

Since human demand for good traits and yield is very high, only a small fraction of the world's approximately 200,000 plant species have, through history, survived the rigorous scrutiny of the domestication process. Around 3,000 species may have at some point been used for food, feed, spices, and materials but only as few as around 200 have ultimately been completely domesticated. Today, humankind is relying solely on 15–20 species for the entire world food production.

Plant mutagenesis is rapidly coming of age in the aftermath of recent developments in high-resolution molecular and biochemical techniques. By combining the high variation of mutagenised populations with novel screening methods, traits that are almost impossible to identify by conventional breeding are now being developed and characterised at the molecular level.

## Mutagenesis

During crop evolution there has been a continuous reduction in genetic diversity as breeders have increasingly focused on so-called “elite” cultivars. This **genetic erosion eventually became a bottleneck and various techniques to induce mutations and artificially increase variation emerged** in the middle of the last century. **Initially, X-ray radiation was used as a mutagen** since **it was readily available** to researchers. In 1927, Muller showed that X-ray treatment could increase the mutation rate in a *Drosophila* population by 15,000%, and a year later, Stadler observed a strong phenotypic variation in barley seedlings and sterility in maize tassels after exposure to **X-rays and radium**. Later, more sophisticated techniques such as **gamma and neutron radiation** were developed at newly established nuclear research centers. During and directly following the **Second World War, radiation-based techniques were complemented by chemical mutagens** that were less destructive, freely available, and easier to work with. Pioneer work in this area was performed by Auerbach and others, who demonstrated an increased mutation frequency in *Drosophila* following exposure to **mustard gas** (War Gas). A few years later, this work was followed by the discovery of **methane-sulphonates** and **other chemical mutagens**, which are still in use today.

The **goal in mutagenesis breeding** is to cause maximal genomic variation with a minimum decrease in viability. Among the radiation-based methods,  **$\gamma$ -ray and fast neutron bombardment now supersedes X-ray in most applications**. Of these,  $\gamma$ -ray bombardment is less destructive causing point mutations and small deletions whereas fast neutron bombardment causes translocations, chromosome losses, and large deletions. Compared to chemical mutagens, both types of radiation cause damage on a larger scale and severely reduces viability.

**Chemical mutagens** have gained popularity since they are easy to use, do not require any specialised equipment, and can provide a very high mutation frequency. Compared to radiological methods, chemical mutagens **tend to cause single base-pair (bp) changes, or single-nucleotide polymorphisms (SNPs)** as they are more commonly referred to, rather than deletions and translocations. Of the chemical mutagens, **EMS (ethyl methanesulfonate) is today the most widely used**. EMS selectively alkylates guanine bases causing the DNA-polymerase to favor placing a thymine residue over a cytosine residue opposite to the O-6-ethyl guanine during DNA replication, which **results in a random point mutation**. A majority of the changes (70–99%) in EMS-mutated populations are GC to AT base pair transitions.

Mutations in **coding regions** can be silent, missense or nonsense. In **noncoding regions**, mutations can change promoter sequences or other regulatory regions, resulting in up- or downregulation

of gene transcription. **Aberrant splicing of mRNA, altered mRNA stability and changes in protein translation** may also occur as a result of mutagenesis.

**Other mutagens** such as **sodium azide (Az)** and **methylnitrosourea (MNU)** are also used and often combined into an Az-MNU solution. Genetically, Az-MNU predominantly **causes GC to AT shifts, or AT to GC shifts**. Thus, **contrary to EMS, a shift can happen in either direction**. All three chemical mutagens are, as can be expected, strongly carcinogenic and should be handled with extreme care.

Through the years, mutagenesis has **generated a vast amount of genetic variability** and has played a **significant role in plant breeding programs** throughout the world. Records maintained by the joint FAO/IAEA Division in Vienna show that **2965 crop cultivars**, with one or more useful traits obtained from **induced mutations**, were released worldwide during the last 40 years. Notable examples are several wheat varieties e.g., **durum wheat used in pasta**, barley including **malting barley, rice, cotton, sunflower, and grapefruit, resulting in an enormous positive economic impact**.