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RNA Interference: New Approach of Gene Silencing in Plants

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ABSTRACT

RNA interference (RNAi) is a naturally occurring mechanism that leads to the "silencing" of genes. In consequence, the respective protein is no longer synthesised. In nature, this mechanism is used for the regulation of specific genes and is also applied as a defence against viruses. RNA interference (RNAi) is a form of post transcriptional gene regulation in which non translated double stranded RNA (dsRNA) molecules called small interfering RNA (siRNA) mediate sequence specific degradation of target messenger RNA (mRNA). 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)]. 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. The natural function of RNAi is referring to the mechanism involved in cellular defense against viruses, genomic containment of retro-transposons, 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). 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.

Key words: *RNA, RNA Interference, Pigments, Viruses.*

INTRODUCTION

Process of RNAi

In general, RNAi is triggered by double stranded RNA, which may be produced naturally in a cell or may enter the cell exogenously. An enzyme, called Dicer, cuts the long double stranded RNA into small pieces of approximately 21 nucleotides length. These small pieces could be miRNA (micro RNA; originating from endogenous long dsRNA) or siRNA (small interfering RNA; originating from exogenous sources). These RNAs then bind to the RNA-induced silencing complex (RISC).

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After binding, one strand of the double stranded RNA is removed, leaving the remaining strand available to bind to messenger RNA target sequences. This strand is complementary to the sequence of the target mRNA. RNA Induced Silencing Complex (RISC) cleaves mRNA or represses their translation by homology dependent mRNA degradation, which effectively silences the gene. The use of RNAi has been extensively reported for modifying plants to enhance their nutritive value, pathogen and pest resistance, decreasing amount of unwanted metabolite production, etc.

Recently two RNAi based crops have been given regulatory approval for commercial production and sale. These are the non-browning Arctic apples and the non-browning Innate potatoes. The firms producing these crops claim that the idea behind producing the non-browning apples and potatoes is not only to improve the look of the product, but it is also intended to increase the consumption of the raw fruits along with reducing naturally occurring carcinogens (as in the case of innate potatoes). While the science behind both these products is a little complicated as both are RNAi based, in simple way it can be put as both apples and potatoes have certain genes suppressed. Both of them, though genetically modified, are grown the same way as conventional varieties. These products are likely to find a place of attraction in the fresh-cut product sales.

Mechanism of RNAi:

1. The entry of long double stranded RNA, such as an introduced transgene, a rogue genetic element or a viral intruder, triggers the RNAi pathway of cells. This results in the recruitment of the enzyme Dicer.
2. Dicer cleaves the dsRNA into short, 20-25 basepairs long, fragments, called small interfering RNA (siRNA).
3. An RNAinduced silencing complex (RISC) then distinguishes between the two siRNA strands as either sense or antisense. The sense strands (with exactly the same sequence as the target gene) are degraded.
4. The antisense strands on the other hand are incorporated to the RISC. These are used as guide to target messenger RNAs (mRNA) in a sequence specific manner.
5. Messenger RNAs (mRNA), which codes for amino acids, are cleaved by RISC. The activated RISC can repeatedly participate in mRNA degradation, inhibiting protein synthesis.

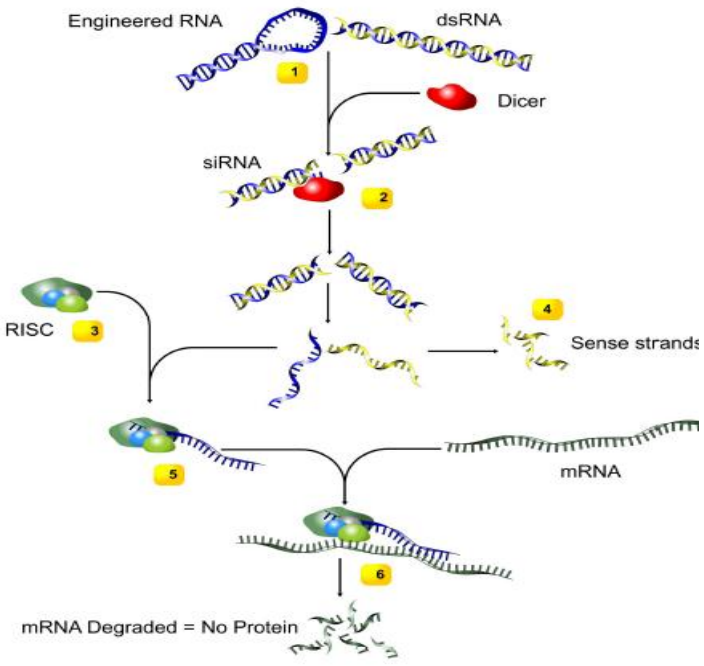

Figure 1. Mechanism of RNAi.

Application of RNAi technology

-In plant system, it provides defense mechanism to protect against infection by viruses, transposons and other insertional elements.

-RNAi also plays a role in regulating development and genome maintenance.

- Development of male sterile plants in rice.

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THE NON-BROWNING APPLES – ARCTIC APPLES

It is a common observation that once an apple is cut or bitten, it turns brown. The reason behind this browning is that when the cells of an apple fruit are damaged through a cut, bruise or bite, some enzymes, known as polyphenoloxidases (PPOs) come in contact with the polyphenolics found elsewhere in the cell. PPO catalyzes the oxidation of polyphenolics to melanins, causing browning.

In healthy apple cells, PPO is present in the plastids, while the phenolics substrates are present in the vacuoles. On cell damage, the cells loose their compartmentalization, and PPO comes in direct contact with its substrate. PPO is involved in the reaction of synthesis of quinones from diphenols. The quinones then undergo non-enzymatic condensation with lesser amounts of amino acids and proteins, to give brown pigmented polymer of lignin-like compounds.

Therefore, PPO is the key enzyme responsible for apple browning. To stop or minimize fruit browning, reducing the activities of PPO has long been considered as an effective approach. The technology of silencing the PPO genes was developed in Australia and licensed by a Canadian firm Okanagan Specialty Fruits Inc. (OSF).

Arctic apples do not show signs of browning

Scientists at Okanagan Speciality Fruits developed these non-browning apples using the technique of RNA interference to block the activity of PPO. They used gene silencing to develop a new type of apple in which a gene from the original apple was deleted. They named these apples as Arctic Apples. Two varieties of apple, namely, Granny Smith and Golden Delicious, have been modified so far using this technique (Image 2).

Image 2: Arctic apples

Strategy to develop non browning apples

Genetic engineering using Agrobacterium based transformation was used to achieve this goal. First and foremost, the genes encoding PPO in apple were identified. Scientists were able to identify ten such genes of their interest. They categorized these genes into four groups. One gene from each group was used for further developments.

DNA fragments were then produced from each of the four genes and combined into a single gene. They named it as PGAS, taking the first letter of each of the gene selected (PPO2, GPO3, APO5 and pSR7). This transgene was incorporated into a vector for further apple transformation. The hybrid gene was placed under the control of the cauliflower mosaic virus 35s promoter (P*CAMV35s*) and nopaline synthase terminator (TNOS). Apple cells were transformed with bacterium *Agrobacterium tumefaciens* carrying vector GEN-03, and then transgenic plants with the expected transgene PGAS selected. The transformed plants were analyzed for reduced or non-browning characteristics in the fruit. On analysis the PPO activity was found to be significantly reduced in these apples.

NON-BROWNING POTATOES – INNATE

The potato that does not brown on cutting was developed by J. R. Simplot Company. They used RNAi to silence 4 different proteins. Scientists at this company engineered a popular potato variety Russet Burbank to contain late blight resistance, low acrylamide potential, reduced black spot, and lower reducing sugars. These potatoes have resistance to the serious disease late blight, caused by fungus *Phytophthora infestans*. In addition, three potato quality issues were addressed: (1) the presence of high amounts of reducing sugars which cause brown spots on accumulation (2) enzymatic browning (3) presence of high level of non-essential free amino acid asparagine which gets oxidized to form carcinogenic compound acrylamide upon baking or frying. Enzymatic browning occurs due to PPO activity as is the case in apples. In potatoes, browning also occurs non-enzymatically because of the partial degradation of starch into sucrose and fructose. Also, these reducing sugars react with amino acids on heating to produce a variety of desired compounds contributing to flavor, but also produce acrylamide which is harmful on consumption.

Scientists at Simplot silenced the invertase gene in potato tubers to reduce the conversion of sucrose to reducing sugars, allowing for storing potatoes at colder temperatures. The company believes that the overall yield improvement lowered fungicide use because of late blight resistance would be beneficial due to lesser harmful impact on the environment due to reduced fungicide use. They also found that low asparagine and low reducing sugars resulted in greater than 70% reduction in acrylamide even after extended cold storage, addressing this potential health risk for consumers and the food industry.

Strategy used

The innate potatoes were developed using Agrobacterium mediated transformation technique. First the plants were transformed to reduce the expression of the gene asparagine synthetase (to reduce amount of asparagine) and PPO (to reduce polyphenol oxidase). These transformed plants were then again transformed with genes to induce late blight resistance and silence invertase. The transformed potatoes were compared for their composition to untransformed ones and were found to show food safety equivalence with the untransformed potatoes (Image 3).

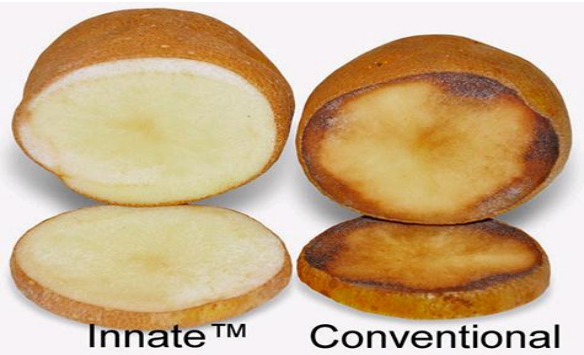

Image 3: Innate potatoes

REGULATORY APPROVAL

Both, Arctic apples and Innate potatoes, have been granted approval for commercial sale in the United States. The Innate potatoes were approved by the United States Department of Agriculture (USDA), Food and Drug Administration (FDA) and Animal and Plant Health Inspection Service (APHIS). Three potato varieties, Ranger Russet, Russet Burbank and Atlantic Potatoes, are known by the trade name Innate. The Arctic apples were approved for sale in the United States by USDA and FDA and in Canada by Canadian Food Inspection Agency (CFIA) and Health Canada.

Now that RNAi based foods are coming to the market, it can be concluded that this kind of genetic modification is new in its kind, because these are not a traits aimed at farmers, but directly at consumers. The earlier commercialized biotech crops were aimed to improve pest resistance or herbicide resistance in crops standing in the field, but these traits will be directly visualized by the commodity consumers.

Application in improvement of nutritional value:

- RNAi technology used to produce cotton seed containing lower level of dcadinene synthase which is key enzyme in gossypol production.
- RNAi method were used in cotton to down regulate two key fatty acid desaturase gene encoding stearoyl acyl careeer protein D9 desaturases and Oleoyl phasphatidylcholine w6 desaturase. Knockdown of these genes in cotton led to increase of nutritionally improved high oleic (HO) and high stearic (HS) cottonseed oil that is more suitable for human consumption.
- In maize, RNAi technology has been used to reduce phytic acid by silencing MRP4 ATP binding cassette (ABC) transporter.
- In soybean, Silencing of Omega3 fatty acid desaturase gene in soybean using RNAi reduce alinolenic acid and improve oil stability and flavour.
- Using RNAi technique, varieties of barley developed which are resistant to BYDV (barley yellow dwarf virus).

Advantages of RNAi

- This technology is highly gene specific.
- High gene silencing efficiency.
- Screening targeted plants takes less time.
- It is highly inducible.

Disadvantages of RNAi

• It does not knockout a gene for 100%.
• siRNA tends to activate unwanted pathways.

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