Technologies for crop improvement in the 21st century

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SUMMARY – Crop yields increased dramatically in the 20th century. That increase has been powered by changes in the genetic potential of the crop and in the way in which it has been managed. Nevertheless, the challenge to feed a world population that is likely to rise to 8 billion is formidable, particularly since recent analyses suggest that the rate of increase in yields of several crops may have dropped over the last decade. This paper discusses some of the technologies that will be available in the 21st century to meet this challenge.

Key words: Genomics, plant breeding, phenotypes, genetic markers.

RESUME – *"Technologies pour l'amélioration des cultures au 21ème siècle". Les rendements des cultures ont augmenté de façon spectaculaire pendant le 20ème siècle. Cette augmentation a été générée par des changements du potentiel génétique de la culture et par la façon de la mener. Néanmoins, il existe un défi immense, qui est d'alimenter une population mondiale qui atteindra probablement 8 milliards d'habitants, en particulier parce que des analyses récentes suggèrent que le taux d'augmentation des rendements de plusieurs cultures pourrait avoir chuté pendant la dernière décennie. Cet article discute certaines des technologies qui seront disponibles au 21ème siècle pour relever ce défi.*

Mots-clés : *Sciences du génome, amélioration végétale, phénotypes, marqueurs génétiques.*

Progress in the 20th century

The 20th century has seen a tremendous increase in crop yields, for example the yields of wheat from the well fertilised plots of the classical experiment on Broadbalk field at Rothamsted have increased four-fold in the century. Detailed analyses (see Evans, 1993, for discussion) have indicated that half of the increase can be traced to improvements introduced through plant breeding and half through agronomy, although each is dependent on the other. There have also been changes in the quality and distribution of crops in Europe. Whereas the UK used to import almost all the wheat needed to make bread, improvements in the bread making quality of British wheat cultivars have dramatically reduced the need for imports. Maize, which was a minority crop in Europe at the beginning of the century, is now grown over large areas. In contrast, oats have decreased markedly in importance. These changes have brought large social gains, (e.g. greater food security, lowering of malnutrition, lower prices freeing up income for other discretionary activities, vastly greater food choices, safer foods) and caused large changes in the environment, in both developed and developing countries, some of which have been judged as harmful.

The rediscovery of Mendel's work on genetics at the beginning of the century, and the steady development of scientific plant breeding based on those principles, has been of vital importance in improving crop varieties. The collection and spread of germplasm around the world and the development of new breeding systems (e.g. hybrid maize) has also had a major effect. The former ensured that all breeders could quickly benefit from advances of others, the latter laid the foundation for a highly profitable commercial plant breeding industry able to invest in crop enhancement. In many instances, major increases in yield have been attained from changes in relatively few genes (for example those involved in straw length and photoperiodism). These have led to significant changes in the distribution of crop dry matter into the harvested part of the plant but have not changed its basic productivity (see Evans, 1993).

Advances in agronomy have stemmed from the increased use of fertilisers, and the development of methods to reduce the competition from weeds, pest and diseases. Crop protection has depended on the developments of the agrochemical industry, which has developed sophisticated chemical syntheses and screening technologies. Mechanisation of agronomy has enabled field operations to be done in an efficient and timely manner which has lessened, but not removed, some of the limitations imposed by the weather.

Physiology and biochemistry have developed as powerful disciplines during the 20th century but only in a limited number of instances have they led to crop improvement. This is because the links between them and the genetics of the processes described have not been established. Their role has rather been to provide possible explanations for the improvements that have been achieved by the breeders. One of the exceptions has been in the field of wheat quality where knowledge of the role of endosperm storage proteins in determining the processing properties of wheat (bread and pasta making) has enabled the development of laboratory tests to enable the breeders to select improved lines (Payne, 1987; Shewry, 1995; Porceddu *et al.,* 1998).

The challenges for the 21st century

The popular conception in the Western world is that we have surpluses of food of virtually every description. Where there are shortages and starvation, this is in large part due to other factors such as war, poverty, political systems or poor distribution. However, as we enter the next century we are faced with a number of challenges to maintain the necessary food production. These include an estimated increase in the world's population to around 8 billion by 2020; the trend to increased meat consumption as societies become more affluent, which in turn increases the *per capita* consumption of crops; global warming, causing more frequent and severe fluctuations in climate, thus increasing the chance of crop failures; a strictly limited availability of land; shortage of the water necessary to support crop growth with irrigation; the need to prevent environmental degradation of that land and water; the need to improve land already partially degraded, for example by salinisation or soil erosion; ensuring that the rate of increase in crop yields continues as it has done in the past; and, the continued need to protect crops from pests and diseases of a diverse and unpredictably changing nature. These factors have been used to make predictions for the future and the outcomes vary from the bleak (Brown, 1994) to the optimistic (Dyson, 1996), however, both of these authors, and most others writing on the subject, emphasise the need for continued agricultural research. If this is not forthcoming, or is not successful, then most would agree that the pessimistic forecasts are the more likely outcome. Because crop plants are the direct or indirect source of virtually all of our food, future success will be critically dependent on the success of crop research to address the challenges outlined above. Fortunately, we are at a stage in biological research where there are tremendous advances being made in our understanding of organisms and these provide opportunities for enhancing the technologies available for crop improvement as outlined below.

Technologies for crop improvement in the 21st century

Analysis of crop performance

Plants have evolved complex genetic systems that enable them to cope with, and adapt to, changes in the environment in order to complete their life cycle. A crop's performance is dependent on the interaction of the crops genetics and the particular environment in which it finds itself. Since the environment changes according to geography and season, a given variety will perform differently from place to place and season to season. That is to say the phenotype of a given crop genotype (or cultivar) can vary markedly according to its interaction with the environment. In the excitement of the tremendous advances in genetics, it is important not to forget the role of the environment in crop performance and that our food comes from successful phenotypes. Nevertheless, the new technologies with the most promise are those that allow us to modify the crop's genetics. Thus, provided that the above complexity is recognised, there is now tremendous potential to analyse the genetic basis of crop performance and from this derive strategies for further advancement. Although this analysis is likely to be iterative and integrated we can consider three basic types of approaches to understanding the genetic basis of crop performance.

Genome sequencing

Major efforts are underway to sequence genomes of a range of organisms including plants. Already the complete genomes of yeast and *C. elegans* have been determined as well as a rapidly increasing number of prokaryotes. Efforts are well underway to sequence the complete genome of *Arabidopsis,* rice and maize and, the complete sequence of chromosomes 2 and 4 have recently been published (Lin *et al.,* 1999; Mayer *et al.,* 1999). The big challenge for biological research in general, and crop improvement in particular, is how to get the most valuable knowledge the quickest from this explosion in

DNA sequence information. Oliver (1996) has outlined some of the methods that are being used in the collaborative yeast project and similar strategies are being developed for other species (e.g. mouse, Brown and Nolan, 1998; crop plants, Cook, 1998). The first approach is usually to search for homology with other known genes. This often leads to a tentative identification of the sequence to a class of genes, however, in all the genomes published so far, a significant number of open reading frames do not have homology to genes of known function; for example, in the *Arabidopsis* sequences of chromosomes 2 and 4 between 40 and 50% of the putative genes do not match a predicted protein of known function (Lin *et al.,* 1999; Mayer *et al.,* 1999). Another approach to understanding the function of specific sequences is to look at their expression under a range of defined conditions. Ruan *et al.* (1998) have measured the expression, during plant development, of 1400 *Arabidopsis* cDNA sequences using micro array technology. These studies, and those in other species, show this has considerable potential as a powerful tool for plant gene discovery and functional analysis as well as aiding the understanding of genetic regulatory networks and gene interaction.

A large scale genomics approach is chiefly being followed in industry and in some national and international programs. It will undoubtedly yield many benefits and, because of the similarities in the sequence of the genes and, in major plant groups such as the grasses, in their position in the chromosome (synteny), these benefits may be applicable over a range of plant species. On the other hand there are also many limitations in the genomics approach. For example, it tends to be driven by data rather than hypotheses, which means it may be linear and unprioritised (i.e. start with the first available sequence and work out the function of the genes one by one). It also faces difficulties in that many important crop traits depend on the interaction between a number of genes or may be encoded by multiple copies of the same gene. In addition, plants are renowned for their ability to compensate physiologically. Thus, generating large populations of plants in which single genes are either knocked out or over-expressed is unlikely to reveal the genetics of processes subject to these phenomena; generating multiple combinations will probably be numerically impossible. Even when the function of a gene sequence has been correctly identified, there remains the problem of finding the better or best alleles. For these reasons, I have argued elsewhere that the current thrust, which is largely based on a genocentric view, needs to be balanced by a matching emphasis on a phenocentric approach (Miflin, 2000a).

Phenotype analysis

Analysis of the phenotype can be considered at both the level of the cell and of the whole organism. At the cellular level the focus is on understanding the transcription of genes into proteins and how this changes from cell to cell and from time to time. The goal is to obtain an overview of the characteristics and activity of every protein of the organism – the proteome. The study of the proteome – called proteomics (see Abbott, 1999, for a survey) should reveal when and how the genes are expressed as may also be expected from transcription analysis. However, because protein protein interactions can also be analysed information on how the products of the genes interact should also be obtained. A start to this type of approach has been made in yeast (Uetz *et al.,* 2000) and have detected over 900 putative interactions amongst 1004 *S. cerevisiae* proteins.

Probably more important in crop improvement is the analysis of the genetics of the phenotypic traits of the whole plant or whole crop. This has been made possible by the recombinant DNA technology which has given rise to a range of methods that allow the genome to be tagged with DNA markers (see Karp *et al.,* 1997; Davis *et al.,* 1999; Vulysteke *et al.,* 1999). The crucial drive is to provide systems that can be automated, using robots, chips, sophisticated analyses (e.g. MALDI-TOF) and computers, so that large populations of plants can be handled in a cheap and routine manner. These developments are essential to enable breeders working with large populations to make maximum and efficient use of the technology. However, the technology is currently being widely used to identify the loci underlying agronomically important phenotypic traits, particularly using wide crosses with wild relatives of crops and moving these into adapted cultivars using marker assisted breeding. Once loci have been identified, map-based cloning can be used to isolate the genes involved.

The practical value of marker technology has been exemplified by the work of Tanksley, McCouch and colleagues using wide crosses of tomatoes and rice. For example, when crosses between a large red-fruited commercial cultivated tomato (*Lycopersicum esculentum*) and a small green-fruited wild relative (*L. peruvianum*) were made and analysed, a number of loci contributing to the variation in yield, colour, shape and weight of fruit were identified (Fulton *et al.,* 1997). Unpredictably, it was found that a significant number (around 25%) of the favourable alleles were present in the wild species. Thus the small green-

fruited relative had alleles that contributed positively to fruit colour, shape, weight and yield. A large experiment has also been done with rice, involving scientists in many centres in different countries, designed to discover quantitative trait loci (QTLs) important in crop performance and to recombine favourable alleles at those loci. *Oryza rufipogon,* a wild relative of rice, was crossed with three elite cultivars adapted to different growing environments in China, Korea and Columbia and 300 backcross lines derived. These lines were tested in a wide range of environments and evaluated for 12 key agronomic traits. Several lines showed superior performance to the parents (Xiao *et al.,* 1998). Subsequent marker analyses showed that not all of the favourable alleles were in the adapted cultivar. *O. rufipogon* contributed alleles that were consistently associated with improvements in yield, quality, maturity and plant height. This work, together with other examples, suggest that reservoirs of genetic diversity that reside in the wild relatives of crop species may contain numerous alleles that can provide the key to future increases in the productivity of a number of crops (Tanksley and McCouch, 1997). This finding probably reflects that, in domesticating our current range of crops, our ancestors only exploited the relatively narrow base of the wild populations available to them in their immediate vicinity. The other great advantage of the marker approach is that it does not need any pre-knowledge of the genetic nature of the trait and is relatively independent of the pre-conceptions of the researchers. It also automatically imposes some prioritisation of loci, genes and alleles.

Marker technology has also allowed the development of the genetic maps of a number of species. Comparison of these maps has led to the surprising realisation of the positional similarity of the genes in different species. In early work, positional similarities were noted between closely related genomes (e.g. between potato and tomato and between the three genomes of cultivated wheat). Subsequent analysis of a wide range of grass genomes showed that the colinearity of grass genomes exists across the Poaceae and that it is possible to develop a consensus marker map for the grasses (see Gale and Devos, 1998). The significance of this synteny is that advances made in one species can be applied to others relatively easily. For example, wheat has the disadvantage of having a very large genome, larger than that of humans. This means that large-scale sequencing would be prohibitively expensive. However, rice has a several fold smaller genome, is currently being sequenced and as genes are identified in rice it should be easy to find the corresponding gene in wheat and determine any differences. Gale and Devos (1998) suggest that the time is fast approaching when the grasses, including all the major cereals can be considered as a single entity and the information on gene structure, gene action, metabolism, physiology and phenotype accumulated over the past century on the different grass species can be pooled.

However, there are limitations in the marker approach. Formally, the results only apply to the cross that is analysed and certain risks are taken in extrapolating these to other genotypes. The analysis can only identify loci that contain different distinguishable alleles in the parents of the cross used – important but invariant loci will not be identified. To some extent these difficulties can be overcome by making several wide crosses (Fulton *et al.,* 1997). However, there may be important traits where there is no difference between alleles at a locus, for example, those determining the rate of photosynthesis in wheat in which there is little variation (see Evans, 1993). Crucially, the identification of loci for traits is only as good as the measurement of the trait – if the measurements are too variable, allelic variation will not be revealed, and if the measurements are inaccurate the wrong loci will be studied. Finally, even though technical advances in analysis have been made the need for, and the time and cost of, generating suitable populations for analysis are significant limitations.

Biochemical engineering

Biochemical studies of plant metabolism have identified proteins crucial in the functioning of most pathways. Many key genes have been isolated by purifying and (partially) sequencing the proteins and then finding the corresponding cDNA and/or genomic sequences. Knowledge of the changes in a specific plant function induced by different treatments has led to the development of methods to isolate genes involved in the metabolic pathways or their associated physiology. Knowledge of physiology and metabolism has also allowed researchers to set up screens to isolate mutants in a specific process. For example, Somerville and Ogren (1979) used the knowledge that the photorespiratory pathway protects plants from damage when exposed to light in the absence of CO_2 to set up a screen for conditional lethal mutations that caused plants to become damaged and eventually die when grown in the absence of CO_2 but which could be rescued in the presence of high levels of CO_2. Many of the mutations were found to be in the enzymes of the photorespiratory pathway and have aided our understanding of the pathway and eventual isolation of the genes. With the availability of tagged mutant populations the use of “clever

screens" based on knowledge of metabolism provides a relatively easy approach to isolating the genes for key steps. However, as in the mutant approaches discussed above, steps which are encoded by multiple copies of genes may not be revealed by this approach.

One area where biochemical engineering has made a great start is in wheat and the modification of the endosperm storage proteins (Shewry *et al.,* 1995, 1997). These proteins have been well characterised and many of the genes for them isolated. Genes for the high molecular glutenin proteins have then been used in transforming bread and pasta wheat and the artificial cereal tritordeum. In each case the transformants have been shown to have altered processing properties (He *et al.,* 1999; Rooke *et al.,* 1999a,b). The results have generally been in line with those expected by current hypotheses for the role of these proteins in processing properties (Shewry *et al.,* 1997) The way is therefore open to make innovative changes in the genes to construct totally new proteins (see the paper by Shewry in this volume for further discussion).

Information and automation technology

The explosion in the genetic information discussed above will only be fully exploited if the best use is made of information generating and handling technologies. Space prevents detailed discussion but is important to stress that there is a range of technologies existing, or likely to be developed, which will greatly aid crop improvement. One dramatic example is the use of micro array technology. Winzeler *et al.* (1998) have recently demonstrated how the yeast genome can be mapped based on probes, derived from the complete yeast DNA sequence, which were synthesised in a spatially addressable fashion, with a combination of photolithography and solid phase chemistry, on to 1.64 cm^2 micro arrays. Based on the hybridisation of DNA, from four segregants of a tetrad derived from a yeast cross, to the 3714 markers on the arrays, they were able to develop a map of the yeast genome at a density which exceeded the traditional map assembled over the last 40 years. Such results were totally dependent on robotics, sophisticated image readers and computer power. Expansion of the technology to crop breeding will generate massive amounts of information that will require intelligent and innovative software to extract the maximum amount of useful knowledge. Similarly, the analysis of the vast number of mutants capable of being generated as a result of the *Arabidopsis* sequence programme will generate masses of data. John Ryals of Paradigm Genetics estimates that 10 terra bytes of data alone will be generated by his company's efforts in this area (quoted in Miflin, 2000b). Fortunately, the rapid development of massive and cheap computational power has more than kept step with the pace of gene sequencing but there is still a tremendous need for the necessary innovative software to analyse, record and compare sequence information, and to generate, maintain and search the sequence databases.

Genetic improvement of crop performance

Plant breeding

Plant breeding will continue to be the major way in which our crops will be improved in the 21st century. Despite all the advances in gene technology and transfer, these new methods can only deal with small numbers of genes, it will still be essential to continue to improve the whole of the rest of the crop genome. Plant breeding will be vastly empowered by marker technologies as these become cheaper and automated. It will assess a much wider range of gene pools than in the past and will have to incorporate the undoubted advances that will come from the introduction of completely novel genes by crop transformation.

Crop transformation

The ability to transform crop plants has developed remarkably since the first transformed plants were reported in 1983 and all crop plants can be transformed with varying degrees of efficiency (see Birch, 1997). However, there is still a need to improve the efficiency of transformation, to limit the presence of unnecessary genes in the products, to direct insertion to specific sites, and to give more control over when, where and how much expression of the transgene occurs. Given the pace of past progress, there is every reason to believe that many improvements will quickly be developed (e.g. see Kunkel *et al.,* 1999; Srivastava *et al.,* 1999).

Transformation allows genes from all organisms to be considered for crop improvement and the early products on the market, conferring resistance to insects or tolerance to herbicides, are largely based on genes derived from microorganisms. The genes used have not all been direct copies and completely new genes have been synthesised in the laboratory. Despite the immense variation in crops and their relatives and the range of genes in the biological kingdom, there may be instances where the required variation is unavailable and where various forms of gene engineering will be employed.

Agronomic improvement of crop performance

This review has concentrated on genetic improvement because that is where I believe the greatest potential lies to improve the yield of crops. This is in part because of the great success that has already been achieved in crop production through agronomy and crop protection. There will be tremendous advances in these areas in the future but these are more likely to address improving the efficiency in the use of fertilisers and crop protection agents and in minimising the side-effects of these on the environment. The developments of combinatorial chemistry and the identification of new target sites from genomics research are likely to enhance the quality of agrochemicals at the farmer's disposal. Sophisticated systems to support decision making, allied to machinery capable of implementing those decisions precisely, particularly in respect to the use of water, fertilisers and crop protection agents, will undoubtedly improve the quality of agriculture but may not greatly enhance its output.

Conclusions

Although society appears to be complacent about food production, except for some concerns about food safety, the predicted growth in world population and the likely effects of climate change will pose some very testing challenges to agriculture, and particularly crop production, during the first part of the 21st century. The great hope for meeting these challenges comes because we are at the beginning of a biological revolution in which we will accumulate a tremendous amount of information about the genetics of plants. The task is to turn this into the knowledge and technologies needed to improve the yield of the world's major crops.

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