Genetic Engineering in Agriculture

By studying this Factsheet the student will gain a knowledge of the applications of genetic engineering in agriculture that are referred to in current syllabuses. These include the production of genetically modified crops to improve yield or flavour, and to incorporate insect resistance and herbicide resistance into crops.

The technique of genetic engineering to produce genetically modified plants

DNA is extracted from the plant which contains the required gene. Alternatively mRNA can be extracted and treated with the enzyme reverse transcriptase to make copy DNA (cDNA) which should contain the required gene. The DNA or cDNA is then treated with a restriction endonuclease (RE) enzyme to split the DNA into short lengths at specific base sequences, thus producing ‘sticky ends’. Figure 1 shows the production of sticky ends.

Exam Hint - Questions on this topic will probably test recall knowledge about the actual techniques of genetic engineering and its agricultural applications, or give data about GM crops for processing and analysis or require discussion about the ethics, advantages and disadvantages of GM crops.

Fig 1. The production of sticky ends

The cut DNA fragments are subjected to gel electrophoresis which separates the fragments according to their size. Large molecules move more slowly through the gel than small ones. The movement of the DNA is induced by passing an electric current across the gel which is submerged by buffer in a gel tank. Since DNA is negatively charged it moves towards the positive electrode.

Samples of the fragments are then treated with a gene probe to determine which fragments contain the required gene. Those which contain the gene will be used to make recombinant DNA. If necessary, large numbers of these fragments can be manufactured by using the polymerase chain reaction.
The required gene must be inserted into the genome (genetic make up) of the plant to be modified by using a suitable vector. The soil bacterium *Agrobacterium tumifaciens*, which causes ‘crown gall’ disease in plants contains a DNA plasmid (known as the Ti plasmid) which will become integrated into a host cell chromosome. There are many strains of Agrobacterium and they will infect over 1000 different species of plants. Thus the Ti plasmid makes an excellent vector.

The Ti plasmid DNA is extracted from the bacteria and treated with the same restriction endonuclease that was used to cleave the original DNA containing the required gene. The split Ti plasmid DNA will thus contain ‘sticky ends’ complementary to those of the original split DNA. When the two samples of DNA are mixed together the complementary bases of the ‘sticky ends’ join by hydrogen bonding, and the sugar-phosphate links can be sealed by treating with the enzyme DNA ligase. Thus recombinant DNA is made by the Ti plasmids and the DNA fragments containing the required gene joining together.

**Exam Hint** - A common error made by candidates is to say that ‘DNA ligase joins the complementary bases together’.

The recombinant DNA plasmids are then mixed with a culture of Agrobacterium tumefaciens in cool calcium chloride solution. This is then quickly heated to 42°C (heat shock) with the result that the bacteria take up the recombinant Ti plasmids. These bacteria can then be cultured in bulk.

**Remember** – a marker gene, such as one that gives antibiotic resistance, is also usually incorporated into the recombinant Ti plasmid. Thus once the plasmids are incorporated into the bacteria, the culture can be treated with the antibiotic and only the bacteria which have taken up the plasmids will survive, allowing further culturing.

The transformed bacteria are then used to infect the plants to be genetically modified. They can be used to infect various types of plant tissue culture which then go on to develop into the genetically modified plants. This is illustrated for callus culture in Fig 2.

**Remember** – a gene probe is a length of DNA which contains the complementary base sequence to the gene being searched for. Once the base sequence of the gene is known, such a probe can be chemically synthesised. The probe is tagged with a radioactive isotope (phosphorus^{32}) so that when it attaches to the gene by complementary base pairing, it can be visualised by autoradiography (X-ray photography).

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**Fig 2. The production of plant cells by callus culture**

The callus is made up of undifferentiated plant cells that can undergo mitosis and differentiate into any tissue. The Agrobacteria, or naked plasmids, can be used to infect the callus cells and the Ti plasmids become attached to a plant cell chromosome. The uptake into the callus can be improved by applying an electrical potential difference across the cell culture. This creates tiny pores in the cell membranes through which the plasmids can enter the cells. The callus can then be induced to develop into shoots and roots by varying the auxin and kinin concentrations. This is illustrated in Fig 3.
GM crops may be developed to grow in arid or desert conditions and the foods produced by GM crops may have a longer storage life.

GM crops may help to reduce some of the problems of famine in the following ways:

- Many human populations in the World have a lack of food and famine is widespread. It is important for humans to produce enough food to feed the whole human population. GM crops may help to reduce some of the problems of famine in the following ways:
  - GM crops may produce a higher yield or a product with greater nutritional content.
  - the foods produced by GM crops may have a longer storage life.
  - GM crops may be developed to grow in arid or desert conditions and so can be grown in areas of famine where there is poor rainfall.

- GM crops which have been made insect resistant should not have yields reduced or quality spoiled by insect attack, and money should be saved by not having to use conventional chemical insecticides. Pollution by chemical insecticides is thus avoided.
- GM crops which have been made virus resistant should also produce better yields and not suffer damage due to viral attack.
- GM crops which are herbicide resistant are labour saving, and thus money saving, because crops which are not herbicide resistant must be weeded by hand. This is not fully efficient and the crop yield may be reduced due to competition from missed weeds or contaminated with products of the weeds.
- GM crops which have been given genes conferring nitrogen fixing ability may improve soil fertility and productivity.

There are however possible drawbacks and risks in developing and growing GM crops. Thus scientists and industrialists should proceed with caution, employ stringent testing conditions and not be governed by the prospect of rapid financial gain. GM crops may, or already do, cause the following problems:

- pollen from GM corn crops may carry insecticides such as the Bt protein (aimed to kill insects such as the European Corn Borer) or insecticidal lectins (designed to kill aphids) from GM crops such as tomatoes, potatoes, sugar cane. This pollen may be blown onto food plants of other harmless or beneficial insects resulting in their death. For instance, in 1998 Bt modified corn was found to be releasing pollen which contained the Bt protein. The pollen landed on milkweed plants, the food plant of the Monarch Butterfly caterpillar. Caterpillars which ate the contaminated milkweed died within four days. The insecticidal lectins found in GM potatoes are also known to harm ladybirds by reducing their lifespan and reproductive capacity. Ladybirds are useful predatory insects that naturally control aphid populations.
- insects are already developing resistance to the GM insecticides in crops. For example, in 1996 in America, over 18,000 acres of Bt crops were destroyed by insects which were no longer affected by the Bt protein.
- disruption to foodchains is likely to occur due to some insect species being destroyed whilst others develop resistance and become overpopulated.
- pollen from GM crops may hybridise into other plants giving them insect resistance or herbicide resistance. Thus they will gain selective advantage and may cause disruption to food chains and the normal balance of nature. Gene flow between fodder beet and wild beet is already known to occur so that the herbicide resistant gene in GM beet may be transferable.

Exam hint – give positive factual statements about the advantages and disadvantages of GM foods. Avoid vague non-scientific statements, such as ‘man should not play at being God/man should not interfere with God’s creation’.

Examples of genetically modified crops
Genes have been inserted into the following plants by genetic engineering:

- a gene into tomato plants to improve the flavour of the tomatoes.
- a gene into tomato plants to lengthen shelflife enabling the tomatoes to be stored for longer periods.
- a gene into tomato and tobacco plants which makes them resistant to tobacco mosaic virus.
- a gene into turnip plants, sugar beet and barley plants which gives them tolerance to the herbicide glyphosate (Roundup). The crops can thus be sprayed with glyphosate to destroy weeds without harming the crop. The gene was obtained from Salmonella bacteria. Similarly, a gene has been inserted into oilseed rape and fodder maize (used to make silage for animal feed) to make it tolerant to the herbicide glufosinate (Liberty).
- a gene into cotton plants and sweet corn plants to enable them to produce an insecticide. This kills any insect which feeds on the crop and so improve yields. The gene was obtained from the bacterium Bacillus thuringiensis which produces a protein (called Bt) which damages the gut of insects thus causing their death. It mainly effects the caterpillars of Lepidoptera (butterflies and moths).
- a gene from snowdrops which produces a lectin that causes resistance to aphid attack has been inserted into a wide range of plants, including potatoes, grapes, oilseed rape, rice, sweet potato, sugar cane, sunflower, tobacco, walnuts and tomatoes.
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Advantages and disadvantages of genetically modified crops & foods.

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Once whole plants are obtained they can be grown and reproduced in bulk.

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- There may be possible risks to animal and human health due to eating some GM products. For instance, GM potatoes containing an insecticidal lectin from jackbean plants when fed to rats has been reported to cause a general reduction in brain, liver and kidney size and the stomach to develop a thicker lining. GM potatoes containing a lectin from snowdrops did not produce these effects.

Practice Questions

1. Genetic engineering is used to ‘improve’ many crop plants. One example is the introduction of a gene into potato plants which gives them resistance to infection by potato leafroll virus (PLRV). The tumour inducing (Ti) plasmid of Agrobacterium tumefaciens is used as a vector. The diagrams below show the Ti plasmid in its natural state and in its recombinant state.

![Diagram showing Ti plasmid in natural and recombinant states.]

2. Bacillus thuringiensis is a bacterium which lives naturally in the soil. It produces a toxic protein, known as Bt toxin, which is poisonous to caterpillars and some other insects. Genetic engineers have inserted the gene for the production of Bt toxin into tomato plants. An outline of the procedure they used is given below.

1. DNA extracted from Bacillus thuringiensis
2. Identify and clone Bt toxin gene
3. Transfer gene into callus of tomato cells
4. Grow cells which have the Bt toxin into tomato plants
5. Tomato plants produced which have cells capable of producing Bt toxin

(a) What is the purpose of inserting the Bt toxin into tomato plants?
(b) Explain how scientists could have identified the Bt toxin gene.
(c) Name one enzyme which would have been used during cloning at stage 2.
(d) The scientists were not sure that all the leaves of the tomato plants would produce the toxin. Outline how this could be investigated in a laboratory.

Answers

1. (a) (i) Extract DNA from potato plant which has shown resistance to potato leafroll virus infection; cut DNA into sections with sticky ends using a restriction endonuclease enzyme; separate DNA fragments by (gel) electrophoresis; (blot DNA onto a (nylon) membrane and) treat with radioactive gene probe; to recognise specific base sequences thus locating DNA fragments with the required gene; locate DNA fragments with X-ray film and collect by washing from nylon; amplify/multiply fragments using the polymerase chain reaction; max 6

(ii) Extract DNA from bacterium and separate plasmid DNA; by (ultra) centrifugation (gel) electrophoresis; treat with same restriction endonuclease to obtain complementary sticky ends; separate fragments by gel electrophoresis; use gene probe to identify and discard fragments containing tumour gene; use polymerase chain reaction to multiply/amplify remaining plasmid fragments; max 4

(iii) Mix plasmid DNA with sticky ends and potato DNA with sticky ends together; treat with DNA ligase to seal ends together; max 2

(iv) Mix plasmids in growing culture of the bacterium; in presence of calcium ions/apply heat shock; max 2

(v) Culture transformed bacteria and potato tissue/callus together; bacteria infect potato tissue and plasmids incorporate into potato cells; using plant cell attachment gene; callus/culture differentiates into new resistant potato plants; can be recognised by effects of marker gene; max 3

(b) Insect resistant plants:
- Fungal resistant plants:
- Pesticide resistant plants:
- Improved flavour tomatoes:
- Increased shelf life tomatoes: (any other correct examples) max 2

Total 19

2. (a) Make them toxic to pests/insects/caterpillars/give them a natural insecticide/reduce use of artificial pesticide; max 1

(b) Identify sequence of amino acids in toxin; use genetic code to identify the base sequences; make complementary radioactive gene probe; to find the required base sequences in the bacterial DNA; max 3

(c) Restriction endonuclease; max 1

(d) Put genetically engineered plant/leaves into a suitable container; set up a similar container containing normal plant/leaves; expose each to a similar number of insects/caterpillars; record leaf damage/consumption/count dead insects (after a few hours); max 3

Total 8

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