BIOTECHNOLOGIES AND THEIR USE IN WHEAT RESEARCH IN ROMANIA

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ABSTRACT

Biotechnology tools seemed a true challenge 50 years ago, but proved to be a huge opportunity for agriculture by the techniques of manipulating the genes of plants, animals, and microorganisms. Remarkable advances were made for the improvement of agricultural species both through traditional breeding and through techniques of molecular biology. Using these methods people manipulate a natural process to produce varieties of organisms that display desired characteristics, such as disease resistant crops or food animals, but there are few major differences between these methods of gene transfer which lie neither in goals nor processes, but rather in speed, precision, reliability and scope. In traditional breeding many crosses are necessary before the "right" chance recombination of genes results in offspring while biotechnological methods can insert individual genes for specific traits directly into an established genome, so by gene transfer can shorten the time required to develop new varieties.

INTRODUCTION

Biotechnology can be defined in many different ways, but for all areas, molecular approaches are used to understand and manipulate a plant genome and so became one of the latest tools of agricultural research. It involves the systematic application of biological processes for the beneficial use.

Biotechnological advances in agricultural production has achieved over the past 50 years through the use of molecular techniques for detecting differences in the DNA of individual plants, which can have many valuable applications to crop improvement.

Biotechnological approaches have the potential to complement conventional methods of breeding by reducing the time taken to produce cultivars with improved characteristics.

While conventional breeding utilizes domestic crop cultivars and related genera as source of genes for improvement of existing cultivars and this process involves the transfer of a set of genes from the donor to the recipient, biotechnological approaches can transfer defined genes from any organism, thereby increase the gene pool available for improvement.

The improvement of wheat by biotechnological approaches primarily involves introduction of exogenous genes in heritable manner and secondarily, the availability of genes that confer positive traits when genetically transferred into wheat.

Wheat received considerable attention over the years from plant breeders with the purpose of increasing the grain yield and to minimize crop loss due to unfavorable environmental conditions.

Wheat improvement by modern technologies requires the delivery, integration and expression of defined foreign genes into suitable regenerable explants. The application of recombinant techniques to improve wheat quality and yield is not only desirable but also has potential to open up new opportunities (Bhala, P.L., 2006).

CIMMYT is a non-profit unit of international agricultural research in crop improvement by biotechnology applications such as: develop wheat with improved levels of resistance to a range of biotic stresses encompasses the utilization of genes conferring "durable resistance". These can be done by identification and combine diverse sources of resistance/tolerance, epidemiology evaluations by monitoring the pathogen distribution and evolution and utilization of molecular markers to characterize the genes conferring slow rusting resistance and tag such genes with molecular markers to enable breeders to manipulate them in breeding material using marker assisted selection strategies.

The *Triticum* collection of Romania comprises 3088 accessions including 1173 accessions kept by the Suceava Genebank, stored under standard conditions under long- and medium-term conservation. *Triticum aestivum* is the dominant species, being the major useful species in the breeding programmes. The collection contains 1271 Romanian accessions and 1559 accessions from other European countries. The composition of the collection according to the status of samples is as follows: breeders' lines 1797, advanced cultivars 826, landraces 251, wild 16, unknown 198. Passport data about the *Triticum* collection are included in the Romanian Catalogue of Plant Genetic Resources, published by the Suceava Genebank (https://www.researchgate.net).

Romanian NARDI Fundulea continues the tradition of agricultural research performed since the end of 19th century, especially in cereals development field, using modern scientific approaches to solve the priority problems of Romanian agriculture. Agricultural research consists in: breeding varieties or hybrids adapted to the Romanian environment, especially for the Southern plains of the country, in cereals (small grains, maize, sorghum), grain legumes, oil crops (sunflower, linseed) and forrage crops; improving crop management practices; recommending integrated plant protection measures; producing basic seed of own cultivars and providing seed to seed multiplying farmers; fundamental research on genetics, biotechnology, plant physiology, biochemistry, etc., to support applied research on breeding and agronomy (Verzea, M., 2007).

In wheat, Fundulea work of 50 years on improving the genetic basis of wheat production in Romania leaded to a genetic progress for yield was earlier estimated at about 50 kg/ha/year or about 1%/year. Some of wheat cultivars released from Fundulea have been grown on more than half of the wheat acreage in Romania and other cultivars were registered and are grown in countries like Canada, Hungary, Turkey, Kyrgyz Republic and Bulgaria. All created cultivars, along with numerous valuable breeding lines and populations, represent a solid basis for further progress in breeding for adaptability, yield, quality and new challenges, both locally and globally.

Agricultural biotechnology can modify plants, animals and microorganisms in order to improve their productivity. Latest biotechnological methods applicable to crop plants are somatic embryogenesis (androgenesis, macrosporogenesis and gynogenesis) and zygotic embryogenesis (zygotes produced by inter-generic hybridization followed by chromosome elimination of the pollen source partner since the first division cycles of zygotes)(Giura, A., 2020). In practical breeding, application of the *Zea* system led to the creation of new and superior homozygous cultivars and brought substantially acceleration of genetic progress using as parents for the next cycle of crossing the best yielding DH lines in course of evaluation (Săulescu et al., 2012).

The acceleration of genetic progress and saving time in obtaining recombinant inbred lines ready for yield evaluation is by producing doubled haploids (DH) needed to reach homozygosity. The application of anther culture method in wheat breeding presented several disadvantages, among which the fact that there were large genotypic differences in the response to anther culture. Other approach in producing wheat haploids is based on sexual hybridization of wheat by maize and spontaneous elimination of maize chromosomes in the early cell division cycles of hybrid zygote (Săulescu et al., 2012).

MATERIAL AND METHOD

This short article is an attempt to present different methods and achievements of using modern technologies in wheat improvement.

RESULTS AND DISCUSSIONS

Over time, two types of wheat have been developed and dominate production: common wheat (*Triticum aestivum*) and is the most cultivated type of wheat, also known as bread wheat, and wheat or durum wheat (*Triticum turgidum*) or pasta wheat.

Romanian biotechnological research and development in molecular genetics of wheat have been slow due to its ploidy level, the size and complexity of genome. Most research is made in institutes in collaboration with universities from all country. Increased genetic diversity can contribute to genetic progress in context of the new challenges imposed by a changing world in a changing climate (Săulescu et all., 2010). *Aegilops speltoides* has been used as a source for introduction of more diversity in the winter wheat (*Triticum aestivum* L.) breeding program (Giura, A. et all., 2019). *Triticum dicoccoides* specie has long been known and characterized by a very high concentration of protein in the grain. Numerous researches have focused on the transfer of this property to cultivated tetraploid and hexaploid grains, as well as the elucidation of genetic control and physiological mechanisms of high protein content (Uauy, C. et al., 2006). It has been shown that most of the increase in protein concentration is under the control of a single gene (Gpc1), which is effective in both the genetic background of tetraploid grains and that of hexaploid grains. For the introduction of the Gpc1 gene transferred from *Triticum dicoccoides*, backcross with adapted winter wheat varieties or lines was used, both in the case of common wheat and in the case of durum wheat. In addition to direct selection for protein content, marker-assisted selection was also used, using markers already described in the literature (Khan, I.A. et al., 2000).

Aegilops squarosa is one of the ancestors of wheat, being the donor of the D genome of hexaploid wheat, but only a small part of the genetic variability of the wild species participated in the spontaneous hybridization. Use of genetic diversity available within the species *Aegilops squarosa* was made by the method of incomplete backcross, with the interleaving of generations of selection for disease resistance.

Thinopyrum intermedium has aroused the interest of wheat breeders, especially because it is resistant to the barley dwarfing and yellowing virus (BYDV), a disease for which there is insufficient resistance to wheat and which is increasingly becoming a threat to wheat cultivation in the conditions of climate change. For the introgression of BYDV resistance genes from *Thinopyrum intermedium*, the method of incomplete backcross with selection performed under very early sowing conditions and marker-assisted selection was used.

The results of breeding experiments for the diversification of disease resistance genes led to the obtaining of lines with resistance to blight transferred from rye, wheat lines with resistance to BYDV transferred from rye, lines with resistance to powdery mildew, rust brown and black rust, as well as for the high production potential, good resistance to yellow dwarf virus or having a behavior similar to the lines that possess resistance transferred from *Thinopyrum intermedium* and lines with increase of early seedling vigor and the modification of plant albedo.

Drought as the major abiotic stresses can reduce yield potential up to 70% in crop plants. The complex nature of this phenomenon could not be deciphered by conventional methods of breeding. Biotechnological approaches have made it possible to understand the processes that underlie plants' responses to drought at the molecular and whole levels of plants. Hundreds of drought stress-induced genes have been identified and some of these have been cloned (Gosal, S.S. et all., 2009).

Molecular markers are being used to identify drought-related QTL and their efficient transfer into commercially grown crop wheat and other species.

Molecular markers increase selection efficiency and marker-assisted selection improved drought responses in wheat. At the association of several SSR markers with membrane stability in a series of doubled haploid (DH) lines derived from a cross between two Romanian cultivars, better performance in membrane stability after water stress (Ciucă Matilda and Petcu Elena, 2009).

Although not a crop plant, *Arabidopsis* has played a vital role in the elucidation of the basic processes underlying stress tolerance and the knowledge obtained has been transferred to a certain degree to important food plants (Zhang, J.Z. et al. 2004). Many of the genes known to be involved in stress tolerance have been isolated initially in *Arabidopsis*. The introduction of several stress-inducible genes into plants by genetic engineering has resulted to increased tolerance of transgenics to drought, cold and salinity stresses (Umezawa et al., 2006).

Plants respond to the changing environment in a complex, integrated way that allows them to react to the specific set of conditions and constraints present at a given time. Therefore, the genetic control of tolerance to abiotic stresses is not only very complex, but is also highly influenced by other environmental factors and by the developmental stage of the plant. The plant’s response to drought is accompanied by the activation of genes involved in the perception of drought stress and in the transmission of the stress signal.

Romania has doubled its cereal production in the last 10 years, and in 2018 production reached a record level. Thus, with a production of 31 million tons, Romania ranked 3rd in terms of the amount of cereals harvested after France and Germany (Eurostat, 2018), although the cultivated area gradually decreased. This is due to many factors: large areas with fertile lands and favourable climate, a series of advances in the science and technology of agriculture, more intensive use of fertilizer and pesticides, new yielding varieties and mechanization. But, breeding for yield stability is important and continue to be as predicted climate changes already brought weather variations from one year to another.

Advances in biotechnology have been made for pest control (Bt crops); *Bacillus thuringensis* (Bt) is a bio-control agent used in the last 20 years. A naturally occurring bacterium that produces protein crystals that is toxic to insects. Bt genes for control of an insect species have successfully been incorporated into tomatoes, tobacco, cotton and corn with good results; weed control (herbicide tolerance - ability of a plant to be unaffected at any feasible rate of herbicide application); disease control (as most of the diseases viruses are spread mechanically or through insect vectors, control efforts have traditionally revolved around the control of vectors and destruction of diseased plant materials.). Developed countries have consistently used plant cells and tissue culture techniques to manipulate crops and other environmentally important plants to achieve maximum productivity which include: micropropagation for clonal and mass propagation of elite crop plants to achieve rapid multiplication and availability as planting materials for increase productivity; meristem or shoot tip cultures of selected superior lines of crops in order to produce disease-free planting stocks; callus cultures, cell suspension cultures and somatic embryogenesis that would produce somaclonal variation and eventually lead to the selection of improved crop lines; raising embryo rescue and culture; fast breeding through anther and pollen culture; embryogenesis of haploid plants (http://hubrural.org).

Molecular genetics uses molecular techniques for detecting differences in the DNA of individual plants and has many applications to crop improvement. The differences are called molecular markers because they are often associated with specific genes and act as "signposts" to those genes. Such markers, when very tightly linked to genes of interest, can be used to select indirectly for the desirable allele and this represents the simplest form of marker-selection (MAS), whether used to accelerate the back-crossing of such an allele or in pyramiding several desirable alleles. Markers can be used for dissecting polygenic traits into their Mendelian components or quantitative trait loci (QTL), thus increasing understanding of the inheritance and gene action for such traits and allowing the use of MAS as a complement to conventional selection procedures. Molecular markers are also used to probe the level of genetic diversity among different cultivars, within populations, among related species, etc. The applications of such evaluations are many, including varietal fingerprinting for identification and protection, understanding relationships among the units under study, efficiently managing genetic resources, facilitating introgression of chromosomal segments from alien species and tagging of specific genes. In addition, markers and comparative mapping of various species have been very valuable for improving the understanding of genome structure and function and have allowed the isolation of genes of interest via map-based cloning (http://www.fao.org/3/y4011e0d.htm).

Restriction fragment length polymorphisms (RFLPs) were the first developed (some 15 years and widely/successfully used to construct linkage maps of various species, including wheat. With the development of the polymerase chain reaction (PCR) technology, several marker types emerged. The first of those were random amplified polymorphic DNA (RAPD), which quickly gained popularity over RFLPs due to the simplicity and decreased costs of the assay. However, most researchers now realize the weaknesses of RAPDs and use them with much less frequency. Microsatellite markers (SSRs), combine the power of RFLPs (codominant markers, reliable, specific genome location) with the ease of RAPDs and have the advantage of detecting higher levels of polymorphism. The amplified fragment length polymorphism (AFLP) approach takes advantage of PCR technique to selectively amplify DNA fragments previously digested with one or two restriction enzymes. Playing with the number of selective bases of the primers and considering the number of amplification products per primer pair, this approach is certainly the most powerful in terms of polymorphisms identified per reaction (http://www.fao.org/3/y4011e0d.htm).

Present agricultural research is a “gene revolution” and followed the 1960’s “green revolution” which helped hundreds of millions by increasing yields of wheat, rice and other crops.

CONCLUSIONS

The potential of biotechnology for advances in agriculture is huge and refers to the creation of new strains of crop plants, new plant and animal diagnostic products, animal vaccines, biological pesticides and herbicides, other biological control agents, and modifications in domestic animals used for food production.

By diversifying the genetic basis of wheat breeding programs with the help of biotechnological methods can increase the chances of genetic progress to meet the new challenges facing agriculture as a result of climate change.

Modern biotechnology is a safe and effective mean which provide productive and better crops.

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