Genetic Engineering:

Application-Plant Biotechnology:
Plant Disease Diagnosis:

In general most the plant diseases can be diagnosed by physical symptoms. However if there is any mixed infections or some rare infectious agents, detection can be made by using ELISA techniques. For this kind of investigations one has to have the required antibodies for each and every infectious agent.

Similarly one can use PCR as the diagnostic tool. To use PCR one has to have specific set of primers for each of the disease causing organisms. Combination of both will certainly provide the information on infectious agents.

Disease Resistant Plants:

In the past agricultural scientists used selective breeding methods for identifying disease resistant varieties and cultivating the same. This process is time consuming and lot of manual work and waiting. If the breeder is lucky he may get a disease resistant plant in about 12 to 20 years or within a year or two; one should be lucky. With the knowledge of molecular technology, it is possible to introduce a specific gene to combat a particular disease.

For example disease resistant against ToMV and ToLCV in Tomato (obtained by G.R.Kantharaj), disease resistant to fungal infection, disease resistance to bacterial infection or and disease resistance to pests.

Resistance to plant viruses:

Disease resistance against plant viruses one can use capsid-mediated resistance, or RNA mediated resistance or one can employ Antisense techniques.

Capsid mediated resistance: Example: Tomato plants resistant to tomato mosaic virus and tomato leaf curl virus. Isolate viral particles. Identify gene or genes for capsid proteins and clone them into a binary vector in such a way the cloned DNA is in reading frame with a promoter.

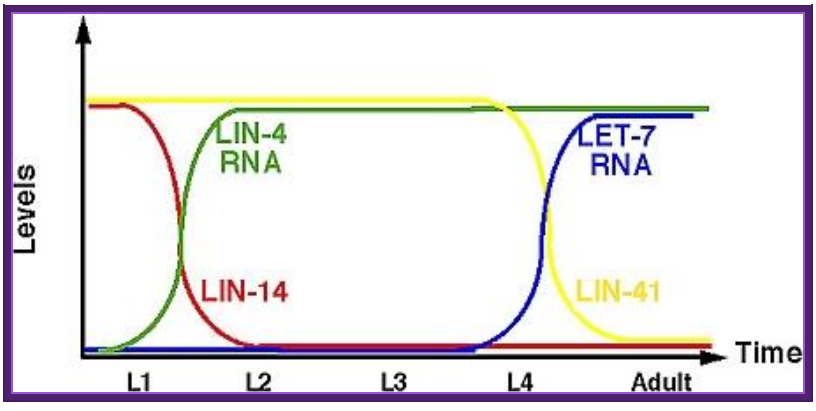
Using the recombinant binary vector, transform the tomato tissue either by Agrobacterium or by gene gun method. Regenerate the tissue into plantlets. Different constructs are used separately to develop two specific transgenic plants. Check for the expression of the capsid protein by western blot. Transgenic plants thus obtained are resistant to ToMV and similarly other plants are resistant to ToLCV. In order to combat mixed infection, which is usually the case, hybridize the two transgenics to obtain resistance to both viruses. It is also very important to check the stability of the insert.

Production of capsid protein provides protection against invading viruses by the binding of capsid proteins to newly introduced viral RNAs, thus the multiplication of the viruses is prevented.

RNA mediated: In this case the capsid gene is truncated in such a way, when this piece of the gene is cloned; the expressed (RNA) or mRNA does not translate to proteins. Continuous synthesis of foreign RNA leads to RNAi interference. The RNAs bind to viral RNAs in sequence specific manner and then they are degraded by RNases. Thus it gives protection against plant viruses. The methodology can be employed for other plant viruses. Plant viruses cause devastating disease and the crop production is reduced in many cases from 60% to 70%. The most important agricultural crop plants are Rice, Sorghum, Potato, wheat, Tomato and many more such plants can developed into transgenics against many viral diseases.

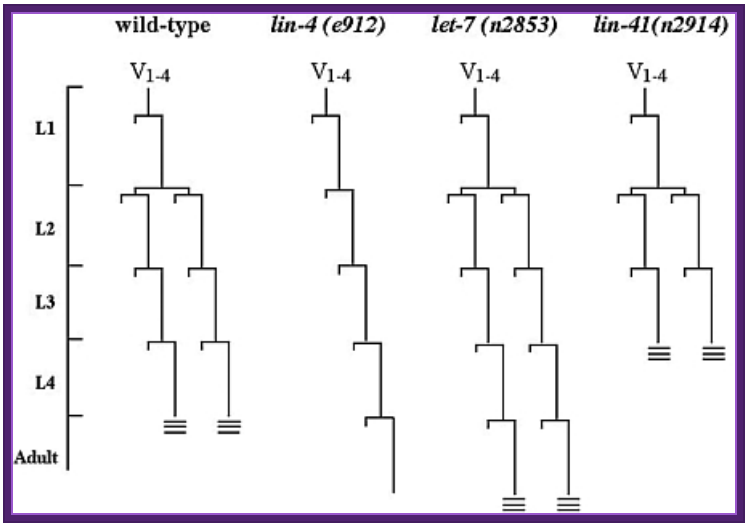
RNAi Method:

This technique is a powerful method by means of which one can combat against any of the known disease. The RNAi modulation was first discovered in C. elegance, a worm. In this process cells generate or made to synthesize a double stranded RNA of 21-23 bp long or more.



Graphic expression representing the expression of different lin RNAs and Let RNAs; https://bcrc.bio.umass.edu

This is subjected processing by an enzyme complex called Dicer. This then taken over by another protein complex called RISC RNA-induced silencing complex, which makes the dsRNA into selective ssRNA. This RNA interacts with cellular RNAs including specific mRNA and searches for complementary bases at the 3'end of the target RNA.

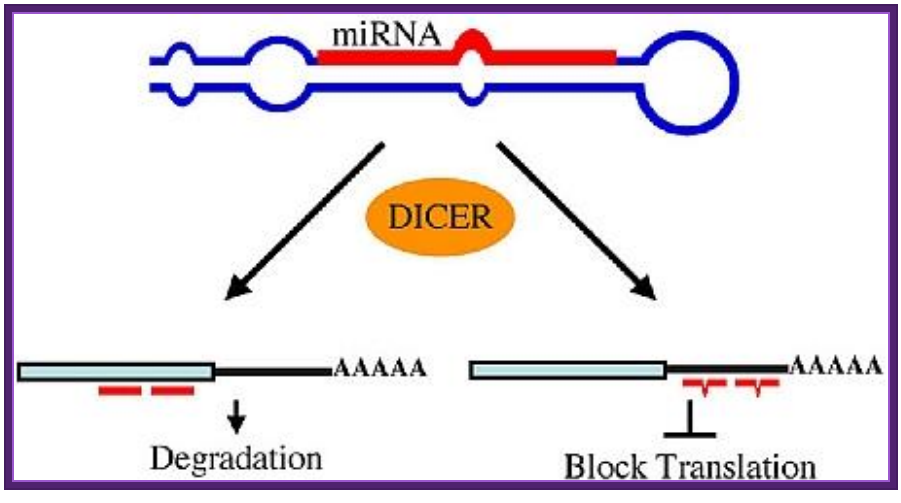


lin-4 and *let-7* are the best understood miRNAs. They control the timing of adult cell fate determination in hypodermal cells by binding to partially complementary sites in the mRNA of key developmental regulators to repress protein expression. For example, *lin-4* is predicted to bind to seven sites in the *lin-14* 3' untranslated region (UTR) to repress LIN-14, while *let-7* is predicted to bind two *let-7*complementary sites in the *lin-41* 3' UTR to down-regulate LIN-41. Two other miRNAs, *lsy-6* and *mir-273*, control left-right asymmetry in neural development, and also target key developmental regulators for repression. Approximately one third of the *C. elegans* miRNAs are differentially expressed during development indicating a major role for miRNAs in *C. elegans* development. Given the remarkable conservation of developmental mechanism across phylogeny, many of the principles of miRNAs discovered in *C. elegans* are likely to be applicable to higher animals; Lineage defects in the seam cells associated with *lin-4*, *let-7* and *lin-41* lf mutants. Seam cell terminal differentiation is represented by 3 horizontal bars.Monica C. Vella, Frank J. Slack; http://www.wormbook.org/

If the target mRNA or any other RNA has perfect matching, the enzymes associated just cleave the target RNA completely (Si technique). If the complementarity is not complete but shows partial, this prevents the target mRNA from translation (miRNA mode). Now this technique has been used in variety system to combat a variety of diseases and even for genetic manipulation by which one can just knock out a gene product, with out

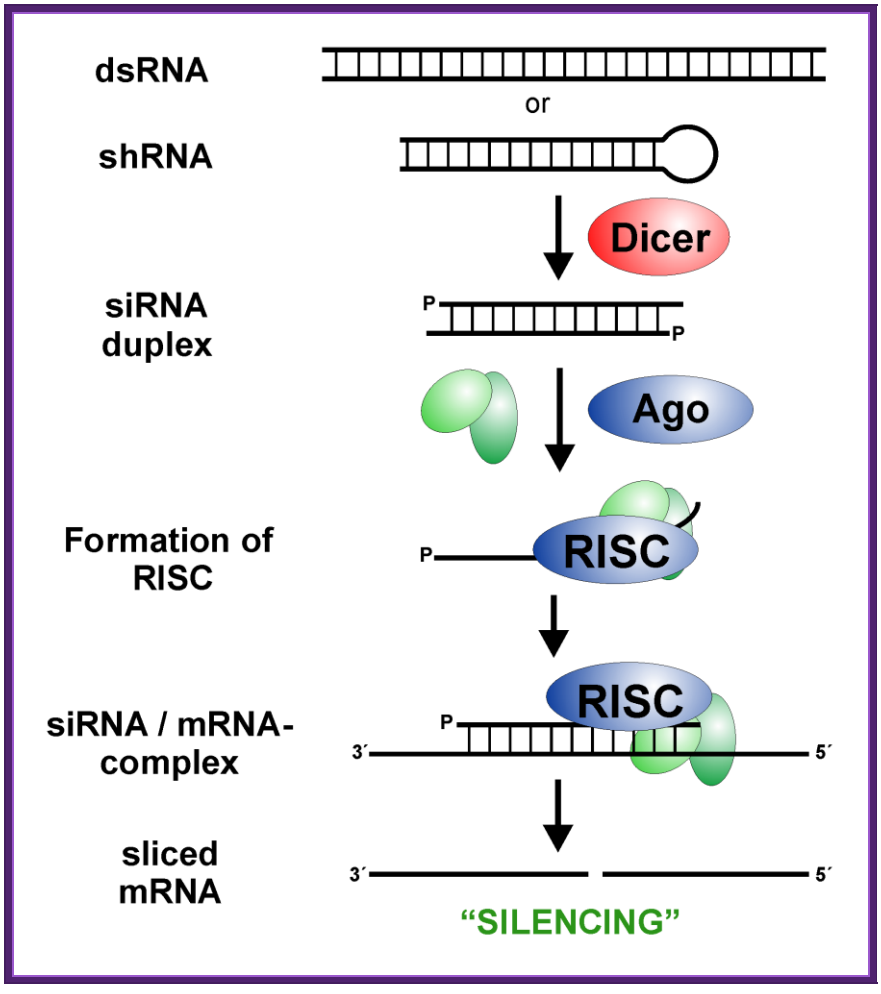
destroying the gene. This technique is some way analogous to antisense RNA technique.

Potential RNA/RNA duplexes between *let-7* and *lin-41*: The position of the *let-7(n2853)* mutation is shown by an arrow below the duplexes; Lin41 mRNA hybridized by let-7 RNAi, which leads either to destruction of lin4 mRNA or its translation is blocked; this happens during the development of the worm. Monica C. Vella, Frank J. Slack; http://www.wormbook.org/

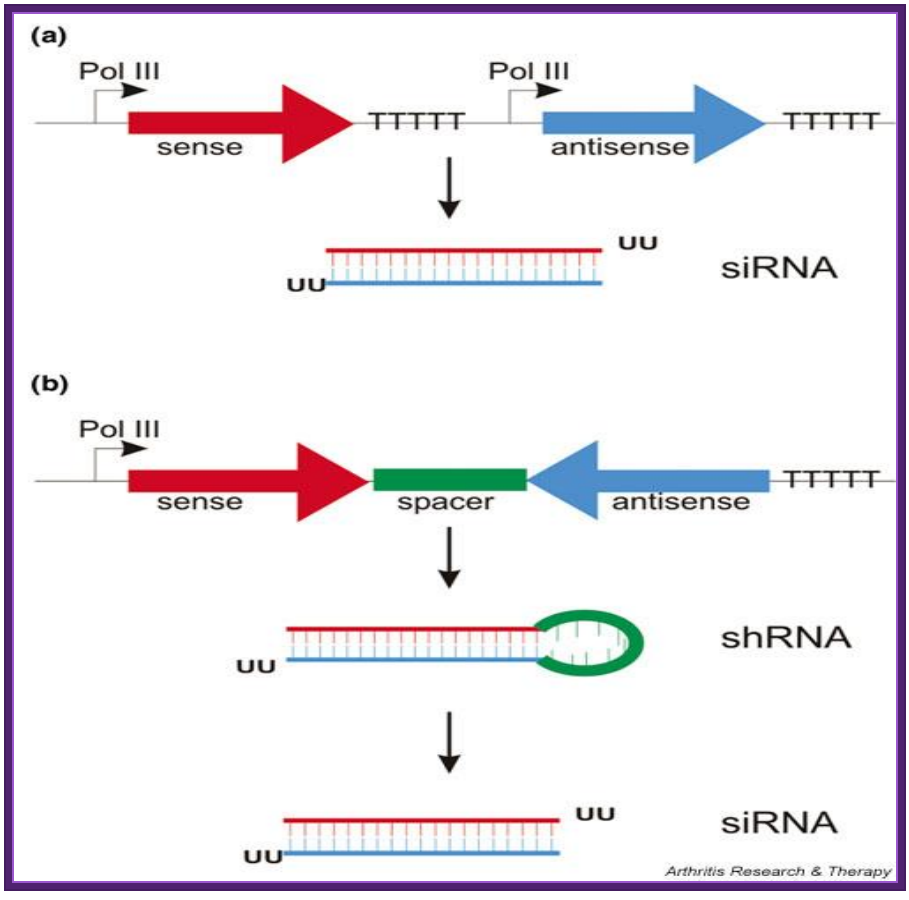


miRNAs use two mechanisms to exert gene regulation. Some animal miRNAs can bind to mRNA targets with exact complementarity and induce the RNAi pathway. miRNAs also bind to targets with imperfect complementarity and block translation. There is no evidence that *C. elegans* miRNAs use the former; **mi RNA production and processing; http://www.wormbook.org/**

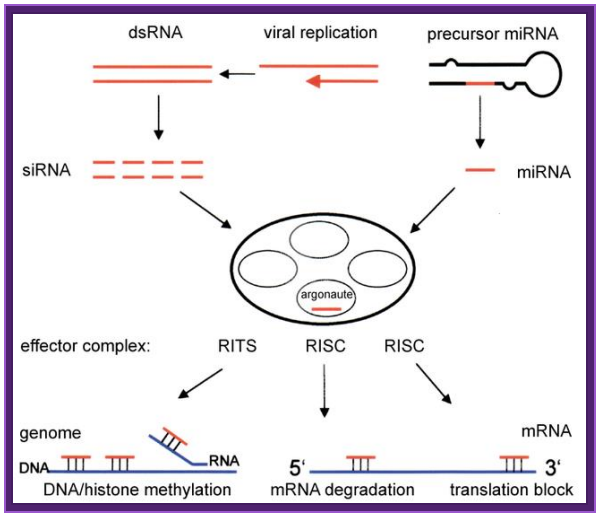
Si or mi RNAs are also copied and amplified by RNA dependent RNA polymerases-RdRP (as shown in the above diagram).



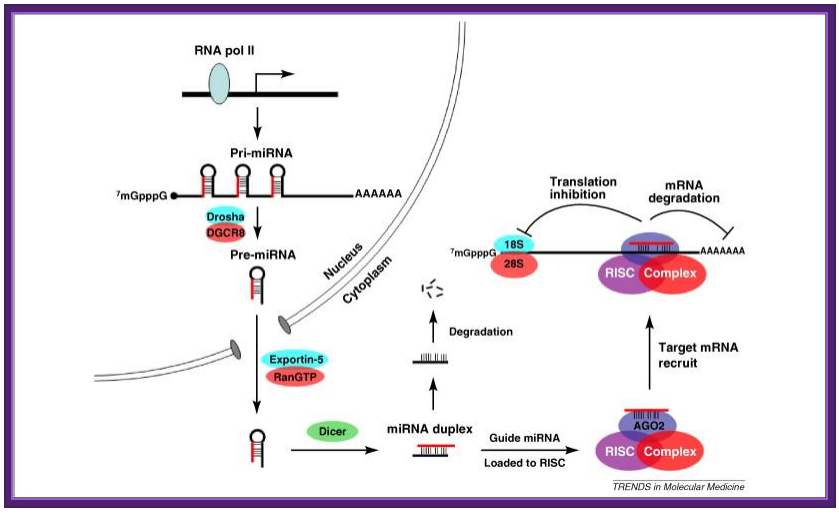
Mechanism of siRNA silencing http://www.uni-konstanz.de/FuF/chemie/jhartig/; http://www.gene-quantification.de/



Pol III generated sh-siRNA; http://www.gene-quantification.de/

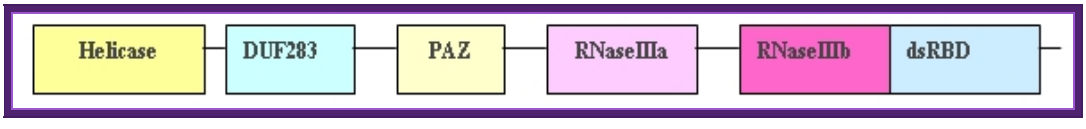


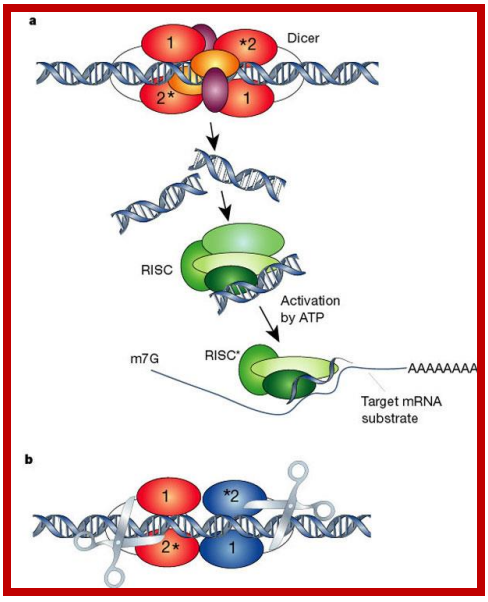
The enzyme dicer trims double stranded RNA, to form small interfering RNA or microRNA. These processed RNAs are incorporated into the RNA-induced silencing complex (RISC), which targets messenger RNA to prevent translation; http://en.wikipedia.org/



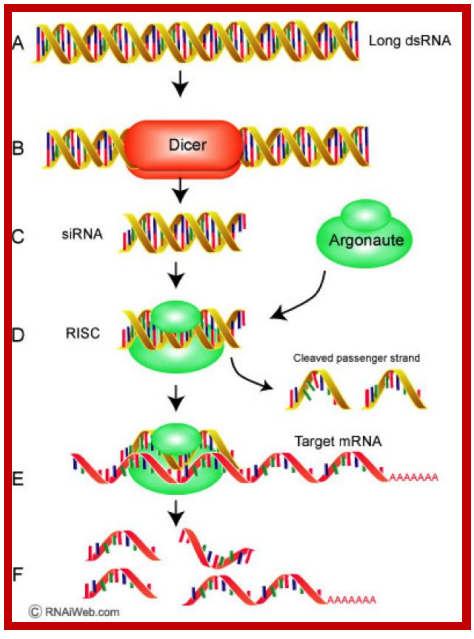
MiR-210;

Hypoxia inducible factors (HIFs) regulate a variety of genes to prepare cells to adapt and survive under a hypoxic environment. Recently, microRNAs (miRNAs) have emerged as a new class of genes regulated by HIFs in response to hypoxia, of which miR-210 is the most consistently and predominantly upregulated miRNA. Functional studies have demonstrated that miR-210 is a versatile gene that regulates many aspects of hypoxia pathways, both in physiological and malignant conditions. Here, we summarize recent findings on the mechanism of hypoxia regulation of miR-210 expression and its multifaceted biological functions in normal physiological and malignant conditions, and discuss the challenges we face in elucidating the biological functions of miR-210 and exploring its potential use for therapeutics; http://www.sciencedirect.com/





Dicer and RISC (RNA-induced silencing complex): a, RNAi is initiated by the Dicer enzyme (two Dicer molecules with five domains each are shown), which processes double-stranded RNA into ~22-nucleotide small interfering RNAs[36]. Based upon the known mechanisms for the RNase III family of enzymes, Dicer is thought to work as a dimeric enzyme. Cleavage into precisely sized fragments is determined by the fact that one of the active sites in each Dicer protein is defective (indicated by an asterisk), shifting the periodicity of cleavage from ~9–11 nucleotides for bacterial RNase III to ~22 nucleotides for Dicer family members[40]. The siRNAs are incorporated into a multicomponent nuclease, RISC (green). Recent reports suggest that RISC must be activated from a latent form, containing a double-stranded siRNAs to an active form, RISC*, by unwinding of siRNAs[41]. RISC* then uses the unwound siRNAs as a guide to substrate selection[31]. b, Diagrammatic representation of Dicer binding and cleaving dsRNA (for clarity, not all the Dicer domains are shown, and the two separate Dicer molecules are coloured differently). Deviations from the consensus RNase III active site in the second RNase III domain inactivate the central catalytic sites, resulting in cleavage at 22-nucleotide intervals. Gregory J. Hannon; http://www.nature.com/



https://rybicki.wordpress.com

Plants have RNA-dependant RNA polymerases (RdRPs) that accentuate the process as they extend the bound guide strand to create more dsRNA that can then re-enter the RNAi cycle. dsRNA delivered to insects by various routes has been seen to induce RNAi, however insects lack RdRPs and therefore require a large constant supply of siRNAs for sustained gene silencing. Herbivorous insects feeding on stably transformed transgenic host plants have been seen to take up the produced dsRNA molecules into their gut cells, causing post transcriptional gene silencing. Generation of these stable transgenic plant lines is a time consuming task, while transient plant transformation offers a faster and more versatile approach, allowing for a number of dsRNA products to be created as a quicker screening method.

Larvae of the tobacco hornworm *Manduca sexta* contain genes that encode for nicotine-catabolising enzymes, rendering them resistant to the toxic nicotine alkaloid produced by their host plant *Nicotiana attenuata*. It was previously seen that some cytochrome P450 (CYP) genes were up-regulated in the larval gut in response to nicotine ingestion (CYP4M1 and CYP4M3 genes) and CYP6B46 was down-regulated when fed on nicotine suppressed plants.

In this paper the tobacco rattle virus (TRV) was used to transiently produce dsRNAs in *Nicotiana attenuata* – this approach was termed plant-virus based dsRNA producing system (VDPS) – in comparison to stably transformed

plants – termed plant mediated RNAi (PMRi) for the silencing of these lepidopteran genes.

They initially checked to see if *M. sexta* could indeed take up the dsRNA and cause PMRi. It was observed that when the larvae were fed on a transgenic plant expressing dsRNA for the CYP6B46 gene, there was CYP6B46 smRNA found in the midgut and a reduction in the CYP6B46 transcript levels was observed, effectively causing silencing of the gene. It was very specific as the transcript levels of a similar gene (CYP6B45 – 80% similarity) was not affected. The VDPS was tested and compared to the PMRi for the same target and produced comparable silencing, that was also highly specific and did not cause any "off-target" effects.

Since the VDPS is a more rapid technique and was seen to be comparable to the PMRi, it was therefore used to screen the other gene targets – CYP4M1 and CYP4M3. Again the smRNAs for each were seen to be present in the midgut of the larvae when fed on the plants and the transcript levels were reduced with high specificity. The reduction of the CYP4M3 transcription levels also caused larval growth to decrease, indicating that this gene may a central role in nicotine tolerance.

The length of the dsRNA is known to have an effect on RNAi experiments and it would be ideal if the lengths were standardised. It is possible that the lepidopteran dicers that function in extremely alkaline environments of the midgut are specialized and possess different dicing properties than the plant dicers; consequently, insect-dicer diced smRNA might be more effective than the plant-dicer diced smRNA in gene silencing in insects.

Plant Dicers (DCLs) are involved in the biogenesis of smRNA by cleaving longer dsRNA. Four different types of DCLs are reported in higher plants. Their function has been found to overlap in plants, suggesting that one DCL can contribute to and/or compensate for the function of the others. Hence, more than one DCL might be involved in processing long dsRNA.

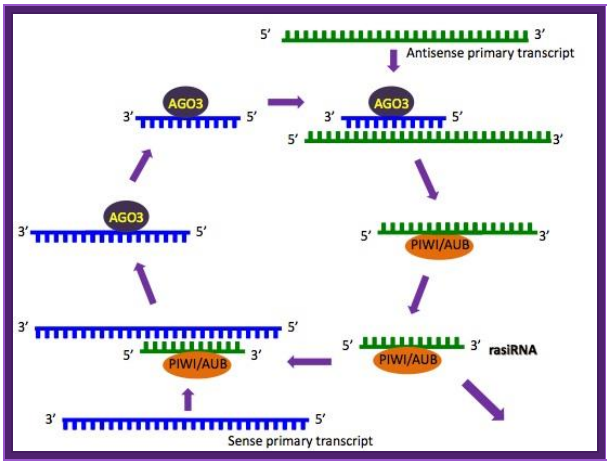
To address this they then silenced different combinations of the four *N. attenuata's* Dicer genes in the transgenic PMRi lines producing CYP6B46 dsRNA. Long CYP6B46 transcript levels in the plants was found to be increased more than 50 fold when the DCL 1,3,4 or DCL 2,3,4 were co-silenced. These then lead to an enhanced silencing effect in the larvae midgut, indicating that there could be a preference for insect diced smRNAs or simply that the larger dsRNAs were more stable and the higher

concentration enhanced the silencing effect. It also suggests that the plant and insect RNAi machinery respond differently to the dsRNA.

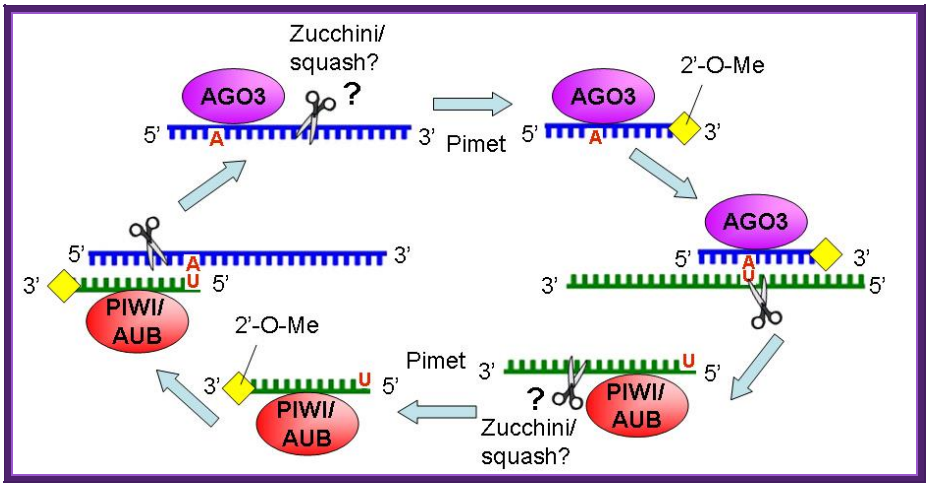
In conclusion PMRi can be a specific and robust system of gene silencing in*M. sexta*. PMRi would be the method of choice for crop protection in countries which allow the growth of transgenic crops. While retaining all the virtues of PMRi, VDPS promises to be a rapid and high throughput alternative, suitable for ecological research.

This article has been a short review of the journal article stated below. For more in depth information on this research, follow the link and download the freely available journal article; Mark Whitehead:

Repeat associated small interfering RNA (rasiRNA); Biogenesis:



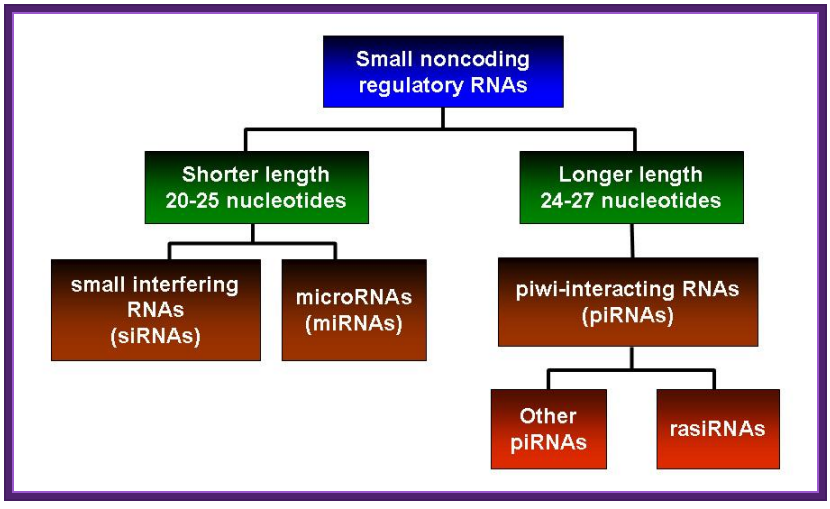
The biogenesis of rasiRNA is independent of Dicer, but does require the Argonaute proteins Argonaute 3 (Ago 3), Piwi and Aubergine which is a Piwi-like protein.[2] The mechanism for rasiRNA biogenesis is a ping-pong mechanism. The Piwi/Aub associated RNA is the rasiRNA. The rasiRNAs match the antisense strand of retrotransposons and repetitive sequence elements (hence the name rasiRNA).[2] The Ago3 associated RNAs are derived from the sense strand. http://en.wikipedia.org/



http://mcmanuslab.ucsf.edu/

rasiRNAs have been identified in plants, *Trypanosoma brucei*, fission yeast *Schizosaccharomyces pombe*, *Drosophila melanogaster* and zebrafish *Danio rerio*. In mammals, rasiRNAs have not been specifically described, but RNAs that are 22-24 nt in length have been reported in the mouse male germline and they associate with murine homolog of piwi which is part of the Piwi Argonaute subfamily. These RNAs have been more generically termed as piRNAs, because even though they share common features with rasiRNAs, they lack sequences sequences matching repetitive elements.

Like miRNAs and siRNAs, rasiRNAs have a strong preference for pyrimidine residues, especially uridine, at their 5′-most position. However, this is only restricted to the antisense rasiRNA strands. Sense rasiRNAs show no bias at the 5′ ends, though they have a strong preference for adenine at nucleotide 10. Also, rasiRNAs have a monophosphate group at the 5′ end, similar to miRNAs and siRNAs. However, unlike miRNAs and siRNAs which have 2′ and 3′ hydroxyl termini, rasiRNAs have a modification at one of the 2 hydroxyl termini.



http://mcmanuslab.ucsf.edu/

Importance of RasiRNA:

While miRNA act in translational repression and mRNA cleavage and siRNA act in mRNA cleavage, rasiRNA act to regulate chromatin structure and transcriptional silencing; In Drosophila, mutations in the Piwi proteins that associate with rasiRNA lead to sterility and loss of germline cells in both males and females. Transposon repression is not affected by the loss of Dicer within the germline cells revealing that this is the target of the rasiRNA pathway. Similar to miRNA and siRNA, the rasiRNA silencing pathway is evolutionarily conserved and homology dependent. When the rasiRNA pathway is not present, germline cells may undergo retrotransposition which are sensed as DNA damage and signal the cell to apoptosis. RasiRNA is key to the regulatory mechanism of many organisms as part of the RNA interference pathway.

RNAi Components:

S.No	Species	Proteins	Number of proteins	Functions	References
1.	*C. elegans*	Dicer	1	Long dsRNA processing	Tabara et al 2002
2.	*Homo sapiens*	Dicer	1	Long dsRNA processing to generate both miRNA & siRNA.	Bernstein et al.,2001
3.	*Drosophila melanogaster*	DCR1 DCR2	2	Generates miRNA Generates siRNA	Lee et al.,2004
4.	*Arabidopsis thaliana*	DCL 1 DCL 2 DCL 3 DCL 4	4	Processing of miRNA Processing of siRNA Processing of rasiRNA Unknown function	Xie et al, 2004 Vazquez et al, 2004 Xie et al, 2004
5.	*Schizosaccharomyces pombe*	Ago1	1	Gene silencing	
6.	*Tetrahymena thermophila*	Argonaute	1	Gene silencing	
7.	*C elegans*	Argonaute/ rde1	24		Carmell et al.,2002
8.	*Drosophila melanogaster*	Argonaute1 Argonaute2 Argonaute3 PIWI Aubergine	5	miRNA accumulation siRNA triggered silencing	Okamura etal.,2004
9.	*Homo sapiens*	Ago1 Ago2 Ago3 Ago4	8	Associate with miRNA& siRNA Exhibit RISC activity _ _	Meister et al.,2004

The table shows proteins involved in RNAi processing

Resistance to fungal diseases: Clone specific Chitinases or and beta-Gluconase genes specific to fungi, into binary vector in proper reading frame. Transform the desired plants. When the genes express the enzymes attack the fungal cell walls and destroy the infected fungi, thus one can obtain resistance to fungi. However these constructs have to be standardized.

Resistance to Bacteria: Bacterial cells have peptido-glycan, which can be degraded by Lysozyme. Similarly Cecropin is another, which punches holes

into bacterial membranes and destroys bacterial cells. By cloning such Lysozyme genes form bacterial or viral source one can develop plant resistant to bacteria. Plants such as citrus members are the most important crops, which are devastated by bacterial agents

Resistance to pests: Cotton, Coffee, Rice and many other important crop plants are infected with pests, which actually attack plants as larvae and bore into plants and kill them. In many situations nearly 50-70% of the cotton crop is lost because stem borers. The stem borers are species specific. For example cotton larvae are killed by some bacterial proteins, which are in crystal form. Bacillus thuringienesis has many strains and each of the strains produce specific proteins. When the larvae eat such proteins, they die. Bacillus thuringienesis mediated cotton is now in production all over the world and cotton GM plants are grown and farmers have been greatly helped and their crop production increased and this benefits them. Only thing that is important is the bacterial strains used should be specific to the peas, other wise many innocuous insects will be killed and it is disastrous to environment.

Terminator Gene by Monsanto:

This gene construct was constructed by Delta and Pineland (USA), patent number 5723765, March 3rd 1998 and now owned by Monsanto, a giant Biotech firm in Louisiana, USA. This technology was designed to produce hybrid seeds for a particular crop and in the second generation one cannot use the seeds by farmers because the seeds if used don't germinate. Thus farmers are forced buy hybrid seeds from the company, which produces them. Though this technology has been banned from production, the scientific technique that is employed in this is very fascinating. This technique can be used for generating any antibiotic or any other genes used as a marker diagnostic gene.

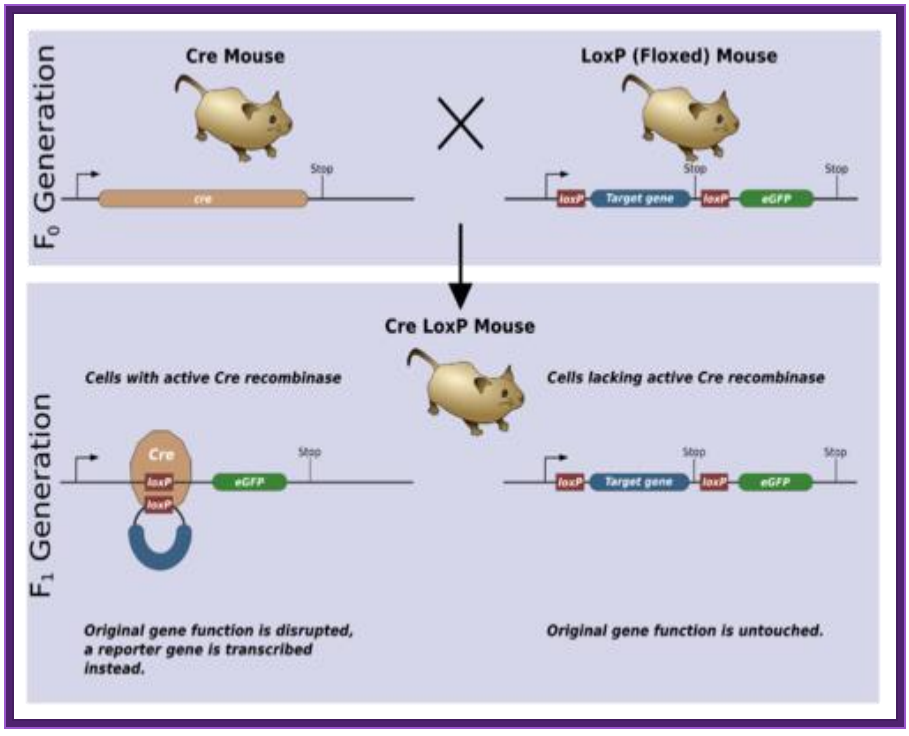
In this technique three gene constructs with three different promoters are employed and some of the gene constructs are regulated.

One gene construct contained a Toxin gene called Cholera toxin. Even an RNase gene or a Barnase gene or Ribosomal inhibitor genes can be used as lethal genes that can kill the cells where they are expressed.

Lethal Gene construct:

A lethal gene, any one of them mentioned above is cloned under the promoter, which is active only during late embryogenesis. But the expression of this gene is blocked by a blocking sequence inserted between the promoter and the lethal gene. The blocking gene is bracketed by lox-P sequences, which can exercised only Recombinase coded for by the gene called Cre. In a transgenic plant this gene is expressed very late in the embryogenesis. Even if it is expressed the Toxin gene is not produce because of the presence of blocking sequence.

But if the tissue also contains a Cre gene and if it is expressed, at this stage of development, a Cre product recombinase uses the lox-P sequences and removes the blocking sequence in such a way the Toxin gene is placed next to the late promoter in reading frame. Interestingly the Cre gene is cloned under a Tet operon promoter, which is blocked by Tet repressor proteins, which is also expressed in the same tissue. The Tet repressor protein binds to Cre- recombinase promoter and inhibits the expression the gene.



e-specific Recombination is also an important process that viruses, such as bacteriophages, adopt to integrate their genetic material into the infected host. The virus, called a *prophage* in such a state, accomplishes this via integration and excision. Using this protocol one can remove a toxic gene used as marker by hybridizing the Cre gene and a desired gene bordered with LoxP sites. In the hybrid plant the Cre enzyme acts at loxP site and remove the unwanted gene. The following is Lox P sequences. In this the middle 8bp is an asymmetric sequence. http://en.wikipedia.org/

13bp **8bp** **13bp**

ATAACTTCGTATA - GCATACAT -TATACGAAGTTAT

—**N-Part of the Gene**--loxP--antibiotic gene--LoxP-C-**Part of the Gene**--

V

Cre Recombinase

V

Functional Gene

If Tetracycline is added to the tissue, the antibiotic binds to the Tet repressor protein and makes it inoperative and it does not bind to the promoter, so the

recombinase gene expresses and the recombinase acts on the lox-P sequences and produces a functional Toxic gene. The Tet repressor protein is expressed under the control of CaMV35S promoter as constitutively expression mode.

I---L-P----I>>>>> Blocking<<<<<I-----Toxin gene-----I Ttr--

Note the promoter in this is late embryo promoter and this will be active only at late embryo development. Other wise it remains silent. Ttr means it is poly (A) signal for transcription termination.

I---Tet P/O—I-------Cre-gene---------I-Ttr-

Tet-P/O; it is Tet-promoter operator, which can be regulated by the Tet-repressor protein. The repressor is from Tn10-tet. This when present it binds to the operator promoter and prevents the expression of Cre-gene.

I—CAMV35S-P---I-----Tet-repressor-----I-Ttr—

CAMV35S-P is a promoter derived from Cauliflower mosaic viral capsid promoter. This promoter is used for constitutive expression.

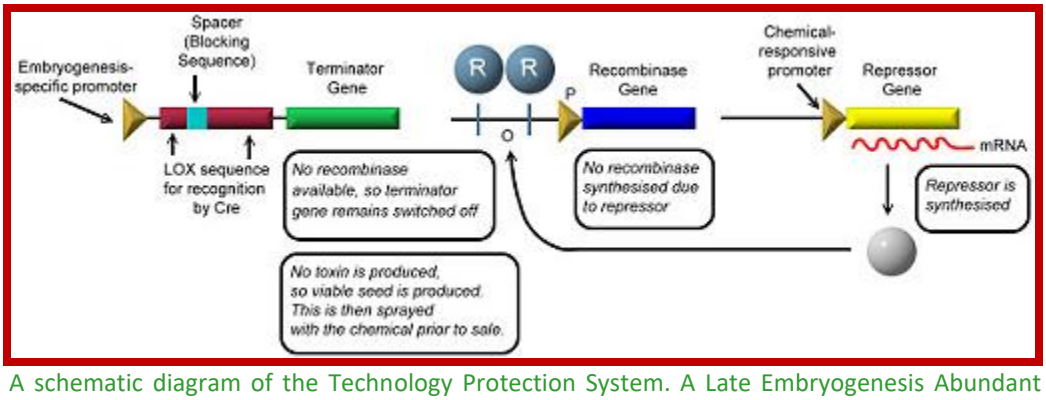
I—L/P---I------Toxin gene----I—Tr-tr-

When all these constructs are added to the plant tissue and the transgenic plant, which is a hybrid for a specific trait, grows and produces the desired product. In this tet-repressor is constitutively expressed and it binds to the Tet-o/p and prevents the expression of recombinase. In the absence of recombinase the blocking segment remains intact and the functional Toxin gene product is not produced.

If such first generation seeds are obtained and treated with tetracycline, during the development recombinase is obtained and the recombinase acts on the Lox-P sequences found at either ends of blocking sequences and cleaves and removes the blocking sequences. Thus the toxin gene is placed under the control of late embryogenesis promoter.

When such seeds are grown the plants develop well and the harvest is obtained. These plants produce seeds. But while the seeds develop the late promoter becomes active and during late embryogenesis the toxin gene is expressed and the tissues are killed. When such seeds are sown the embryo won't develop. So the farmer is forced to go back to the hybrid seed producer every time.

This very fascinating and brilliant technology, but look at the company's intent behind it. Interestingly the same technology can be used for making a gene inactive at specific stage of development. For example most the GM plants have antibiotic genes. When the plants develop and seeds or fruits produce the KAN^+ or $Hygromycin^+$ genes are present. Consumers won't be happy and certainly it is not desirable. If gene constructs are made to eliminate the antibiotic gene late in the development, the plant products are desirable and bring no harm to the consumers. If such toxin genes are placed in between LoxP direct repeats and these plants are hybridized with plants containing Cre gene, the loxP is subjected Cre recombinase and the toxin gene is eliminated. To day this techniques has been employed in the production o GM plants.



A schematic diagram of the Technology Protection System. A Late Embryogenesis Abundant (LEA) promoter regulates the expression of a LOX sequence (itself containing a recognised blocking sequence), followed by a Ribosomal Inactivating Protein ("terminator") gene. Further down the chromosome is a Cre Recombinase gene, under the control of a tet promoter. A tet repressor gene prevents transcription of the Recombinase gene by synthesising a blocking protein. When tetracycline is applied, repressor gene transcription is stopped. This causes transcription of the Cre Recombinase gene, which produces Cre. Cre recognises the Cre blocking sequence in the LOX sequence and splices

LOX from the genome, thus placing the Ribosomal Inactivating Protein under the direct control of the Late Embryogenesis Abundant promoter. During late embryogenesis, the Ribosomal Inactivating Protein "Terminator gene" is expressed, leading to the abortion of all embryos.; http://www.adonline.id.au/

Post Harvest Technology:

This post harvest techniques are virtually non-existence not in practice by our Indian farmers. However they have developed few protocols such as storing food grains in deep pits (Hagev in Kannada) in the earth or in large wooden containers (Panata in Kannada). At the time of storing farmers used mix the grains with lot of Neam leaves. Now scientists have found that Neam leaves and seeds have insecticidal property.

Greatest flaw in storing fleshy fruits is that they cannot stored for a long time and they either have to be disposed or they have to be consumed before they rot. But our farmers have developed certain post harvest technology of hastening the ripening process of raw fruits. Toady we know the relevance and scientific mechanism involved in this process which was used by plantation owners (small and big) since thousands of years.

Farmers at eve of village shanty market, they used to harvest plantains bunches. Then they are kept in a big oval shaped earthen pot (designed for this purpose) which contains a kiln like opening at the bottom. The top is sealed with soil paste. In the evening the some cow dung both dry and semi dry type are placed. Then it is lit with fire. This burning produces black smoke. When the smoke fills up the earthen container to the brim, they close the kiln and the top is also sealed with wet mud. Leave the whole thing the entire night. When the earthen pot opened in the morning, one finds all green colored fruits have turned into beautiful yellow color. Even harvested green mangoes were buried in paddy or eleucine or sorghum harvested stocks (Banave in Kannada). This was also found to hasten the process of ripening and producing beautiful color.

Today the knowledge of fruit ripening is known. As the raw fruits, fleshy and green, as fruit start aging it produces ethylene by gene activation. Ethylene activates the ethylene producing genes; so more and more of ethylene is produced. Ethylene in turn activates many genes responsible for degrading chlorophylls and genes responsible for the synthesis of flavones and color pigments. At the same time few more genes are activated for degradation pectin, cellulose and hemicelluloses. Another set of genes that are activated is responsible for converting stored food materials into sweet sugars. In this process of ripening fruit becomes soft, sour or tasteless content becomes sweeter and green color changes into lovely orange or reddish orange color. The biochemical process of ripening reaches a crescendo resulting in a climacteric process and has triple effects.

Almost all fleshy fruits are perishable and cannot be kept for a long time, so they have a very short shelf life. Farmers do suffer from this. Longer shelf life is advantageous for the farmers to keep them for longer period and they can sell their products as and when they get good prices, otherwise they are all in the mercy of commission agents and Dallals; the money suckers and bloodsuckers make money and farmers/growers are left high and dry. This is phenomenon all over the world.

Ethylene synthesis multistep biochemical pathway where several enzymatic and steps are involved. To start with L-Methionine is converted to S-adenosine Methionine. Then it is converted to 5′ Methyl adenosine And 1-aminocyclopropane -1-carboxilic acid, which when oxidized liberate $CH_3=CH_3$ and CO_2 and Ammonia. The enzyme responsible for the synthesis is ACC synthase. Plant molecular biologist have constructed a gene for ACC synthase and cloned into a binary vector under a promoter that is activated only when the fruit is ripening. The gene is cloned in such a way its orientation is reversed. When this gene is expressed the RNA that is transcribes has nucleotide sequences complementary to that of ACC synthase mRNA. When the anti sense RNA is expressed in sufficiently large amounts the antisense RNA hybridizes with ACC mRNA and makes it untranslatable.

So no ethylene is produced. Thus automatically fruit ripening is delayed. This is because; ethylene production is not completely stopped. This has a great advantage for one can keep the fruits for a long period of time and also allow the fruit to ripe slowly. Such fruits were first produced in USA and introduced in Chicago and Los Angeles market as Flavr Savr fruits by Calgene and a slight variant in UK by Zeneka. People actually lapped up the fruits. The same technology can be applied to banana, apple, orange and other fleshy fruit plants.

Antisense RNA technology can also be applied to delay senescence of cut flowers. Cut flowers some-how rot in their stacks, this is due to ethylene effect. If the same anti sense mRNA for ACC synthase is used it is possible to keep cut flowers for a longer period of time?

Post harvest technology can also be applied to protect food grains from insect attack. Similar to Bt gene products one has to find such toxins to specific insects. Their genes can be cloned into the required plants. So they are over expressed late in the grain filling. When such grains are stored, even if insects feed upon they die. One has to take care of such proteins are not harmful to humans and other animals that eat them.

When did genetically modified foods originate?

Between 1997 and 1999, gene-modified (GM) ingredients suddenly appeared in 2/3rds of all US processed foods. In 2003, countries that grew 99 % of the global transgenic crops were the United States (63 %), Argentina (21 %), Canada (6 %), Brazil (4 %), China (4 %), and South Africa (1 %) and today the Grocery Manufacturers of America estimate that 75 % of all processed foods in the U.S. contain a GM ingredient.

Between 1995 and 2005, the total surface area of land cultivated with GMOs had increased by a factor of 50, from 17,000 km² (4.2 million acres) to 900,000 km² (222 million acres), of which 55 percent were in

Brazil. In the US, by 2006, 89 % of the planted area of soybeans, 83 % of cotton, and 61 % maize were genetically modified varieties.

Genetic engineering is inherently dangerous, because it greatly expands the scope for horizontal gene transfer and recombination." - Dr. Mae-Wan Ho; then today Russia, China and other countries are growing transgenic plants and they are consumed (some food crops), till now there is not a single instance where people who ate them and/ or who grew them suffered from cancer. In nature there are many instances where the there is a horizontal gene transfer. Most the natural crops are derived from cross pollination among the population of plants and animals. Precaution should be taken by plant scientists who does this kind of work should take care of removing the antibiotic marker or similar genes should be removed by Cre mechanism or by any such process. People who are against such crops should realize that when they eat animal meat and plants, they do consume large number proteins and they are digested to their respective amino acids. Benefits of GM plants is overwhelmingly good for the world population for the yield of the crops is going down and the cultivable lands are grabbed by the industrialists and the farmers sons are settling in cities. What to do with food generating crop plants?

Transgenic Male Sterile Plants:

Production of male sterile lines of specific species is an invaluable tool in the hands of plant breeders. Such male sterile lines are always being the females and the hybrids with other pollen donors can be selected and the desired genes can be transferred. And the genes transferred will be known. The offspring's of such breeding with male sterility will also be male sterile. In such situation one has to resort to restorer lines for making them bisexual.

Technically one has to use toxic genes like cholera toxin, RNase or Barnase genes. Any one of them can be used. Such genes have to be cloned into a

binary vector under the control of anther specific promoter. If the gene is expressed where the anther is maturing the expressed products ablaze the tissues and no pollen grains are produced. Thus the plant becomes male sterile line.

Fast growing plants: Biology news net. "Gene Discovery suggest ways to engineer fast growing plants"

That genome-wide search in the roots of the familiar laboratory plant Arabidopsis and subsequent screening of mutant lines turned up a single gene, which the researchers call UPBEAT1 (UPB1). Further study showed that UPB1 controls the gene expression of enzymes known as peroxidases.

They then showed that these peroxidases control the balance of free radicals between the zone of cell proliferation and the zone of cell elongation where differentiation begins. (Although free radicals are probably most familiar as agents of stress to be combated with antioxidants, Benfey's noted that the balance of free radicals has also been implicated in the control of a similar transition from proliferation to differentiation in animals.

"It's possible that by manipulating a single gene, you could get a plant with rapid growth," Benfey's said. Interestingly, UPB1 appears to act independently of plant hormones that play well-known roles in the balance between cell division That genome-wide search in the roots of the familiar laboratory plant Arabidopsis and subsequent screening of mutant lines turned up a single gene, which the researchers call UPBEAT1 (UPB1). Further study showed that UPB1 controls the gene expression of enzymes known as peroxidases.

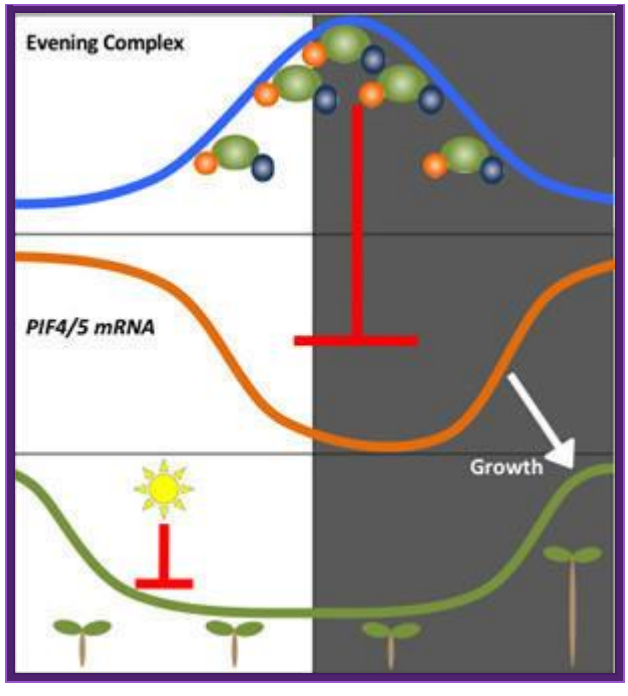
When the researchers experimentally disrupted UPB1 activity in the plant root, it altered the balance of free radicals such that cells delayed their differentiation and continued growing. Those plants ended up with faster-

growing roots, having more and larger cells. When UPB1 activity was artificially increased, the growth of plant roots slowed

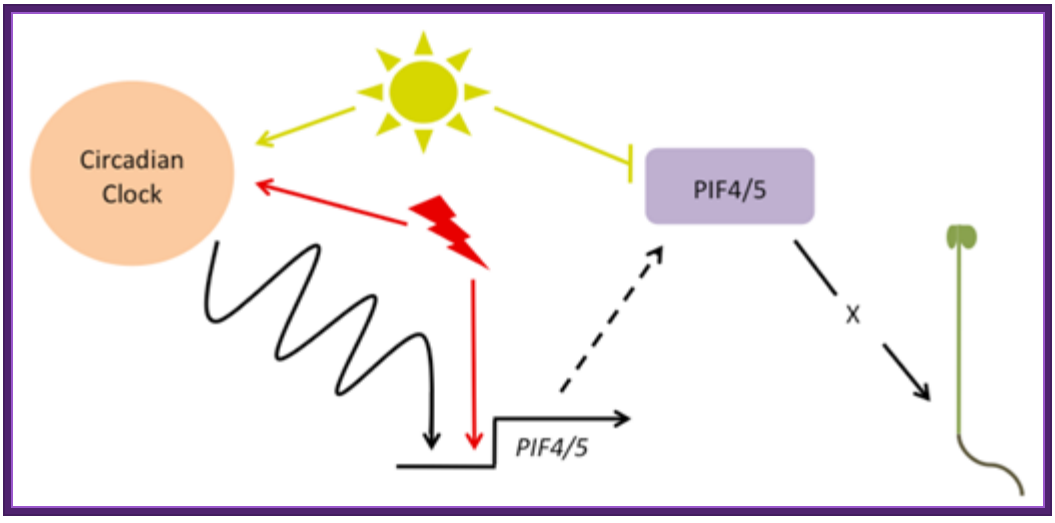
"It's possible that by manipulating a single gene, you could get a plant with rapid growth," Benfey said. Interestingly, UPB1 appears to act independently of plant hormones that play well-known roles in the balance between cell division and differentiation.

Biologists Discovered an 'Evening' Protein Complex That Regulates Plant Growth (Science Daily july 13, 2011)

Observers of nature have long known that crops like corn and sorghum grow taller at night. But the biochemical mechanisms that control this nightly stem elongation, common to most plants; have been something of a mystery to biologists. It is now known (Nature) that the "evening Complex" controls the growth of plants in the evening.



The researchers demonstrated that the evening complex puts the brakes on the activity of two genes in plants--PIF4 and PIF5--that are important in promoting plant growth. (Credit: Yhew Pongsawakul); http://franciscofuentescarmona.blogspot.com/



Schematic of the external coincidence of Arabidopsis stem growth. Current knowledge of this system suggests that the development of the Arabidopsis stem is controlled through the transcription factors PHYTOCHROME INTERACTING FACTOR 4 (PIF4) and PIF5. These genes integrate pathways from the circadian clock and the external environment, notably light (yellow sun) and temperature (red bolt) signalling. PIF4/5 have been shown to be regulated by LUX ARRHYTHMO (LUX) from the circadian clock, whilst temperature increases the PIF mRNA abundance and light (through PHYB - the primary regulator of red light signals) inhibits the accumulation of protein. University of Edinburgh; Dr Karen Hallidayhttp://hallidaylab.bio.ed.ac.uk/

Biologists show how this protein complex is intricately coordinated through the biological clock with the genes that promote stem elongation in a way that could enable plant breeders to engineer new varieties of crops that grow faster, produce greater yields of food or generate more biomass per acre of land for conversion into biofuels.
UCSD scientist discovered three genes responsible for such growth (in Arabidopsis). "This protein complex is clearly acting like the brakes on growth," said Kay. "So when we mutate any one of these genes the plants elongate much more."

"What we show in our paper is that the evening complex binds to the promoters of PIF4 and PIF5 and, at the end of the day and through the early part of the night, prevents the plants from growing," said Kay. "And when the levels of the evening complex begin to drop, PIF4 and PIF5 are expressed and drive plant expression programs that support stem elongation, and the brakes on plant growth are taken off."

Improvement of Timber yielding plants:

Phytohormones such as Auxins and Cytokinins control growth of plants in height and thickness of stems. Gibberellins also control the height of the plants. Gene for the synthesis of Auxins and Cytokinins has been isolated. Certain transgenic plants with IAA and IPA genes have been obtained. But unfortunately the level of hormone synthesis was not under the control. In spite of this, plants showed very bushy characters. If the level of hormone production is properly controlled, it is possible control the growth of the plant. Thus many tall tree plants can come to maturity in short period of time instead of taking 50 to 75 years characters and the character of timber can also be improved.

If such timber yielding plants also contain antifreeze genes the plant do not suffer from frostbite. Such transgenic plants have been produced and perhaps they are in use. The gene for anti freezing protein has been isolated from certain fishes.

By genetic manipulation it is possible to generate new color or a combination of colors in horticultural plant, which are in demand for their colorations. It has been done in the case of petunia.

Advances in Tree Genetic Engineering in China:

Su Xiao-hua[1], Zhang Bing-yu, Huang Qin-jun, Huang Lie-jian and Zhang Xiang-hua

Wood quality improvement;

At present, the genetic engineering of lignin biosynthesis appears to be a promising forest biotechnological application to improve wood quality. Because the economy of pulping processes depends almost

entirely on the efficiency of removing lignin which encrusts the wood fibers. Thus, one way to improve the efficiency of pulping is to genetically reduce the quantity or to alter the quality of lignin in pulpwood species. Lignin biosynthesis was the most peculiar biological process involving various components of wood. Genetic manipulation of lignin is largely a matter of manipulating genes encoding lignin pathway-specific enzymes. It takes a long time to reach the breeding goals in traditional breeding program, because multi-genes controlled most wood traits. At present, transgenic method is of great expectations. So, the genetic engineering of lignin biosynthesis has bright prospects in its application in tree breeding for pulping and papermaking industries. Research in this field should be strengthened, to breed new varieties for commercial plantations for pulp in Yangtze and Yellow River systems.

Genetically Engineered "Forests"- Monday August 31, 2009.

Genetically engineered Eucalyptus is resistant to cold (USDA). Loblolly is one of the dominant native trees in this part of the continent (USA). Improved varieties of loblolly pine should mature in 18 yrs rather than 26 years, and eucalyptus in 4 rather than 7!

Forest plants should have durability and strong and should grow faster, each 50 ft in two to three years. The timber quality should be good; these can be achieved with thinking and innovative concepts.

Pharma-Plants:

Plants can produce a variety of organic compounds if their genes are properly regulated. With the introduction specific genes one can make the plants to produce a desired compound, which can be made either secrete out of tissues or it can be made to store in vacuoles or chloroplasts. Many biopolymers such as poly hydroxyl butyrate (PHBs) are produced by gene manipulations.

- Edible vaccines have been produced against cholera toxin genes. The said bacterial gene is cloned into a binary vector and placed under a general promoter or it can be placed under inducible promoter. Here the toxin gene is not cloned in its entirety, but only specific segment of the gene is chosen which gives maximum immunity. Such vaccines are called subunit vaccines.
- Cholera toxin is a multimeric protein consisting of one 27KD protein and 5 11.6KD subunits, which are non-covalently linked to one another dough like structure). This multimeric binds to gangliosides that are present on epithelial cell surface. Antibodies against such proteins prevent the binding of toxins to cell surfaces.

When such gene fragments, which produce a part of the protein, which act good inducers of immunity, can be introduced into plants, so as to express in a particular tissue, such as a fruit or a tuber. In the Texas A & M University, cholera toxin gene has been introduced into potato plant and the gene was made express in the tuber. By eating the tubers, by Galt system one can develop immunity against cholera disease by a process called passive immunization. People are working on developing a variety of subunit vaccines in plant and the product to be expressed in fruits such as tomato, apple or Banana, for these fruits are more palatable for eating than the tubers.

- Plant scientists have developed transgenic plants, which produce specific antibodies, which are called plantibodies. Advantage of producing antibodies in plants is one can scale up the production of the tissue by single cell culture or callus culture. If the genes are clines as inducible, it will be great. It is not only Antibodies; one can produce many many important medicinal products in plants. One can use plants as biopharmaceutical living factories.

- In Scrip's Institute, Lajolla, California scientists have developed antibodies when added to tooth pastes against specific bacteria one can prevent tooth infection and decay in children. Jack Johnson et al has reported from Perdue University that they have cloned gp4 of HIV

viral gene into cowpea mosaic viral genome and introduced into cowpea plant. The produce was capable of neutralizing HIV infection.

- Certain strains of E.coli produce heat labile enterotoxins, which cause Diarrhea. If such enterotoxins gene is used to produce proteins they can act as active oral antigens or immunogens. These are called efficacious vaccines have the potentiality the mucosal immune system leading to the production secretary Immunoglobulin type A (Ig-A) this kind of immunization is achieved by oral than 'parenateral antigen' delivery. So the delivery of this enterotoxins protein or any other pathogenic proteins (including subunits) orally makes it as a very potent oral vaccine against many such diseases causing organisms.

- Gene encoding LTB or LTB-fusion protein with SKDEL signal sequence for targeting to microsomal membranes are cloned into a binary vector and then such genes are transferred into potato and Tobacco plants. ELISA has detected the expressions of such proteins. The amount of proteins accumulated is found to be 14ug per gram of total soluble Tobacco leaf protein and 100ug per gram of soluble protein in potato microtubular proteins.

-
- When such protein extracts of LTBs were orally administered to human trail specimens with a dosage of 12.5ug for 4, 21 and 25 Th day, the mucosal antibodies were detected at 30 to 35 days. This clearly demonstrates that plant derived proteins have immunogenic properties.

The above said examples are typical paradigms for a vast variety of recombinant DNA products that can be produce in plants in large quantities which have an applied value on large scale.

Plant based Biopolymers:

Poly R-3 Hydroxy Butyrate called PHBs and other related polyhydroxy alkanoates are aliphatic polyesters. These have Thermoplastic properties of industrial applications.

Alcaligenes eutrophus and few other bacteria produce polyhydroxy alkanoates as carbon source when grown on excess carbon diet. The PHBs are synthesized and accumulate as granules of 0.2 to 1um size. Often accumulation is up to 80% of the dry weight the cell protein.

The pathway of the synthesis is: –

Acetyl Co-A --E1→ --E2--→ ---E3→ PHB

E1 = 3-Ketothiolase (PHB-A gene).
E2 = acetyl Co-A reductase (PHB-B gene).
E3 = PHB synthase (PHB-C gene).

The said genes were isolated and cloned independently into PBI 121 binary vectors under the control of CAMV35S promoter. However the genes are cloned in such a way the gene product was targeted into chloroplasts. The signal sequences used for this were from small subunit RuBpCASE (Ribulose bi phosphate carboxylase). The said genes were used obtain transgenic plant for each of the genes. Then they are crossed to each other to combine all these genes to be in the same plant. Such plants showed the expression of the cloned products accumulated in chloroplasts of older leaves. More to it was the enzymes were able to synthesize PHBs to such an extent that 14% of the dry weight of the chloroplasts was found to be PHBs. PHBs are not utilized as carbon source by plants. Such PHBs can be used as thermoplastics and they are environmentally friendly and they are biodegradable.

An advantage against cotton fibers is that cotton fibers exhibit wrinkles, but if the PHBs are expressed in such a way they are to be with the core of the cotton fibers. Such fibers are free of wrinkles.

Agricetus Inc in Middleton, Wisconsin, has worked on such polymers. Maliyakal Ejohn in Agricetus has succeeded in gene engineering of cotton plants and produced cotton plants, which produce PHBs along with cellulose fibers.

Alpha Trichosanthin: Alpha Trichosanthins (THS) are inhibitors of ribosomes. They are produced by Trichosanthin kirilowii maximowicz. The product is monomer 27KDs. It binds to 28s RNA and it inhibits translation when administered in higher doses. This protein is synthesized as pre-protein with 19 amino acid residues signal sequence and secreted after the cleavage of signal sequences. The secreted product is an n active protein. It is believed that it inhibits HIV replication in CD4 lymphocytes as well as macrophages; hence this protein has great potentiality as therapeutic agent.

This gene has been cloned under the control of a promoter of sub genomic coat protein of TMV. By genetic manipulation a hybrid of TMV RNA and an mRNA for Trichosanthin was obtained. When this hybrid RNA was inoculated into Nicotiana banthamiana species, recombinant viral particles were produced. These viral RNAs produced more viral particles and they were found spreading to other parts of the plant. At the same time they also generated Trichosanthins in plenty. The amount of this protein produced in the plant in this mode was found to be 2% of the total soluble proteins. This production was estimated to be highest amount of protein produced by a transgenic plant.

List of genetically modified foods: *Mavis Butcher* - Published: 2009-09-22

It's virtually impossible to provide a complete list of genetically modified food (GM food) in the United States because there aren't any laws for genetically modified crops. While engineering a plant with a desired gene we use a marker gene like an antibiotic gene or any other that is used to know whether the desired gene is introduced into plant tissue. But there technologies that can be used eliminate such marker

genes, thus the agricultural plant thus modified are safe. There are people who have idea about genetic engineered plant, simply raise nonsense problems without knowing what is good and bad about genetically engineered plants. Some estimates say as many as 30,000 different products on grocery store shelves are *"modified."* That's largely because many processed foods contain soy. Half of North America's soy crop is genetically engineered! Do you the most intelligent persons think Americans are fools to eat such plants; ask how many Americans have died by eating such plants.

Rapeseed - Resistance to certain pesticides and improved rapeseed cultivars to be free of erucic acid and glucosinolates. Glucosinolates, which were found in rapeseed meal leftover from pressing, are toxic and had prevented the use of the meal in animal feed. In Canada, where "double-zero" rapeseed was developed, the crop was renamed "canola" (Canadian oil) to differentiate it from non-edible rapeseed.

Honey - Honey can be produced from GM crops. Some Canadian honey comes from bees collecting nectar from GM canola plants. This has shut down exports of Canadian honey to Europe.

Cotton - Resistant to certain pesticides - considered a food because the oil can be consumed. The introduction of genetically engineered cotton plants has had an unexpectedly effect on Chinese agriculture. The so-called Bt cotton plants that produce a chemical that kills the cotton bollworm have not only reduced the incidence of the pest in cotton fields, but also in neighboring fields of corn, soybeans, and other crops.

Rice - Genetically modified to contain high amounts of Vitamin A. Rice containing human genes is to be grown in the US. Rather than end up on dinner plates, the rice will make human proteins useful for treating infant diarrhea in the developing world.

Soybean - Genetically modified to be resistant to herbicides - Soy foods including, soy beverages, tofu, soy oil, soy flour, and lecithin. Other products may include breads, pastries, snack foods, baked products, fried products, edible oil products and special purpose foods.

Sugar cane - Made resistant to certain pesticides. A large percentage of sweeteners used in processed food actually comes from corn, not sugar cane or beets. Genetically modified sugar cane is regarded so badly by consumers at the present time that it could not be marketed successfully.

Tomatoes - Made for a longer shelf life and to prevent a substance that causes tomatoes to rot and degrade.

Corn - Resistant to certain pesticides - Corn oil, flour, sugar or syrup. may include snack foods, baked goods, fried foods, edible oil products, confectionery, special purpose foods, and soft drinks.

Sweet corn - genetically modified to produces its own insecticide. Officials from the US Food and Drug Administration (FDA) have said that thousands of tons of genetically engineered sweet corn have made their way into the human food supply chain, even though the produce has been approved only for use in animal feed. Recently Monsanto, a biotechnology food producer, said that about half of the USA's sweet corn acreage has been planted with genetically modified seed this year.

Canola - Canola oil. May include edible oil products, fried foods, and baked products, snack foods.

Potatoes - (Atlantic, Russett Burbank, Russet Norkatah, and Shepody) - May include snack foods, processed potato products and other processed foods containing potatoes.

Flax - More and more food products contain flax oil and seed because of their excellent nutritional properties. No genetically modified flax is currently grown. An herbicide-resistant GM flax was introduced in 2001, but was soon taken off the market because European importers refused to buy it.

Papaya - The first virus resistant papayas were commercially grown in Hawaii in 1999. Transgenic papayas now cover about one thousand hectares, or three quarters of the total Hawaiian papaya crop. Monsanto donated technical knowhow to Tamil Nadu Agricultural University, Coimbatore, for developing a papaya resistant to the ring spot virus in India.

Squash - (yellow crookneck) - Some zucchini and yellow crookneck squash are also GM but they are not popular with farmers.

Red-hearted chicory - (radicchio) - Chicory (Cichorium intybus var. folio sum) is popular in some regions as a salad green, especially in France and Belgium. Scientists developed a genetically modified line of chicory containing a gene that makes it male sterile, simply facilitating the production of hybrid cultivars. Today there is no genetically modified chicory on the market.

Cotton seed oil - Cottonseed oil and linters. Products may include blended vegetable oils, fried foods, baked foods, snack foods, edible oil products, and small goods casings.

Tobacco -The Company Vector has a GMO tobacco being sold under the brand of Quest® cigarettes in the U.S. It is engineered to produce low or no nicotine.

Meat - Meat and dairy products usually come from animals that have eaten GM feed.

Peas - Genetically modified (GM) peas created immune responses in mice, suggesting that they may also create serious allergic reactions in people. The peas had been inserted with a gene from kidney beans, which creates a protein that acts as a pesticide.

Vegetable Oil - Most generic vegetable oils and margarines used in restaurants and in processed foods in North America are made from soy, corn, canola, or cottonseed. Unless these oils specifically say "Non-GMO" or "Organic," it is probably genetically modified.

Sugar beets - May include any processed foods containing sugar.

Dairy Products - About 22 percent of cows in the U.S. are injected with recombinant (genetically modified) bovine growth hormone (rbGH).

Vitamins - Vitamin C (ascorbic acid) is often made from corn, vitamin E is usually made from soy. Vitamins A, B2, B6, and B12 may be derived from GMOs as well as vitamin D and vitamin K may have "carriers" derived from GM corn sources, such as starch, glucose, and maltodextrin.

Vaccine antigen - production in transgenic plants, Stable integration of a gene into the plant nuclear or chloroplast genome can transform higher plants, into bioreactors for the production of subunit vaccines for oral or parental administration.

How can the public make informed decisions about genetically modified (GM) foods when there is so little information about its safety?

According to the FDA and the United States Department of Agriculture (USDA), there are over 100s plant transgenic plant varieties that have completed all of the federal requirements for commercialization.

2003: GM crops cover more than 60% of US Crops ~ 110 million acres, and 167 million acres worldwide (Argentina is next biggest grower of GM crops) today (Summer 2007...). Do our Indian top specialists think it is a bad idea to use genetically transformed plants for agriculture? China is using transgenic crops uninhibitantly. All over the world statics' show- Only Indian agricultural scientists and some big bosses in Central govt., preventing the introduction of human consumable crop plants.

~40% of all corn is genetically engineered

~80% of all soybeans are genetically engineered

~75% of all cotton is genetically engineered.