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The Role of Biotechnology in Plant Breeding

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Abstract

The global food and nutrition crisis is primarily the result of the loss of agricultural productivity caused by global climate change. Moreover, a growing global population drives up food demand, aggravating the issue of food scarcity. Despite initiatives to lessen food shortages, millions of people still do not receive the required nourishment. Plant breeding methods are primarily concerned with genetic advancement of crops by selection, crossing of superior genotypes and their screening. The introduction of desired traits through utilizing the available variability by introduction, selection and hybridization methods, can lead to the development of improved crop varieties. The traditional techniques produce advanced cultivars with desirable characteristics, but it takes longer (6 to 12 years) to complete the process. Modern biotechnological technologies, such as plant tissue culture, molecular markers, genetic transformation, hybrid generation, nano-biotechnology, and genome editing tools such as zinc-finger and CRISPR-Cas9, developed in the last two decades, have improved our understanding of the genetics of characteristics and enabled traditional breeding to produce new varieties faster.

Keywords: Biotechnological technologies, nono-biotechnology, zinc-finger, CRISPR-Cas9

Introduction

We will concentrate on the recent developments and spin-off applications of plant tissue culture and molecular biology in plant breeding. As far as we can evaluate, these are triple. First, plant cell tissue culture has its most important application in the rapid amplification of genotypes. Second, biotechnology offers the opportunity to guide and sustain the crosses and the screening of the progeny. Thirdly, biotechnology can contribute to the introduction of new markers and characteristics, which cannot easily be crossed in and which have proven to be useful and valuable. This can be done by the selection of mutations and by gene transfer experiments.

Plant tissue culture

The growth of plants and plant parts, such as shoots or roots, as well as differentiated or undifferentiated plant cells on synthetic media in aseptic conditions, has become a very important technique in plant breeding. The in vitro culturing allows a long-term maintenance of genotypes and, at the same time, a rapid propagation from a limited amount of material. Vasil and Vasil (1980) published a table of 352 species in which adventitious buds, shoots, embryos, and whole plants were produced aseptically on synthetic media. However, regeneration is still a bottleneck for many species. Therefore, more research on the in vitro culturing is a necessity.

Rapid in vitro application

The *in vitro* culturing has become especially important for the rapid economical propagation of ornamentals as well as for potato, fruit, and forest trees. This subject has recently been reviewed by Bornman (1993). The *in vitro* amplification of clones is also used during breeding to assess and/or to reproduce the qualities of certain clones in the field. The example shows that *in vitro* propagation allows for retaining clones in vitro while evaluating the best hybrid vigor effect of different genotypes in combination with a given male-sterile plant. To make the *in vitro* amplification method also valuable for field

crops, the production of synthetic seeds via somatic embryogenesis has been proposed to overcome the labor-intensive transfer from *in vitro* to *in vivo* (Gray, 1990). However, a general remark is that more research is needed to evaluate the genome stability during the different in vitro manipulations.

Long-term storage

Long-term storage of living collections would be particularly advantageous to preserve germplasm of heterozygous and polyploid varieties, for which the maintenance of seeds is not possible. Long term storage demands an establishment of conditions with a minimal risk for soma clonal variation. A good method would be the cryo-preservation of meristems, as all metabolic functions are arrested at -196°C. However, until now, this is only possible for a limited amount of plant species (50 according to Chandel and Pandey, 1991).

***In vitro* culture as a source of variation**

Mutations can be either obtained spontaneously, by specific mutagenic treatments, or by tissue culturing. Especially, specific point mutations have proven to be valuable in certain crops, such as fruits (e.g., tomato) and ornamentals. Whereas the genome instability can represent a problem during the rapid *in vitro* propagation of genotypes, the increased mutation frequency during *in vitro* culture, called soma clonal variation, is nowadays also being exploited (Scowcroft, 1985). The soma clonal variants are screened for desirable phenotypes either *in vitro* or in the field. By now, soma clonal variation is included in the breeding program of particular crops such as tomato and other vegetables. Also, the production of polyploid varieties by colchicine treatment is successful, especially for vegetative and fodder crops. Plant cell tissue culture is also an obligatory step in many of the biotechnological procedures aiming at the transfer of genes via methods other than fertilization. In particular, there are the techniques of somatic hybridization by protoplast fusion and of gene transfer. The production of haploids obtained by androgenesis or by parthenogenesis is by itself not directly of practical use. However, the doubled haploids are nowadays frequently used as such (wheat, barley) and in hybrid breeding programs. It allows obtaining homozygotes from a cross much faster, so that the selection for a stable, uniform, and discernible variety with a new combination of the parents' traits can occur much faster. Along the same lines, the shortening of the life cycle by floral induction in vitro could contribute considerably to the shortening of breeding programs.

Use of molecular markers in plant breeding

Besides the in vitro tissue culturing, recent developments in molecular biology have gained impact in plant breeding for the diagnosis of varieties and for the selection of breeding populations. The methods to reveal molecular markers are nearly all based on electrophoresis systems. Migration polymorphisms of proteins or DNA fragments result in discernable patterns. These can be diagnostic on the level of families, species, varieties, or ecotypes, depending on the assay or probe used.

Protein polymorphisms

On the protein level, there are primarily the sodium dodecyl sulfate protein-denaturing gel electrophoresis (SOS-PAGE) systems and the isoenzyme characterization methods. Although SOS-PAGE is a very powerful method for the typing of prokaryotic strains, it has only limited applications for plant breeding. However, it could become one of the fastest methods to compare or type seed stock varieties. The main problem of SOS PAGE is that for all other applications, the complex banding pattern is very much influenced by external, non-genotypic parameters, such as age, all types of stresses, and environmental circumstances. The isoenzyme method is very reliable and is useful in certain species for identification and breeding experiments. However, the number of markers is woefully limited. However, we provide a reliable map for the different chromosomes.

DNA polymorphisms

Direct DNA screening not only provides a series of new markers, but also has the advantage over immunological and enzyme-based procedures (gene product level), that is possible to detect differences or alterations without the need to express the effect of the difference. In other words, DNA markers give direct information about the genotype composition independent of whether the alleles are dominant or recessive. The number of assays in which we find polymorphic markers is steadily increasing. All are based on different electrophoretic banding patterns, which in their turn are based on different lengths and/or compositions of the revealed fragments. The DNA-banding patterns are either generated by (i) restriction enzyme endonucleases, (ii) the polymerase chain reaction (PCR) of primer-based

amplified-DNA segments, or (iii) a combination ofboth. The polymorphisms are then generated at the level of the presence or absence of either a restriction site or primer-binding sites. which is based on the review of Rafalski and Tingey (1993). First, we will give an overview of the different possible approaches, and second, we will describe some possible applications in plant breeding.

Gene transfer

The basic requirement for breeding is genetic diversity. This can be broadened by the induction of mutations and, more recently, by defined gene transfer from one genotype to another between species, families, and kingdoms. It is often desirable to change only one specific trait in a specific variety, without disturbing the rest of the genome. In this case, either the induction of mutations or the transfer of specific genes may be preferable, depending on whether the gain or loss of a function is aimed at. The relatively new methods of gene transfer will not replace any of the existing methods aimed at creating new gene combinations. They do, however, extend the tools that can be used in breeding programs. The best trial design has to be chosen dependent on the aim. the crop and the available facilities and materials.

Conclusion

Together, these applications are theoretically very powerful. The *in vitro* culture has already proven to have many applications in different breeding strategies. Also, the molecular diagnosis will undoubtedly enhance the screening power dramatically to find certain wanted recombinant varieties. However, the disadvantages are that they are labor-intensive procedures with expensive consumables. Therefore, a common effort of many different research groups is obligatory for the construction of chromosome maps and the development of the most efficient diagnostic procedures. Only then will biotechnology be able to sustain plant breeding goals and prospects successfully and competitively.