Some New Techniques for Sugarbeet Improvement*

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From a host of recent reports and recommendations (e.g., 1, 2) has come the expectation that contemporary analytic biology will contribute to the goals and methods of agricultural research. Can molecular biology be utilized for the solution of problems in agricultural plant biology? Will a correlation of in vitro events with the responses of crop plants in the field allow a better understanding (and perhaps more importantly, allow manipulation) of the biological processes underlying crop productivity? There are several possible responses to these questions, all of which have been expressed in one form or another during the numerous recent debates concerning the potential of increasing agricultural productivity. The first response points out that our current levels of crop productivity were achieved in the absence of a direct knowledge of molecular mechanisms, and that there is no reason to believe this knowledge would enhance productivity. A second response, the direct opposite of the first, asserts that only by a complete molecular analysis of the processes underlying crop productivity is there any hope of manipulating the components of yield in a rational way. A third and more realistic response suggests that a molecular analysis will be of importance in manipulating some biological processes but will not be a panacea for all the problems of agricultural biology.

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Plant breeding is an ancient science. The origins of our current crop species are buried in prehistory; all evidence indicates that most crop species were domesticated during the Stone Age. Crop species arose from native wild species that underwent natural hybridization resulting in increased genetic variability and subsequent selection for desirable phenotypes by prehistoric peoples. Methods of reaping and sowing in the field, or methods for storage or preparation, can be effective selection screens for plants just as growth on a Petri plate is for a bacterial colony. The early plant breeders searched for, recovered, and propagated genetic variants or recombinants which displayed desirable traits under certain environmental conditions. The transformation of wild species into crop plants was accomplished in the absence of modern science, or of any knowledge of Mendelian genetics.

Contemporary plant breeders employ essentially the same strategy with great success. Their approach involves the production of populations with a broad genetic base followed by selection at the whole plant level for recombinants with desirable alterations. Genetic manipulation is practiced without knowing the biochemical basis of the separate components which comprise the character being modified. Selection for traits such as final yield is practiced at the endpoint of the complex biological processes which produce a whole plant. Mendelism and a knowledge of genetic transmission provide a conceptual basis for what is occurring during the breeder's genetic manipulations (3).

In most current breeding programs, the availability of genetic variability is not the limiting factor in crop and variety improvement. There is a wide range of genetic diversity in the surviving natural populations of most crop species. The focus of breeding efforts is centered on selecting the desirable recombinant types that emerge from any particular cross or segregation population. Currently, the assays of agronomic or horticultural utility and the subsequent selections are based on observations of whole plant phenotypes. Consequently, only major alterations can be recognized. These alterations appear as statistically significant changes in characteristics of bulk populations. Assaying at the endpoint of a
number of complex biochemical, physiological, and developmental processes hides many potentially useful recombinants in the complexity of the buffered processes producing whole plant (4).

The complexity of plant biology and of productivity is expressed in the genetics of agriculturally important traits. The majority of these traits appear to be controlled by "polygenes," and their transmission is analyzed by quantitative methods. The quantitative inheritance of these traits is a reflection of the complex biological processes which underly their expression and of the lack of well defined genetic variants with which to analyze them. Quantitative inheritance is a phenomenon involving naturally occurring genetic variability and complex biological end products. There is no reason to expect that mutants affecting these processes could not be produced once their individual components are identified, nor that the genetics and biochemistry of such traits would be any different from that found in other organisms (e.g., metabolic pathways). For the time being, however, the plant breeder has little choice but to use the phenotype of the endpoint as the basis for selection. Significant progress could be made in the improvement of breeding techniques if it was possible to establish reliable physiological or biochemical assays at critical points in a number of the component processes of agronomic traits. Examples of such processes are: nitrogen metabolism, photosynthesis, water relations, mineral nutrition, and tolerance to environmental stress. With these critical processes individually analyzed and assayed, genotypes demonstrating optimal performance at different steps in a process could be combined to produce a new, highly productive cultivar.

Recent advances in molecular biology have provided methods of genetic manipulations which should be applicable to the improvement of plant species. This is certainly an exciting prospect. Despite the rapid expansion of our knowledge of basic genetic and biochemical mechanisms in lower organisms, this knowledge has had no direct impact on plant improvement. This lack of impact may be ascribed in large part to conceptual and experimental differences between the disciplines of molecular biology and plant breeding.
Molecular biology is comprised of two basic elements: the reductionistic world-view of basic science and a powerful set of analytical experimental tools. One example of this approach was the use of defined genetic variants combined with precise biochemical methods to elucidate the mechanisms that regulate metabolic pathways in a variety of organisms. In contrast, plant improvement as currently practiced has, of necessity, a more holistic approach. Plant breeders have to operate within difficult constraints. They have little choice in either their experimental materials or the problems which confront them. No strong correlations have been established between yield and any of the individual physiological or biochemical processes that contribute to the final product. The experimental and technological requirements of plant breeding and the constraints of the plant system are different from those imposed by molecular biology. The question is, can the novel methods of "genetic engineering" defined in microbial systems really be applied to plant improvement?

There are several approaches to extending the techniques of molecular biology from microbial investigation to application for crop improvement: one of these involves cellular manipulations. Cellular manipulations hold the potential for developing an experimental system for crop species suitable for more refined analytical techniques. Using single somatic cells as experimental organisms, it is possible to achieve mutant production, analysis, and hybridizations not possible using whole plants. Such techniques may permit important cellular processes to be characterized to the extent that useful, directed modification is possible.

Work focused upon the manipulation of sugarbeet cells cultured in vitro has not been extensive. There is the current realization that such work could be productive, and that sugarbeets are an attractive species for the development of cell culture techniques. It is now possible to initiate and maintain callus cultures from various parts of the sugarbeet plant (5). From these callus cultures, it is possible to produce suspension cultures of sugarbeet cells proliferating in a liquid medium (4). Regeneration of entire sugarbeet plants from callus cultures has proved to be a
difficult goal, but it has been observed recently (5, 8). Regeneration of whole plants from single cells has not been reported.

Although many tools of the molecular biologist are now available to the plant geneticist, some limitations prevent their application to breeding problems, particularly to sugarbeet. The first problem with these approaches is a technical one. Regeneration of whole plants from single cells is essential for application of the technology of \textit{in vitro} genetic manipulation to higher plants. However, this step has only recently been accomplished with several major food crops, and it is not yet possible with sugarbeet. The second problem arises from the real needs of the plant breeder. In most instances, the availability of genetic variability is not the limiting factor in crop improvement; the ability to recognize and recover useful recombinants sets the limit. Hence the production of genetic variability via cellular mutation, or hybridization, provides no uniquely useful tool at present. The third problem results from the development biology of agronomic and horticultural characters. Many agronomic traits are tissue-specific; their expression is found in only one or a few tissue within the plant and is often not found in cells cultured \textit{in vitro}. If a particular trait is not expressed in culture, there is no reason to expect that the trait can be altered and screened for \textit{via in vitro} methods. The fourth problem involves the genetics of agricultural traits. Mutant selection systems and DNA manipulations allow modification of single gene traits. Most agronomic and horticultural traits, as they are now defined, are polygenic in inheritance. Small additive, stepwise modifications would be difficult to recognize. Currently, genetic modification of crop plants, using cellular manipulations, should prove appropriate in cases where the alteration involves single-gene traits which are not tissue-specific, and for which there are good selective techniques. These are indeed rare instances. The technology involved in these approaches will almost certainly be improved to overcome the limitations discussed above. However, at present, single-gene traits which are not tissue-specific are rare, as are appropriate selective systems. Possible examples of such traits would include disease resistance, or tolerance to ion toxicity, but the range is limited.
It would appear that molecular biology is not yet directly relevant to crop improvement. The difficulty is that current efforts have attempted to transfer the experimental results directly (i.e., defined genetic manipulation) without also extending the reductionistic approach of molecular biology. The immediate need is not to find new ways to generate genetic variability but to find new ways to screen critically the variability already provided by nature and to identify the biochemical, physiological, and developmental components of traits which determine plant productivity. Once individual components and the rate limiting steps of important traits are identified, designing methods of selection for altered traits are possible.

Effective genetic manipulation of traits affecting plant productivity requires identification of the relevant metabolic processes and specific rate-limiting steps. Many such traits including drought tolerance, total yield, time of maturity and temperature tolerance are complex quantitative traits under the control of multiple genes (polygenes). The final phenotype is separated from the basic biochemical steps, the units of selection, by several levels of biological organization and environment-genotype interactions. Unfortunately, the definition of polygene and statistical methods for its analysis are not compatible with the analytical approaches of molecular genetics. Likewise, biochemical approaches have been frustrated by the complexity of quantitative traits, even when they include the analysis of divergent genotypes. Despite considerable effort, no strong correlation has yet been found between final yield and the productivity of any distinct biochemical pathway (1, 4). The problem is that any metabolic reaction can affect the final productivity in a given environment. The question is, which reactions or steps actually do affect productivity?

Whatever the eventual role of molecular biology in plant production, it is essential to begin to approach the classical holistic descriptions, or plant productivity, with reductionistic and analytical tools. In this process, a number of traditionally disparate biological disciplines can be brought to bear on the unique and complex problems of agricultural plant biology.
LITERATURE CITED


