APPLICATION OF MOLECULAR BREEDING TO CROP IMPROVEMENT

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INTRODUCTION

Since the latter part of the 1970s, scientists have proposed that genetic engineering, based on the new recombinant DNA technology, would have a profound impact on plant breeding. Indeed, even more today than a decade ago, technological advances in the field of plant molecular biology make it possible to consider seriously the role molecular genetic techniques will play in commercial breeding programs. This paper presents a brief review of plant breeding strategies and discusses the feasibility of incorporating recombinant DNA technology into conventional plant breeding programs.

BREEDING STRATEGIES

Classical Breeding. Breeding strategies differ for ornamental plants that are seed propagated and ones that are vegetatively propagated. For seed-propagated species the genetically improved cultivar must be either a true breeding inbred or a hybrid cultivar comprised of inbred parents. Breeding programs of these types typically include years of backcrossing and/or selfing to obtain true breeding parents. Hybrid seed production usually is more advantageous than inbred seed as the hybrid is protected from further sexual propagation and generally has more vigor. However, the production of hybrid seed cultivars can be labor intensive and thus costly.

Vegetative propagation is also labor intensive and costly; however, the high cash value of the crops often insures economic feasibility for this mode of propagation. Whereas seed propagation requires true breeding parents, this is not necessary for vegetatively propagated plants. Vegetative propagation leads to a perpetuation of the same genotype with great precision, and an indefinitely large number of genetically identical plants can be obtained irrespective of the degree of heterozygosity. This manner of breeding makes it possible to screen many thousands of highly heterozygous plants in order to find the single most desirable phenotype from a cross and evaluate it in performance trials. A limitation of this manner of breeding is that, due to the high degree of heterozygosity, it is difficult or impossible to produce isogenic lines.

Classical breeding methods have been very successful in providing the market with a great variety of ornamental species with beneficial attributes. However, in spite of the achievements, classical breeding has limitations. Foremost among these is that the breeder is unable to alter traits in a directed manner; genotypes are seriously disrupted by crossing. Breeders cannot manipulate genes independently; and, if linkage is tight, the breeder may never be able to backcross in a certain cultivar to achieve a particular phenotype.

Another limitation confronting the breeder is the limited number of traits within the gene pool of any species. For example, no plant species possesses the genetic capacity for producing the full spectrum of flower colors nor resistance against all diseases. There are some traits which the breeder will never be able to manipulate through a sexual cross.

Mutation Breeding. Mutation breeding has been used as an approach to introduce new traits by changing a single gene or a few genes (1, 8). The spontaneous mutation rate in nature is very low, on the order of 10^{-6} to 10^{-7}, for any particular gene. Although in some cultivars the spontaneous mutation frequency can be high enough to find sports or naturally occurring mutations. Chemical and physical techniques are used to increase the mutational frequency to a level that permits detection in a manageable number of populations. Chemical mutagenesis has had limited success and is most useful with seed-propagated species rather than vegetatively propagated species. Ionizing radiation, Xrays, and gamma rays have been used successfully for mutation breeding in vegetatively propagated crops. The majority of commercially successful introductions in the ornamental industry have been flower color mutants and form and size mutants.

Despite the successes of mutation breeding, the method is not always reliable nor predictable. In many instances the high frequency of deleterious mutations, the presence of chimeras and the widespread occurrence of gross chromosomal changes has made the establishment of stable breeding lines difficult and time consuming. Somaclonal variation has been suggested as an alternative to mutagen use (3). Plants regenerated from somatic cells via tissue culture can express genetic variability, either transiently (epigenetically) or in a stable fashion. If the mutations are stable and desirable, they could be used in a breeding program.

Molecular Breeding. Molecular breeding, the application of recombinant DNA technology to conventional breeding, is seen as the means to introduce more precision into plant breeding programs. Recombinant DNA technology permits direct gene transfer and enables the breeder to introduce single genetic changes into superior cultivars and to preserve intact the elite genotype. For seed-propagated crops this capability may possibly decrease the number of years, which is typically 10 to 15 years, needed to produce superior cultivars. This molecular approach would eliminate linkage problems and allow the breeder to cross sexual barriers in search of new genetic variation.

Technology advancements make direct gene introductions feasible, but there are significant limitations to molecular breeding, one of which is the expense of the technology. While significant progress has been made in gene isolation and manipulation, there is a paucity of cloned and characterized genes that would have a direct impact on such major agricultural issues as disease and pest resistance, stress resistance, control of maturity and flowering, control of morphological traits, and yield. With classical breeding techniques it is possible to manipulate a large number of genes (polygenic traits) simultaneously. This is especially important when breeding for inheritance of genes that control quantitative traits such as yield, vigor, and maturity. With molecular breeding it is an impossible task to manipulate multiple genes; current technology constraints limit molecular breeding to single gene changes.

The number of economically important crop species that can be efficiently transformed and regenerated is limited at this time. However, the transformation and regeneration of recalcitrant plant species will most likely be accomplished in the near future by one or more of several recently developed technologies such as microprojectiles (6). The recent report of *Agrobacterium tumefaciens*-mediated transformation and regeneration of chrysanthemum (9) demonstrates that molecular breeding may be practical in the future breeding of important ornamental crops

Traditional plant breeding has already been impacted by molecular techniques with the advent of restriction fragment length polymorphism (RFLP) analysis. The development of RFLPs offers a tremendous potential to construct detailed linkage maps, to identify quantitative trait loci (QTL), to analyze genome organization, and in cultivar fingerprinting as a means of identifying and protecting proprietary germplasm (4, 7). An application of this technology to floriculture is the construction of an RFLP linkage map in rose (5).

IMPORTANT TRAITS FOR CROP IMPROVEMENT

In general, the most valuable phenotypic traits in ornamental species include resistance to diseases and pests, productivity, plant growth and habit, flowering response, flower color and shape, and postharvest physiology attributes. Resistance to diseases and insect pests is undoubtedly the phenotypic trait that is the most important to the industry. For the ornamental industry the aesthetic quality of the plants is the measure used to gauge yield. Chemical control is becoming increasingly more scrutinized by both regulatory agents and the public. In the future the industry may be limited to a much smaller number of chemicals with reduced effectiveness. The establishment of effective integrated pest management programs and the breeding of resistant cultivars is essential to the industry.

Increased resistance to several plant viruses has been demonstrated by the engineering and expression of viral coat protein in transgenic plants (13). *Bacillus thuringiensis* (BT) toxin expression in transgenic plants is seen as a means to control those insect pests which are affected by the toxins (2). Both of these technologies could be extremely useful in ornamental plants as a means to effect virus and insect control.

Phenotypic traits such a senescence, flower morphology, flowering, and growth habit are controlled in part by plant hormones. The biochemistry and physiology of hormonal responses are not well documented. Among the many hormone biosynthetic genes only 1-aminocyclopropane-1-carboxylic acid (ACC) synthase has been cloned (12). In those plant species in which senescence is controlled by ethylene, it may be possible to extend vase life by engineering ethylene resistance.

Flower color manipulation is the area that molecular breeding in ornamental plants may have an immediate impact. The reason for this is that the biochemistry and genetics of the anthocyanin pathway have been well characterized. In addition several genes in the pathway have been cloned and characterized. Several successful approaches for manipulating flower color in *Petunia* × *hybrida* have been reported (10, 11, 14).

SUMMARY

Since the beginning of this century plant breeding programs have introduced a tremendous amount of genetic diversity into crop species from which superior genotypes have been selected. Molecular breeding is not envisioned as an alternative to conventional breeding, rather, it is perceived as yet another tool for the breeder to create and analyze genetic diversity in a more directed fashion. Clearly there are still technological hurdles that limit the role of molecular techniques in ornamental breeding programs. A decade ago plants could not be transformed routinely, yet the past ten years have witnessed significant technological achievements that give hope that this technology will have a role in future plant breeding programs

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