IN THE NAME OF GOD

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Genetically Modified Techniques, transgenic products

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What are GMO techniques?

- The term genetically modified (GM), as it is commonly used, refers to the transfer of genes between organisms using a series of laboratory techniques for cloning genes, splicing DNA segments together, and inserting genes into cells. Collectively, these techniques are known as recombinant DNA technology

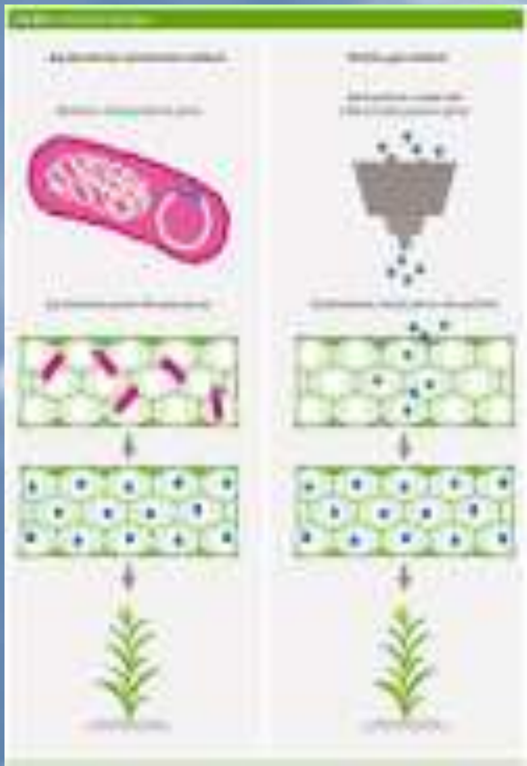
➢• What are transgenic products?

➢• The engineered organisms scientists produce by transferring genes between species are called transgenic. Several dozen transgenic food crops are currently on the market, among them varieties of corn, squash, canola, soybeans, and cotton, from which cottonseed oil is produced.

What type of technology is used in genetically modified food?

- **recombinant DNA technology**
- The technology is often called "modern biotechnology" or "gene technology", sometimes also "recombinant DNA technology" or "genetic engineering". Currently available GM foods stem mostly from plants, but in the future foods derived from GM microorganisms or GM animals are likely to be introduced on the market.

➢How do you genetically modify food?



➢GM is a technology that involves inserting DNA into the genome of an organism. To produce a GM plant, new DNA is transferred into plant cells. Usually, the cells are then grown in tissue culture where they develop into plants. The seeds produced by these plants will inherit the new DNA

➢How does genetic modification work?

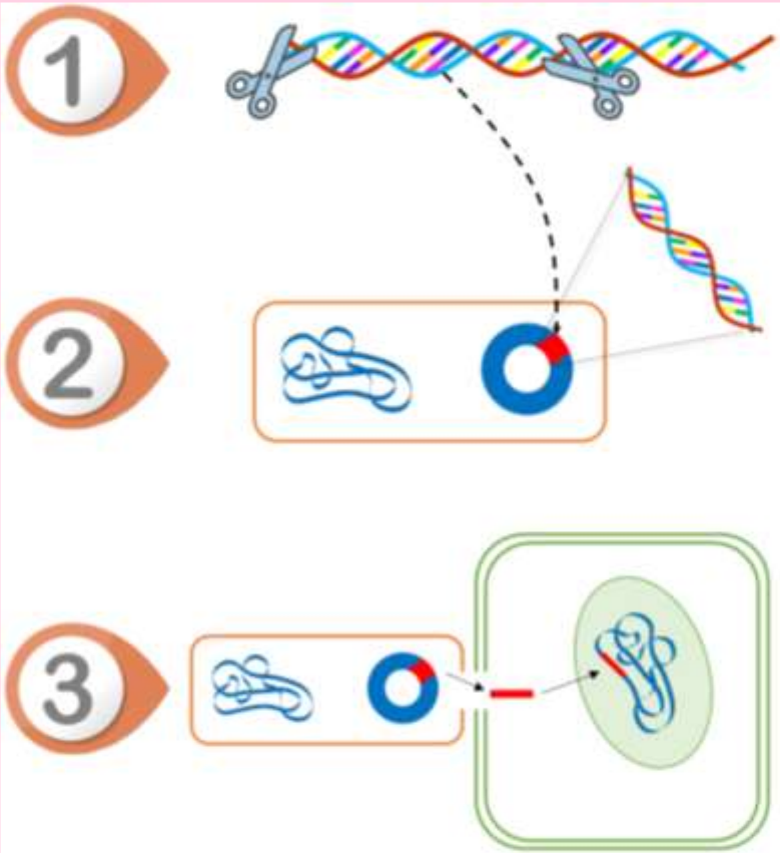
➢**1. First, a gene of interest is selected.**

This is then transferred to a plant cell, usually through one of two main methods:

➢**2. being fired from a device known as a ‘gene gun’ into the cell;**

➢**3. being inserted into a specialised piece of DNA in bacteria that are capable of transferring this information to plants (see image).**

➢How does genetic modification work?Continued



A gene of interest is located and cut out (either from the same species or a different one).

The gene is inserted into a specialised piece of DNA called a ‘plasmid’ in a bacterium.

The bacteria that are selected have the ability to transfer a piece of their DNA into plant cells. By replacing this DNA with the gene of interest, it is incorporated into the plant’s DNA.

Which is the first transgenic plant?

The world's first genetically modified crop is the **tobacco plant**, which produced in 1982; this plant was antibiotic-resistant. But China became the first country which launched the first transgenic plant at commercial level in the year 1992, that virus-resistant tobacco plant.

History of genetic engineering

Human-directed genetic manipulation of food began with the domestication of plants and animals through artificial selection at about 10,500 to 10,100 BC. The process of selective breeding, in which organisms with desired traits (and thus with the desired genes) are used to breed the next generation and organisms lacking the trait are not bred, is a precursor to the modern concept of genetic modification (GM). With the discovery of DNA in the early 1900s and various advancements in genetic techniques through the 1970s, it became possible to directly alter the DNA and genes within food.

➢History of genetic engineering Continued

➢Genetically modified microbial enzymes were the first application of genetically modified organisms in food production and were approved in 1988 by the US Food and Drug Administration. In the early 1990s, recombinant chymosin was approved for use in several countries. Cheese had typically been made using the enzyme complex rennet that had been extracted from cows' stomach lining. Scientists modified bacteria to produce chymosin, which was also able to clot milk, resulting in cheese curds.

➢History of genetic engineering Continued

➢The first genetically modified food approved for release was the Flavr Savr tomato in 1994. Developed by Calgene, it was engineered to have a longer shelf life by inserting an antisense gene that delayed ripening. China was the first country to commercialize a transgenic crop in 1993 with the introduction of virus-resistant tobacco. In 1995, Bacillus thuringiensis (Bt) Potato was approved for cultivation, making it the first pesticide producing crop to be approved in the US. Other genetically modified crops receiving marketing approval in 1995 were: canola with modified oil composition, Bt maize/corn, cotton resistant to the herbicide bromoxynil, Bt cotton, glyphosate-tolerant soybeans, virus-resistant squash, and another delayed ripening tomato.

➢History of genetic engineering Continued

- ➢With the creation of golden rice in 2000, scientists had genetically modified food to increase its nutrient value for the first time.[45]
- ➢By 2010, 29 countries had planted commercialized biotech crops and a further 31 countries had granted regulatory approval for transgenic crops to be imported. The US was the leading country in the production of GM foods in 2011, with twenty-five GM crops having received regulatory approval. In 2015, 92% of corn, 94% of soybeans, and 94% of cotton produced in the US were genetically modified varieties

➢History of genetic engineering Continued

➢**The first genetically modified animal to be approved for food use was AquAdvantage salmon in 2015. The salmon were transformed with a growth hormone-regulating gene from a Pacific Chinook salmon and a promoter from an ocean pout enabling it to grow year-round instead of only during spring and summer.**

➢**A GM white button mushroom (Agaricus bisporus) has been approved in the United States since 2016.**

➢**The most widely planted GMOs are designed to tolerate herbicides. The use of herbicides presents a strong selection pressure on treated weeds to gain resistance to the herbicide. Widespread planting of GM crops resistant to glyphosate has led to the use of glyphosate to control weeds and many weed species, such as Palmer amaranth, acquiring resistance to the herbicide.**

History of genetic engineering Continued

- In 2021, the first CRISPR-edited food has gone on public sale in Japan. Tomatoes were genetically modified for around five times the normal amount of possibly calming GABA. CRISPR was first applied in tomatoes in 2014.

Genetic engineering techniques process

- Creating genetically modified food is a multi-step process. The first step is to identify a useful gene from another organism that you would like to add. The gene can be taken from a cell or artificially synthesized, and then combined with other genetic elements, including a promoter and terminator region and a selectable marker. Then the genetic elements are inserted into the targets genome. DNA is generally inserted into animal cells using microinjection, where it can be injected through the cell's nuclear envelope directly into the nucleus, or through the use of viral vectors.In plants the DNA is often inserted using Agrobacterium-mediated recombination, biolistics or electroporation. As only a single cell is transformed with genetic material, the organism must be regenerated from that single cell. In plants this is accomplished through tissue culture. In animals it is necessary to ensure that the inserted DNA is present in the embryonic stem cells. Further testing using PCR, Southern hybridization, and DNA sequencing is conducted to confirm that an organism contains the new gene.

Genetic engineering techniques process continued

Traditionally the new genetic material was inserted randomly within the host genome. Gene targeting techniques, which creates double-stranded breaks and takes advantage on the cells natural homologous recombination repair systems, have been developed to target insertion to exact locations. Genome editing uses artificially engineered nucleases that create breaks at specific points. There are four families of engineered nucleases: meganucleases, zinc finger nucleases, transcription activator-like effector nucleases (TALENs), and the Cas9-guideRNA system (adapted from CRISPR). TALEN and CRISPR are the two most commonly used and each has its own advantages. TALENs have greater target specificity, while CRISPR is easier to design and more efficient

Genetically modified crops

- Genetically modified crops (GM crops) are genetically modified plants that are used in agriculture. The first crops developed were used for animal or human food and provide resistance to certain pests, diseases, environmental conditions, spoilage or chemical treatments (e.g. resistance to a herbicide). The second generation of crops aimed to improve the quality, often by altering the nutrient profile. Third generation genetically modified crops could be used for non-food purposes, including the production of pharmaceutical agents, biofuels, and other industrially useful goods, as well as for bioremediation. GM crops have been produced to improve harvests through reducing insect pressure, increase nutrient value and tolerate different abiotic stresses. As of 2018, the commercialised crops are limited mostly to cash crops like cotton, soybean, maize/corn and canola and the vast majority of the introduced traits provide either herbicide tolerance or insect resistance.

➢Genetically modified crops

➢Adoption by farmers has been rapid, between 1996 and 2013, the total surface area of land cultivated with GM crops increased by a factor of 100. Geographically though the spread has been uneven, with strong growth in the Americas and parts of Asia and little in Europe and Africa in 2013 only 10% of world cropland was GM, with the US, Canada, Brazil, and Argentina being 90% of that. Its socioeconomic spread has been more even, with approximately 54% of worldwide GM crops grown in developing countries in 2013. Although doubts have been raised, most studies have found growing GM crops to be beneficial to farmers through decreased pesticide use as well as increased crop yield and farm profit.

➢Fruits and vegetables

➢**Three views of a papaya, cultivar "Sunset", which was genetically modified to create the cultivar 'SunUp', which is resistant to Papaya ringspot virus.**

➢**Papaya was genetically modified to resist the ringspot virus (PSRV). "SunUp" is a transgenic red-fleshed Sunset papaya cultivar that is homozygous for the coat protein gene PRSV; "Rainbow" is a yellow-fleshed F1 hybrid developed by crossing 'SunUp' and nontransgenic yellow-fleshed "Kapoho". The GM cultivar was approved in 1998[88] and by 2010 80% of Hawaiian papaya was genetically engineered.**

➢Fruits and vegetables

➢The New York Times stated, "without it, the state's papaya industry would have collapsed". In China, a transgenic PRSV-resistant papaya was developed by South China Agricultural University and was first approved for commercial planting in 2006; as of 2012 95% of the papaya grown in Guangdong province and 40% of the papaya grown in Hainan province was genetically modified. In Hong Kong, where there is an exemption on growing and releasing any varieties of GM papaya, more than 80% of grown and imported papayas were transgenic.

➢In February 2013, BASF withdrew its application. In 2014, the USDA approved a genetically modified potato developed by J. R. Simplot Company that contained ten genetic modifications that prevent bruising and produce less acrylamide when fried. The modifications eliminate specific proteins from the potatoes, via RNA interference, rather than introducing novel proteins.

➢As of 2005, about 13% of the Zucchini grown in the US was genetically modified to resist three viruses; that variety is also grown in Canada

➢

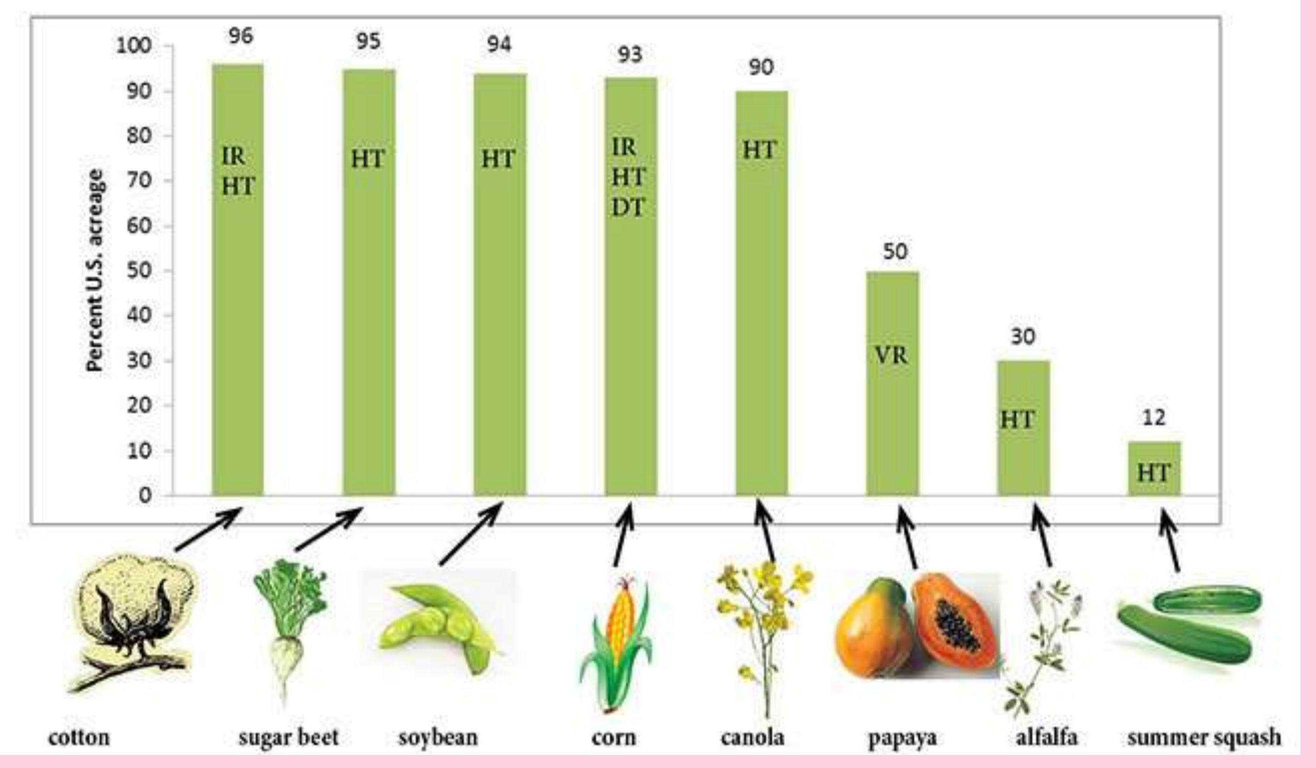
Rice

➢Golden rice is the most well known GM crop that is aimed at increasing nutrient value. It has been engineered with three genes that biosynthesise beta-carotene, a precursor of vitamin A, in the edible parts of rice. It is intended to produce a fortified food to be grown and consumed in areas with a shortage of dietary vitamin A, a deficiency which each year is estimated to kill 670,000 children under the age of 5 and cause an additional 500,000 cases of irreversible childhood blindness. The original golden rice produced 1.6µg/g of the carotenoids, with further development increasing this 23 times. In 2018 it gained its first approvals for use as food.

Derivative products Sugar

- The US imports 10% of its sugar, while the remaining 90% is extracted from sugar beet and sugarcane. After deregulation in 2005, glyphosate-resistant sugar beet was extensively adopted in the United States. 95% of beet acres in the US were planted with glyphosate-resistant seed in 2011. GM sugar beets are approved for cultivation in the US, Canada and Japan; the vast majority are grown in the US. GM beets are approved for import and consumption in Australia, Canada, Colombia, EU, Japan, Korea, Mexico, New Zealand, Philippines, the Russian Federation and Singapore. Pulp from the refining process is used as animal feed. The sugar produced from GM sugar beets contains no DNA or protein – it is just sucrose that is chemically indistinguishable from sugar produced from non-GM sugar beets. Independent analyses conducted by internationally recognized laboratories found that sugar from Roundup Ready sugar beets is identical to the sugar from comparably grown conventional (non-Roundup Ready) sugar beets.

GE Crops that have been commercialized in US



What Are the Disadvantages of GMOs?

- It can be dangerous to other insects that are important to our ecosystem. ...
- It sparks concerns on changing the field of agriculture. ...
- It can damage the environment. ...
- It causes unwanted residual effects. ...
- It can create more weeds. ...
- It threatens crop diversity. ...
- It has trade issues.

➢What are the Benefits of GE foods?

- More nutritious food
- Tastier food
- Disease- and drought-resistant plants that require fewer environmental resources (such as water and fertilizer)
- Less use of pesticides
- Increased supply of food with reduced cost and longer shelf life
- Faster growing plants and animals
- Food with more desirable traits, such as potatoes that produce less of a cancer-causing substance when fried
- Medicinal foods that could be used as vaccines or other medicines

➢What are the Benefits of GE foods?-Continued

- **GE can help produce more food and/or enhance quality to feed growing world population**
- **Reduces: yield loss, crop damage from weeds, diseases and insects, drought**
- **More recent crops/under development: improved nutrition, taste, quality, shelf life**

Are GE foods safe?

- **100s of studies have shown that GEs do not present any health risk**
- **No documented instance of harm to human health from GE (since 1994, first GE seeds)**
- Because each genetically modified food (GMF) is different from the next, each new food should undergo safety assessments to weigh up both the benefits and any risks.

Health and safety concerns

All of the few GMFs available on the market today have been rigorously tested and been deemed safe. There is currently no evidence that any of these have caused any health issues in humans.

In Europe, the European Food Safety Authority carefully assesses any new GMF to make sure that it is safe to consume and retests them every ten years.

Detection of genetically modified organisms

Testing on GMOs in food and feed is routinely done using molecular techniques such as PCR and bioinformatics.
In a January 2010 paper, the extraction and detection of DNA along a complete industrial soybean oil processing chain was described to monitor the presence of Roundup Ready (RR) soybean: "The amplification of soybean lectin gene by end-point polymerase chain reaction (PCR) was successfully achieved in all the steps of extraction and refining processes, until the fully refined soybean oil. The amplification of RR soybean by PCR assays using event-specific primers was also achieved for all the extraction and refining steps, except for the intermediate steps of refining (neutralisation, washing and bleaching) possibly due to sample instability. The real-time PCR assays using specific probes confirmed all the results and proved that it is possible to detect and quantify genetically modified organisms in the fully refined soybean oil. To our knowledge, this has never been reported before and represents an important accomplishment regarding the traceability of genetically modified organisms in refined oils.

➢Gene editing

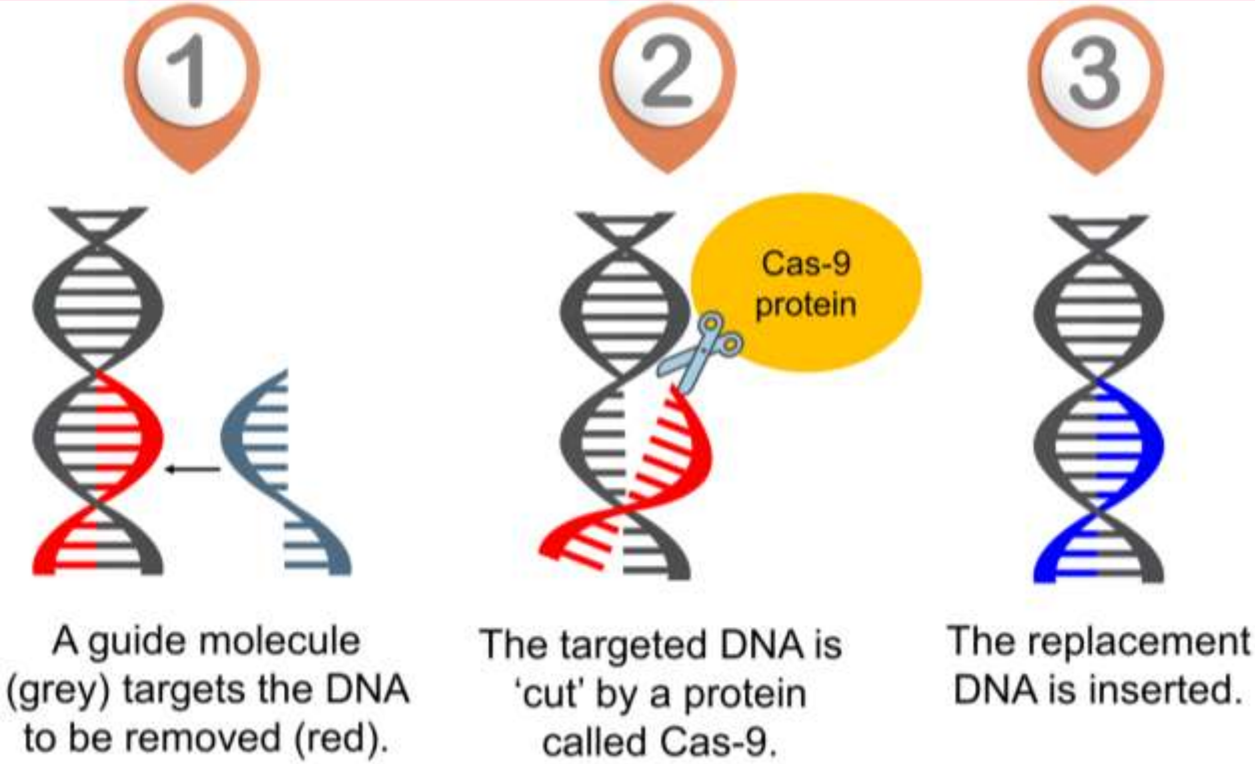
Recent advances in science now allow for a new way to modify genes.

This technology is known as gene editing.

Gene editing allows for specific and targeted changes to an organism's DNA.

Gene editing is very similar to the 'cut and paste' function on a computer.
A specialised protein is used to cut the DNA, and the desired replacement piece of DNA can be inserted into the cut.

➢Gene editing - Continued



This diagram shows an example of one kind of gene editing (CRISPR/Cas9)

➢Different types of gene alterations

➢ Genes can be altered in different ways to produce different effects. These include:

- Gene knockout – a gene is made completely inactive.
- Gene knock-in – a gene is replaced by another one.
- Gene knockdown (or gene silencing) – a gene is made partially inactive.

A food example of gene knockdown is a 'nonbrowning apple', which produces lower amounts of an enzyme that causes browning when the fruit is cut or bruised.

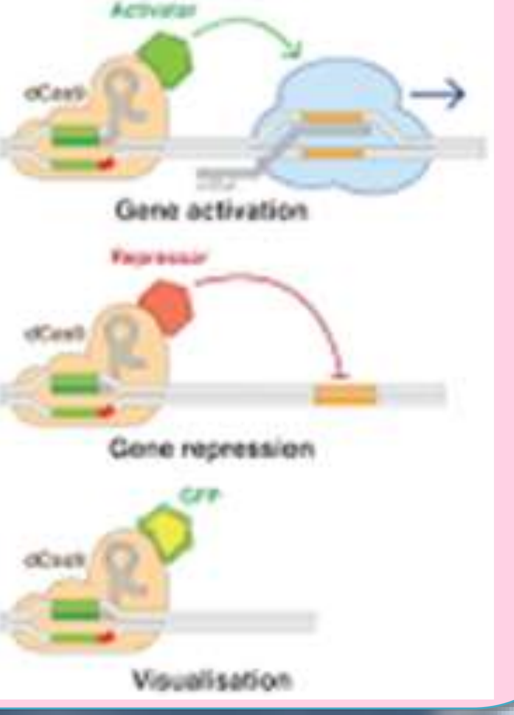
There are currently very few food products available that use gene editing, due to the new technologies still being perfected, consumer concerns and legal and financial barriers to creating these foods.

Genetic engineering techniques

Traditionally the new genetic material was inserted randomly within the host genome. Gene targeting techniques, which creates double-stranded breaks and takes advantage on the cells natural homologous recombination repair systems, have been developed to target insertion to exact locations. Genome editing uses artificially engineered nucleases that create breaks at specific points. There are four families of engineered nucleases: meganucleases, zinc finger nucleases transcription activator-like effector nucleases (TALENs), and the Cas9-guideRNA system (adapted from CRISPR). TALEN and CRISPR are the two most commonly used and each has its own advantages. TALENs have greater target specificity, while CRISPR is easier to design and more efficient.

➢• How does Crispr CAS work?

CRISPR-Cas9 was adapted from a naturally occurring genome editing system in bacteria. The bacteria capture snippets of DNA from invading viruses and use them to create DNA segments known as CRISPR arrays. ... If the viruses attack again, the \bacteria produce RNA segments from the CRISPR arrays to target the viruses‘ DNA.



➢Step-by-Step Guide on Using CRISPR:

- ➢1. Decide which gene to modify (cut, activate or inhibit). ...
- ➢2. Decide which endonuclease protein to use. ...
- ➢3. Design the gRNA to target the gene of interest. ...
- ➢4. Assemble the gRNA Expression Vector in your browser. ...
- ➢5. Assemble the plasmid at the bench! ...
- ➢6. Engineer the Cells!

➢: How does CRISPR-Cas9 edit genes?

➢Steps and Procedure of CRISPR-CAS9:

➢• Selecting an organism:

➢• Selecting a gene or target location:

➢• Select a CRISPR-CAS9 system:

➢• Selecting and Designing the sgRNA:

➢• Synthesizing and cloning of sgRNA:

➢• Delivering the sgRNA and CAS9:

➢• Validating the experiment:

➢• Culture the altered cells:

➢What exactly is CRISPR and how does it work?

➢CRISPR/Cas9 in its original form is a homing device (the CRISPR part) that guides molecular scissors (the Cas9 enzyme) to a target section of DNA. Together, they work as a genetic-engineering cruise missile that disables or repairs a gene, or inserts something new where the Cas9 scissors has made some cuts.

➢How does CRISPR-Cas9 edit genes?

➢CRISPR/Cas9 edits genes by precisely cutting DNA and then letting natural DNA repair processes to take over. The system consists of two parts: the Cas9 enzyme and a guide RNA. Rapidly translating a revolutionary technology into transformative therapies.

➢Summary

- Genetic modification provides a method for making desirable changes to a crop more rapidly than conventional selective breeding.
- Genetically modified foods (GMFs) may have useful functions such as drought resistance, having higher nutrient content or pest and herbicide resistance, which can be particularly important in developing countries.
- Criticisms of GMFs include concerns about health risks, concerns about harm to other species where new crops are planted, religious or moral objections and concerns about how farmers may be affected.
- To date, no health risks have been identified from any available GMFs.
- Gene editing is a new approach for modifying genes that is more specific and targeted than genetic modification techniques.

با تشکر از توجه شما