The Potential Impacts of Apomixis: A Molecular Genetics Approach

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Introduction

Any volume purporting to describe the impacts of plant molecular genetics can currently deal only with anticipated impacts, rather than actual and measurable effects. It will still be years until a substantial change in agricultural practice and economic return ensue from these research efforts. Thus, it is relevant here to speculate on and outline strategies for achieving the best possible outcome from development of a new technology. In this chapter, we will describe some of the avenues that may be productive for development of apomixis as a powerful new technology, and speculate on impacts that could be achieved with proper attention (Bicknell, 1994c; Jefferson, 1992)

What is Apomixis?

Apomixis is the naturally occurring ability of many plants to produce unreduced seeds parthenogenetically-without fertilization of the female gamete. Apomixis exists in hundreds of plant species distributed among many plant taxa, and is accomplished through a wide range of mechanisms (Gustafsson, 1946; Richards, 1986; Asker and Jerling, 1992).

Very few crop species have substantial apomictic characters. Over the last thousands of years today's crop species have been chosen from among the numerous edible or fibrous proto-crops by farmers. The criteria for such selection almost certainly would have involved the ability of the proto-crop to segregate variation-to improve under mass selection-the very property that apomixis in most of its guises prevents. Thus our small collection of modem day crops probably represents a biased population in favor of sexuality.

The Impact of Plant Molecular Genetics
BWS Sobral, Editor

Potential Impacts of Apomixis

When apomixis is generated with a very high degree of flexibility, the impacts on agriculture could be profound in nature and extremely broad in their scope. A few of the changes that could ensue are:

- immediate fixation of heterozygous genotypes, including those made through wide crosses, allowing single plant evaluation and making possibilities for new breeding strategies and methods in sexual and vegetatively propagated crops;
- plant breeding could become readily responsive to microenvironments, cropping conditions, pathogen populations, and markets, stimulating diverse strategies for agroecosystem management and optimization;
- preparation of very large numbers of hybrid cultivars from almost every crop species into which the trait is introduced;
- propagation of hybrid seed directly by the farmer without the need for inbreds or male steriles and without recourse to frequent seed purchases;
- true-seed propagation of traditionally vegetatively-propagated crops, with concommitant elimination or reduction of disease, substantial increases in germplasm flows, and potential expansion of growing regions;
- elimination of anthesis and fertilization-related crop losses (eg. anthesis drought, heat stress, pollinator failures, submergence or pathogenesis), a major cause of reductions in crop yield and reliability;
- substantial increases in yield in some crops due to increased photosynthate availability through elimination of male flowers/flower parts;
- breeding specifically for endosperm performance in locally adapted varieties without compromising agronomic traits in nonautomous apomicts;

This list of possible impacts is so striking, and so comprehensive that apomixis, when developed to achieve the goals on the list, may well be the most important target for concerted international agricultural research.

Molecular Biology as an Approach to *De Novo* Development of Apomixis

The introduction of apomixis into a target species can be achieved by two possible mechanisms, introgression from an apomictic relative, or transgenesis of an engineered genetic construct. Traditional approaches for developing apomictic crops involve the introgression of apomictic traits or tendencies from wild or related material into crop species. Results have, in general, been disappointing and limited in their potential. Existing apomictic tendencies, available in the gene pools of crop relatives, are neither farmer nor breeder-controllable in a useful manner, are often inefficient (with limited penetrance), are frequently associated with pseudogamy, requiring pollination, and of questionable generality. What is the likelihood then, of finding a truly useful mode of apomixis in a wild relative of a crop?

Unlike introgression, molecular biology offers the potential to develop one or more generic mechanisms for apomixis, crafted to allow field-level control of the trait which is then applied to numerous species. In a more fundamental sense, molecular biology may also provide an avenue for elucidating the underlying biology of the phenomenon, and for tailoring it for human requirements, and for the vicissitudes of the environment and circumstances.

Although molecular biology and genetic engineering are often presented as powerful new tools to introduce an existing trait into sexually incompatible species, the real power of these approaches lies elsewhere. The ability to understand the underlying mechanism(s) of the biological processes involved through combined molecular, cellular, and genetic analysis opens up numerous possibilities for experimentation, enhancements and alterations. These need not, and most probably will not, arise through simple introduction of preexisting alleles or genes from other sources. Rather, adjustments and modifications will be made to native processes by molecular intervention.

In just the last few years, for example, conditional nuclear male sterility has been genetically engineered into numerous crops by transforming plants with chimeric genes that direct and limit the expression of toxic gene products into the male flower parts. This gives rise to a dominant nuclear male sterile phenotype with efficiencies approaching 100%. When combined with a genetically engineered restorer line that encodes a specific inactivator of the toxin, a universal and completely reversible two line male sterility system is achieved. There is every reason to believe that this mechanism, with few variations or exceptions, will be applicable to most crop species. Currently it is effective in both monocots and dicots, both autogamous and allogamous species. This has been carried out largely by teams in the private sector with the goal of developing commercial and proprietary hybrid production for many crops (Mariani et al, 1991). However, while elegant, it is only the beginning of what can be done by adjusting the sexual systems of plants through molecular intervention. Hybrids, while alone very important, are greatly increased in value if the hybrid trait can be fixed in a heterozygous form and thus breed true. More important, an ability to perform single-plant level evaluation would eliminate the major bottleneck of hybrid cultivar development.

Achieving such modifications in transgenic plants will probably need the full arsenal of existing molecular methodologies for ectopic and heterochronic gene expression and will doubtless use dominant suppression mechanisms such as antisense and cosuppression.

Integrated Strategy to Develop Apomixis Through a Transgenic Mechanism

Envision a scenario in which a single apomictic gene cassette that produces several linked phenotypes can be generated through molecular biology. This cassette would function as a dominant trait in virtually all crops to which it is introduced in order to:

(1) block entry of the embryo mother cell into meiosis, and thus to produce a fully functional diploid female gamete;

(2) block the development of male sexual structures;

(3) allow fully autonomous development of the embryo and endosperm without pollination;

(4) have this condition be fully penetrant; and

(5) have the condition be dominant but conditional, when the default state is apomictic, but upon application of a nonproprietary, inexpensive compound, the trait is fully suppressed so that crosses can be performed in either direction.

This scenario is the best case situation, and it should be borne in mind for the research and development of apomixis.

In nature, most apomixis coexists with some degree of sexuality. There are, however, very few, if any, cases in which there are defined and manipulable conditions to interchange these two states at will. Thus, while environmental extremes such as day length or heat/cold stress could have influence on the balance between sexuality and apomixis, these do not tend to be either universal (i.e., most day length dependencies would rule out controlled apomixis in the tropics, where its introduction could be of such value), nor controllable (heat shocking a field is not a realistic option).

Yet to maximize the benefits to the agricultural community, it must be possible to introduce apomixis into a wide spectrum of genetic backgrounds, ideally retaining the ability to cross with related germplasm, then apomictically fix any genotype for subsequent tests and multiplication. The ability to suppress conditionally the apomictic trait should also be available to the small farmer and to the resource-limited breeding and crop management community.

To maximize the utility of the trait and its acceptance in the farming community, it should probably have virtually complete penetrancc; apomixis must not be a tendency but a strict and obligate feature of the plant's reproduction until, as noted above, that trait is suppressed to make new combinations. There may be situations in which this is not the case, but in most scenarios, it would be most likely essential.

While many apomicts require pollen either to fertilize the central nuclei to form a functional triploid endosperm and/or to stimulate the development of the embryo and endosperm (pseudogamous apomicts), there exist a substantial number of apomictic plants that have absolutely no dependence on pollination whatsoever (autonomous apomicts). These include the well characterized genera *Taraxacum* (dandelions) and *Hieracium,* with their many agamospecies.

The advantages of autonomous apomixis are substantial, most prominently in the avoidance of threats to crop production associated with failures in pollination and/or fertilization. The events of sexual hybridization, male flower development, pollen formation and shedding, pollen transport, and fertilization combine to present a window of vulnerability in cropping systems. This vulnerability often results in catastrophic and unpredictable yield losses in difficult cropping systems and environments, and in some crops leads to suboptimal performance even in benign environments. Heat, cold, or drought stress, water stresses, submergence, pollinator

failures (for instance from promiscuous insecticide usage), and other environmental and biotic factors can have enonnous effects on pollen formation, viability, spread, and effectiveness. For instance, a fairly brief period of excessive heat and drought during this window of vulnerability can destroy maize silks and prohibit reasonable seed set, even if the crop has been well managed throughout its life. In rice, moderately low temperatures during pollen meiosis can result in drastically reduced fertility. Thus, autonomous apomixis would render many cropping systems robust and reliable whereas under current practice they are fragile and risk prone.

The importance of this feature will clearly differ between crops. In self fertile forage crops, in which harvest is associated with the mature plant and pollination is relatively assured by the structure of the flower, pseudogamous reproduction is quite an acceptable option. In self fertile grains, however, although pollination is relatively reliable, the harvest is principally associated with the endosperm tissue. Variation in the genotype of that tissue is reflected in yield, both with respect to the quality and quantity of grain harvested. This would be of particular concern if apomixis is widely used to capture heterosis from highly heterozygous populations through single plant selection. Autonomous endospermy would allow the composition of the endosperm to be addressed through direct selection, and the improvements made could be retained in a highly heterotic genetic background.

The increasing vertical integration of agricultural production, and the concomitant privatization and centralization of agricultural research, instills legitimate fears that key methods and opportunities could become unavailable through intellectual property restrictions. This concern is voiced by plant breeders in the private and public sectors alike, as well as by agricultural policy makers and the general public. If apomixis technology is patented and restricted to just one entity, rather than widely licensed to all potential users, its application for broad environmental and economic benefit could be greatly reduced. This restriction would be particularly unfortunate, because apomixis, more than any other innovation mooted in agriculture could have a profound decentralizing and diversifying effect.

Components of an Integrated and Effective Research and Development Strategy for Apomixis

Several steps are seen as being necessary to achieve an adequate understanding of the processes that underlie apomixis, and the introduction of an inducible (or suppressible) form of the trait into target species:

(1) Detailed molecular and cellular analysis of a model apomict;

(2) Genetic screens for apomictic mutants in *Arubidopsis thaliana;*

(3) Analysis of plant genes by complementation of meiotic mutants in yeasts;

(4) Development of tools to delimit and control gene expression within target plants; and

(5) Development of tools for field level control of transgene expression.

Most studies of apomixis have focused on describing the cellular mechanisms employed, or on the ecological implications of the trait for different species. While the diversity of these data can be valuable, it is often difficult to rationalize disparate pieces of information from widely different systems. There is, therefore, a need to develop a model system to study apomixis. Before proposing any candidates, however, it is helpful to consider the features required, with particular regard to a molecular study of the trait.

Ideally, a model plant should be easily cultivated, both *in vivo* and *in vitro,* and be a perennial that is easily propagated vegetatively, to permit the maintenance of sterile or self-incompatible sexual biotypes. Small stature, a short generation time, abundant seed set and a simple mechanism for hybridization facilitate the analysis of inheritance and the rapid turnover of experimental populations. Both sexual and apomictic biotypes need to be available, preferably employing autonomous endospermy to avoid difficulties associated with pseudogamy. Apomixis needs to be easily assessed, preferably in a format that can be quantified, to facilitate the evaluation of allelic differences and of the additive and epistatic influences of modifier loci. Of the mechanisms of apomixis reported, apospory appears to be the most appropriate for the initial study, although comparative analyses of diplosporous and adventitious embryogenic systems at a later time would help to identify elements common to natural apomictic systems and regions of control that overlap with sexuality. Apospory appears to be relatively simply inherited, at least in the small number of cases studied, which should simplify the molecular analysis. It is also typically a facultative mechanism in which both zygotic and clonal seeds can be harvested from the same plant. Facultative systems have the advantage that the selective inactivation of either the sexual or apomictic developmental pathway through mutation is likely to lead to the exclusive expression of the other. When apospory is conferred by the inheritance of a dominant allele, mutation results in the expression of sexuality, the recessive condition. This provides an invaluable internal control since the formation of sexual seed indicates that megagametogenesis and embryogenesis remain functional.

There are also a number of requirements which would specifically assist the advancement of a molecular research program. A model system for this type of study must be amenable to genetic transformation to permit the introduction of marker genes, mutagenic sequences, and the reintroduction of putative control sequences. It is preferable that it have a small genome and ideally already be characterized with respect to morphological and molecular markers (deletions, translocations, RFLPs, RAPDs, transposons, etc.), to facilitate the localization of critical loci. For commercial reasons it would also be advantageous, although not essential, to use a crop species to facilitate the transfer of research findings into practical outcomes.

Although more than 300 flowering plants from more than 35 families have been described as apomictic no species meets all of the above criteria. Model

systems, of course, have been proposed previously. Most have been monocotyledonous species that are either important forage crops such as *Petznesetum* (Ozias-Akins et al, 1993) and *Bruchiaria* (Borges do Valle et al, 1994), or wild relatives of grain crop species, such as *Tripsacum* (related to maize), Elymus (wheat), and *Panicum* (Millet). Monocotyledons, however, are typically difficult to transform, imposing limitations on the approaches that can be taken to identify and isolate the genetic elements involved. Some dicotyledonous apospotic species have been used as model systems. Rutishauser (1948) studied *Potentilla* (Rosaceae) and Nogler (1984) conducted a series of elegant experiments on *Ranunculus auricomus* (Ranunculaceae). Unfortunately both of these species are pseudogamous, presenting difficulties associated with pollination and the subsequent need to prove clonal seed formation. Autonomous endospermy is not common among aposporous apomicts, but is known in several genera of the *Aster*aceae. Within that family, the taxon that appears to be best suited for use as a model system for a molecular study of apomixis is *Hieracium* subgenus *Pilosella*, a compilation of over 60 apo-species native to Eurasia and North America.

Hierucacium species display many of the features listed above. Most are small herbaceous perennials that are easily propagated and maintained in the greenhouse. Yeung (1971) reported that *Hierucium jloribundum* can be induced to flower after exposure to five or more days of continuous light. Similar findings have been reported for *H. robustum* (Bergstrom, 1969) and *H. boreale* (Philipson, 1948). It is interesting to note that *Hieraciutn* does not flower in response to gibberellic acid application, unlike many other Long Day rosette species (Peterson and Yeung, 1972). Using day-length-extension lighting in the greenhouse, plants of *H. pilosellu* and *H. aurantiacum* can be encouraged to flower throughout the year. Seed sets within 3-4 months of germination, allowing 3-4 generations per annum.

Hieracium seed develops by facultative apospory coupled to autonomous endospermy. Pollination is therefore not required for the formation of clonal seed, and apomixis can be scored by seed set after the exclusion of pollen. The capit*ulum* of *Hieracium* is a compound inflorescence containing 60 to 120 individual florets. Decapitation of the immature bud removes both anthers and stigmas, which prevents sexual seed formation but not clonal seed set (Ostenfeld, 1906). Gadella (1991) has reported that many of the sexual forms of *H. pilosellu* that he has studied were self-incompatible tetraploids. Self-incompatiblity greatly simplifies hybridization in a plant that bears such small flowers. In common with almost all apomictic plants, *Hierucium* species are typically polyploid. Reported examples range from 3 to 8X (Tutin et al, 1976).

The cellular mechanism of apomixis in *Hieracium* is relatively well documented (Skalinska 1971, 1973; Skalinska and Kubien 1972). Until recently, however, very little information has been available on the experimental manipulation of these plants. The apparent suitability of *Hierucium* for use as a model system has stimulated an effort in our group to develop the methods necessary for a molecular analysis of apospory. A range of tissue culture techniques have now been described, including methods for micropropagation and shoot regeneration from

leaf tissue (Bicknell, 1994a). An efficient genetic transformation system has also been developed (Bicknell and Borst, 1994b). and activity and inheritance have been demonstrated for chimeric genes conferring resistance to spectinomycin, kanamycin, and hygromycin, susceptibility to 5 tluorocytosine, and for β glucuronidase activity. Introduced activator transposable elements from maize have been demonstrated to move in this system (Bicknell, 1994d). One interesting outcome of this work has been the use of introduced dominant marker genes to quantify segregation and recombination in facultatively apomictic biotypes of *Hierucium.* Chimeric heterologous antibiotic marker genes are dominant, highly penetrant, and can be scored all together at the seedling stage. Inheritance can then be confirmed by Southern blot or polymerase chain reaction (PCR) analyses. Inheritance of a dominant positive marker sequence, such as kanamycin resistance, from a paternal parent can be used to quantify recombination. Similarly, the loss of a negative selection marker, such as 5-fluorocytosine sensitivity, in the maternal parent can be used for a measure of segregation. Current work on *Hierucium* focuses on the development of a two element transposon tagging system in this plant, to facilitate the isolation of the genetic control elements involved in the expression of apomixis (Bicknell, 1994c).

Genetic Screens for Apornictic Mutants in Arabidomis thaliana

The small crucifer *Arabidopsis thaliana* has now become the most important model for plant genetic research. Its genetics and molecular biology have been developed to a remarkable degree of sophistication, allowing the rapid isolation of mutants in numerous processes and the subsequent cloning of the relevant genes. This has been largely due to the small size of its genome, its short generation time, and the substantial critical mass of research now being conducted using this species.

One consequence has been the often unspoken adoption of the *Arabidopsis* genome as the representative genetic compliment of a flowering plant. Apomixis is a developmental process and therefore will not be fully understood until it is considered within the context of the total developmental program operating in tlowering plants. As most of our understanding of plant developmental genetics relates to the biology of *Arabidopsis,* it will probably be necessary to conduct comparative studies with this plant early in the development of any new research strategy.

A.M. Chaudhury and his colleagues at the Commonwealth Scientific and Industrial Research Organization (CSIRO) Division of Plant Industry are conducting experiments to obtain and analyze apomictic mutants of *Arubidopis.* Using well chosen combinations of visible and male-sterile mutations as the parental material in a screen for apomictic characters, Chaudhury has obtained a set of *Arubidopsis* mutations with promising components of apomixis including several fertilization independent seed (fis) mutants. Substantial further analysis is underway (Chaudhury, 1995).

MOLECULAR GENETIC ANALYSIS OF CANDIDATE GENES AFFECTING OVULE DEVELOPMENT

Understanding of the molecular processes that control the formation of flowers and the various floral organs has increased considerably over the past few years. Homeotic genes play an essential role in these developmental processes. Analysis of homeotic mutants in Arabidopsis and *Antirrhinum* has resulted in a powerful genetic model for floral organ development (Weigel and Meyerowitz, 1994). Following this paradigm, Angenent and Dons at the Centre for Plant Breeding and Reproduction Research (CPRO-DLO) in the Netherlands are taking an interesting approach to obtaining and understanding genes involved in ovule development in *Petunia.* They have isolated a series of genes belonging to the MADS box homeotic gene family, and they have been analyzing these by ectopic and heterochronic transgene expression. These genes are designated floral binding protein (fhp) genes and have been shown to be involved in the formation of the floral organs. (Angenent et al, 1993).

It is clear that understanding the molecular regulation of ovule development and the formation of the embryo sac might open new ways for the induction and control of apomixis. Recently two MADS box genes (fbp 7 and fbp 11) that determine the identity of ovules within the pistil of *Petunia hybrida* have been characterized (Angenent et al, 1995). Both genes are expressed only in the developing ovules. Knocking out the genes or causing ectopic expression of the genes in transgenic plants clearly reveals that expression of these genes is necessary for proper development of functional ovules.

These results are very encouraging and may provide insights to further unravel the genetic control of processes taking place in ovules, especially the formation of the embryo sac. Since MADS box genes encoding transcription factors control a number genes, it seems likely that isolation of such target genes will bring us closer to genes directly involved in embryo sac formation, and thus the control of apomixis.

Analysis of Plant Genes by Complementation of Meiotic Mutants in Yeasts

Because apomixis is fundamentally a trait affecting the meiotic process, and in particular the points of entry and exit from meiosis, it would be sensible to make use of the wealth of information already obtained from genetic and molecular studies of the yeasts, *Saccharomyces cerevisiae* and *Schizosaccharomycespombe.*

These systems have allowed the characterization of numerous genes and gene products associated with the decisions to enter and exit meiosis. These include mutants affecting sporulation *(spo)* (Honigberg. and Esposito, 1994), cell division control (cdc), meiotic induction *(ime)* (Kassir et al, 1988) and even apomictic strains that produce true diploid spores (Bilinski et al, 1989). Many of the well-characterized effects are mediated by protein kinases operating in cascades (Yoshida et al, 1990).

The degree of conservation in function, and indeed often in primary protein

structure, between yeasts and plants is likely to be substantial, given the critical role of meiosis in evolution. Studies conducted by Hirt, Heberle-Bors, and their collaborators *in* Vienna have clearly shown that plant genes can complement cell cycle defects in yeasts (Hirt et al, 1991, 1992, 1993; Jonak et al, 1993), and other groups are making substantial headway in isolating genes associated with cell-cycle regulation (Hemerly et al, 1992; Kobayashi et al, 1994).

Characterization of plant genes isolated by complementation of yeast meiotic lesions, and yeast genes expressed in plants may be very valuable in illuminating the parallels and differences in the entry and exit points of meiosis. This in turn could lead to new avenues to inhibit entry to or precociously exit from a meiotic phase, the essence of apomixis.

Tools to Delimit and Control Gene Expression within Target Plants-Cell Type Specificity

All transgenic strategies will hinge upon an ability to tightly control the expression of introduced genes within particular target cells and to particular times of development. This will require the isolation and characterization of promoters and other controlling sequences that are specific in their action to nucellar cells, megaspore mother cells, and other cells of the embryo sac. Methodology for isolation and characterization of cDNAs specific to particular tissue types in plants has been well described (Koltunow et al, 1990); however, the difficulties in preparing pure populations of megaspore mother cells are substantial. Whereas even a few years ago this task would have been umnanageable due to the need to isolate large quantities of homogenous material from which to prepare cDNA libraries or probes, the advent of PCR now allows true micro scale amplification of mRNA populations from even single cells. For example, a recent publication describes work in which a large cDNA library has been developed from only about 100 egg cells of maize (Dresselhaus et al, 1994).

Using RNA mediated Arbitrarily Primed PCR (Welsh, et al, 1992), or RAP PCR (also called RNA display) (Liang and Pardee, 1993), it is also possible now to compare extremely small amounts of RNA (in the nanogram range) without library generation and subtraction. This opens possibilities for using single or a few excised nucellar or embryo mother cells at particular stages, or from apomictic and non-apomictic siblings, to establish the differential patterning and allow subsequent gene isolation. Heterochronic or ectopic expression of these candidate genes, or suppression of their activity through antisense or cosupprcssion approaches, can then be a particularly valuable avenue to explore function.

In recent studies at Cold Spring Harbor Laboratory, Dr Ueli Grossniklaus has developed a large set of Ds-mediated enhancer trap mutant lines *of Arabidopsis* in which a promoter-less or promter-deficient GUS gene is inserted in single copies into the genome. After extensive screening of stained and cleared ovules, Grossniklaus has found lines in which GUS is expressed in a variety of cells and stages of the ovule, including some lines in which the insertion causes single cells in the embryo sac to express GUS (U. Grossniklaus, personal communication). This extremely powerful approach, in one step, allows not only

tants to be generated which are defective in particular aspects of female gametophytc gene function, but also the ready analysis of gene expression of the insertion, and a routine cloning to rescue the inserted gene for subsequent analysis. This brute-force approach seems certain to give us a vast suite of necessary tools as well as mutants to investigate and manipulate ovule and female gametophyte development and genetics, and hence to obtain and control apomixis.

Development of Tools for Field Level Control of Trunsgene Expression and Environmental Limitation of the Trait

Responsible development of apomixis must involve the use of mechanisms to ensure both the environmental and ecological restriction of the trait. This could probably be achieved through concomitant male sterility, engineered as early in the development of the male floral organs as possible. This trait could best be envisioned as being facultative, so that on demand, the trait could be passed to sexually related species through conventional breeding methods.

It is important to ensure that the apomictic trait is expressed as the default condition, but suppressible at the will of the farmer or breeder. Molecular mechanisms need to be developed that will allow breeders to switch the trait off to perform a sexual cross, then release the switch to allow reversion of the Fl to apomictic mode. Thus, systems comprising compounds that can be applied to induce or repress defined, corresponding promoters need to be developed.

lnteresting candidates would be compounds that are not present in plants, are not toxic, and that have a well characterized ability to induce transgenes. Such systems, showing varying degrees of success, have been developed using induction by copper ions (Mett et al, 1993), or glucocorticoids (Schena et al, 1992).

A particularly good example of the type of approach showing promise for controlling complex field traits is afforded by the excellent work of Gatz and her colleagues in engineering the bacterial tetracycline repressor/operator interaction in transgenic plants (Gatz and Quail, 1988; Gatz et al, 1992; Roder et al, 1994; Weinman et al, 1994). In these studies, Gatz et al have generated a set of molecular tools by which transgene action can either be induced (in which the tet repressor functions as a negative regulator) or repressed (in which the tet repressor is fused to a transcriptional activator, and is hence a positive effector) through the action of exogenously applied tetracycline, with a dynamic range of up to 1000 in some cases. While the tetracycline system is by far the best developed, there is no reason to expect that other systems cannot be developed to provide field level control of transgene action as well.

Some Challenging Questions about Apomixis

The Physical Nature of the Lesion

Is there a chance that apomixis is not encoded by a gene *per* se? What if apomixis is the result of structural features of a chromosome(s) that cause inappropriate or

precocious entry into a pathway of megaspore mother cell formation and exiting of meiosis‘? What if it is a structure that interferes with the initiation of meiosis? For instance, one can envision a binding site for a key protein that has too high an affinity and thus titrates the protein before it can build to a sufficient level to initiate the meiotic process. Is it conceivable that there is not any gene expression in a traditional sense associated causally with apomixis. Variants on this model could also explain the tendency to find apomixis preferentially in polyploids and its tendency to be a dominant trait. If this were so, then differential gene expression screens would probably not show a causative mRNA expressed, and hence would not be productive.

Misleading Single locus Behaviour

Apomixis has seemingly occured in many diverse plant taxa independently, and because of the diversity of mechanisms and phenotypes (autonomous or pseudogamous, aposporous or diplosporous, facultative or obligate etc), it seems very possible that this trait is not produced by a simple mutation of a single target gene. However, in the majority of the few well-studied apomictic systems, apomixis does seem to segregate as a single dominant locus.

It is very possible that apomixis is a result of several different mutations that independently produce deleterious effects but which, in combination, would produce the requisite failure of the meiotic process with a concommitant ability to precociously enter embryogenesis. Because of the independent deleterious effects of either of these traits alone, one would anticipate that propagation of apomixis would often, if not always, behave genetically as a single locus. Segregation of either lethal or sublethal trait would guarantee the elimination of the alleles from any sexual population, placing strong selection pressure to ensure cosegregation or pseudolinkage. This could occur by any of a variety of mechanisms such as association on a balancer chromosome that is recombinationally deficient due to its structure. Thus, the appearance of apomixis as a single mutation, locus, or linkage group is almost certainly to be expected no matter how many actual genes are involved in the process. This caveat could prove very troublesome to approaches relying on gene disruption and isolation through transposon insertion, for example.

Summary

No technological intervention in such a fundamental activity as agriculture can ever be socially or environmentally neutral. The form that the development of apomixis takes will greatly influence who stands to benefit from the innovation. When it is achieved, however, the degree to which agriculture and society will change will be almost beyond comprehension. The advent of apomixis will make the advances and the problems of the Green Revolution seem tractable and easy to predict. Because of the changes in agricultural practice that could ensue, the

magnitude, diversity, and types of socio-economic impacts must be considered soon. It is only through foresight that we can encourage a responsible shaping of the research and development strategies. The development of policies and infrastructures suitable to cope with an innovation of this magitude in anticipation of its arrival, rather than ex *post facto* is essential and will greatly enhance its utility and minimize its adverse effects.

While it is clear that apomixis has the potential to have substantial impacts on agriculture, it is important to consider the optimum criteria for the development of the trait, to focus research efforts, and target the most appropriate crops. Social, agricultural, and environmental benefits will not necessarily flow directly from the simple invention or discovery of an apomixis gene. A concerted and ethical effort should be made to ensure that maximum social and environmental good occurs from the innovation.

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