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Review article

Application of RNA interference in plants

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ABSTRACT

Gene silencing can occur either through repression of transcription, termed transcriptional gene silencing (TGS), or through mRNA degradation, termed post-transcriptional gene silencing (PTGS). TGS results in reduction of transcription whereas PTGS results in sequence specific mRNA degradation in cytoplasm without dramatic changes in transcription of corresponding gene in nucleus. Both TGS and PTGS are used to regulate endogenous genes. Interestingly, mechanisms of gene silencing also protect the organism's genome from transposons, viruses. In this paper, molecular aspects and mechanisms of gene silencing in plant were reviewed. Finally, its applications were discussed.

Keywords: Gene silencing, miRNA,siRNA

Abbreviations: miRNA- microRNA ; PTGS- post-transcriptional gene silencing-; RNAi -RNA interference ; siRNA- small interfering RNA; TGS- transcriptional gene silencing

Introduction

RNA silencing is a novel gene regulatory mechanism that limits the transcript level by either suppressing transcription (TGS) or by activating a sequence- Specific RNA degradation process [PTGS/RNA interference (RNAi)] (Agrawal *et al*., 2003). During the 1990s, a number of gene silencing phenomena that occur at the post-transcriptional level were discovered in plants, fungi, animals and ciliates (Baulcombe, 2000; Matzke *et al*. 2001). The silencing effect was first observed in plants in 1990, when the Jorgensen laboratory introduced exogenous transgenes into petunias in an attempt to up-regulate the activity of a gene for chalcone synthase, an enzyme involved in the production of specific pigments (Agrawa *et al*., 2003; Napoli *et al*., 1990). Unexpectedly, flower pigmentation did not deepen, but rather showed variegation with complete loss of color in some cases. This indicated that not only were the introduced trangenes themselves inactive, but that the added DNA sequences also affected expression of the endogenous loci (Hannon, 2002). This phenomenon was referred to as "co-suppression" (Napoli *et al*., 1990; Campbell, 2005). A similar phenomenon in the fungus *Neurospora crassa* was named quelling (Romano *et al*., 1992; Cogoni *et al*., 1996; Fire *et al*., 1998) identified a related mechanism, RNA interference (RNAi) in animals. The natural function of RNAi is referring to the mechanism involved in cellular defense against viruses, genomic containment of retrotransposons, and post-transcriptional regulation of gene expression. RNAi can specifically silence individual genes, creating knockout phenotypes, either in transformants that can produce the required hairpin RNAs, or upon infection with recombinant RNA viruses that carry the target gene (VIGS, viral-induced gene silencing) (Tenea, 2009). RNAi is a multistep process involving the generation of small interfering RNAs (siRNAs) *in vivo* through the action of the RNase III endonuclease 'Dicer'. The resulting 21- to 23-nt siRNAs mediate degradation of their complementary RNA (Zou *et al*., 2005; Shi, 2003). Here, the mechanism of RNAi was reviewed and then some of its applications in plants discussed (Table 1).

Mechanism of RNAi

There are two small RNAs in the RNAi pathway: small interfering RNAs (siRNAs) and microRNAs (miRNAs) that are generated via processing of longer dsRNA and stem loop precursors (Bernstein *et al*., 2001; Hammond *et al*., 2000; Stevenson, 2004). Dicer enzymes play a critical role in the formation of these two effectors of RNAi (Elbashir *et al*., 2001). They can cleave long dsRNAs and stem loop precursors into siRNAs and miRNAs in an ATP-dependent manner, respectively. The biogenesis of miRNAs is a multi-step process (Hammond *et al*., 2001). A primary miRNA transcript (pri-miRNA) (Yin *et al*., 2002), which is frequently synthesized from intronic regions of protein-coding RNA polymerase II transcripts (Novina *et al*., 2002; Tijsterman *et al*., 2004), is first processed by a protein complex containing the double-strand specific ribonuclease Drosha in the nucleus to produce a hairpin intermediate of 70 nucleotide (nt) (Hamilton *et al*., 1999). This precursor miRNA (pre-miRNA) is subsequently transported by Exportin-5/Ran-GTP (Hamilton *et al*., 1999; Mette *et al*., 2000) to the cytoplasm where it is cleaved by another dsRNA specific ribonuclease, Dicer, (Jones *et al*., 2001; Tang *et al*., 2007) into miRNA duplexes. After strand separation of the duplexes, the mature single-stranded miRNA is incorporated into an RNA-induced

silencing complex (RISC)-like ribonucleoprotein particle (miRNP) (Hamilton *et al*., 1999; Wassengger *et al*., 1994; Mette *et al*., 2000). This complex inhibits translation or, depending on the degree of Watson-Crick complementarity, induces degradation of target mRNAs (Jones *et al*., 2001; Tang *et al*., 2007). Exogenous synthetic siRNAs can also be incorporated into the RNA-induced silencing complex (RISC), thereby bypassing the requirement for dsRNA processing by Dicer. siRNAs are incorporated into the multi-protein RISC. A helicase in RISC unwinds the duplex siRNA, which then pairs by means of its unwound antisense strand to messenger RNAs (mRNAs) that bear a high degree of sequence complementarity to the siRNA **(**Stevenson, 2004). An as yet unidentified RNase (Slicer) within RISC proceeds to degrade the mRNA at sites not bound by the siRNA. Cleavage of the target mRNA begins at single site 10 nucleotides upstream of the 5'-most residue of the siRNA–target mRNA duplex (Elbashir *et al*., 2001). Although the composition of RISC is not completely known, it includes members of the Argonaute family (Hammond *et al*., 2001) that have been implicated in processes directing post-transcriptional silencing **(**Stevenson, 2004).

Application of RNAi for improvement of crop and plant nutritional value

Lysine synthesis is strongly regulated by a feedback inhibition loop in which lysine inhibits the activity of dihydrodipicolinate synthase (DHPS), the first enzyme on the pathway specifically committed to lysine biosynthesis. Genetic mutations in the tobacco DHPS gene, rendering its encoded DHPS lysineinsensitive causes lysine overproduction in all plant organs. However, although high lysine levels in seeds are beneficial, increases in the level of this amino acid in vegetative tissues are undesirable, because high levels of lysine cause abnormal vegetative growth and flower development that, in turn, reduces seed yield (Frankard *et al*., 1992; Negrutiu *et al*., 1984). Because lysine accumulation in plants is negatively affected by its catabolism (degradation), constitutive knockout of lysine catabolism using a gene insertion knockout approach accelerates lysine accumulation in seeds when combined with the seed-specific expression of a feedback-insensitive DHPS (Zhu *et al*., 2003). However, seeds of plants that have accumulated elevated lysine levels germinate poorly because the excess lysine levels produced in the seeds are not efficiently degraded during seed germination (Zhu *et al*., 2003). Reduction of lysine catabolism specifically during seed development by an RNAi approach indeed improves seed germination (Zhu *et al*., 2004; Tang *et al*., 2007). In another study, RNAi has been successfully used to generate a dominant high-lysine maize variant by knocking out the expression of the 22-kD maize zein storage protein,a protein that is poor in Lysine content (Segal *et al*., 2003)and RNAi generates quality and normal maize seeds with high levels of lysine-rich proteins (Tang *et al*., 2004).

Barley and Rice

Bayer Crop Science has acquired an exclusive worldwide license to develop, market, and sell selected crop plant varieties in which the RNAi technology has been successfully applied by the CSIRO scientists. Using this technique this group has developed varieties of barley that are resistant to BYDV (barley yellow dwarf virus) (Wang *et al*., 2000). Their results showed that the barely plants developed through RNAi technology are resistant to viral infection while the control plants became infected with the yellow dwarf virus. Kusaba and his team (Kusaba *et al*., 2003) have made significant contribution by applying RNAi to improve rice plants. They were able to reduce the level of glutenin and produced a rice variety called LGC-1 (low glutenin content 1). The low glutenin content was a relief to the kidney patients unable to digest glutenin. The trait was stable and was transmitted for a number of generations. They showed that the procedure may apply to both monogenic and polygenic agronomic characters (Williams *et al*., 2004).

Banana

Another instance where RNAi may be fruitfully applied is in the production of banana varieties resistant to the Banana Bract Mosaic Virus (BBrMV), devastating the banana population in Southeast Asia and India (Rodoni *et al*., 1999). In certain years, the entire banana crop in certain areas is lost due to the attack by the above virus. The BBrMV infects banana plants destroying the fruit producing bract region, rendering them useless to farmers. The virus is spread by small plant eating insects called aphids, as well as through infected plant materials. The problem is further compounded when further banana crops are raised in the infected field because the infection spreads from the previous diseased crop. However, by carefully designing an RNAi vector aimed at silencing the Coat Protein (CP) region of the virus, scientists may be able to develop a banana variety that is resistant to BBrMV and yet safe to eat. The CP region of the different strains of virus is highly conserved and as such silencing of this gene in other varieties of banana will not pose a problem. Another novel approach here would be to utilize an inducible promoter system in order that dsRNA is produced only upon infection and not constitutively (Williams *et al*., 2004).

Cotton

Another nutritionally important crop is cotton. Cotton is mainly used for fiber production, and is an important crop not only in developed countries, but also in many developing countries where malnutrition and starvation are widespread (Sunilkumar *et al*., 2006). In these developing areas, the cottonseeds that remain after fiber extraction could be extensively used as sources of protein and calories, but they are largely underutilized because they contain a toxic gossypol terpenoid. Gossypol is also produced in vegetative cotton tissues where it protects cotton plants from insects and other pathogens (Sunilkumar *et al*., 2006). Hence, the suppression of its production should occur specifically in cottonseeds. Indeed, transgenic cotton plants expressing a RNAi construct of the d-cadinene synthase gene of gossypol synthesis fused to a seed-specific promoter caused seed-specific reduction of this metabolite, while its content in non-seed tissues was comparable to the control plants (Sunilkumar *et al*., 2006). These cotton plants are thus expected to have similar insect and pathogen resistance to that of wild type cotton, but to produce seeds with higher nutritional value (Tang *et al*., 2007).

Jute

A possible application of RNAi involves the down regulation of a key enzyme in the biosynthetic pathway of lignin in the two economically important Corchorus species, namely, C. capsularis and C. olitorius. The enzyme 4-coumarate: CoA ligase (4-Cl) is one of the key enzymes in the early stages of lignin biosynthesis. This makes it a promising target for regulating the quantity of lignin, produced in the jute plant.

With the availability of the sequence of the 4-Cl gene, it would be possible to create a transgenic jute variety expressing the RNAi construct to down regulate the quantity of 4-Cl mRNA thereby reducing the lignin production. With this approach, it would also be possible to vary the quantity of lignin synthesis by the help of different promoters and altering the length of interfering RNA. Thus RNAi technology may prove to be a powerful molecular tool by generating jute varieties with low lignin content, allowing for easier, environmentally friendly and cost effective processing of fiber for the production of various economically important commodities such as high quality paper and cloth (Williams *et al*., 2004).

Increasing grain amylose conten

Foods rich in inefficiently digested carbohydrates, such as fiber, are considered to be health promoting (Williams, 1995). The major nutritional source of plant-derived carbohydrates is starch, which is composed of amylopectin and amylose polysaccharides, synthesized by two competitive pathways. Yet, in cooked plant foods undergoing cooling before eating, amylose molecules tend to efficiently form digestion-resistant complexes that are part of healthy dietary fiber (Crowe *et al*., 2000). Aiming to increase the relative content of amylose in wheat grains, a RNAi construct designed to silence the genes encoding the two starch-branching isozymes of amylopectin synthesis, were expressed under a seed-specific promoter in wheat (Regina *et al*., 2006). This resulted in increased grain amylose content to over 70% of the total starch content (Tang *et al*., 2007).

Latyrus sativus

In Ethiopia, Bangladesh and India, the people in the lower socioeconomic class use a leafy vegetable known as Lathyrus sativus. It is a leguminous crop and contains a neurotoxin called β-oxalylaminoalanine-L-alanine (BOAA) (Spencer *et al*., 1986). People consuming this vegetable suffer from a paralytic disease called, lathyrism. The disease paralyses people both temporarily and permanently, however the effects can be somewhat reduced if the plant is boiled prior to consumption. Paralysis in the limbs is a known symptom of BOAA, yet people still consume this vegetable in times of famine. This is an instance where RNAi technology can be used to silence the gene(s) responsible for production of BOAA. There may be one difficulty; in that the BOAA genes may be linked to genes, which confer immunity to this unique crop or impart drought and flood tolerance. Bringing down the levels of BOAA to a safe concentration, rather than totally silencing the concerned genes, may overcome this obstacle (Williams *et al*., 2004).

Tomato

A significant part of human diets both in developed and developing countries is composed of vegetables. Among the vegetables, tomato fruits are relatively rich in a number of vitamins as well other health promoting metabolites, such as flavonoids and carotenoids, including the strong antioxidant carotenoid, or lycopene, which provides the tomato fruit with its typical red color. Carotenoids are synthesized by the same biosynthetic pathway that synthesizes chlorophyll, and it has been shown that genes controlling the light-mediated regulation of the photosynthetic machinery also influence tomato fruit quality by altering the levels of carotenoids and flavonoids (Adams-Phillips *et al*., 2004). The tomato high pigment (hp-2) phenotype, which accumulates elevated levels of carotenoids and flavonoids, is due to mutations in the regulatory gene DE-ETHIOLATED1 (DET1), which represses several light-dependent signaling pathways (Levin *et al*., 2003; Mustilli *et al*., 1999). Despite their positive effects on fruit quality, hp-2 mutants generally possess abnormal growth and various vegetative phenotypes and so are transgenic plants in which the DET1 gene is constitutively silenced (Davuluri *et al*., 2004; Levin *et al*., 2003; Mustilli *et al*., 1999). Yet, RNAi-mediated suppression of DET1 expression under fruit-specific promoters has shown to improve carotenoid and flavonoid levels in tomato fruits with minimal effects on plant growth and other fruit quality parameters (Davuluri *et al*., 2005) . This exemplifies again how coupling the highly efficient RNAi gene suppression machinery with tissue specific promoters provides a highly valuable trait that is impossible to obtain by conventional breeding (Tang *et al*., 2007).

Coffee

10% of the coffee on the world market is shared by decaffeinate coffee (DECAF). Decaf is wanted by buyers which are sensitive to caffeine. A standard cup of filter coffee generally contains between 60mg and 150mg of caffeine, while concentrations in instant coffee are generally lower. A single espresso contains about 100mg, while decaffeinated coffee has about 2mg to 4mg per cup. Caffeine is a stimulant of the central nervous system, the heart muscle and the respiratory system, and has a diuretic effect. Its adverse side-effects include insomnia, restlessness and palpitations. Decaf is obtained from natural coffee by several ways: by using water or solvent extraction. RNAi technology has enabled the creation of varieties of *Coffee* that produces natural coffee with low or very low caffeine content, thus by-pass the need of extraction (Van Uyen, 2006).

Flower color modification of plants by RNAi-mediated gene silencing

Gentian

Gentians, *Gentiana triflora*, *Gentiana scabra* and their interspecific hybrid, are one of the most popular floricultural plants in Japan, and more than half of gentian production is from the Iwate prefecture. Gentians come into bloom from early summer to late autumn in Japan, and are often used as ornamental cut flowers. Genetic engineering approaches are being applied to several ornamental plants (Forkmann *et al*., 2001; Tanaka *et al*., 1998; Tanaka *et al*., 2005). For example, Florigene Ltd. and Suntory Ltd. have developed blue-flowered carnations using genetic engineering, and they are commercialized in North America, Australia and Japan (Tanaka *et al*., 2005). We have also produced white-flowered transgenic gentians by suppressing the chalcone synthase (*CHS*) gene using antisense technology (Nishihara *et al*., 2006). In this case, only 3 of 17 independent transgenic lines displayed white-flowered phenotypes, but other transformants did not lead to successful suppression of *CHS* gene expression. Moreover, no transgenic gentian plants with suppressed expression of other anthocyanin biosynthetic genes, such as dihydroflavonol 4-reductase (*DFR*) and flavonoid 3_,5_-hydroxylase (F3_5_H) genes, have so far been obtained by antisense and sense suppression technology (Nishihara *et al*., 2005). The low frequency of down-regulation of the targeted genes was thought result from the use of both the cauliflower mosaic virus (CaMV) 35S promoter and antisense gene suppression technology. Gene silencing by RNAi was also utilized to modify the flower

Table 1. Application of RNAi interference in plant systems

Application	Case study	Authors
Increasing the level of lysine	Reduction of lysine catabolism and improving seed germination generating a dominant high-lysine maize variant by knocking out the expression of the 22-kD maize zein storage protein	Zhu *et al*., Tang *et al.,* Segal *et al.*
Barley and Rice	Resistance of barley to BYDV and producing a rice variety called LGC-1 (low glutenin content 1) by RNAi technology	Wang *et al*., Kusaba *et al*. Williams *et al.*
Banana	Production of banana varieties resistant to the Banana Bract Mosaic Virus (BBrMV) by RNAi	Rodoni et al.
Cotton	Transgenic cotton plants expressing a RNAi construct of the d-cadinene synthase gene of gossypol synthesis fused to a seed-specific promoter caused seed-specific reduction of Gossypol	Sunilkumar et al.
Jute	Generating jute varieties with low lignin content by RNAi technology	Williams et al.
Lathyrus sativus	RNAi construct designed to silence the genes encoding the two starch-branching isozymes of amylopectin synthesisRNAi technology can be used to silence the gene(s) responsible for production of BOAA	Regina et al.
Tomato	RNAi-mediated suppression of DET1 expression under fruit-specific promoters has recently shown to improve carotenoid and flavonoid levels in tomato fruits with minimal effects on plant growth	Williams et al.
Coffee	RNAi technology has enabled the creation of varieties of *Coffee* that produces natural coffee with low or very low caffeine content	Davuluri et al.
Flower color modification		
Gentian	Producing white-flowered transgenic gentians by suppressing the chalcone synthase (*CHS*) gene using antisense technology	Van Uyen
Blue Rose	Producing blue transgenic rose by knock -downing the cyanidin genes in rose and carnation by RNAi technology and introduce delphinidin genes	Nishihara et al.
Flowering time		Van Uyen
Healthier oil	Using RNAi to silence the gene in cotton which codes for the enzyme that converts oleic acid into a different fatty acid	Waterhouse et al.
Pest control	Combining Bt technology with RNAi would both enhance product performance and further guard against the development of resistance to Bt proteins	Goldstein et al
Wood and fruit quality	-Down regulation of lignin biosynthesis pathways. -Producing transgenic hypoallergenic apples and a possible solution for the undesirable separation of juice into clear serum and particulate phase by using RNAi	Hu *et al*., Teo *et al*., Amancio José de Souza *et al*.

color in some plant species, including petunia, torenia and tobacco plants (Tsuda *et al*., 2004; Nishihara *et al*., 2005; Nakamura *et al*., 2006; Nakatsuka *et al*., 2007a; Nakatsuka *et al*., 2007b; Nakatsuka *et al*., 2008).

Blue Rose

Among anthocyanins precursors for all plant pigments, there are cyanidin, pelargonidin and delphinidin. The cyanidin gene is responsible for a synthethic pathway that leads to formation of red pigment and a correspondent Delphinidin gene is the key gene for formation of blue color. Scientists at Florigene (Australia) and Suntory (Japan) have been successful in knock -downing the cyanidin genes in rose and carnation by RNAi technology and introduce delphinidin genes, which, in natural condition, are absent in these two important cut flowers. The result has been spectacular to the flower industry, as from as far as 1840, the Horticultural Societies of Britain and Belgium has offered a prize of 500 000 francs to the first person to produce a blue rose (Van Uyen, 2006).

RNAi and Flowering time

One of the important aspects of crop production is flowering time. For instance, if a cereal crop flowers too early, it may have not yet made sufficient energy stores to fuel its maximum grain production. Similarly, if it flowers too late in the season there may be insufficient time to produce a good yield. So, being able to control flowering time in plants could be a very useful tool in horticulture and agriculture. In *Arabidopsis*, there is a gene called FLC which represses flowering and we have used RNAi to switch it off and bring on flowering. This clearly shows that the technology has the potential to regulate flowering time in crops **(**Waterhouse *et al*., 1998).

Healthier oil

An application of RNAi in plants that is much closer to agricultural use is the silencing of genes involved with seed-oil production. Some seed-oils are much better for human health than others, and some oils are more stable at high temperatures than others. It all depends on the fatty acid composition of the oil. For example palm oil is very high in palmitic acid which makes it stable at high temperatures but also unhealthy for human consumption, as it raises LDL cholesterol levels. Olive oil, on the other hand, is high in linoleic acid which is much healthier for human consumption, but it is not stable at high temperatures and therefore not good for frying. The best oil for heat stability and with no negative effects on cholesterol levels is one which is high in oleic acid. We have used RNAi to silence the gene in cotton which codes for the enzyme that converts oleic acid into a different fatty acid. This has altered the seed-oil from being around 10% to an impressive 75% oleic acid. If these plants were used in agriculture it would produce two crops, fiber and seed-oil, for the price of one **(**Waterhouse *et al*., 1998).

Pest control

The digestive in an insect or a nematode is very different from the digestive system in mammals, and we now know that dsRNA designed to suppress specific genes in some pests,can be provided in the diet to suppress or kill those pests. The sequence specificity of RNAi presents the opportunity to selectively target some pest species while sparing desirable species. Unlike some chemical pesticides, RNAi in plant is not expected to have any effect on non-target insects and nematodes, birds, reptiles, fish, or mammals. All transgenic-plant-mediated insect control technology is based on the plants producing proteins derived from a specific type of bacteria, *Bacillus thuringiensis* or Bt. Insect resistance to Bt has not been a significant problem to date, but risk of development of resistance to these proteins can be further reduced with the use of non-Bt-crop or natural refuges of non-resistant plant hosts. Combining Bt technology with a second, independent mode of insect control via RNAi would both enhance product performance and further guard against the development of resistance to Bt proteins (Goldstein *et al*., 2009).

RNAi and Wood quality and Fruit quality

During chemical pulping of wood, one of the most expensive and environmentally hazardous processes is to separate lignin from cellulose and hemicellulose (Pilate *et al*.,2002). The production of plant material with lower contents of lignin would mean a significant reduction of cost and pollution to the paper industry. One of the approaches to obtain reduced lignin forest trees has been the down regulation of lignin biosynthesis pathways (Hu *et al*., 1999). The main genes involved with genetic transformation targeting lignin reduction are 4-coumarate: coenzyme A ligase (*Pt4CL1)* (Hu *et al*., 1999), cynnamyl alcohol deshydrogenase (CAD - the final enzyme in the biosynthesis of lignin monomers) (Baucher *et al*., 1996) and caffeate/5-hydroxyferulate O-methyltransferase (COMT-enzyme involved in syringyl lignin synthesis) (Lapierre *et al*., 1999). In some woody 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). Apples containing reduced amounts of the Mal d 1 allergen were obtained by the expression of an intron spliced hairpin RNA containing Mal d 1-specific inverted repeat sequences (Gilissen *et al*., 2005). According to these reports, it is possible to produce transgenic hypoallergenic apples using RNAi. Research on leaf sorbitol silencing suggests that sorbitol distribution affects fruit quality such as starch accumulation and sugar-acid balance (Teo *et al*., 2006). 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 (Guo *et al*., 2005). In this case, RNAi could be used to achieve this goal (Amancio *et al*., 2007).

References

Adams-Phillips L, Barry C, Giovannoni J (2004) Signal transduction systems regulating fruit ripening. Trends in Plant Science 9: 331–338.

Agrawal N, Dasaradhi PVN, Mohmmed A, Malhotra P, Bhatnagar RK, Mukherjee SK (2003) RNA Interference: Biology, Mechanism, and Applications. Microbiology and Molecular Biology Reviews 67: 657-685.

Amancio José de Souza; Beatriz Madalena Januzzi Mendes; Francisco de Assis Alves Mourão Filho (2007) Gene Silencing: Concepts, Applications, and Pespectives In Woody Plants 64(6):645-656.

Baucher M, Chabber B, Pilate G, Vandoorsselaere J, Tollier MT, Petit-Conil M, Cornu D, Monties B, Van Montagu M, Inze D, Jouanin L, Boerjan W (1996) Red xylem and higher lignin extractability by down-regulating a cinnamyl alcohol dehydrogenase in Poplar. Plant Physiology 112: 1479-1490.

Baulcombe D (2000) Unwinding RNA silencing. Science 290: 1108-1109.

Bernstein E, Caudy AA, Hammond SM, Hannon GJ (2001) Role for a bidentate ribonuclease in the initiation step RNA interference. Nature 409: 363–366.

Campbell TN (2005) Choy FYM, RNA Interference: Past, Present and Future. Curr. Issues Mol. Biol 7: 1-6.

Cogoni C, Irelan JT, Schumacher M, Schmidhauser T, Selker EU, Macino G (1996) Transgene silencing of the *al-1* gene in vegetative cells of *Neurospora* is mediated by a cytoplasmic effector and does not depend on DNA-DNA interactions or DNA methylation. EMBO J. 15: 3153-3163.

Crowe TC, Seligman SA, Copeland L (2000) Inhibition of enzymic digestion of amylose by free fatty acids in vitro contributes to resistant starch formation. Journal of Nutrition 130: 2006–2008.

Dandekar AM, Teo G, Defilippi BG, Uratsu SL, Passey AJ, Kader AA, Stow JR, Colgan RJ, James DJ (2004) Effect of down-regulation of ethylene biosynthesis on fruit flavor complex in apple fruit. Transgenic Research 13: 373-384.

Davuluri GR, van Tuinen A, Mustilli AC, Manfredonia A, Newman R, Burgess D, Brummell DA, King SR, Palys J, Uhlig J, *et al.* (2004) Manipulation of DET1 expression in tomato results in photomorphogenic phenotypes caused by posttranscriptional gene silencing. Plant Journal 40: 344–354.

Davuluri GR, van Tuinen A, Fraser PD, Manfredonia A, Newman R, Burgess D, Brummell DA, King SR, Palys J, Uhlig J, *et al.* (2005) Fruit-specific RNAi-mediated suppression of DET1 enhances carotenoid and flavonoid content in tomatoes. Nature Biotechnology 23: 890–895.

Elbashir SM, Martinez J, Patkaniowska A, Lendeckel W, Tuschl T (2001) Functional anatomy of siRNAs for mediating efficient RNAi in Drosophila melanogaster embryo lysate. EMBO J. 20:6877-6888.

Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC (1998) Potent and specific genetic inteference by double-stranded RNA in Caenorhabditis elegans. Nature 391:806-811

Forkmann G, Martens S (2001) Metabolic engineering and applications of flavonoids. Curr Opin Biotechnol 12: 155–160.

Frankard V, Ghislain M, Jacobs M (1992) Two feedbackinsensitive enzymes of the aspartate pathway in Nicotiana sylvestris. Plant Physiology 99: 1285–1293.

Giliseen LJWJ, Bolhaar STH, Matos CI, Rouwendal GJA, Boone MJ, Krens FA, Zuidmeer L, Van Leeuwen A, Akkerdass J, Hoffmann-Sommergruber K, Knulst AC, Bosch D, Van De Weg WE, Van Ree R (2005) Silencing of the major apple allergen Mal 1 by using RNA interference approach. Journal of Allergy and Clinical Immunology 115: 369-384

Goldstein DA, Songstad D, Sachs E, Petrick J (2009) RNA interference in plants. Monsanto. http://www.monsanto.com

Guo W, Duan Y, Olivares-Fuster O, Wu Z, Arias CR, Burn JK, Grosser JW (2005) Protoplast transformation and regeneration of transgenic Valencia sweet orange plants containing a juice quality-related pectin methylesterase. Plant Cell Reports 24: 482-486.

Hamilton AJ, Baulcoumbe DC (1999) A species of small antisense RNA in posttranscriptional gene silencing in plants. Science 286(5441):950–952.

Hammond SM, Bernstein E, Beach D, Hannon GJ (2000) An RNA-directed nuclease mediates post-transcriptional gene silencing in Drosophila cells. Nature 404:293-296.

Hammond SM, Boettcher S, Caudy AA, Kobayashi R, Hannon GJ (2001) Argonaute2, a link between genetic and biochemical analyses of RNAi. Science 293: 1146-1150.

Hannon GJ (2002) RNA interference. Nature 418: 244-251

Hu W, Harding SA, Lung J, Popko JL, Ralph J, Stokke DD, Tsai C, Chiang VL (1999) Repression of lignin biosynthesis promotes cellulose accumulation and growth in transgenic trees. Nature Biotechnology 17: 808-812.

Jones L, Ratcliff F, Baulcoumbe DC (2001) RNA-directed transcriptional gene silencing in plants can be inherited independently of the RNA trigger and requires Met1 for maintenance. Curr. Biol. 11: 747–757.

Kusaba M, Miyahara K, Lida S, Fukuoka H, Takario T, Sassa H, Nishimura M, Nishio T (2003) Low glutenin content 1 : a dominant mutation that suppresses the glutenin multigene family via RNA silencing in rice. Plant Cell 15: 1455-1467.

Lapierre C, Pollet B, Petit-Conil M, Toval G, Romero J, Pilate G, Leple J, Boerjan W, Ferret V, De Nadai V, Jouanin L (1999) Structural alterations of lignins in transgenic poplars with depressed cinnamyl alcohol dehydrogenase or caffeic acid *O*-methyltransferase activity have an opposite impact on the efficiency of industrial kraft pulping. Plant Physiology 119: 153-164.

Levin I, Frankel P, Gilboa N, Tanny S, Lalazar A (2003) The tomato dark green mutation is a novel allele of the tomato homolog of the DEETIOLATED1 gene. Theoretical and Applied Genetics 106: 454–460

Matzke M, Matzke A, Pruss G, Vance V (2001) RNA-based silencing strategies in plants. Curr. Opin. Genet. Dev. 11: 221-227.

Mette MF, Aufsatz W, Vander Winden J, Matzke , A,Matzke AJ (2000) Transcriptional silencing and promoter methylation triggered by double-stranded RNA. EMBO J. 19: 5194–5201.

Mustilli AC, Fenzi F, Ciliento R, Alfano F, Bowler C (1999) Phenotype of the tomato high pigment-2 mutant is caused by a mutation in the tomato homolog of DEETIOLATED1. Plant Cell 11: 145–157.

Nakamura N, Fukuchi-Mizutani M, Miyazaki K, Suzuki K, Tanaka Y (2006) RNAi suppression of the anthocyanidin synthase gene in *Torenia hybrida* yields white flowers with higher frequency and better stability than antisense and sense suppression. Plant Biotechnol 23: 13–18.

Nakatsuka T, Abe Y, Kakizaki Y, Yamamura S, Nishihara M (2007a) Production of red-flowered plants by genetic engineering of multiple flavonoid biosynthetic genes. *Plant Cell Rep* 26: 1951–1959.

Nakatsuka T, Pitaksutheepong C, Yamamura S, Nishihara M (2007b) Induction of differential flower pigmentation patterns by RNAi using promoters with distinct tissue-specific activity. Plant Biotechnol Rep 1: 251–257.

Nakatsuka T, Mishibaa KI,Abe Y, Kubota A, Kakizaki Y, Yamamura S, Nishihara M (2008) Plant Biotechnology, Flower color modification of gentian plants by RNAi-mediated gene silencing. Plant Biotechnology 25:61-68.

Napoli C, Lemieux C, Jorgensen R (1990) Introduction of chimeric chalcone synthase gene into *Petunia* results in reversible cosuppression of homologous genes in trans. Plant Cell 2: 279-289.

Negrutiu I, Cattoir-Reynearts A, Verbruggen I, Jacobs M (1984) Lysine overproducer mutants with an altered dihydrodipicolinate synthase from protoplast culture of Nicotiana sylvestris (Spegazzini and Comes). TAG Theoretical and Applied Genetics 68: 11–20.

Nishihara M, Nakatsuka T, Yamamura S (2005) Flavonoid components and flower color change in transgenic tobacco plants by suppression of chalcone isomerase gene. FEBS Lett 579: 6074–6078.

Nishihara M, Nakatsuka T, Hosokawa K, Yokoi T, Abe Y, Mishiba K, Yamamura S (2006) Dominant inheritance of white-flowered and herbicide-resistant traits in transgenic gentian plants. Plant Biotechol 23: 25–31.

Novina CD, Murray MF, Dykxhoorn DM, Beresford PJ, Riess J, Lee SK, Collman RG, Lieberman J, Shankar P, Sharp PA (2002) siRNA-directed inhibition of HIV1 infection. Nature Med. 8: 681−686.

Pilate G, Guiney E, Holt K, Petit-Conil M, Lapierre C, Leple J, Pollet B, Mila I, Webster EA, Marstorp HG, Hopkins DW, Jouanin L, Boerjan W, Schuch W, Cornu D, Halpin C (2002) Field and pulping performances of transgenic trees with altered lignification. Nature Biotechnology 20: 607-612.

Regina A, Bird A, Topping D, Bowden S, Freeman J, Barsby T, Kosar-Hashemi B, Li Z, Rahman S, Morell M (2006) High-amylose wheat generated by RNA interference improves indices of large-bowel health in rats. Proceedings of the National Academy of Sciences of the USA 103: 3546–3551.

Rodoni BC, Dale JL (1999) Harding RM Characterization and expression of the coat protein-coding region of the banana bract mosaic potyvirus, development of diagnostic assays and detection of the virus in banana plants from five countries in Southeast Asia. Archives of Virology 144: 1725-1737.

Romano N, Macino G (1992) Quelling: transient inactivation of gene expression in *Neurospora crassa* by transformation with homologous sequences. Mol. Microbiol 6: 3343-3353. Segal G**,** Song, Messing J (2003) A new opaque variant of maize by a single dominant RNA-interference-inducing transgene. Genetics 165: 387–397.

Shi Y (2003) Mammalian RNAi for the masses. Trends Genet 19: 9–12.

Spencer PS, Roy DN, Ludolph A, Hugon J, Dwivedi MP, Schaumburg HH (1986) Lathyrism: evidence for role of the neuroexcitatory aminoacid BOAA. Lancet 2(8515): 1066-7.

Stevenson M (2004) Therapeutic Potential of RNA Interference. The new England journal of medicine 351:1772-1777

Sunilkumar G, Campbell LM, Puckhaber L, Stipanovic RD, Rathore KS (2006) Engineering cottonseed for use in human nutrition by tissue-specific reduction of toxic gossypol. Proceedings of the National Academy of Sciences of the USA 103: 18054–18059.

Tanaka Y, Tsuda S, Kusumi T (1998) Metabolic engineering to Modify Flower Color. Plant Cell Physiol 39: 1119–1126

Tanaka Y, Katsumoto Y, Brugliera F, Mason J (2005) Genetic engineering in floriculture. Plant Cell Tiss Org Cult 80: 1–24.

Tang G, Galili G (2004) Using RNAi to improve plant nutritional value: from mechanism to application. TRENDS in Biotechnology 22(9) 463-469.

Tang G, Galili G, Zhuang X (2007) RNAi and microRNA: breakthrough technologies for the improvement of plant nutritional value and metabolic engineering. Metabolomics 3:357–369.

Tenea GN (2009) Exploring the world of RNA interference in plant functional genomics: a research tool for many biology phenomena, Centre of Microbial Biotechnology pp. 4360-4364.

Teo G, Suziki Y, Uratsu SL, Lampien B, Ormonde N, Hu WK, Dejong TM, Dandekar AM (2006) Silencing leaf sorbitol synthesis alters long-distance portioning and apple fruit quality. Proceeding of the National Academy of Sciences of the USA 103 :18842-18847.

Tijsterman M, Plasterk RH (2004) Dicers at RISC; the mechanism of RNAi. Cell 117 (1): 1-3

Tsuda S, Fukui Y, Nakamura N, Katsumoto Y, Yonekura-Sakakibara K, Fukuchi-Mizutani M, Ohira K, Ueyama Y, Ohkawa H, Holton TA, Kusumi T, Tanaka Y (2004) Flower color modification of *Petunia 83ybrid* commercial varieties by metabolic engineering. Plant Biotechnol 21: 377–386.

Van Uyen N (2006) Novel approaches in plants breeding RNAi technology. Proceedings of International Workshop on Biotechnology in Agriculture 12-16.

Wang M, Abbott D, Waterhouse P M (2000) A single copy of a virus derived transgene encoding hairpin RNA gives immunity to barley yellow dwarf virus. Molecular Plant Pathology 1: 401-410.

Wassengger M, Heimes S, Riedel L, Sanger HL (1994) RNA-directed *de novo* methylation of genomic sequences in plants. Cell 76: 567–576.

Waterhouse PM, Graham MW, Wang MB (1998) Virus resistance and gene silencing in plants can be induced by simultaneous expression of sense and antisense RNA. Proc Natl Acad Sci USA 95: 13959–13964.

Williams CL (1995) Importance of dietary fiber in childhood. Journal of the American Dietetic Association 95: 1140–1146

Williams M, Clark G, Sathasivan K, Islam AS (2004) RNA Interference and its Application in Crop Improvement. Plant tissue culture and Biotechnology 1-18.

Yin JQ, Wan Y (2002) RNA-mediated gene regulation system: now and the future. Int. J Mol. Med. 10 (4): 355-365

Zhu X, Galili G (2003) Increased lysine synthesis coupled with a knockout of its catabolism synergistically boosts lysine content and also trans-regulates the metabolism of other amino acids in Arabidopsis seeds. Plant Cell 15: 845–853.

Zhu, X, Galili G (2004) Lysine metabolism is concurrently regulated by synthesis and catabolism in both reproductive and vegetative tissues. Plant Physiology 135: 129–136.

Zou GM, Yoder Mervin C (2005) Application of RNA interference to study stem cell function: current status and future perspectives. Biol. Cell 97: 211–219.