

Role of Gene Silencing In Agriculture



Biotechnology

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ABSTRACT

Gene silencing play role of suppressing gene expression in plants. The ability of gene silencing has produced transgenic plants able to switch off endogenous genes and invading nucleic acids. Mechanism for gene silencing through at the transcriptional level (TGS) or at the post-transcriptional level (PTGS). It play a key role in many essential cellular processes. RNA interference (RNAi) is an evolutionarily conserved mechanism that causes sequence specific gene silencing. Though biologically evolved to mediate resistance to both endogenous and exogenous pathogenic nucleic acids and to regulate the expression of protein coding genes, it is being successfully used as a tool for functional genomics. This powerful Agricultural tool has provided plant breeders and researchers with great opportunity to accelerate breeding programs and developmental studies in agriculture.

INTRODUCTION

Gene silencing is describing as epigenetic processes of gene regulation. Gene silencing is a technique used to turn down or switch off the activity of genes by a mechanism other than genetic modification. That is, a gene which would be expressed (turned on) under normal circumstances is switched off by machinery in the cell. The discovery of mechanism of RNA interference by Prof. Andrew Fire and Prof. Craig Mello in 1998, gave them the Nobel Prize in 2006. Their ever-growing list of potential applications has contributed to the rapid growth of this technique and to the understanding of its biology.

Gene silencing (GS) is defined as a molecular process involved in the down regulation of specific genes, The mechanisms of Gene silencing that suppress gene activity in plants has extended that control of gene expression (Mansoor et al., 2006). Currently, there are several routes of GS identified in plants, such as: transcriptional gene silencing and post transcriptional (PTGS or RNAi) gene silencing, microRNA silencing and virus induced gene silencing. All these pathways play an important role at the cellular level, affecting gene regulation and protection against viruses and transposons (Waterhouse et al., 2001). The post-transcriptional gene silencing involves breakdown of the mRNA itself by various techniques like Ribozymes, antisense RNA, DNazymes and RNA interference (RNAi). Among all these techniques RNA interference has emerged as most potent tool to effect targeted gene silencing and is being used to determine the function of genes which are expressed in a constitutive or cell-fate dependent manner.

Discovery of gene silencing

Jorgensen and colleagues in (1990) were trying to dark purple colour by introducing a chalcone synthase gene in *Petunia* under a strong promoter, they found that instead of getting dark purple colour most of the flowers lost their colour. They termed this phenomenon of suppression of both the homologous endogenous gene and the transgenic copy of the gene as co-suppression. This phenomenon of suppression of endogenous gene by transformation with homologous sequences was also observed in the fungi *Neurospora crassa* where it was called as quelling (Hammond et al., 2001). Guo and Kamphuis (1995) tried to knock down *par-1* gene expression in *C. elegans* by antisense RNA, they found similar loss of expression in their sense RNA controls also. In 1998, Andrew Fire, Craig C. Mello and colleagues for the first time showed potent and specific genetic interference by double-stranded RNA in the nematode *Ceanorhabditis elegans*. The RNAi tool has also been used in *Drosophila* to mediate specific gene silencing in the flies, embryo extracts as well as cultured cells.

Transcriptional gene silencing

Transcriptional gene silencing is the result of histone modifications. Methylation of DNA and remodeling of chromatin play significant role in transcriptional gene silencing (TGS). Increase in DNA methylation possibly induces formation of heterochro-

matin, It promotes protein binding that recognizes methylated cytosine, leading to chromatin remodeling which is associated to TGS thus avoiding the binding of transcription factors (Wasenegger, 2002).

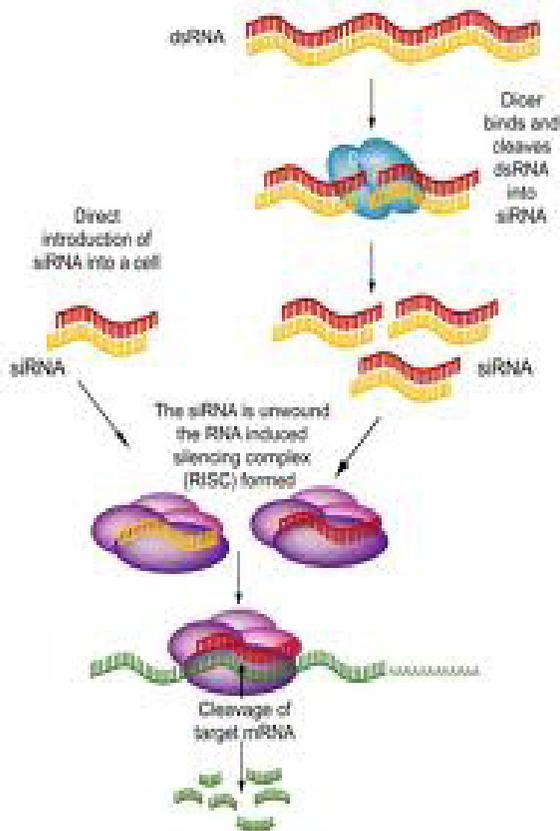
In plants, genes insert into the genome at randomly by recombination so that the number of inserted copies, their chromosomal location and their local arrangement vary between one transformant and another. An inverse correlation between copy number and the level of gene expression which responsible for increasing the number of copies of a particular gene can lead to gene silencing. Genes within loci are hypermethylated and have an increased resistance to DNase I digestion. It is assumed that TGS occurs in pairing between closely linked copies that leads to the formation of secondary DNA structures that attract methylation and heterochromatin components. However, these analyses did not determine whether hypermethylation is a cause or a consequence of TGS. Occasionally, single copies of a gene are subject to TGS. It is assumed that this results from large discrepancies between the GC content of the gene and that of the surrounding genomic sequences leads to silencing.

Post-transcriptional gene silencing

Post-transcriptional gene silencing is the result of mRNA of a particular gene being destroyed or blocked. The destruction of the mRNA prevents translation to form an active gene product. A common mechanism of post-transcriptional gene silencing is RNAi.

Mechanism of Gene Silencing

The RNA interference pathway starts with the presence of dsRNA in the cytoplasm that vary in length and origin. The active molecules which effect the RNA interference-mediated gene silencing are siRNAs (small interfering RNAs). So the first step in the RNAi process is the cleavage of long dsRNA into 21- to 25-nucleotide long pieces of siRNAs. This process occurs in the cytoplasm and is catalyzed by the enzyme Dicer. These siRNAs are incorporated into the multiprotein silencing complex called RNA-induced silencing complex (RISC). The siRNA duplex is then unwound and the RISC complex is remodeled in an active conformation. The next step of the process is the recognition of the mRNA to be degraded. This recognition step is mediated by antisense RNA which acts as guide strand for the RISC complex mediated endonucleolytic cleavage of the homologous mRNA. According to them the target mRNA is cleaved in the centre of the region recognized by the sequence complimentary guide siRNA, 10-12 nt from the 5' end of siRNA. The last step in the RNAi process is the amplification of the siRNA molecules itself. It has now been well characterized that a next generation of siRNAs is generated from the priming of the RNA dependent RNA polymerase (RdRp) on the target mRNA by existing siRNAs, (Denli and Hannon, 2003).



APPLICATIONS

Recent studies of gene silencing in crops have revealed two RNA-mediated epigenetic processes, RNA-directed RNA degradation and RNA-directed DNA methylation. These natural processes have provided new avenues for developing high-throughput technology for gene suppression in plants.

Virus induced gene silencing for gene function studies in plants

VIGS can be defined as the silencing of endogenous plant genes initiated by recombinant viral vectors (Ruiz et al., 1998). It is a technique designed to suppress gene expression and study gene function in plants. The model includes viral dependent initiation and viral independent maintenance of VIGS. The approach consists of cloning and inserting plant endogenous gene sequences in recombinant viral vectors, which are then inoculated in a young plant, and in a few weeks natural defense mechanisms of the plant directed at suppressing virus replication also result in specific degradation of mRNAs from the endogenous plant gene that is targeted for silencing. VIGS is rapid (3-4 weeks from infection to silencing), does not require development of stable transformants, allows characterization of phenotypes that might be lethal in stable lines, and offers the potential to silence either individual or multiple members of a gene family. tool for assessing and characterizing the function of plant genes, (Fitch, 1992). In early approaches on VIGS studies, gene sequences were individually subcloned into viral genomes, and plants were physically inoculated using viral RNA produced by in-vitro transcriptional reactions. It is an easier method for plant infection would be the use of agroinfiltration of viral cloned vectors (Robertson, 2004). VIGS protocols can be optimized by the insertion of viral RNA genomes in binary vectors for agroinfiltration. VIGS is a powerful tool due to its fast initiation of silencing in intact wild-type or transgenic plants. It is possible to silence genes in mature plants using this technique. Therefore, no plant transformation is necessary since the silencing is induced by the viral infection. The limitation of this approach resides in the viral host range, pattern of viral spreading, and symptoms caused by the infection, (Ratcliff et al., 2001).

Gene silencing and plant disease resistance

Crops are particularly susceptible to virus diseases. Once infected by viruses, these plants must be rouged. Virus disease resistance in plants may be achieved by several approaches, including coat protein mediated protection, antisense RNA, replicase mediated protection, PTGS, and pathogen derived resistance (PDR). The concept of PDR suggests that the resistance to a determined pathogen could be obtained from its own genetic material. Currently, RNAi is the major strategy in plant transformation for virus resistance. *Citrus tristeza virus* (CTV) -resistant citrus plants, expressing the coat protein of the virus has been obtained. In this work, virus resistance was related to the accumulation of the transgenic viral protein in the plant tissue. The mechanism proposed was pathogen derived resistance. Signs of PTGS could be observed within the resistant plants such as high level of transgene transcription in the nucleus, low levels of transgene mRNA in the cytoplasm. PTGS seems to be the best explanation for these results since resistant citrus plants had multiple copies of the transgene, low levels of the corresponding mRNA, methylation of the silenced transgene, (Fagoaga et al., 2006).

Slow fruit tomato softening

Enzyme polygalacturonase (PG) degrades pectin which leads to softening of fruits and deterioration in fruit quality. Transgenic tomato contains antisense construct of the gene coding PG, show a drastically reduced expression of PG and markedly slower fruit softening. In USA, transgenic tomato marketed under the name of 'FlavrSavr'.

This technology use to suppress ethylene biosynthesis in tomato. ACC synthase is responsible for the ethylene biosynthesis so the antisense construct of ACC synthase reduces the ethylene production to <1% of the normal. So the fruit remain green and hard on the plant and accumulate more sugars and organic acids that make the fruit sweeter and richer in taste. The new transgenic variety 'Endless Summer' developed by gene silencing technology.

Changed fatty acid composition of Brassica oil

The enzyme stearoyl- ACP desaturase catalyzes the conversion of stearoyl - ACP to oleoyl - ACP, which is the first desaturation step in seed fatty acid biosynthesis. Transgenic *Brassica rapa* and *Brassica napus* plants containing the antisense gene construct of stearoyl - ACP desaturase encoding gene have been produced, this is linked to seed specific promoter (napin or ACP promoter) to ensure its expression only in seeds. So the transgenic plant showed the highly reduced stearoyl - ACP desaturase activity, a dramatic increase in the level of stearic acid (from <2% to up to 40%), and an associated decline in oleic acid level in their seeds.

Gene silencing for male sterility

Flavonoids are essential for pollen development and function and its deficiency prevents pollen maturation. Chalcone synthase (CHS) is key enzyme of flavonoid biosynthesis. In *Petunia* antisense construct of the gene encoding CHS has been transferred, these plants shows negligible CHS activity and non-functional pollen. These pollen grains become functional when flavonoids apply. So it is applied during pollination of the CHS antisense male sterile (MS) lines to obtain 100% male sterile progeny.

APPLICATIONS OF GENE SILENCING IN ANIMAL AGRICULTURE

Recently aided by genomic selection tools, has been successful in achieving remarkable progress in livestock improvement. However, genetic selection has led to decreased genetic diversity and, in some cases, acquisition of undesirable traits. Future increases in productivity may be dependent upon the acquisition of genetic traits not currently encoded by the genomes of animals used in standard agricultural practice, thus making classical genetic selection impossible. The technology of activating the RNAi pathway and the application of this technology to enhance livestock production through increased production efficiency and prevention of disease. An increased demand for

sustainable food production is at the forefront of scientific challenges (Elbashir, 2001).

GENE SILENCING AND WOOD QUALITY, FRUIT QUALITY AND OTHER TRAITS

The production of plant material with lower contents of lignin would mean a significant reduction of cost and pollution. One of the approaches to obtain reduced lignin forest trees has been the down regulation of lignin biosynthesis pathways (Hu et al., 1999). In some plants, self-incompatibility stands as a major problem in fruit set and breeding programs. Broothaerts et al. (2004), reported the production of transgenic apple trees able to self pollinate and develop fruit. This break through was achieved by silencing of the S-gene responsible for self-incompatibility. The self-compatible transgenic plants lacked the pistil S-RNase protein, which is the product of the S-gene.

Fruit quality has also been addressed by silencing experiments. Several characteristics are involved in fruit quality. Transgenic apple fruits silencing key enzymes involved in autocatalytic ethylene production were significantly firmer and displayed an increased shelf-life (Dandekar et al., 2004). In *Citrus*, the down regulation of putative thermostable pectin methylesterase genes is projected as a possible solution for the undesirable separation of juice into clear serum and particulate phase. In this case, RNAi could be used to achieve this goal. (Guo et al., 2005).

Significance of Gene silencing

1. Gene silencing represents an "immune defense" of the genome. Close to 50% of our genome consists of viral and transposon elements that have invaded the genome in the course of evolution.
2. Gene silencing represses protein synthesis and regulates the development of the organism.
3. Gene silencing protects against viral infections, in plants, worms and flies. Its relevance, for vertebrates including humans is to be clarified.

4. Targeted gene silencing can be used to study the function of individual genes, in cultured cells as well as in transgenic organisms.
5. Gene silencing via specifically designed oligonucleotides might be a useful approach in future gene therapy.
6. Gene silencing secures genome stability by keeping mobile elements silent.
7. Gene silencing keeps chromatin condensed and suppress transcription, and regulates the activity of genes in the immediate vicinity of the condensed blocks of chromatin, through histone modification.

Disadvantages

It is still difficult to express aRNA only in targeted tissues. as it is right now, aRNA sometimes binds to mRNA that is not its target. Precision gene therapy using aRNA needs to be improved because the Antisense technology has not been perfected. Uptake of aRNA is still imprecise. Expensive and needed in large doses. Synthetic oligonucleotides have sometimes aptamers effects and may cause toxic side effects. We should be able to deliver intact Antisense RNA or DNA as a chemical to all affected cells within a patient and not just to a few cell in culture.

Conclusions

Fast developments in Gene silencing technique have made its use for crop improvement. Gene silencing has already begun to revolutionize the functional genomics and experimental biology in organisms. It can however, also be used as a potential biological warfare tool to switch off the expression of any gene in an organism. Keeping such multifaceted risks aside, the enormous potential of RNAi can be used for the service of mankind. Today dsRNA is used as a powerful tool to experimentally elucidate the function of essentially any gene in a cell.

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