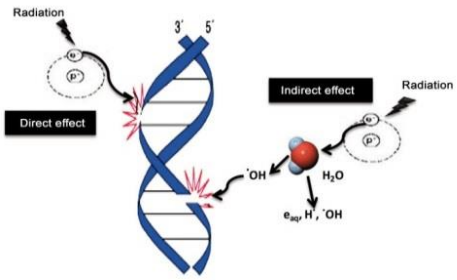
The *Arabidopsis thaliana* PsbS mutant

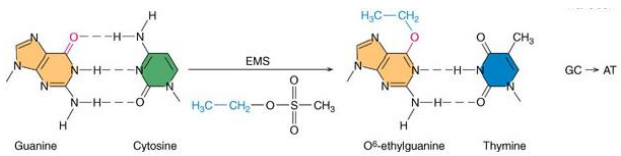
The same mutant produced five times, but which ones are within the scope of the European GMO legislation?

What is the function of PsbS?
PsbS is a protein which is involved in photosynthetic light harvesting and has been characterized as a 'safety valve'. Plants that lack the protein show reduced fitness and seed production under natural conditions. Mutant plants that fully lack the protein or produce a dysfunctional protein have been obtained in different ways.

A. The radiation mutant

The first PsbS mutant was made by exposing Arabidopsis plants to fast neutrons. The fast neutrons generate damage in the DNA that is repaired by the cells own DNA-repair machinery. During this repair, the whole PsbS gene was deleted and PsbS is therefore not present in the plant. However changes in other genes may also have occured following the radiation.



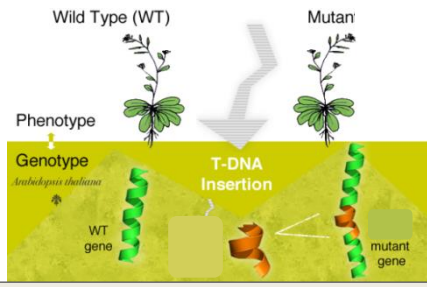


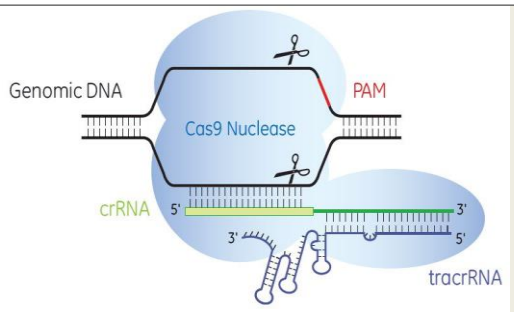
B. The chemically induced mutant

The second PsbS mutant was made by exposing Arabidopsis plants to the chemical mutagen EMS. One letter in the gene for PsbS was changed leading to a dysfunctional PsbS protein.

C. The T-DNA mutant

The third PsbS mutant was made by transferring so-called T-DNA from the soil bacterium *Agrobacterium tumefaciens* to Arabidopsis plants. The T-DNA has inserted into the PsbS gene leading to a disruption of the gene. The result is that the PsbS gene is no longer functional.





D. & E. The modern genome edited mutant

The most recent technology to generate PsbS mutants is the so-called CRISPR/Cas mediated genome editing. This CRISPR/Cas system generates two double strand breaks close to each other at predetermined locations in the PsbS gene. The DNA-repair machinery repairs the break, deleting the DNA between the breaks leading to a dysfunctional PsbS gene. The intermediate mutant that still contains the DNA for producing the CRISPR/Cas complex that generates the double strand break is called mutant D. The final mutant E is produced from mutant D after a round of spontaneous fertilization. One quarter of the offspring no longer contains the genes for the complex and these are selected a mutant E. They contain no foreign DNA and only differ from wild type Arabidopsis by a small deletion in the PsbS gene.

A GMO or not a GMO?

Mutants A and B are not within the scope of the European GMO legislation. Mutant C is, even though T-DNA sequences are shown to naturally occur in crops like tobacco and sweet potato, considered a GMO. But what about mutant D and E? Mutant D still contains foreign DNA and is therefore considered a GMO. Mutant E does not contain foreign DNA and only lacks a number of DNA base pairs in the PsbS gene. Does this removal of a few base pairs constitute a novel combination of genetic material? Probably not. When compared to the mutants A and B it would be illogical to subject the genome edited mutant E to the requirements of the GMO legislation.