Engineering apomixis in crops: A challenge for plant molecular biologists in the next century

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Researchers in plant sciences and agriculture, all over the world, are currently greatly interested in the induction of apomixis in elite hybrid lines. Very simply, apomixis means reproduction without sexual recombination. Apomixis is an area of research that is extremely significant for the agriculture of tomorrow. With recent advances in our knowledge of cellular and molecular biology and establishment of sophisticated recombinant DNA techniques, we are poised now to engineer apomixis into elite hybrid lines so that clonal seeds can be had and benefit of hybrid vigour maintained and passed on to progeny making it easy for farmers to grow a desired crop. The article summarizes our knowledge of apomixis in plants and points out promising approaches for further research in this area. A two-pronged approach is suggested for research. In one, already being followed in certain laboratories, apomictic lines are expected to be generated by induction of mutations by EMS or other agents – tagging can also be employed which can give a clue about the identity of the apomixis gene. In another and a complementary approach, use could be made of modern molecular biology techniques such as subtractive hybridization for cDNA clones and differential display of messenger RNA for identifying the apomixis gene as also the genes that control mitosis-to-meiosis transition in ovules. This article thus also gives an overview of our knowledge of cell cycle controls as well as identifies the immediate problems that need to be addressed to induce apomixis at will.

ALTHOUGH apomixis has been known for many years[1-3], it is only recently that its potential to transform the agricultural scene is being widely realized. Indeed, according to one view[4], exploitation of this single trait in plant breeding can usher in *the greatest change in agricultural practice since the dawn of cultivation.* Apomixis is asexual reproduction that occurs in the ovule of flowering plants, leading to production of fertile seeds (historically, not only somatic embryogenesis, but even budding related modes of reproduction have been included under apomixis and from a molecular biology viewpoint, there can be a basic commonality in all these routes to embryo development). As to the significance of apomixis, plant breeders, employing hybridization, have combined genes from diverse plants, including wild species. Nevertheless, it is necessary that a prized hybrid be multiplied in a way that the progeny is *identical* to the parent, i.e. the selected hybrid. In crops, hybrids are highly prized on account of their 'hybrid' vigour, but their multiplication through seeds poses a serious problem, and is at present almost impossible, since normal sexual reproduction involves segregation and recombination of chromosomes, leading to a mixed progeny. If, however, some way could be found of stopping reduction divisions in the megaspore mother cell and obtain embryos, say parthenogenetically without fertilization (or alternatively from ordinary nucellar cells), a particular hybrid can be 'fixed' with seeds which will form plants true to the parental type. This can be a great boon to agriculture.

Only a few years ago, it would have been impossible to envisage any practical approach to make clonal seeds. Of late, there has been an explosion of knowledge in the area of molecular control of cell division. Many genes controlling mitosis and meiosis have been identified in yeast and animals. Recently, a meiosis gene in mice has been knocked out by homologous recombination, leading to direct parthenogenetic development of embryos. And, if the necessary genes controlling mitosis and meiosis can be identified in plants as well, apomictic

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development of embryo could be achieved. Since apomictic plants are derived from embryos that have the genetic constitution of the female parent, they can greatly facilitate hybrid seed production and be valuable because heterosis will be perpetuated through successive generations via seed. Thus, a prized hybrid, once produced, can be multiplied *vegetatively* via apomictic seeds at no additional cost to the farmer. The hybrid will not lose its genetic make-up – unavoidable in sexual crossing – and will *breed true*. The farmer can also be spared incurring expenditure on purchasing expensive hybrid seeds which normally he must procure every year from seed companies (specializing in maintaining male sterile and restorer lines and related techniques) and which is on average at least 10 times costlier. It is this attractive prospect that has awakened world-wide interest in introducing apomixis in crops[5–10]. Apomixis can also be useful in a crop like potato where currently bulky and perishable tubers are the common means of multiplication.

Research on apomixis: The past and the present

Since the principal interest of scientists from an agricultural viewpoint is to obtain 'clonal' seeds without fertilization, we confine our review to 'apomixis' (*sensu stricto*). Apomixis of this kind, i.e. through seed, occurs naturally in many plants. One of the earliest recorded instances is of *Hieracium* (Compositae)[11]. Indeed, Gregor Mendel was among the first to study inheritance in this plant for corroborating the laws he had deduced from the study on pea plants. However, the results in *Hieracium* were very different from those in the pea plants (very puzzling then but quite understandable now), leading to considerable controversy between him and Carl Nageli, another well-known botanist of those times and a specialist of *Hieracium*, regarding the general validity of his laws! Usually, apomicts have been identified in populations because of the unusual uniformity observed in the progeny to the maternal parent. In many other plants, another type of apomixis leading to formation of seeds with multiple embryos has been observed as well. Such observations were followed up by detailed cytological and embryological studies (in addition to the references already cited, see refs 11–19). Apomixis is especially prevalent in polyploids; auto as well as allopolyploids, wherein meiosis is very frequently disturbed because of difficulties in pairing and segregation of chromosomes—apomixis bypasses sexual reproduction and enables these plants to reproduce. Many wild species belonging to Gramineae, Compositae and Rosaceae show apomixis. Some species of Rutaceae, Liliaceae, Urticaceae and some other families where diploidy is observed also show this trait. Overall approximately 300 species belonging to nearly 40 families of plants show apomixis which is basically of three types.

Mitotic and meiotic diplospory

Among polyploids, the commonest way for the origin of an apomict is the failure of female meiosis. In the normal sexual mode of reproduction in higher plants, the megaspore mother cell in an ovule undergoes meiotic divisions forming typically a set of four megaspores. The nucleus in one of the megaspores undergoes three mitotic divisions leading to the formation of an 8-nucleate female gametophyte, and consequently all nuclei of the embryo sac including the egg cell are haploid to start with. In *mitotic diplospory*, however, the megaspore mother cell in the ovule may not enter meiosis at all e.g. in *Antennaria* (Compositae). On the other hand, in *meiotic diplospory* the megaspore mother cell does initiate meiosis and passes through typical stages like leptotene and zygotene (diakinesis is also often observed) but the meiotic programme is aborted soon resulting in a 'restitution' nucleus that restores the original unreduced chromosome number e.g. *Taraxacum* and *Ixeris* (Compositae). In either case, the resulting embryo sac and the egg cell are typically diploid and the embryo then arises without fertilization.

Apospory

Here, the ovule forms accessory embryo sacs from nucellar cells neighbouring the original archesporial or the megaspore mother cell—i.e. we have in these plants a multicellular archesporium. Often the original embryo sac with the haploid nuclei degenerates, and embryos then form spontaneously from the egg cells of the additional embryo sacs with unreduced nuclei. Examples are *Hieracium* (Compositae) as also many grasses such as *Panicum*, *Pennisetum* and *Poa*.

Adventive embryony

Here the embryos arise from ordinary cells of the nucellus (with unreduced chromosome number) lining or close to the embryo sac, and rarely even from the integument. *Citrus* (Rutaceae) is a classical example of this. The nucellar cells do not form additional embryo sacs, but instead form embryos directly. In grasses also, e.g. in *Poa pratensis* (Gramineae), supernumerary embryos arise often by growth of nucellar cells into the embryo sac.

Some further remarks

From the various examples of apomixis cited above, it is clear that the absence of meiosis does not prevent formation of the embryo sac or development of the embryo. Surprisingly, in many apomicts not only does

fertilization of the unreduced egg not occur, but apomictic embryo begins its development even before anthesis. However, another form of apomixis is represented by haploid parthenogenesis—where meiosis does occur, but the embryo may develop without fertilization[20]. Since the discovery of anther or pollen culture as a means of inducing haploidy, this has been a widely accepted approach towards generating haploids—but, it may be preferable if seeds could be had of the desired lines, with parthenogenetic haploid embryos.

An additional noteworthy point is the two pathways of reproduction, sexual and apomictic, are not mutually exclusive and often co-exist in the same plant and even in a given ovule in that the nucellar embryos may exist along also with a zygotic embryo, or aposporous embryo sacs may grow by the side of a normal embryo sac. Although there are examples of plants displaying near 100% (obligate) apomixis, often it is facultative and the equilibrium between the two modes may be modified by environmental factors[21], such as photoperiod and stress. Almost nothing is known of the molecular basis of facultative behaviour, but control of reproduction at will – whether via the sexual or apomictic mode in a given plant – will give a powerful handle to many researchers.

Endosperm development – can be spontaneous in some apomicts

An endosperm is obligatorily required for nutrition and normal growth of embryos in any developing seed. Typically, when the mature embryo sac is formed, three of the eight nuclei form the egg apparatus (comprising two synergids and the egg cell), three others at the opposite end form antipodals, and one nucleus from each pole moves to the central area to form polar fusion nucleus. Of the two sperms formed in the mature pollen, the nucleus of one fuses with the polar fusion nucleus initiating simultaneous development of the endosperm, which is triploid. Interestingly, the endosperm develops autonomously in many apomicts, being derived from the fusion of unreduced polar nuclei (e.g. in Compositae). In apomict grasses, however, pollination is necessary for formation of the endosperm following the fusion of unreduced polar nuclei with a sperm nucleus (the phenomenon is called 'pseudogamy' since true fertilization, that is of the egg, does not take place).

Genetic basis of apomixis – probably a single gene controls apomixis

The genetic basis of apomixis is of great significance for any research aiming to transfer 'apomixis' to nonapomictic plants[9], since if many genes are involved, the apomictic trait will be difficult to engineer. Broadly, three sets of genes appear to be involved in female meiosis and reproduction for: (i) the progress of normal meiosis, (ii) the mitosis of megaspore nucleus followed by embryo sac development, and (iii) development of the embryo. Typically, it is the failure of meiosis during megasporogenesis, and the initiation of mitosis in the unfertilized diploid egg which result in apomixis. However, in apospory and adventive embryony, renewed mitotic activity of nuclei in nucellar cells is responsible for apomixis. Clearly, not only may apomixis have arisen parallely in different plant families, but the precise genetic basis of apomixis may be different in various types of apomixis.

A consideration of various biochemical events underlying mitosis and meiosis indicates that scores and even hundreds of genes must be involved in these processes and, on account of the existence of various types of apomixis, the deciphering of the mechanisms of apomixis may seem a herculean task, let alone engineering such a trait. But, fortunately, the basic genetics is not as complex because, generally, there are critical nodal points in development and key regulatory genes for these nodal points are few. Given the knowledge of existence of homeotic genes in animals and lately also in plants, one may speculate that for initiating each of the processes (i), (ii) and (iii) mentioned above, there may be a separate regulatory gene of this kind. Although the genetics of apomixis has not been thoroughly investigated, it does appear that only one or two genes may control this trait[3,7]. At least in *Pennisetum*, *Panicum*, *Ranunculus* and *Citrus*, apomixis is controlled by a single gene or locus (however, according to a recent study in *Tripsacum*[22], the single locus may comprise several extremely tightly linked genes). Nevertheless, independent evolution of apomixis has, apparently, led to some diversity in the mechanism of apomixis even in plants that show the same type of apomixis (e.g. diplospory). Thus, although in certain plants apomixis appears to be governed by only one gene and it is inherited as a dominant trait, in some other plants it has been reported to be recessive, pointing to the involvement of at least two types of genes.

Introgression of apomixis and mapping the apomictic gene

Because only a few genes (probably a single key gene) seem to be involved in the control of apomixis, many breeders have been encouraged to introgress the apomictic trait in crops from wild relatives. Although almost all the studies to date have relied on classical and empirical breeding methods, such as repeated back-crossing by a donor or the gene, some notable progress has been made using these methods. Examplewise, the aposporous trait

found naturally in *Pennisetum squamulatum* is being introduced into *Pennisetum glaucum*, a cultivated species, by Ozias-Akins and co-workers[23]. One resulting line has the apomictic gene located on a supernumerary *squamulatum* chromosome. Fortunately, RFLP markers are now available for several important crop plants and efforts are being made also to develop RFLP maps. Two molecular markers co-segregate with apomixis, thereby giving a valuable opportunity to locate the apomictic gene and work on this is being actively pursued by this group in USA. Similar efforts are underway to transfer the apomictic trait also across genera, for example in maize[24] from *Tripsacum*, a wild relative, and in wheat[25] from *Elymus* as well as to isolate the gene for apomixis. To summarize, there is some progress towards transferring apomixis from wild to cultivated species and also identifying gene loci responsible for apomixis. Nonetheless, so far, the identity of the genes or of the products and their function(s) are unknown and this is clearly a task for the future.

Molecular biology strategies for induction of apomixis

Although, as mentioned above, empirical studies are being done by plant breeders to transfer apomixis from the wild relatives, the major strategies for the development of apomictic lines will now be based on the use of either (i) the mutational approach in which induction of a mutation is followed by eventual isolation of the apomictic gene by transposon tagging, or (ii) the biochemical approach that will utilize the knowledge gained by a systematic study of the molecular biology of apomixis. Whichever approach is employed, any heritable change will require later the use of the entire arsenal of modern gene transfer techniques. Focusing attention on the 'apomixis' gene, it is reasonable to assume that it must basically be one concerned with the control of mitosis and meiosis. Recombinant DNA techniques could be adopted for inhibiting meiosis, which is a key step in sexual reproduction, and allowing diploid egg cells to form embryos without fertilization. Alternatively, one may induce resumption of nuclear divisions or mitoses in nucellar cells around the embryo sac to form embryos, either directly as in *Citrus* (via nucellar embryony) or indirectly through formation of additional embryo sacs and unreduced eggs as in grasses (via apospory).

The approach of inducing mutations and isolation of the apomixis gene by transposon tagging

Using the approach of mutations first, since apomicts are fairly common among wild plants, it is reasonable to assume that mutations could be also induced for producing new apomictic lines. Conversely, an apomict could also be mutated to render it sexually fertile. Techniques now exist whereby mutation of a gene can be induced by insertion of T-DNA or a suitable transposon. The gene can then be easily identified by hybridization of the restricted DNA with a labelled probe employing the T-DNA or the transposon. Here, work on *Arabidopsis thaliana*, a wild but a model plant, would be most useful. In fact, methods for isolation of desired genes via transposon tagging are already well advanced and are being further perfected[26]. Nevertheless, a major problem is how to screen for a rare apomictic mutant. Using the classical histological technique one would have to examine sections of ovules for presence of multiple megaspores or supernumerary embryo sacs or signs of formation of restitution nuclei indicative of development of diploid embryo sacs. Recently, confocal microscopy has been employed for following the female gametophytic development in *Arabidopsis*[27].

Another convenient procedure of selecting mutants by simple visual screening has been developed. This method relies on screening for silique size[28,29]. Ordinarily in plants, without fertilization, not only do ovules fail to form seeds but even fruits remain small. An apomictic mutant may however form near-normal sized siliques (in such experiments, it is best to use lines that are male-sterile so that sexual reproduction is avoided totally and one is reasonably sure that one is dealing with a putative apomict). Some progress in this direction has already been made and mutants have been obtained where to some extent the endosperm and the seed develop autonomously[28,29], even though embryo development is partial or lacking altogether (when an embryo does develop, it is arrested around the globular stage). Although the mutants obtained so far may not necessarily relate to the same gene(s) that control apomixis, this represents valuable direction of work and cloning of the genes responsible for such mutations should not only advance our knowledge of the regulation of embryo and endosperm development, but lead to the isolation of the 'apomixis' gene itself.

The second approach – manipulation of mitosis and meiosis and need for research on molecular biology of cell division

The second major approach for engineering apomixis in crops is to manipulate the biochemical machinery that regulates mitosis and meiosis. If one could identify the gene(s) controlling the mitosis → meiosis switch, one could engineer the antisense version of the appropriate gene and ablate the gene in a desired hybrid, for inducing apomixis. Clearly, a thorough knowledge of cell division controls is required. In fact, recently, there has been an explosion of knowledge in this area—utilizing yeasts as research material. Although a detailed review on cel

cycle control is outside the scope of this article, one can briefly state that it is now known that the basic driving force or 'engine' in nuclear or cell division is a serine–threonine type protein kinase, product of a gene called *cdc*2 or *CDC*28 or sometimes p34 kinase gene (though in recent years there is a move now to designate the gene as CDK or Cyclin Dependent Kinase gene). Incidentally, the multiplicity of nomenclature arises because different investigators employed different systems for its nomenclature not knowing that all are related to the same key gene or enzyme.

The genetic studies

The advances in our present knowledge of protein kinases and genes coding them is a consequence of the integration of results of studies from two areas: (i) genetics of cell division, and (ii) general biochemistry. The genetic studies that started in the seventies, mainly conducted on the two yeasts – the baker's yeast, *Saccharomyces cerevisae*, and the fission yeast, *Schizosachharomyces pombe*, led to the isolation of various temperature-sensitive mutants: the *CDC* series in baker's yeast and *cdc* series in fission yeast[30–33]. The mutants were arrested in various steps of cell division and could be differentiated and identified by such criteria as size of cells at the time of arrest and the ploidy state. The most important of these were the *CDC*28 and *cdc*2 in the two yeasts and concerned the same key gene since the defect in one yeast could be complemented by transfection of wild-type DNA of the other.

The biochemical studies

The biochemical studies have been conducted on animal systems such as *Xenopus*, clam, sea urchin and HeLa cells. The earlier studies (utilizing a variety of mammalian systems) led to the discovery of phosphorylation as an important regulator of cellular metabolism in which attachment of a phosphate group to a protein by kinases or its removal by specific phosphatases plays a key role in all living organisms from yeast to plants and animals (classical studies of Krebs, Fischer and others between 1960s and 1980s). Considerable research has also taken place in the area of signal transduction, uncovering the role of several messengers in which Earl Sutherland, Michael Berridge, Robert Irvine and Y. Nishizuka and their associates have made important contributions, identifying cyclic nucleotides, Ca^{++}, IP_3 and DAG as second messengers. These advances are now common knowledge[34].

The cell division cycle kinase(s) and cyclins regulating cell division

Focusing attention on cell division, between 1970s and early 1980s, it was discovered that cell extracts from mature frog eggs (well on way to development) had the ability to induce divisions if injected in immature oocytes. The putative signal, named then as MPF (Maturation Promoting Factor), has turned out to be a protein kinase complex of the serine–threonine class[35,36]. The current view is that this protein kinase is a kind of 'master switch' that controls cell division from its start to finish, and one of the most significant achievements of the last decade is that the *CDC*28 and *cdc*2 encode kinases that are not only homologous between the two yeasts, but homologous also to the kinase in the MPF complex. The master enzyme apparently works through either direct phosphorylation of key substrates or phosphorylation of transcription factors or still other kinases ('slave kinases' to use the terminology of Timothy Hunt[37], employed in an address and one of the pioneers of such studies) that may finally work on a multitude of ordinary proteins and enzymes. Together, the phosphorylations are responsible for such events as dissolution of nuclear membrane, decondensation or condensation of chromosomes, duplication of DNA, transcription, chromosomal movements, and cytokinesis (for meiosis, proteins and enzymes are needed also for pairing of chromosomes, formation of synaptonemal complexes and recombinational events). An important point, however, is that the master kinase brings about its effects by associating with a special protein called cyclin of which there are several kinds. Cyclin is one part of the MPF complex[38], the other being the kinase. Depending on the phase of a cell cycle, a particular cyclin may be synthesized or degraded and hence this name for these proteins. In human cells, evidence has come for the existence of nearly a dozen cyclins. Not only are the various cyclins considered to be responsible for ordered completion of different phases of the cell cycle (such as G1 to S or G2 to M transitions), but cyclins also provide the basis of specificity of master kinase action in targetting different substrates at various points in the cell cycle[39,40]. It appears that the site for steric complementarity for protein–protein interaction between a particular substrate and the enzyme is provided jointly by a cyclin and a *cdc* kinase.

But CDC kinases and cyclins themselves are under further control

The summary above, however, gives only the barest outline of the rapidly expanding field of molecular control of cell division. More facts are emerging every day. Like cyclins, there is a multiplicity in *cdc/CDC* kinases; while in yeast a single such kinase is sufficient for all cell cycle transactions, in humans as many as 8 kinases have been identified. Also, the *cdc*2/*CDC*28-type kinases and cyclins, are really a part of a rather complex network. The *CDC*28/*cdc*2 kinases and cyclins, them-

selves, are under the control of other kinases and phosphatases which regulate the central *cdc/CDC* kinases positively or negatively, depending on the environment and whether a particular cell is or is not programmed to further divide[40]. Many of these kinases are located downstream of various other signaling cascades operating through second messengers, i.e. Ca^{++}, cAMP, cGMP and IP_3, or through the MAP kinase cascade connected often to cell-surface receptors which respond to signals such as hormones circulating in the blood-stream or to external factors like light, touch or wounding in plants. Further, the synthesis and degradation of *CDC* kinases and cyclins too is under regulatory controls.

Biochemistry of meiosis and inducing parthenogenesis in frog oocytes by knock-out of a meiotic gene

From a biochemical viewpoint, induction of apomixis can be achieved either by inhibiting meiosis (as in mitotic or meiotic diplospory) or promoting mitosis (as in nucellar polyembryony). Unfortunately, the biochemistry of meiosis, in contrast to mitosis, is understood rather poorly. In budding yeast a number of genes have been identified, e.g. *RME*, *IME* and *UME* series (*RME* and *IME* for *Regulator* and *Inhibitor of Meiotic Expression* and *UME* for Unscheduled Meiotic Expression), that code for transcription factors or related proteins that bind to DNA directly or indirectly and control expression of early meiotic genes[41,42]. Similarly, in fission yeast a couple of key *MEI* genes have been identified[41,43]. But in higher organisms one has hardly any idea of the mitosis → meiosis switch. Nevertheless, the studies on meiosis in mouse have already brought results which are of great relevance to plant scientists. Recently, parthenogenetic activation of egg has been achieved by engineering a mutant *c-mos* gene replacing the wild type *c-mos* by the technique of homologous recombination[44,45]. The *c-mos* gene (originally identified as an oncogene) also codes for a protein kinase of serine–threonine class and its role is to prevent parthenogenetic development of the unfertilized egg[46].

Getting back to plants—the current status and research needed

In examining the current status of our knowledge in plants, despite extensive genetic work, such as done on maize[47], very little is known yet about the molecular biology of the meiotic process in plants, regardless of whether male or female meiosis is considered. Nevertheless, there has been considerable activity in the area in recent years and employing a variety of methods, but principally by the use of heterologous or PCR-generated probes and screening of cDNA libraries, homologues of *CDK* as well as cyclin genes have been found in plants[48,49]. In fact, like in animals, there is a multiplicity of both *CDK* and cyclin genes as found in *Arabidopsis* and maize. Very recently, evidence has also appeared for the existence of homologous genes that code for proteins similar to the retinoblastoma protein and other suppressors of *CDK* activity[50]. Although no one has yet been able to correlate the onset of meiosis to the presence or absence of any specific *CDK* or cyclin (or any inhibitor), the stage is now set for research on apomixis by use of molecular biology techniques. Several directions of research in this area can be suggested. It is most important to determine the key players of mitosis and meiosis and, in particular, the mechanism that controls switching from mitosis to meiosis. In budding yeast, the three key genes involved in this switch are *RME*1, *IME*1 and *UME*6. The last one codes for a DNA-binding protein which by itself acts as a repressor of early meiotic genes (yet allowing mitosis). When the *IME*1 gene is active, however, its protein product associates with that of the *UME*6 gene, turning the complex into an activator. Correspondingly, in fission yeast, the most important genes are *MEI*2 and *MEI*3. Though the work on the yeasts has created a puzzle of its own (the genes in two yeasts are quite different!), attempts can now be made for identifying homologous genes in plants such as *Arabidopsis thaliana* (a recent report[51] suggests the presence in this plant of a *MEI*2 homologue). Key genes could be identified in plants by preparing cDNA libraries of ovules undergoing meiosis and those yet at the archesporial stage (or even younger) and use of subtractive hybridization or the more recently developed 'differential display' technique for detecting unique mRNAs or their cDNAs. Research of this nature could be undertaken also on plants such as rice and wheat (as also on any apomict relatives). However, there is considerable wisdom in casting our net wider and take up work on natural apomicts even if they are weeds and in taxa where both ordinary and apomictic lines are available (e.g. *Hieracium, Pennisetum*), subtractive hybridization and differential display of mRNAs could be applied for directly isolating the desired gene[52,53]. In any event, as would be obvious, all such work (whatever be the chosen material) would finally converge on identification of the same key genes controlling apomictic behaviour. And while the mutational approach with transposons may be rewarding in the short term, a sound understanding of the biochemistry of onset of meiosis as well as mitosis (as in adventive embryony in *Citrus*, or a grass such as *Poa*) is required for proper manipulation of this trait in the long run.

Conclusion

Clearly, there is considerable interest in engineering of apomixis now. Our main conclusion, however, is that

the understanding of the mechanism of apomixis will also be greatly aided by research into the basic biochemistry of mitosis and meiosis. This is because it is extremely likely that in most instances apomixis is the result of activation or inactivation of a gene coding for a cell division kinase or a cyclin (or a regulatory molecule that interacts with either of them) which may disturb female meiosis. In animal systems, it has become possible to induce parthenogenetic development of an embryo from an oocyte by knock-out of a gene that is required for progress of normal female meiosis. This approach can also be extended to plants. Of course, in other plants, for example those showing nucellar polyembryony, resumption of mitotic activity is responsible for apomictic reproduction. Finally, it is suggested that while research is initiated on key genes controlling mitosis, meiosis, and thus apomixis, parallel work needs to be undertaken on developing more sophisticated transformation techniques such as targeting genes at particular loci by homologous recombination.

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