ORIGINAL RESEARCH PAPER

Genetics

KEY WORDS:
characterization, sewage, municipal waste, domestic fertilizer, sampling.

RNA INTERFERENCE:- A NOVEL STRATEGY IN CROP IMPROVEMENT

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INTRODUCTION:-

RNA interference (RNAi) is a natural cellulardefensemechanism involved in regulation of geneexpression. It involves double stranded ribonucleic acid (dsRNA) that canspecifically silence the expression of two genes with sequences, which are complementary to the dsRNA. The components of double stranded RNA initiate the process of RNAiwhen an antisense RNA strand is activated which targets a complementary gene transcript such as a messenger RNA for cleavage by a ribonuclease. RNAi has been shown to be an important regulator of gene expression in many eukaryotes including plants, animals and humans. In 2006, the Nobel prize was awarded to Andrew Z. Fire and Craig C. Mello , for unravellingthe mechanism of gene silencing by double stranded RNA, based on their studies in nematode worm Caenorhabditiselegans. It is also known by other names like 'post transcriptional gene silencing', 'transgene silencing' and 'quelling'. Around 1990, Scientists working on petunia plants (Napoli et. Al 1990)4 reported some unexpected genetic expression. The goal of their experiments was to breed petunia plants to increase the colour intensity of the flowers. For the target to be achieved, they introduced cloned gene encoding a key enzyme for red pigmentation of flowers. Surprisingly, many of the petunia plants with additional copies of this gene showed petals which appeared fully white or partially white instead of the expected colour. The scientists, based on their evidences, explained the phenomenon as post transcriptional gene inhibition or "co-suppression of gene expression". Similar observations were recorded in few years later in viruses but the molecular mechanism remained unknown until the recent land mark discovery by Fire and Mello.

Major Componants of RNAi Mechanism:-

1. Dicer :-Double-stranded RNA triggers processed into siRNAsby enzyme RNAseIII family, specifically the Dicer family. Dicer family proteins are ATP-dependent nucleases.These proteins contain an amino-terminal helicase domain, dual RNAseIII domains in the carboxy-terminal segment, and dsRNA-binding motifs.They can also contain a PAZ domain, which is thought to be important for protein-protein interaction.Dicer homologs exist in many organisms including C. elegans, Drosphila, yeast and human.

2. RISC complex:-RISC (RNA induced silencing complex) is a large (~500-kDa) RNA-multiprotein complex, which triggers mRNA degradation in response to siRNA.

Cellular Mechanism of RNAi:-

RNA interference is an RNA dependent gene silencing mechanism that includes the endogenously induced gene silencing effects of miRNA as well as silencing triggered by foreign dsRNA. The dsRNA binds with a protein complex DICER which is an endonuclease, which cleaves it into short fragments with 20 to 25 base pairs with a few unpaired overhung bases at both ends. The short dsRNA fragments produced by DICER, is called small interfering RNAs (Si RNAs). Thus, miRNA and SiRNA share same cellular machinery as well as functional analogy. These fragments (SiRNA or miRNA) integrate with another active protein complex RISC (RNA induced silencing complex). The catalytic active component of the RISC complex is known as argunaute protein which acts as endonuclease and mediates siRNAinduced cleavage of the target mRNA strand. Consequently, one of the RNA strands (antiguidestrand or passenger strand) is degraded while the other is selected as a guide strand which remains bound to RSIC complex. When a complementary mRNA is located by an RSIC bound guide strand it binds to it and is cleaved and degraded. The expression of the gene corresponding to the mRNA is silenced. It has been established that RNAi mechanism provides genomic stability, especially in plants, keeping the transposon production under control.

Application of RNAi in Agriculture:-

RNAi holds potential too in genetic engineering of crop plants particularly, targetting to reduce level of natural toxin products. The techniques are advantageous in plants for their stable and heritable RNAi phenotype. The application of RNAi proved successful in a major way, in cotton plant. The cotton seeds are rich in dietary protein but unsuitable for human consumption because of its toxic terpenoidproduct, gossypol. RNAi has been used to produce cotton stocks with seeds containing lower levels of delta-cadinene synthase, a key enzyme in gossypol production, without affecting the enzyme's production in other parts of the plant, where gossypol is important in preventing damage from plant pests. By using the same technique it has been possible to reduce linamarin content, cyanogenic substance in cassava plants. Efforts have been also directed to improve many other useful crop plants, keeping in view a specifictarget as above, by use of RNAi.

Conclusions :-

1. It was recently discovered that small RNAs correspond to centromer heterochromatin repeats. RNAi regulates heterochromatic silencing faster identification of gene function.

2. Protects the genome from viruses.

3. Powerful for analyzing unknown genes in sequence genomes.

4. efforts are being undertaken to target every human gene via miRNAs

5. Gene therapy:-down-regulation of certain genes/mutated alleles

6. Cancer treatments:-
   - –knock-out of genes required for cell proliferation
   - – knock-out of genes encoding key structural proteins

7. Gene silencing is a convenient approach in species or varieties for which exhaustive mutant collections are not yet available.

8. Gene Knockout:
-Introduce dsRNA as hairpin RNA (hpRNA) to silence some specific gene.
-Trick the plant in to shutting down or re-routing specific molecular pathways to alter biological activities.
e.g. -flowering time, leaf shape, yield index, oil quality

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