Association Française des Biotechnologies Végétales

INFORMATION SHEET
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UNDERSTANDING THE VALUE OF MUTATIONS IN PLANT BREEDING

LIFE EXISTS ONLY BECAUSE OF MISTAKES

Life has been based on the existence and reproduction of nucleic acids, essentially DNA, for more than 3 billion years. DNA comes in the form of two complementary polymers, the strands of the "double helix". The order in which thousands, millions, or billions of nucleotides follow one another, symbolized by the 4 letters A, T, G, C, along these strands is the genetic message, the genome, of the organism carrying them.

The mechanisms that allow identical DNA to be reproduced at each cell division are not foolproof. In nature DNA is also subject to physical hazards, such as various radiations, or chemical hazards -- molecules produced by the cell or applied to the cell that contains the DNA. By more or less complex processes, these hazards cause modifications of the DNA sequence, so-called spontaneous mutations. It is a truism to say that the past and present diversity of life forms is the result of an infinite succession of these errors, of these mutations, from the very beginning: without them, the initial molecules would have remained confined to the domain of chemistry and inanimate matter.

MUTATIONS SURROUND US, BUT THEY ARE OFTEN INVISIBLE

We are used to thinking that spontaneous mutations are rare: common experience comforts us in the belief of the immutability of species, which has been the dogma over the centuries, except probably for the gardener or the breeder. Modern DNA sequencing tools allow objective measurement of the frequency of mutations. The child's genome is half that of each parent, but includes about 70 mutations that occurred before the parents' germinal cells were united. Our body contains tens of thousands of billions of cells, and in all these copies of our genome, very few are free from mutations. The farmer harvests as many mutations in a hectare of wheat as there are genes in this species, and around the world, each of these genes mutates hundreds of millions of times.

However, most of these mutations are silent due to the low proportion of coding sequences in the genome and the characteristics of the genetic code and proteins. But it is these spontaneous mutations which build the genetic variation of species and it is through them that adaptation to variations in ecosystems is achieved for all species. This variation is exploited by breeders for the creation of new varieties. But the breeder can only exploit a tiny part of this variability for purely material reasons. The bacteriologist can manipulate billions of bacteria in a test tube, or an adventitious plant species can develop a mutant resistant to a herbicide if the herbicide is used on thousands of hectares where billions of seeds of this species are found. But by studying, observing, selecting a few thousand individuals, which is a lot for the traits he cares about, the breeder only exploits a thousandth, or less, of the variability of the species. This is the reason why the breeder has been using mutation breeding (or induced mutagenesis) by various means, for more than a century, to access greater variability.

THE NATURE OF SPONTANEOUS MUTATIONS IS EXTREMELY VARIABLE

Replication errors or exposure to hazards can have various consequences for the DNA molecule: nucleotide substitutions; adding (inserting) or removing (deleting) adjacent nucleotides; the elimination of a fragment of a chromosome or even an entire chromosome (aneuploidy); the insertion of other endogenous DNA sequences such as chloroplast DNA fragments or even an entire mitochondrial genome; rearrangements of genome fragments relative to one another such as reversing the orientation of a sequence on one chromosome or moving a fragment to another site in the genome (translocation) on the same or another chromosome; the insertion of transposons or retro-transposons which inactivate or modify the expression of a gene and which have played an important role in the domestication and diversity of cultivated plants.

INDUCED MUTAGENESIS IN PLANTS DEVELOPED IN THE 20TH CENTURY

As early as 1908, Stuart Gager exposed *Datura stramonium* flowering plants to radium radiation to discover that these treatments gave birth in the offspring to plants with new characteristics. In 1928 Stadler described how he obtained the first barley mutants. During the first half of the 20th century the use of X-rays or gamma rays was used to induce the appearance of new traits. From the second half of the century, various mutagenic substances were used with the hope, which never materialized, that their chemical nature could direct the variation of certain genes and therefore certain traits. A certain deregulation of DNA repair systems or control of transposable elements under stress conditions in tissue culture *in vitro* promotes the appearance of mutations among regenerated plants. This process has been used since the 1970's. Mutagenesis has thus become a common tool in plant breeding. Based on non-exhaustive statements from breeders around the world, in 2015 there were more than 3,200 varieties derived from mutagenic treatments, mainly by radiation, among 170 species. These include field crops (wheat, rapeseed, cotton, barley, rice, soybeans ...), vegetables (beans, peas, sweet potatoes ...), fruit trees (apple, banana ...) and a large number of ornamental species (chrysanthemum, rose ...).

INDUCED MUTATIONS ARE OF THE SAME NATURE AS SPONTANEOUS MUTATIONS

Compared to spontaneous mutations, the use of mutagenic treatments considerably increases the frequency of mutations, commonly by a factor of 1000, which reduces in the same proportion the number of individuals needed to be observed to select the desired mutation. These mutagens "damage" DNA, causing in a second phase further mutations if they are not perfectly repaired. The mutation is the result of an interaction between the inducer (the mutagen), the target DNA sequence, and the reaction of the cell's DNA repair systems. We can thus clearly see the intrinsically random nature of the mutations that occur within the genome, random by the modifications of the sequence and random by their position on it. During a mutagenic treatment, spontaneous mutations are added to induced mutations, in a very small proportion. As the DNA modifications caused in both cases can be the same, there is no signature indicating the origin of a mutation, whether spontaneous or induced. There is only a greater likelihood that it was caused by treatment rather than being spontaneous. A mutation "occurs", we do not "manufacture" it, unlike a transgene that "we build".

PLANT MUTAGENESIS IN VITRO HAS BEEN PRACTICED FOR A LONG TIME

To take advantage of large numbers in a reduced volume, as practiced by microbiologists, mutagenesis and selection of plant cells *in vitro* appeared in the scientific literature as early as the end of the 1960's. Indeed, this was the time when regeneration of plants from single cells began to be mastered. In tobacco, plants resistant to certain herbicides were thus obtained in 1973 from cells treated *in vitro* and various morphological mutants were obtained from the culture of irradiated microspores in 1974. An important contribution of these techniques for vegetatively produced plant varieties was to obtain plants mutated in all of their cells unlike chimeras obtained after treatment of buds *in vivo*, thanks to the regeneration from a single cell of plant tissue cultivated *in vitro*. Chrysanthemum mutants were obtained from irradiated leaf explants in 1975, a variety of sugar cane resistant to a virus in 1974, varieties of bananas with larger bunches cultivated since 1984, rapeseed resistant to the imidazolinone herbicide family were obtained in the late 1980's, marketed after 1995 in Canada and subsequently in Europe. It should be noted that in the latter case, mutations of the same gene have been obtained spontaneously in this species. Plants resistant to the same herbicides were already obtained in 1982 by *in vitro* culture selection in corn, after mutagenic treatment on seeds in wheat and rice in the 1990's, spontaneously in a wild sunflower in 1996, allowing the use of these herbicides on all of these crops. In other words, the same mutations are obtained in different species, of which nothing would enable one to devise the origin, whether spontaneous, induced, *in vitro* or *in vivo*.

CONCLUSION: THE CIRCUMSTANCES OF MUTAGENESIS DO NOT CHANGE THE NATURE OF THE MUTATIONS

In plant breeding, mutagenesis induced *in vivo* has been used since the beginning of the 20th century and mutagenesis *in vitro* since the middle of the 20th century. These two sources of genetic variation have led to the production of plants with interesting agronomic or quality traits, and subsequently to the commercialization of more than 3000 varieties worldwide, without distinction regarding the source of their genetic variation.

Plant cells can be subjected to the action of mutagens in different circumstances, depending on the organ where they are found, inflorescence, pollen, bulb, seed, buds or branches, whether on the plant, or isolated in the form of *in vitro* micro-cuttings or *in vitro* cell culture. There is no specific signature of such circumstances of mutagenesis. No mutation is distinguished from another by the fact that it is induced or spontaneous. To make a distinction on this criterion is without scientific foundation and is therefore arbitrary. Such would be the case were a differentiated regulation established for example between mutants obtained *in vitro* and mutants obtained in other circumstances.

For further reading:

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