNA dependent RNA polymerase / cRNA model is that it explains the strong sequence specificity of post transcriptional gene silencing.

7. SILENCING CHARACTERISTICS IN TRANSGENIC PLANTS

In plants, mainly micro RNAs are produced from precursor RNA which is necessary for biogenesis of siRNAs and in regulation of gene expression, miRNA is involved by base-pairing with target RNAs leading to their cleavage in plants. An important regulatory role of micro RNAs could be conserved in other plant species too which are under consideration and other important class of small RNAs is siRNAs that are involved in epigenetic modification (Miki and Shimamoto, 2008). The initiation stage of transgene silencing process has been involving in the generation of double stranded RNA and still under debate about important major factors in transgenic perspective. Therefore, against RNA silencing, the transgenes are generally more sensitive than endogenous genes in plants. And most of viral RNA's contain specific sequences for triggering transgene (De Haan et al., 1992; Lindbo et al., 1993; Mueller et al., 1995).

8. CONCLUSION

The epigenetic effects as well as silencing mechanism, the role of transgenic technologies have played a major role in achievements and behind various types of gene silencing mechanism involved in siRNA and miRNA. And the various observations correlated with gene silencing independent pathways. The RNA dependent RNA polymerase model gives many predictions related to production of cRNAs and to generate the mutants in that RNA dependent RNA polymerase genes are knocked out by these plants and also induce the post transcriptional gene silencing process.

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