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GENETIC VARIATION, DEVELOPMENT AND AVAILABILITY OF USEFUL GERMPLASM FOR PLANT BREEDING

INTRODUCTION

Since the dawn of neolitic revolution the main source of food supply for human societies was the utilization of genetic variability among plant species chosen for cultivation. The continuous process of selecting the best seeds from the best plants in each generation led to the development of cultivars adapted to prevailing cultivation practices. These cultivars gradually were more and more distant from original parental species and now are almost totally dependent on farming for their survival. This empirical process of "selecting the best plants and seeds" was the main practice of plant breeding till modern times, marked by the rediscovery of Mendel's laws of inheritance at the beginning of twenty century.

The improvements of cultivars measured by yield increase were very slow during this first stage of plant breeding; during the long periods there was no increase in yield at all. However, the main cultivated species were domesticated and established in agriculture during this period. The dimension of changes introduced is best illustrated when parental diploid species are compared to hexaploid bred wheat or teosinte plant with cultivated maize.

The success of a breeding programe in meeting the various objectives determined by cultivation practices and crop utilization methods is dependent upon two main factors:

– availability of necessary genetic variation,

– ability to manipulate it to produce a stable new cultivar.

The possibilities of improvements within these both factors were very limited during the pre–science period of plant breeding; practically the "breeders eye" was the only tool available for variability assessment and

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his intuition was a main guide in introducing selected trait into new cultivar.

The establishment of professional plant breeding enterprises in Europe in the second half of nineteen century and the rediscovery of Mendel's laws at the beginning of 1900s, were the backbones of modern plant breeding. Since than breeding was more and more based on scientific principles, which increased the possibilities for the breeder in both aspects of his activity. But mass and pure line selection in landraces, consisting of genotypic mixtures were most popular breeding techniques until the 1930s. At that period breeders were restricted to use practically only the genetic variability within the cultivated species and easily crossed related species. The application of genetics and statistics in plant breeding supported the visual selection of desirable traits in cultivar development started the acceleration of progress in plant breeding measured by increase in yielding ability of successively released cultivars. This started in 1930s in maize breeding with the development of commercial hybrid varieties (so called double cross hybrids), followed since 1960 by utilization of single cross hybrids. Pedigree selection methods, backcross and bulk selection techniques led to improvement in yielding ability of new released cultivars of other crops (Sanchez–Monge, 1993).

Scientific advances in plant breeding which took place since 1930s together with the development of modern cultivation technologies (tractors, mineral nitrogen fertilization) culminated in the so–called "Green revolution" which allowed cereal production to keep pace with the high average population growth rate of 1,8% since 1950. In effect, there are 150 million fewer hungry people in the world today than it was 40 years ago despite there are twice as many human beings. But it is also the other side of the coin: high – input crop production inherently linked to "green revolution" in some regions led to environmental damages. This indicates that there are limited possibilities to increase food production along these lines. But we need to produce food for additional two billion people by the early part of the 21^{st} century. According to Norman Borlaug, the father of "green revolution" we need to apply in plant breeding the new techniques offered by the development of molecular biology to boost yields of crops feeding the world (Borlaug, 2000).

In this paper I intend to summarize very shortly so called conventional approaches to identify or develop and utilize genetic variability in plant breeding and will concentrate my lecture on possibilities and constrains of molecular techniques in detection, assessment and development of new genetic variation for utilization in plant breeding.

NATURAL SOURCES OF GENETIC VARIATION

Genetic variability expressed in the form of visual differences in plant morphology, development pattern or reaction to biotic and abiotic stresses was the only variation upon which breeders could work during the pre–Mendelian era. Only very few agronomically important plant characters are controlled by simple genetic mechanism and are there–fore relatively easy detected and selected; most plant traits important for grower are very complex and difficult to select for. This was the main reason for very slow progress in cultivar improvement observed before modern times. In spite of this, however, some important goals were reached before the rediscovery of Mendel's laws and establishment of genetics as a science; for example, the need for pollination for fruit de–velopment in dates was recognized some nine centuries before Christ, in France, in the 17th century several varieties of "heading lettuce" were developed and some of them are still in cultivation. A French family of seed growers Vilmorins established the first seed company in 1727 and some years later Luis de Vilmorin was the first, to use a progeny test, evaluating the strain by the study of its descents. The most spectacular achievement of pre–mendelian plant breeding is development of sugar beet. Margraaf in 1747 discovered that the roots of fodder beets con–tained 6% of sucrose, through mass selection procedures applied by Archard sucrose content was increased to 11% at the beginning of 19th century and the application of pedigree method by Vilmorin resulted in further sucrose content increase to 16% by 1811 (Rees, 1993).

After the rediscovery of the work of Mendel Bateson coined the name "genetics" for the new science in 1906. Modern plant breeding is essen–tially an applied genetics but its scientific basis is boarder and includes concepts and tools of cytology, systematics, physiology, pathology, ento–mology, chemistry and statistics just to mention the most important disciplines. Genetics has given to breeding a better knowledge of the processes involved in the mechanism of development of variability and the important information how to identify, regulate and utilize such variability in breeding work. This knowledge allows the management and protection of plant natural resources, which are the basis for agri–cultural development, and a reservoir of genetic adaptability.

There are three main processes generating genetic variability in na–ture:

- recombination of genes in the process of sexual reproduction,
- spontaneous mutations and polyploidy,
- spontaneous hybridization between related plant taxons.

These processes constitute what was described by Darlington as "ge–netic system", which regulates the variability of populations. Nuclear and cytoplasmic mutations, their combination and recombination, mod–ifications in breeding behavior, structural and numerical changes in chromosomes embrace the spectrum of changes by which genetic vari–ability is generated. This variability allows plant species to evolve and adapt to various environments.

In domesticated species, selection pressures imposed by variety of factors related to their cultivation and utilization during 10 000 years of agriculture led to development of plethora of new forms, even a new species. We are now in the position to explain in genetic terms how such variation is generated and what factors influence its exploitation by the breeder. But still the variability from which the varieties are constructed originates from two main sources: from farm crops produced home and abroad and from crosses made by the breeder. For identification and utilization of plant genetic resources by breeders the two concepts developed after Mendel were very important:

– Vavilov's concept of centers of origin (centers of diversity) of cultivated species,
– De Wett's concept of gene pools,

These concepts allowed for systematic collection and utilization of genetic resources in modern plant breeding projects.

Since humans began to cultivate wild plants having food value the evolutionary processes were set up, controlled by natural and artificial selection which screened the existing genetic variability produced by mutation and recombination. In effect, a bounty of cultivars adapted to their local conditions were created. As the first crops expanded into new regions first through migration movements of early farmers and then along the trade routes they encountered wide differences in climate, soil and other environmental factors. Geographical barriers often separated populations of cultivars leading to the development of numerous, locally adapted landraces. Until very recently the above developments favored constant increase of diversity while the major contributor to increases of food supply was the extension of arable land, leading sometimes to severe disruption of the environment, e.g. Middle East deserts.

The emergence of modern plant breeding in late XIX century supported by rediscovery of Mendel's laws of inheritance and development of science–based breeding methods led to broad distribution and cultivation of new cultivars, which replaced a vast assortment of heterogeneous and primitive landraces. This process accelerated very much during recent decades in developed and developing countries, both west and east and resulted in a profound erosion of genetic diversity among crops. The modernization and introduction of modern cultivars into the agriculture of countries regarded as centers of origin and centers of diversity of major crops is dangerously limiting variability of crops and its related species. The introduction of improved high–yielding varieties into agriculture is a key element for feeding and survival of growing human population therefore the collection and preservation of representative samples of landraces and related species is an urgent task of agriculture services in all countries. On this stored and preserved variability further improvements of plant breeding depends. The other source of variation is opened by modern science – artificially induced variability and variation developed with the use of modern methods of gene manipulations on molecular level. Despite this division made for clarity of our discussion both sources of genetic variation are mutually interdependent.

INDUCED GENETIC VARIATION

Since any attempt to improve crops requires genetic variability it was obvious that as soon as it was found that some physical and chemical factors could increase the mutation rate in plants, induced mutations were used in plant breeding. In the late 20–ties of the 20^{th} century it was established that ionizing radiation discovered at about the onset of that century, caused heritable alternations, called mutations in plants (Stadler, 1930). Today a number of effective physical and chemical mutagens are available for generating variability and a whole system of induction and selection of artificially obtained variability through mutation breeding is utilized.

The effect of mutagens either physical or chemical is unspecific; both groups of mutagens generate two types of heritable changes; so called point mutations when one character is changing for example the colour of flowers or shape of leaves, sometimes the change occurs in one gene but more often among several closely linked genes. Another type are structural mutations when changes are observed on the level of the whole chromosomes or chromosome segments. Specific example of this type of mutation is multiplication of the whole genome called polyploidization. Initially, as it always is with new tools and methods, breeders were very enthusiastic, and overestimated the possibilities of what was called mutation breeding. While in crosses between cultivars or landraces the recombination is restricted to a relatively small fraction of the whole plant genome, mutations, whether spontaneous or induced, may affect any of 100 000 or so genes in nuclear genome and in addition also the ones located in cytoplasmic organelles. Even more, mutagenesis may alter even those genes that do not show any segregation after a cross, so called "house keeping genes". Thus, mutagenesis is a powerful tool for generating genetic variation. But the breeders soon realized also its limitations. The first limitation is imposed by the pre–existing genome: genes that do not exist in the genome of a given crop neither can be mutated nor eliminated. The second important limitation comes from the fact that the action of a mutagen cannot be directed to a specific gene. The third limitation, very important for plant breeder comes from the fact that most alterations randomly induced in a genome of an advanced cultivar, having a combination of good traits, will cause disturbances in this balanced system. The result is that majority of induced genetic variability is found in an unfit individuals. However, favorable induced changes can be incorporated through crossing and selection into a new improved genotype. In conclusion, one can say that induced mutations are important tools for generation of variation supplementing the existing genetic resources.

More then 1500 cultivars of different crops were developed and released through mutation breeding and more than 90% of them are based on mutants induced by X or gamma – rays. Useful induced mutations incorporated in cultivars are easily recognizable types, like causing changes in plant architecture, flowering time, flower shape or color and so on.

A major contribution of mutagenesis to plant breeding progress, however, is its use for the advancement of genetics. The mechanism of genetic system in plants and the way it works was elucidated with the use of induced mutations. Induced chromosome aberrations, which were relatively easy to observe were crucial in the development of cytogenetical location of agronomically important genes on chromosomes. (Micke and Donini, 1993).

The development and application of induced mutations in plant breeding occurred simultaneously with emergence and rapid progresses in molecular biology. It started in 1944 when it was found that DNA is a material carrier of genetic information. Next important discovery was the elucidation of the double helix structure of the DNA molecule in 1953. This, together with the cracking of the genetic code in 1966 was the fundamental breakthrough in biological sciences in the last century with far–reaching consequences for gene manipulation in living organisms. The ability to isolate genes (1973) and development of *in vitro* tissue culture techniques since 1950 created possibilities for application of DNA recombination methods for development of new genetic variability.

It can be stated that it is a very fortunate development that rapid genetic erosion caused by necessary expansion of modern agricultural practices could be alleviated by the development of induced genetic variability. Such a development has been possible thanks to new methods of molecular biology.

But the recombinant DNA methods applied in plant breeding in what we call agrobiotechnology, despite obvious promises for further improvement of cultivars, is also vigorously opposed by some people organized in so called environmental groups. Another controversial issue emerging together with new methods of gene manipulation in plant breeding is a question of patenting and ownership of genetic resources; very important issue for commercial plant breeding companies. In order to assess benefits and risks associated with the application of recombinant DNA methods for generation of useful genetic variability we need to look on this problem in the global context of world food supply at the early twenty first century.

THE IMPACT AND CONSEQUENCES OF GENETIC ENGINEERING ON CROP GENETIC VARIABILITY

The genetic variability, or say it more fashionable, biodiversity maintenance and preservation should be looked on in the context of human population size and necessary food production in agriculture.

There are two contradictory views of the relationship between food production and population growth; one that of Thomas Malthus claims that food supply is the driving variable and population growth depends on it; the other is of Ester Boserup who sees it in an opposite way; with population growth being the driver of agricultural development. In the past and present history one can find data supporting both views; but in any case increased food production must match population growth if we want to avoid disasters.(Siedow, 2001).

As it was said earlier, till the emergency of industrialized era, the driving force for increased food production was the expansion of area of cultivated land; it was the main force in development of medieval Europe and than colonization of Americas, Africa and Australia. New agronomic practices based on scientific principles were developed during the 19th century and the first half of the 20th century and culminated in the "green revolution" which is one of the greatest achievements to feed the world. Great increase in crop production was achieved by increased input of the energy into crop production (mechanization, mineral fertilizers and chemical protection against diseases and pests) linked with the development of genetically improved cultivars able to efficiently utilize those inputs for yield production. Owning to this, cereal production has kept pace with the average population growth rate of 1.8% since 1950; today 370kg of cereals per person are harvested as compared to only 275 kg in the 1950s. Similar progress was noticed in other food crops – 20% since 1960s (Ortiz, 1998).

But the technological progress driven by the forces of technological change, economic growth and world trade is a prime cause for depletion of water, wilderness destruction, water quality problems and accumulation of pesticide residues all of them reducing biodiversity. The recent improvements in agricultural efficiency brought by developing technology are well illustrated by comparing the figures from 1961 to 1993; during that period world population was almost doubled without mass starvation and barely detectable increase in cropland. Yield increase matching the increase of population saved us from Malthus trap so far. This is and must remain a major technological achievement of the last century.

The total estimated land use as farmland in 1993 constituted 36% of land surface (excluding polar caps) and farming is the largest land management system on the earth. If the crop technology had been frozen at 1961 levels to feed the 6 billion populations of 2000 we would need to increase the cropland by 850 million ha of additional land of the same quality (Trewavas, 2001). It is obvious that such surplus of land is not available. And what is more important, to conserve the present ecosys–tems and genetic variability, increased food production must be limited to the cropland currently in use or even reduced in some regions.

The FAO median population assessment is for 7.5 billion in 2020, the increase in the next 20 years is expected to be 2 billion. Much of this population growth will occur in the cities of the developing world. To meet the food demand, the world farmers will have to produce 40% more grain in 2020 than in 1995 and more than 80% of this increase must be obtained by increased yield (Pinstrup–Andersen, et all, 1999). To feed the increase in population expected by the year 2050 with traditional agriculture would require 3–fold increase in land under crops. Tropical forests, much of the remaining temperate forests and most of the re–maining wilderness would disappear – unacceptable scenario. Based on the opinion that in the developed world there is a surplus of food pro–duction some people claim that the world already produces enough food to feed everyone if the food were equally distributed (Conway and Toenniessen,1999). Besides that equal distribution is a myth this is simply not true, bearing in mind that about 73 million people, equiva–lent to the current population of the Philipines, will be added to the world population on average every year between 1995 and 2020 with 97,5% of this increase taking place in developing world.

Although increased efficiency, as a strategy to reduce adverse impact on the environment is a dogma in industrial sector, it is often vigorously contested for agriculture, forestry and other land–based human activi–ties. Often for some green organizations improving efficiency in agricul–ture contradicts their desire to impose some less–efficient, supposedly ecological solution. However, the consequences of less–efficient agricul–ture will be a disaster to biodiversity not to mention its effect on social and political scene. To summarize what is said above: we desperately need increased efficiency in food production and the world has or soon will have the agricultural technology available to feed 8.3 billion people expected in the next quarter of this century; the pertinent question is whether farmers will be permitted to use that technology. I hope they will, despite the fact that extremists in the environmental movement, largely from rich developed and well–fed world seem to be doing every–thing to stop scientific progress in food production. The new technology of genetic manipulation could improve our ability to produce enough food for increasing population and at the same time could allow us to save wilderness and environment protecting genetic variability of plants and animals.

We have examples from the past that many technological develop–ments allowing increase in yields at the same time were saving wilder–ness. Dispensing with pesticides would require at least 90% more cropland to maintain present yields, without mineral fertilizers we will need extra 600 million ha. In general, without technologies of "indus–

trial" agriculture applied in last decades, current food production would only have been achieved by plowing up an extra 2000 million ha.

In conclusion; we need to apply a new methods of food production offered by technological development, recombinant DNA methods included, in order to meet the food demand created by population growth predicted to take place in the first decades of 21 century. Furthermore, only the intensification of food production could allow to alleviate the pressure on expansion of cropland thus giving us a chance to save wilderness and biodiversity for generations to come. We must remember that the vital basics of life are warmth, food security, freedom from disease and long life. These basics require a high standard of living achieved by so called developed world and people from developing countries are prepared to ignore the environmental aspects of industrialization until the basics are achieved. No government is going to agree to rules and conditions protecting the environment but keeping their population poor and it is certainly a hypocrisy for the rich industrialized nation to advocate and impose such constrains on others who have not yet achieved the appropriate level of living standard. Therefore abandoning of technology, advocated by extremist technophobes from anti–GMO groups is not an answer to environmental problems connected with intensive and effective food production. Contrary, we need to improve the technology to remove or reduce the hazards. Only this will ensure continued benefit both to the mankind and the environment. The application of biotechnology in agriculture will hopefully provide the possibilities for doubling of food production till 2025 with minimum impact on the environment – a further development already called "double green revolution".

When discussing impact of agrobiotechnology on the environment we must realize that agriculture, by definition, had, has and will have a profound impact on the environment, the expansion of crop cultivation over the millennia has destroyed millions of hectares of forestland around the world. Almost all species cultivated in this part of the world are alien species introduced into non–native environment disrupting local fauna and flora. If one looks around countryside in Poland and in fact in all Europe and the world, one sees the landscape, which is anthropomorphic, changed profoundly by human activity directed to produce food, feed and fiber. Living in harmony with nature, a myth advocated by some new–age groups, is a possibility that disappeared some 5000 or 10 000 years ago. Therefore the reasonable approach to assess the potential hazards to human health and an adverse effect on the environment involved with utilization of genetically modified cultivars is to view it in the context of our experience with traditional variety development.

The emerging gene–technology offers, theoretically at least, possibilities for development of unlimited combination of genes and characters, which might be utilized for breeding of new and resource efficient cultivars necessary for increased food production in sustainable way, saving wildlife and biodiversity by restricting cropland extension. Typical questions asked about the impact of transgenic crops on environment are:

1. Do GM crops reduce crop biodiversity?
2. Could these crops lead to the development of "supper weeds"?
3. Are we introducing these crops into our environment without fully understanding the consequences of such action?
4. What about genetic pollution?
5. Can the transgens be transferred to other organisms including humans and animals? (Prakash, 2001).

There is a large bibliography dealing with various aspects of problems addressed in those questions and it would be unwise to make here an attempt to answer them in detail. But I think that we can summarize that most risk issues related to currently used transgenic cultivars are not unique when viewed in the context of agriculture development through millennia and modern plant breeding during the past century. What more, a case by case rigorous testing and regulatory framework developed for GM crops led to a confidence that transgenic cultivars may pose no new or increased risk that could not be identified and prevented. Risks from transgenic crops should be monitored and measured but concerns about these risks should be balanced against benefits and against risks associated with alternative options.

When we consider biodiversity we must admit that since the beginning in Neolithic times crop production was based on cultivation of land and sowing of seeds of one species. In this sense agriculture inherently acts to reduce biodiversity. In recent times the success of high–yielding varieties, developed by modern breeding enterprises narrowed substantially the genetic variation found till now in major crops. It is no doubt that saving and preserving genetic variation should be a major task of conservation services around the globe. Biotechnology offers a possibility to reverse this trend of genetic erosion: many old crop varieties were discarded because their resistance to diseases was overcome by new pathogenic strains, gene manipulation techniques opens a possibility to incorporate into this old varieties resistance genes needed, leaving intact all other agronomic properties thus making the "revival" of cultivars possible. Sequencing of genomes of crop plants has opened new frontiers in conservation of plant diversity. Isolation of agronomically important genes and sequencing them in many species can generate revolutionary changes in gene bank procedures; large cold stores and tanks with cryopreserved seeds might be, at least in part, replaced by DNA sequences stored electronically. Genomics will accelerate the utilization of genes available in these gene–banks through transformation without barriers between species and genera. Genetic engineering, often viewed as a threat to biodiversity when strategically applied offers a possibilities for efficient food production for increasing human popula–

tion and also opens a new ways for preservation and maintenance of biodiversity. It is not to say that genetic engineering if not wisely used could not pose some risks and hazards to human health and environment, but such hazards are involved in almost all technologies developed by humans and it is the way they are applied and utilized that decides on its benefits or hazards. All technologies are burdened with some problems simply because we are not perfect but the answer to this is not discard the technology altogether (often practically impossible task) but to improve these technologies.

Let me quote at the end of my lecture a statement I have heard long ago, which contains in condensed form an optimistic approach to our future, which I dare to share:

"Adding more people causes problems, but people are also the means to solve these problems. The main fuel to speed our progress is our stock of knowledge, and the brake is our lack of imagination. The ultimate resource is people – skilled, spirited and hopeful people who will exert their wills and imaginations for their own benefit and inevitably they will benefit not only themselves but the rest of us as well".

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