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Perspective and Prospect

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Perspective and prospect

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Introduction

Plant breeding is concerned with the production of improved varieties of plants by the development of superior genotypes. The nature of the improvements that are sought will not in general depend on genetical considerations, though in some circumstances it may be influenced by them. Rather it will depend on such things as the requirements, preferences or even idiosyncrasies of the consumer, on economic considerations and on the agronomic needs and practices of the grower. Alternative ways of meeting these requirements must be considered alongside the approach through plant breeding. All these must be taken into account by the plant breeder in setting his targets, which must pay due regard to feasibility and also have a sufficient element of prediction, or prophecy, in them to allow for the necessary lapse of time between setting up the breeding programme and having the finished variety ready for the market.

Having set his targets, the breeder must ask how he can best proceed towards achieving them; which features of the plant's physiology offer him the best prospect of raising its production under the various circumstances in which it is likely to be grown; how he can secure the best distribution of assimilates to the economically important part of the plant as opposed to parts not so economically utilizable; how losses arising from the depredation of pests and diseases can be minimized, and so on. The targets must, in fact, be expressed in terms of more detailed definable characters that the breeder will seek to adjust, and which can be followed relatively easily in single individuals, or small families, as a basis for effective comparison and hence meaningful selection. In this the breeder must seek the cooperation of physiologist and pathologist, and also, because of changing practices of agriculture, of agronomist and agricultural engineer.

The part that the physiologist can play, and is indeed already coming to play, in plant breeding emerges clearly from the papers presented by Professor Cooper and Mr Bingham. The latter also refers to the problems of breeding for resistance to pests and diseases, and particularly to the transience so frequently met in the disease resistance with which the breeder endows his varieties. The ability to recognize durable disease resistance, other than by the belated test of its survival in practice, is surely one of the greatest needs of plant breeding today, and one that can be met only with the cooperation of pathologists. Engineering problems raised, for example, by the mechanical harvesting of root and fruit crops are not mentioned; but here, too, breeder and engineer must work together to overcome them.

Plant breeding and genetics

The technology of plant breeding thus transcends genetics when setting its targets, assessing their feasibility, and defining the characters with which the breeder must concern himself. At the same time, since the breeder is seeking to achieve his ends by adjustment of the genotype, his approach must be through applied genetics, and any advance in genetical science will thus be of interest to him as prospectively offering means of refining or extending the methodology on which he can draw for his purposes. The question has, however, frequently been asked, and is indeed still asked on occasion, 'What *has* genetics contributed to plant breeding?' To begin answering this question we must go back to the turn of this century.

Although hybridization was not unknown to the breeders of production crops in the nineteenth century, it appears to have played little part in their work. Their emphasis was on the method of selection, and even in the 1890s the dramatic improvements made by Hjälmar Nilsson in the wheat, barley and oats of Sweden were achieved through single-ear selections from whatever variants presented themselves in the materials available to him. There seems to have been little appreciation of hybridization as a means of deliberately bringing together in one variety desirable features from two or more parental lines. All this had changed by 1906 when, as Dr G. D. H. Bell, F.R.S. has kindly drawn to my attention, R. H. Biffen wrote, 'As recently as six years ago the chances of . . . (obtaining the type of barley we desire by cross-breeding) . . . were so small that the attempt was barely worth the making. Since then thanks to the discoveries of Gregor Mendel . . . we can with reasonable certainty of obtaining the results we require begin our labours. The whole aspect of plant-breeding has been changed and where formerly all was chaos we can now perceive order and the action of definite laws. A few years ago cross-breeding was a gamble of the wildest description, now we can calculate the result of combining any two given parents with well-nigh mathematical accuracy.' He also wrote, 'I can foresee the time coming when we shall be able to unite in one variety the best quality, the best cropping power, the best straw and so on, which we can find in all the numerous varieties now in existence.' Such was the impact of Mendel's findings when, with the principles of segregation and recombination, they gave plant breeders a firm foundation for their practice. Techniques of selection were still important, but now the breeder could with confidence seek to construct the superior genotype for his selection to pick out. And as such, this development can fairly be regarded as the beginning of the manipulation of genetic systems in plant breeding.

This deliberate construction of desirable genotypes is illustrated in a different way by the revolutionary development of hybrid corn, which came along some years later in the U.S.A. Can we doubt that this, too, with its successful combination of controlled uniformity and the heterosis characteristic of hybrids in this crop, stemmed from an appreciation of Mendel's principles, fortified perhaps by Johannsen's experimental demonstration of pure lines and their properties? Hybridization to bring together, after segregation and recombination, desirable features from several parental lines into a single variety or line, and hybridization of chosen inbred lines to give F_1s for immediate use in production are the foundation of our present methods of plant breeding, which Professor Williams describes and discusses.

While Mendel's principles were, of course, his most important legacy to us, he also gave a precise prescription for bringing together (or separating) genes whose individual effects on the phenotype could be recognized without ambiguity. This led to high hopes, no doubt made all

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the higher by findings such as that of Biffen when he showed in 1907 that rust resistance in wheat could segregate from susceptibility in the manner of a single gene difference – hopes that plant breeding would be reduced to little more than an exercise in Mendelian manipulation. Disappointment was thus all the greater when these hopes foundered on the rock of quantitative variation, which appeared not be to capable of Mendelian analysis or even at first of Mendelian interpretation: most of the variation that the plant breeder has to deal with is of this kind. It has taken a long time to show unequivocally that heritable quantitative variation is as dependent on nuclear genes as the simpler Mendelian differences are; to erect on this foundation a methodology of biometrical genetics that enables us to analyse the variation into its non-heritable and various heritable components; and to set out their implications for the work of the plant-breeder. Professor Jinks, however, tells us of the power that biometrical genetics has now come to achieve in tracing the causation of such basic phenomena as heterosis, and in predicting the outcome of breeding programmes. It can thus help the breeder not only to interpret results that he obtains, but also to plan the strategies of his breeding programmes; and it is all the more useful for these purposes in that it deals with the totality of variation in any character with which he is concerned.

Chromosomes, genomes and recombination

Biometrical genetics does not depend for its analytical capacity on assigning to individual chromosomes the genes that contribute to quantitative variation or on assessing the contributions that individual chromosomes make to the totality of the variation observed: indeed, the methodology is of general use and is applicable to all species just because of its capacity for dealing with the totality of variation in a population or a cross, without any such initial partitioning. At the same time, the value of being able to assess the contributions of individual chromosomes can be of great value in facilitating not only the analysis, but even more the synthesis, of desirable combinations of genes, as has long been apparent from studies with *Drosophila*. Facilities for assaying the individual chromosomes were unique to *Drosophila* until relatively recently. Now, however, they are available also in wheat, as Dr Law and Professor Driscoll emphasize to us. We can ascertain how the variation of a character in wheat is distributed between chromosomes and between genotypes; the chromosomes can be deliberately put together to give superior genotypes; valuable blocks of genes can be moved from one variety to another; and special types, such as male-steriles, can be made where they are needed. The technique of manipulating the chromosomes is not the same as in *Drosophila* in that it depends on following and managing them by cytological rather than genetical means; but it is equally effective. At present its use is confined in the main to wheat and its relatives, though, as Dr Thomas tells us, it is becoming increasingly possible in oats, with correspondingly valuable results. Chromosome manipulation, as we see it in these cereals, is indeed a powerful tool for the breeder wherever it can be employed, though its application will necessarily be restricted to species that have chromosomes capable of being followed cytologically as individuals, and whose constitution is such that they can tolerate any genic unbalance consequent on aneuploidy.

The chromosome analysis of wheat has revealed the special action of a gene, or genes, borne on chromosome 5B, in restricting pairing and hence recombination to homologues: in the absence of this chromosome, or when its effect is suppressed, pairing and recombination takes place between homoeologues as well as between homologues. Dr Riley (with Dr Law and Dr Chapman) describes how this can be used to incorporate into bread wheat, carrying the normal 42 chromosomes, desirable characters, such as rust resistance and baking quality, from *Aegilops* spp. In emphasizing the importance of recombination for the management of the variation upon which selection is practised, he notes that at times the breeder's requirements make it necessary to aim for reduction of recombination but at other times for its increase, and he points out that, as a result of the cytogeneticist's work, the breeder now has the means of intervening, to his own benefit, in the genetic control of recombination.

Turning to a different type of manipulation, of chromosome sets rather than of individual chromosomes, the ability to provide haploid individuals when wanted can be a powerful aid to the assessment of quantitative variation, and also to the identification and fixation of desirable recombinants. Haploidy can arise, and indeed be induced, in a variety of ways that are listed and discussed by Dr Hermsen (with Dr Ramanna). It may arise after polyembryony or as a result of pseudogamy. It can be induced in barley by crossing with other species of *Hordeum*, the chromosomes of the alien species being eliminated during embryo formation, and it can be induced elsewhere by pollen culture. No matter how they are obtained, the haploids can easily be rediploidized whenever this is desired and they will then give fully homozygous lines for use whether as varieties in their own right or as 'inbred' parents for intercrossing in a hybrid breeding programme. Nor is the value of the technique restricted to its use in diploid species: it can equally be used for at least the partial analysis, and subsequent resynthesis, of polyploids like the potato.

Polyploids

Chromosome studies early showed that many of our crop plants, including some of the most important, are polyploids, most commonly allopolyploids (or amphidiploids if the term is preferred), though the grass *Dactylis glomerata* may be an autotetraploid. With the discovery of the capacity of colchicine for doubling the number of chromosomes, it became possible to make polyploids at will, whether autopolyploids by the direct doubling of existing species or allopolyploids by doubling hybrids between species capable of being crossed. High hopes were entertained of induced polyploids, especially allopolyploids, as a means of plant improvement, but only limited success has been obtained so far. Autopolyploidy has proved of some interest with ornamental plants in which the value of the increased size that it often engenders can more than offset the reduction in fertility that also results. This could make it of some value in forage crops, too.

In allopolyploidy, the combination of different genomes is likely to bring about greater adaptability rather than, of itself, to produce increase in yield or quality, as Dr Breese (with Dr Lewis and Dr Evans) points out. Furthermore, bringing together sets of genes that have not been adjusted by natural selection to work harmoniously with one another may well result in genic unbalance and hence a certain amount of trouble, which must be removed by selective breeding. For these various reasons a newly synthesized polyploid grain crop is likely to require considerable attention from the breeder before it becomes commercially utilizable, and experience with triticale confirms this expectation. Herbage grasses, as Dr Breese further observes, appear to offer the greatest prospective rewards from hybrid polyploids, for flexibility is a prime requirement in swards while the harvest depends on the vegetative growth rather than seed production (though, of course, enough seed must be obtainable for the commercial

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propagation of the polyploid). Dr Breese and his collaborators have in fact produced agronomically successful tetraploid hybrids between the two diploid grass species *Lolium perenne* and *L. multiflorum*, and though not autotetraploid these hybrids are not full allopolyploids either: some pairing occurs between homoeologues, although it is preferentially between homologues. This preference in pairing is subject to genic control and may be enhanced by selection, so raising the possibility of moving towards the genetic stability of a true allopolyploid. B chromosomes are also known to increase preferential pairing in other tetraploid hybrids between *Lolium* species, so offering a further means of adjusting them to the breeder's requirements.

Other allopolyploids between species of *Lolium* and *Festuca* may well prove to have more value for the transfer of desirable genes and gene complexes from one parent species to the other than as production crops in their own right. It is clear, however, that artificial polyploids have at last come to stay, at least in grass breeding, and basic problems of gene action, particularly in relation to quantitative variation, would now well repay study in them.

New variants

Heritable variation is the plant breeder's raw material, and if it is inadequate for his purposes and is not readily available in other ways, he may seek to induce it by means of radiation or chemical mutagens. Professor Nilan reviews the use of induced changes of both genes and chromosomes in crop plant breeding. There can be no doubt that such gene mutations can be of great value in the investigation of the biochemistry and physiology of important characters and of the genetic structure underlying their manifestion. Neither can there be any doubt of the value, or prospective value, of induced gene mutation to the plant breeder under special circumstances. They have been the basis of the successful development of increasingly high levels of antibiotic production by the fungi on which the antibiotic industry is based: indeed with imperfect fungi their use is unavoidable. They also offer a prospectively valuable approach to, for example, the production of new forms of the standard varieties of top fruit, such as the apple, differing from the parent form in, say, having a size or structure of tree that facilitates harvesting or in being self-fertile, but yielding fruit that is still of the same kind as the parent and so avoids the hazard inevitably met by a grower who seeks to introduce, necessarily at considerable expense, a recognizably new variety in a field where consumer preference is both strong and conservative. As a general adjunct to the breeding of regular crop plants, however, its value is less clear to me. Indeed, mutations of major effect appear seldom to be of a kind that the breeder would regard as prospective raw material not obtainable more usefully in other ways. Furthermore, if we may judge by findings from *Drosophila*, induced genic mutations are not likely to be a very useful means of augmenting the selectable quantitative variation upon which depends so much of the breeder's progress.

Mutagens also, of course, bring about structurally changed chromosomes. As Professor Nilan points out, these can be of great value in the genetic analysis of crop plants and in chromosome manipulation, as for example in Professor Driscoll's production of male-steriles in wheat.

During the course of this symposium, reference has been made to a further, and prospectively most valuable, technique for manipulating genetic variation by the use of ionizing radiation. With his collaborators, Professor Jinks (who, however, does not himself describe the technique) has been pollinating flowers of *Nicotiana rustica* with pollen that has been subjected to a near-

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lethal dose of radiation, and which came from a different line of this species. When germinated, the seed obtained in small quantities from these pollinations produced plants just like their mothers in nearly all respects, but showing evidence of having derived single marker genes from their fathers (Virk *et al.* 1977). There was evidence too, in other cases, of genes affecting quantitative variation having been transmitted in the same way. Given that these paternal genes become stably incorporated into the genotype of the progeny plants, as results obtained by Pandey (1975, 1978) suggest can happen, this technique would offer a way of transferring desirable genes from one line to another with little disturbance of the rest of the genotype and much more quickly than can be achieved by the customary repeated backcrossing. Further results from the use of irradiated pollen from a different species suggest that this approach may also offer the very valuable possibility of transferring desirable genes in the same way between species that do not hybridize effectively by normal pollination.

The value of mutagens lies in their ability to increase the frequency of changes in the nuclear materials. It has, however, long been known that abnormalities of nuclear behaviour can arise as a result of both gene mutation and general upset of genic balance (see Darlington 1932, 1939; Rees 1961). The regular behaviour of nucleus and chromosomes that we normally see must thus have been achieved and maintained by adjustment of the genotype through natural selection. Even so, upsets do occasionally occur, as for example when normally diploid plants produce a tetraploid sector, which is, however, usually contained and limited by competition from surrounding cells of the diploid constitution. In discussing the significance for the plant breeder of spontaneous nuclear upsets, Dr Bennett points to both the nuisance value of their consequences for the maintenance of standards of genetical purity in commercial crops, and the possibilities that they open up for the breeder. He cites three examples of instability that can have direct consequences for the breeder. One is the result of a gene mutation, *tri*, in barley, and the others are the outcome of the genic unbalance consequent on wide outcrossing, namely selective chromosome elimination in *Hordeum* species crosses and the case of the amphidiploid triticale. It is of special interest that, as Dr Bennett tells us, improvement in the quality of seed in triticale has been achieved by a cytogenetic approach.

Dr Durrant is concerned with genic rather than chromosomal instability. In the light of the known instabilities of major genes in *Antirrhinum* and other plants he discusses the possibility of similar instabilities in the genes mediating quantitative variation, where their consequences would be virtually impossible to distinguish from variation arising from other more readily recognizable causes such as segregation or the impact of the environment. He considers whether the remarkable phenomenon of ‘conditioning’, which he discovered in flax and which has since been observed in *Nicotiana rustica*, could be the expression of environmentally induced instabilities of this kind. We still have not come to a full understanding of this tantalizing phenomenon, though our information about it continues to increase. It is currently known only in these two species of plant, but a fuller understanding might show how it could be traced elsewhere and possibly put to good use in breeding production crops.

Tissues, cells and protoplasts

The need for sexual propagation by seed, or even clonal propagation by special organs such as tubers, necessarily imposes constraints on the isolation and exploitation of new variants, especially somatic variants, as well as on their induction. It limits, too, even the range over

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which wide hybridization can be attempted. To remove, or at least ease, these constraints could greatly facilitate the manipulation of genetic systems, and it could also be of help to the plant breeder in other ways.

Professor Davies tells us how this is now being attempted by cell and tissue culture. In doing so he cites a dozen different ways in which the plant breeder can be served by these techniques, ranging from the storage and rapid multiplication of valuable genotypes in a disease-free condition, to the isolation of somatic variants that could otherwise be lost through cell competition and including, for example, the investigation of conditioning. He quotes examples where this approach has already met with success, and we may note also the remarkable series of variants, many of prospective practical value, obtained by Shepard *et al.* (1980) from the cloning of isolated protoplasts of the potato Russet Burbank, whose use in normal breeding programmes is limited by its sterility.

There are, of course, limitations to this approach. Some are analytical in that, of itself, the isolation and propagation of variants by cell and tissue culture, leading to the regeneration of whole variant plants, cannot regularly tell us the causal nature of the variation, whether nuclear or extranuclear, and whether capable or incapable of transmission through seed. This difficulty could, however, generally be overcome by the direct test of breeding from the regenerated plants. Other limitations are technical, including the need for being able to regenerate complete plants from the tissues or cells. At present this can be done for only a restricted range of species, but the problems will no doubt be overcome in time by experimental persistence and growing physiological know-how in species that are of importance to the breeder.

Professor Cocking confines his discussion to the prospective value of protoplasts in genetic manipulation. While noting the successful use of protoplasts for recovering variants in the potato (already mentioned above), he points especially to two other potential uses of protoplasts. One is in the production of somatic hybrids obtained by fusion of protoplasts from the two parental lines, so freeing hybridization from constraints imposed by the sexual system. Indeed, somatic hybrids have already been made between species that will not cross sexually, as well as between others that will. The second potential use that he stresses is in the transfer of genes between species, whether by a technique parallel to that with the use of heavily irradiated pollen or by transformation by using *Agrobacterium* plasmids. It is not yet known whether hybrid polyploids made somatically would have properties different from those of their sexually produced counterparts, but there is no compelling reason *a priori* why they should. Transformation by infection with suitably engineered plasmids, carrying for example the *nif* system of genes, may, however, have wide possibilities of great importance.

The use of protoplasts, like that of cells, is, of course, constrained by the need to regenerate whole plants from them, but the recent regeneration, achieved with the use of young immature leaf material in *Sorghum* (Wernicke & Brettell 1980) encourages the belief that it will soon also be achieved in our cereals.

The molecular approach

The rise of molecular genetics has given us a growing understanding of DNA in all its variety of forms, as well as a growing range of techniques for both analysing and reorganizing it. In doing so it has opened new and wider, even if more distant, prospects for the manipulation of the genetic materials in the amelioration of our plants for the purposes of production. Nor are these advances confined to the DNA of the chromosomes: that of chloroplasts and mitochondria come into the picture, too, with a further prospective widening of the understanding of the materials that the plant breeder, implicitly or explicitly, is using; of the problems that he may have to face in using them; and of the ways in which he may hope to exploit them. Three of the papers reflect this molecular approach, each in its own way.

Professor Rees (with Dr Narayan) is concerned with what he aptly describes as the astonishing variation in the amounts of chromosome DNA in species of higher plants. Much of the variation is due to amplification of sequences: the proportion of repetitive DNA rises disproportionately as the total increases, in some cases to as much as 70%. The function of this repetitive DNA is unclear. It does not affect the viability of, for example, gametes; but it does affect the distribution of chiasmata in the chromosomes and hence the pattern of recombination. It carries few genes coding for proteins, but it appears to contribute to quantitative variation. Can its manipulation be put to use in plant breeding?

Dr Flavell has been cloning DNA of various kinds and various origins. Such cloning, as he points out, makes possible the physical mapping of chromosome material, whether from the nucleus or elsewhere, irrespective of whether or not it is the seat of variation detectable by normal genetic methods. It has already permitted a molecular analysis of the telomeric heterochromatin in rye, whose adjustment, as we have already seen, produces a reduction in the incidence of shrivelled grain in triticale. And the cloning of specific sequences from the mitochondrial DNA of maize has provided means of recognizing cytoplasms associated with male sterility even where the presence of restorer genes prevents manifestation of the sterility. One of the cytoplasms now capable of recognition in this way has been widely used in hybrid corn breeding and has, as a result, led to a disastrously widespread loss of resistance to Southern corn blight, a fungal disease. Dr Flavell finally emphasizes the possibilities that cloning opens up for the modification of plant genotypes by the insertion of specific genes into them.

In a *tour de force* of coverage, clarity and realism, Professor Postgate (with Dr Cannon) turns to the biological fixation of nitrogen in relation to green plants. It might be raised by breeding more effective strains of *Rhizobium*, and other commensal microorganisms might have *nif* genes inserted into them. But the really fundamental approach is through the introduction of the *nif* complex, which includes some 17 genes, into the green plants themselves and securing their expression there. This raises formidable problems not only in relation to the physiology and action of the genes themselves, but also of securing their entry into the higher plant cells by means of a vector that would either maintain a satisfactory numerical stability in the somatic cells of the higher plant and also pass through seed, or preferably would bring about the incorporation of the *nif* complex into the plant's chromosomes. Initial entry would presumably be into protoplasts and the need for regenerating whole plants from them would again arise. Professor Postgate does not disguise the magnitude of these problems and he estimates that it will take a decade or more before we can know whether such engineering is possible. At present, however, he sees no reason to doubt its feasibility.

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Conclusion

After the establishment of Mendel's basic principles the notion of deliberately producing superior genotypes by bringing together desirable genes through hybridization, segregation and recombination, took on vigorous life in the minds of plant breeders. Such has been its success and so widespread is it that it is now regarded as conventional plant breeding. It has had its difficulties, notably in the utilization and management of quantitative variation, but this is now yielding to new and appropriate methods of genetic analysis.

Later developments of genetical science are coming to have their impact too. The rise of cytogenetics, in which Professor Darlington, who introduces this symposium, played such a fundamental part, has led to chromosome manipulation, and this is already achieving its successes. The induction of changes in genes and chromosomes by the use of ionizing radiations and other mutagenic agencies is offering us a variety of means for producing genetic changes where we need them, for facilitating chromosome manipulation and for transferring particularly desirable genes expeditiously from one form, or even species, into another. Now the techniques of tissue, cell and protoplast culture, and, still more fundamentally, those of molecular genetics, are opening up yet further prospects and promising yet further rewards. Beyond even these we can now begin to discern, however dimly as yet, the possibility of deliberately manufacturing genes themselves, with properties that we can specify in advance.

Some new techniques will fall by the wayside, and all will raise their problems which must be overcome. We can, however, see a progression of methodologies from the conventional, which are now accepted as normal practice, through the feasible, which are beginning to record their successes, to the notional, where the problems are still awaiting solution. But can we doubt that the phenominal and accelerating progress of genetical science, supported by that in cognate disciplines, will turn the notional of today into the feasible of tomorrow and the conventional of next week or the week after?

Some of the advances, achieved and prospective, will be of general application; some, however, will be restricted to particular kinds of crop, to particular kinds of genotype or even to particular species, according to the needs that these reveal or the opportunities that they offer. Some advances will depend on developments in physiology or pathology as much as genetics. But all will, in their own ways and their own times, come to open up new prospects for the plant breeder. At the same time, such is the complexity of the plant breeder's work, of the different skills that he must practice and of the judgements that he must make, that while genetics and geneticists may serve him, and serve him well, they will not replace him.

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