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Crop improvement in the 21st century

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Abstract

Crop yields increased dramatically in the 20th century as recorded on Broadbalk or in world averages. The vast majority of that increase has occurred since the last world war and 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. What are the opportunities to meet this challenge and to continue to improve the yields of our crops? Improvements in agronomy are likely to be more concerned with efficiency and elegance rather than in major breakthroughs. More sophisticated crop protection chemicals designed on the basis of vastly increased screening potentials and (at last?) possibilities of rational design will be supplemented by a battery of decision support systems to aid management choices which can be precisely implemented. Genetic improvement is the area in which to look for the major breakthroughs. The broad potential of recombinant DNA technology will provide the possibility of both molecular analyses of crop productivity and ways in which it may be possible to improve that productivity. The goal of analysis may be approached in three ways: starting at the beginning by generating complete sequences of the plant genome; starting at the end by genetic analysis of phenotypes using genetic marker technology; or, starting in the middle, by metabolic analysis. Improvements may be obtained by re-assorting what has been achieved through enhanced breeding technologies, by randomly induced change, and by generation of totally new possibilities through biochemical engineering. Examples of all approaches will be given. The onset of genomics will provide massive amounts of information, but the success will depend on using that to improve crop phenotypes. The ability to meet the challenges of the 21st century will depend on the ability to close that 'phenotype gap'.

Key words: Phenotype gap, plant genome, genomics, marker technology, trait analysis phenocentric.

Progress in the 20th century

The 20th century has seen a tremendous increase in crop yields. This can be illustrated from the records (Fig. 1) of the Broadbalk experiment at Rothamsted which has changed over the years to introduce new practices and cultivars as indicated (Rasmussen *et al.*, 1998, and references therein). From these it is possible to understand the reasons for the improvements in yield. More detailed

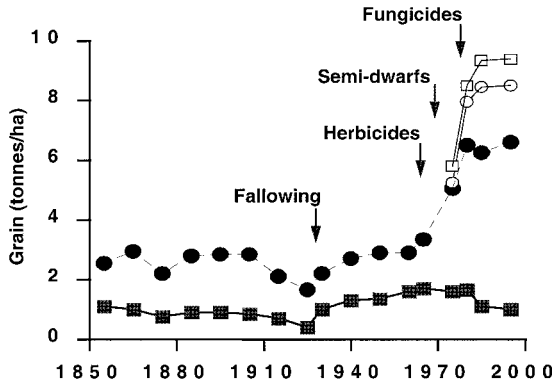

Fig. 1. Yields of wheat in the classical Broadbalk experiment at Rothamsted. The introduction of new technologies is indicated by the arrows. All plots received P and K and the additional fertilizer treatments for the different plots are as follows: (□) wheat grown in rotation and given 96 kg N ha^{-1} plus FYM; (○) wheat grown in rotation and given 144 kg N ha^{-1}; (●) wheat grown continuously and given 144 kg N ha^{-1}; (■) wheat grown continuously with no added N. The figure is adapted from Rasmussen *et al.* (Rasmussen *et al.*, 1998).

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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. These improvements have brought large social gains, for example, in greater food security, lowering of malnutrition, lower prices freeing up income for other discretionary activities, vastly greater food choices, and safer foods, and have caused large changes in the environment, in both developed and developing countries, some of which have been judged as harmful. They have also caused large changes in the structure of agrarian societies in all countries. In parallel, the industrialization of society has led to the congregation of the majority of the population in towns and cities. Together these factors have disrupted the erstwhile close relationship between man and his food.

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 improved 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 the advances of others, the latter laid the foundation for a highly profitable commercial plant breeding industry. In many instances, major improvements in yield have been attained from changes in relatively few genes (for example, those involved in straw length and photoperiodism). These have led to major changes in the distribution of crop dry matter into the harvested part of the plant, but have not changed the basic productivity of the plant (Evans, 1993).

Advances in agronomy have stemmed from the continued use of fertilizers, based on Lawes and Gilbert's work in the previous century, the true value of which could only be realized in the presence of suitable varieties and in the absence of competition from weeds, pest and diseases. The latter protection of crops has depended on the developments of the agrochemical industry which has developed sophisticated chemical syntheses and screening technologies.

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. This situation is likely to change in the future.

The challenges for the 21st century

The popular perception in the Western world is that there are 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 the next century approaches, there are a number of challenges to be faced 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, hopefully, improvement of land already partially degraded, for example by salinization 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 these authors, and most others writing on the subject, emphasize 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 our food, future success will be critically dependent on the success of crop research to address the challenges outlined above. Fortunately, biological research is at a stage 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

Introduction—phenotypes

Crops, unlike animals, stay in one place and are therefore at the mercy of the environment in which they find themselves. As a consequence they have evolved complex genetic systems which enable them to cope with, and adapt to, changes in the environment in order to complete their life cycle. 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. Farmers are concerned with the yield of the crop phenotypes that grow in their fields, whereas plant breeders are seeking to improve the genotypes of their cultivars. This apparent paradox is resolved

by testing their newly created genotypes in a wide range of environments and over a number of years before finally selecting and releasing a new cultivar. Breeders of a range of crops in most agricultural environments have devised technologies for crossing and testing that have achieved the success referred to above. In the excitement of the tremendous advances in genetics across all organisms it is important not to forget the role of the environment in crop performance and that food comes from successful phenotypes.

Analysis of crop performance

Provided that the above complexity is recognized, there is tremendous potential to analyse the genetic basis of crop performance. Although this analysis is likely to be iterative and integrated, three basic types of approaches to understanding the genetic basis of crop performance can be considered.

Starting at the beginning—genomics. Major efforts are underway to sequence genomes of a range of organisms including plants. Already the complete genomes of yeast and *Caenorhabditis elegans* have been determined as well as a rapidly increasing number of prokaryotes. Efforts are well underway to sequence the complete genomes of *Arabidopsis*, rice and maize and, early in 1998, 1.9 Mb of contiguous sequence from chromosome 4 of *Arabidopsis* was published (Bevan *et al*., 1998).

Analysis of these sequences to suggest functions for the genes is now the new challenge and can be achieved in a number of ways. 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, but 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* sequence about 14% of the putative genes do not match a predicted protein of known function (Bevan *et al*., 1998). In any event, whether a tentative function is assigned by homology or not, it is still necessary to carry out a functional analysis in which the gene is either over-expressed or its activity reduced or supressed in some way. A number of approaches to gene suppression have been developed and include co-suppression or antisensing of a defined sequence.

However, transformation of specific sequences can only deal with relatively small numbers of genes. Populations of mutants, in which a series of gene knockouts occur, are being developed so as to deal with the identification of a large numbers of genes. These are then screened for changes in phenotype (i.e. changes in function in a defined environment) and any changes identified traced back to the specific DNA sequence that has been mutated.

This careful linear approach will undoubtably yield much valuable information. It is, however, limited in a number of ways. Firstly, the large number of genes involved ensure that a major effort is required and it is difficult to prioritize which should be done first. Secondly it deals with first order identification where a single function is linked to a single gene—it will reveal little where the function is dependent on the interaction between a number of genes. Thirdly, a function may be discovered, but there is no identification of favourable alleles. Fourthly, plants have ways of compensating for disruption of the function of a gene; these may be at the genetic level, with multiple genes having the same or compensating functions, or at the physiological level, where plants are known to be able to adapt their metabolism to maintain a virtual constancy of phenotype. Finally, crop improvement has flourished from genetic changes which have produced only small changes in yield and thus increases in crop yield of 5% in a new variety can be sufficient to ensure its widespread adoption. However, the ability to identify genes and characterize alleleic variation that causes such small changes in phenotypic performance in a laboratory or greenhouse environment is a major challenge.

Another approach to understanding the function of specific sequences is to look at their expression under a range of specific conditions. Ruan *et al*. have measured the expression, during plant development, of 1400 *Arabidopsis* cDNA sequences using micro array technology (Ruan *et al*., 1998). This study, and those in other species, show that the approach has considerable potential as a powerful tool for plant gene discovery, functional analysis and the understanding of genetic regulatory networks and gene interaction.

Starting at the end—trait analysis. Recombinant DNA technology, besides generating powerful technologies to determine gene sequences, has revolutionized approaches to providing genetic maps to which regions of the chromosome specifying particular traits can be assigned. These technologies, with a bewildering numbers of acronyms, are described in many reviews (for example, see Karp *et al*., 1997). They depend on discovering and exploiting changes in DNA sequence caused by base changes, deletions or additions of bases, or by variation in the number of repeats of short sequences. The differences are revealed by use of restriction enzymes and/or the polymerase chain reaction (PCR) and usually requires separation of DNA on gels. However, new technologies based on micro arrays (Winzeler *et al*., 1998; Edwards, 1999). are promising to remove the need to use gels, enable the use of robotics and thus lead to a major improvement in time and cost. This will vastly increase the number of plants that can be

analysed, a vitally important factor for plant breeders who work with large populations.

Trait analysis usually depends on making crosses between two individuals, that differ in their phenotypes in ways that are potentially interesting for the traits under study, and then deriving a population of recombinant inbred lines. The individual lines in the population are analysed for the traits of interest under one or more relevant environments and the segregation of these traits compared with the segregation of DNA markers. The results allow the assignment of those regions of the chromosomes (loci) which contribute most to the variability in traits. Once the loci have been assigned and the most beneficial alleles at each locus identified, they can be recombined by making the requisite crosses and selecting, by marker technology, those offspring which have the desired combination of alleles. This technology has been in commercial application in a limited manner since 1990 and, besides its use in conventional breeding, has proved particularly useful in moving transgenes into a number of different adapted genotypes from the initial transformants.

Marker technology is powerful in being able to analyse the genetic basis of traits and identifying allelic variants which are beneficial. It can do this without needing any pre-knowledge of the genetic nature of the trait and is relatively independent of the pre-conceptions of the researchers. Recently, Tanksley and colleagues have reported results from wide crosses of tomatoes and rice which have provided some very important results. 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 genes that contributed positively to fruit colour, shape, weight, and yield. Similar results showed alleles in the wild rice *Oryza rufipogon* which were able to confer considerable increases in yield over those in cultivated rice (Xiao *et al*., 1996). This work shows, not only that the wild relatives of commercial crops contain alleles of loci of importance for a number of crop traits, but provides a very effective method for incorporating them into the crops. The results probably reflect that, in domesticating the current range of crops, man's ancestors only exploited the relatively narrow base of the wild populations available to them in their immediate vicinity.

Although breeders can make direct use of the results of marker technology without detailed knowledge of the genes involved, it is nevertheless valuable to resolve the loci down to their component genes and to isolate and sequence those genes. This map-based cloning of genes has been successful in a number of cases and is becoming easier with the development of different genomic libraries of crops in a range of yeast and bacterial based vectors.

The development of the genetic maps of a number of species has led to the surpising realization of the positional similarity of the maps in different species. In early work, positional similarities were noted between closely related genomes, for example, 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 (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, which is the major European cereal, 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 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 (Gale and Devos, 1998).

The application of marker technologies has already made major impacts on our understanding of the genetics of crop plants and on the activities of commercial breeders. It is also being used by biochemists and physiologists to understand the genetics of metabolic processes. New advances will make the technologies cheaper and more effective. The use of markers has the advantage that the traits for which genetic information is required can be prioritized and investigated without needing to sequence and analyse the whole genome. They also allow traits which are controlled by several loci to be analysed (so called quantitative trait loci or QTLs) and favourable alleles at each locus to be identified. These alleles can then be combined by simple crossing and the most favourable combination assembled almost as if the trait were governed by a single locus. However, there are limitations; formally the results only apply to the cross analysed and certain risks are taken in extrapolating them to other genotypes; only loci that contain different distinguishable alleles in the parents of the cross will be revealed—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). 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.

Starting in the middle—metabolic analysis. 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. A good example of this approach is that of Zhang and Forde (2000) to the study of genes important in a plant's acquisition of nitrate. One advantage of this often difficult approach is that it allows the isolation of genes for enzymes which are encoded by more than one gene and which may not be revealed by mutant screens. Again the use of micro array technology (Ruan *et al*., 1998) to study the expression of the genes in relation to physiological processes is likely to identify key genes.

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 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 (Somerville and Ogren, 1979). 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. A similar mutant approach, but chiefly using brute force screening, allowed Somerville and Browse to identify the key steps in fatty acid biosynthesis and the subsequent isolation of the genes (Browse and Somerville, 1991). Based upon this knowledge, and aided by some good biochemistry, Kridl, Davies and others (Hawkins and Kridl, 1998; Voelker *et al*., 1996) at Calgene have generated a series of transgenic varieties of canola which produce oils with modified fatty acid profiles. Some of these are already in commercial use.

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.

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 of the possibilities, even if it were possible to do so. Nevertheless, it is important to stress that there are a range of technologies existing or likely to be developed which will greatly aid crop improvement. The use of micro array technology has been mentioned above. Winzeler *et al*. have recently demonstrated how the yeast genome can be mapped based on probes, derived from the complete yeast DNA sequence, which were synthesized in a spatially addressable fashion, with a combination of photolithography and solid phase chemistry, on to 1.64 cm^2 micro arrays (Winzeler *et al*., 1998). Based on the hybridization 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 which will require intelligent and innovative software to extract the maximum amount of useful knowledge. Similarly, 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

Moving genes around: Genetic improvement of a crop performance can be achieved by introducing improved alleles at existing loci through conventional crossing, aided by marker and other technology, and by adding new loci by transformation. At present it is not possible to use transformation to replace alleles at existing loci, although the expression of genes at existing loci can be blocked by introducing antisense or co-suppression constructs.

The ability to transform crop plants has developed remarkably since the first transformed plants were reported in 1983. However, there is still a need to develop improved methodology, for example, to improve the frequency of transformation, to increase the number of genes that can be transferred, to allow better control of the expression of the transferred genes, and to enable the genes to be inserted at defined positions. Given the pace of past progress, there is every reason to believe that many of the necessary goals will be achieved.

Sources and nature of genes. As described above, there have been spectacular recent advances in demonstrating how alleles from wild relatives of crops which have the potential to improve crop performance can be identified and transferred into adapted germplasm. This is likely to

provide a major stimulus to the use of genetic material from very diverse sources of crop relatives for incorporation into new crop cultivars.

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 based on genes derived from microorganisms. The genes used have not all been direct copies of those in the microorganism, in one case the complete gene has been synthesized in the laboratory to produce the exact protein sequence of a cleaved *Bacillus thuringiensis* insect toxin (Kozeil *et al.*, 1993).

Despite the immense variation in crops and their relatives, or the range of genes in the biological kingdom, there may be instances where the required variation is apparently unavailable. This limitation may be overcome by various forms of biochemical engineering, for example, in generating modified active sites in enzymes or in modifying the structure of wheat storage proteins for bread making by changing their potential to form disulphide cross links. Newer techniques (for example, phage display technology to generate novel antibodies) have, and will be, developed for evolving new genes in bacteria, or in test tubes, for eventual use in plants.

So far the major targets for transformation to improve crops have been related to crop protection, either to enhance the possibilities of using broad spectrum herbicides, or to provide resistance against insects. Genes have also been introduced that affect quality traits such as fruit ripening, fatty acid composition of oils and starch properties. Traits that are more complex, for example, broad spectrum disease resistance, or which may require several genes to bring about the desired changes, are difficult to achieve and will take further research. Whilst an almost limitless number of structural genes to express different proteins are available, there are currently considerable limitations on the availability of promoters and regulators to ensure the reliable expression of the genes at the desired times during development and in the desired organs and tissues. There is also a need for much more understanding as to how complex regulatory and signalling controls work and can be modified. At present, determining what might be achieved in crop improvement through transformation is only at the very early stages.

Agronomic improvement of crop performance

This review has concentrated on genetic improvement because that is where 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 fertilizers and crop protection agents, and in minimizing 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, fertilizers and crop protection agents, will undoubtably 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. A biological revolution is beginning during which a tremendous amount of information about the genetics of plants will be accumulated in the next ten years. The task is to turn this into the knowledge and technologies needed to improve the yield of the world's major crops. Yield is dependent on crop phenotype, in which the interaction between genotype and environment is crucial. Currently, basic research is placing its emphasis on genomics and genetic discovery. This is leading to a genocentric view of priorities (Fig. 2) in which the focus is on finding a function for each of the genes in the genome. While this is necessary for basic discovery it is not sufficient for crop improvement. The danger is that, as work progresses systematically through finding out the function of individual genes, the complex interactions between individual genes and multiple environments, and between multiple genes, may either be left until later or not even elucidated

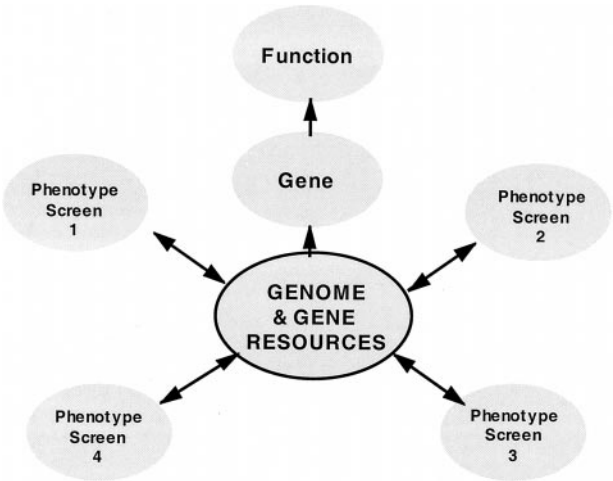

Fig. 2. The genocentric view looking for the function of all the genes in the genome.

by this approach. Undue or sole emphasis on genomics will lead to an ever increasing gap between the genetic information acquired and an understanding of the phenotype, a 'phenotype gap' (the phrase 'phenotype gap' has been used by Brown in reference to the mouse genome, (Brown and Peters, 1996; Brown and Nolan, 1998) but applies even more to plants), with exploitation following even further behind (Fig. 3).

Thus in the author's opinion, to improve crops and to meet the challenges ahead, the genotypic view and emphasis on genomics needs to be balanced by a phenocentric approach (Fig. 4) and an emphasis on phenomics. Thus it means placing emphasis on discovering those genes that are most important in determining the phenotypes required in agriculture. This will be dependent on having screens for a range of traits, for example, in the context of this meeting, stress tolerance, that powerfully discriminate between genotypes and which can be applied to the large populations of mutants of *Arabidopsis* and other species that are being generated. In addition, screening for and measuring important phenotypic traits is also crucial for the full exploitation of the opportunities offered by marker technology. Knowledge of the physiology and biochemistry of plants will be extremely important in devising and interpreting screens.

The application of marker technologies to the re-domestication of crops by exploiting the potential goldmine of favourable alleles existing in the wild relatives of crops provides the best relatively short-term opportunity for achieving the necessary advances in crop performance. Whilst there are and will be tremendous benefits brought to crops via transformation of a small number of genes important for crop protection, for crop quality and for crop stature and development, the major benefits from the technology will be for the more long-term future and will depend on developing a full understanding of the interaction of the genes with the crop environment.

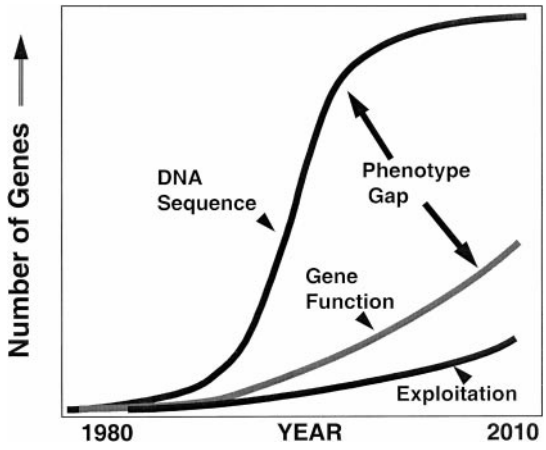

Fig. 3. The phenotype gap. The pace of determining DNA sequence is currently much faster than that of determining function in the organism or in exploiting the genes in crop improvement.

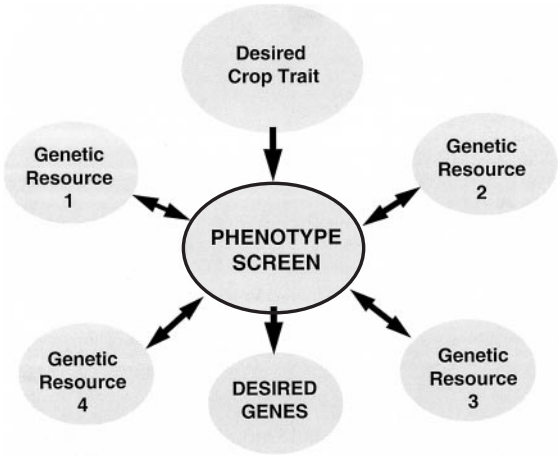

Fig. 4. The phenocentric view looking for the genetic basis of desired crop traits.

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