Why is transferring apomixis to crops still a dream?

Enrico Perotti^{1,2}, Daniel Grimanelli^{1,3}, Peter John², David Hoisington¹ and Olivier Leblanc^{1,3}

¹ CIMMYT, The International Maize and Wheat Improvement Center, A.P. 6-641, Mexico DF 06600, M?xico.

www.cimmyt.org Email perotti@rsbs.anu.edu.au

² The Research School of Biological Sciences, Australian National University. GPO 475, Canberra 2601

³ IRD, Institut de Recherche pour le D?veloppement; Mexico DF 06600, M?xico.

Abstract

Apomixis, or asexual reproduction through seeds, is a natural phenomenon that holds the potential of increasing dramatically the world food production. Apomixis produces progeny that are clones of the mother, a feature allowing the fixation of any favourable genetic combination. Apomixis is a complex phenomenon that displays a wide variety of developmental modifications of sexuality. Despite this complexity, genetic studies show that apomixis is inherited as one or two dominant mendelian traits located on a recombination-less chromosomal segment. Apomixis is typically facultative as only a proportion of the progeny are apomictic while others are sexually derived. The incidence of apomixis depends on the global genetic background and does not seem to be linked to the apomixis locus. For more than four decades, scientists have strived in vain to transfer the trait to commercially important plants through conventional backcrossing schemes. The failure to recover apomictic crops seems to be a direct consequence of the genetic complexity of apomixis. Although apomixis is complicated, it has arisen naturally in many forms, most if not all of which depend on the plasticity of plant reproductive development. Increased understanding of reproductive machinery in plants should enable the engineering of apomixis in sexual crops.

Media summary

Although apomixis could impact crop production, conventional breeding methods have failed to deliver. Engineering of apomixis could be the outcome of enhanced knowledge of sexual reproduction.

Key Words

Apomixis, sexual reproduction, wide hybridisation.

Introduction.

In the next 20 years, the world population will increase to about 8 to 10 billion, putting more and more pressure on agriculture and natural resources (Toniessen 2001). Moreover, although the world produces more than enough to feed the entire population, more than 840 million people in developing countries go hungry every day (www.fao.org). Ensuring food availability for the future generations is a complex challenge that will require political as well scientific breakthroughs.

One of the latter could be apomixis. Apomixis is a naturally occurring way of asexual reproduction through seeds. Apomictic individuals contain a genome derived entirely from the female parent (gynogenetic clone) which could confer crucial advantages in plant performance and yield if the maternal genome contains a beneficial combination of genes. Apomicts produce a majority of gynogenetic clones in combination with some sexual progeny. Historically, apomixis has been classified into two main types depending on the origin of the embryos: adventitious embryony and gametophytic apomixis.

In adventitious embryony, embryos differentiate from somatic cells within the ovary to produce maternal clones. Usually the sexual pathway is unmodified and normal development of the seed requires double fertilization (Wakana and Uemoto, 1988). Mature seeds thus contain a sexual embryo together with maternal ones.

In gametophytic apomixis, the maternal progeny are the product of a parthenogenetical development of unreduced megagametophytes. These functional unreduced megaspores differentiate either from a megaspore mother cell which failed to enter meiosis (diplospory) or a somatic nucellar cells that begins gametogenesis in absence of sporogenesis (apospory). Although apomictic species can be normally classified in one of the classes of apomixis, numerous variations in the modalities of diplospory and apospory exist (Nogler, 1984; Crane, 2001).

As apomixis produces genetic clones of a given individual, it has a great potential for agriculture. If apomixis is transferred to crops it will allow the fixation of heterosis or any given genetic combination. This should accelerate breeding procedures and possibly increase diversity. Harnessing of apomixis would permit breeding for specific micro-environments ("niche breeding") and if freely available, would contribute to providing food security for farmers in developing countries.

For more than four decades, these characteristics of apomixis have stimulated scientists to attempt to transfer the trait to crops through introgression from wild apomictic relatives (wide crosses).

Initial genetic work suggested that a dominant single allele controlled apospory and diplospory (reviewed in Grimanelli *et al.*, 2001). This lead to the postulation that apomixis is a simple trait. Failure of fertilization and parthenogenesis was thought to be a pleiotropic effect of the absence of meiosis. Although not much was known on the molecular basis of the trait, the hypothesis of a monogenic control of apomixis stimulated the development of backcrossing schemes between apomictic relatives of maize, pearl millet and wheat. To date, all attempts to transfer apomixis through backcrossing have failed but the reasons for making the attempts are more valid than ever.

In this paper, we attempt to summarize why transfer of apomixis to crops has not yet been successful.

Genetic control of apomixis

Apomixis is wide spread and occurs in more than 400 species that are found in 40 angiosperm families (Nogler 1984, Carman 1997). Apomixis is restricted to polyploids and is generally a dominant trait only affecting female reproduction. The components of apomixis comprise the absence of meiosis (apomeiosis), embryogenesis in absence of fertilization (parthenogenesis) and functional endosperm development (Richards 1986). Although apomixis has been classified in two types there are numerous variants of the phenomenon (Nogler 1984, Crane 2001). The processes of apomeiosis, gametogenesis and embryogenesis vary between apomictic species as well as in individuals within a single genotype. This complexity in classifying the different mechanisms of apomixis is confirmed when genetic analysis of apomictic development is performed.

Reports on the genetic control of apomixis are often contradictory and show no clear consensus on the number of genes involved in the phenomena. Most analyses state that apomeiosis is controlled by a single dominant mendelian factor that is generally located in a chromosomal segment not subject to recombination (Grimanelli *et al.* 2001). The precise size and number of genes located in this region are not known. The fact that most apomicts studied to date have suppressed recombination suggests that apomixis or apomeiosis is controlled by a tightly linked gene complex. The picture is less clear for parthenogenesis as in some systems it co-segregates with apomeiosis while in others the two traits segregate independently (Grimanelli *et al.* 2001).

Apomixis is typically a facultative phenomenon as apomicts produce variable proportions of sexually derived progeny. These proportions seem to depend on the genetic background. In apomictic maize-*Tripsacum* hybrids the facultative nature of apomixis depends on the maize genome (Leblanc personal communication). Modifiers seem to modulate the expression of apomixis and, as normal sexual pathways remain in most if not all apomicts, it is plausible that the phenomenon has evolved from sexuality and thus apomixis is basically a modification of sexuality.

Necessary modifications to normal plant sexual reproduction include not only the formation of an embryo without involving a male gametic nucleus but also proliferation of endosperm tissue. Not much is known on endosperm development in apomicts. This normally triploid tissue, usually derived from fertilization of the central cell, plays an essential role in sexual reproduction of angiosperms.

Although some apomicts have evolved to develop endosperms autonomously the majority of apomicts are pseudogamous, meaning endosperm development requires fertilization. With a few exceptions, the maternal and paternal genomic contributions to the endosperm differ from that found in sexual plants. This alteration in parental contributions is due to the presence of an unreduced central cell that is either fertilized (pseudogamous) or not (autogamous) by a normal reduced male gametophyte. In many plants, and specifically in cereals, genome dosage in the endosperm is critical for normal seed development. In maize normal endosperm formation requires a maternal paternal ratio of 2 to 1. Any dosage deviation produces defective kernels.

The genome dosage requirement of the endosperm is a strong barrier that hampers the *de novo* appearance of apomixis and impedes its transfer to major crops. In spite of this requirement, apomicts produce viable endosperm in various ways by modifications in fertilization, gametogenesis or through development of immunity to genome dosage effects (Grimanelli *et al.* 2001, Koltunow and Grossniklaus 2003). The genetic control of these circumventions of adverse effects from altered ratios of male and female genomic contributions is unclear.

The origin of apomixis

The origin of apomixis is still a mystery as it is difficult to conceive how various deleterious traits such as apomeiosis, parthenogenesis, endosperm formation can coevolve to produce a viable apomict (Mogie 1992).

Based on the initial findings that apomixis was inherited in a simple manner, it was postulated that a single master regulator was responsible for the phenomenon (Grimanelli *et al.* 2001). Recent data demonstrating the complexity of apomixis suggests that the single master regulator model is not generally appropriate.

Most apomicts are either polyploids or segmental polyploids suggesting that ploidy levels could be a key component of apomixis.

Two models have been proposed concerning the role of polyploidy in apomixis: the genome asynchrony model and the ploidy regulation model.

The ploidy regulation model

This model postulates that the expression of genes that are involved in sexual reproduction is dosage dependent. If this is correct, when ploidy increases, the expression profiles of certain genes, especially those involved in reproduction will be modified thus producing apomixis.

Recent studies in yeast, maize, *Arabidopsis* and wheat suggested that the expression of a number of genes was diminished or activated upon polyploidization (Grimanelli *et al.* 2001, He *et al.* 2003). Moreover, Quarin *et al.* (2001) showed that genome doubling of a sexual *Paspalum* produced an apomictic autotetraploid. This suggests that in certain cases polyploidy is sufficient to produce apomixis. However, only a minority of the 70% of all plants that are thought to be polyploids are apomictic, therefore polyploidy is probably crucial but not in itself sufficient for production of viable apomicts.

The genome asynchrony model

In 1918, Ernst proposed that apomixis resulted from the hybridization between distinct albeit related species. If this is correct, apomixis may result from the hybridization of two related species with different

reproductive conduct and timing (Carman 1997). Asynchronous misexpression of the parent genes in the hybrid could then lead to all the components of apomixis.

This model is supported by data demonstrating apomictic components in allopolyploids produced from distinct sexual parents (Carman 1997). It is also been corroborated by recent cytological studies in apomictic *Tripsacum* showing global alteration in sporogenesis, gametogenesis and embryogenesis have (Grimanelli *et al.* 2003).

The two models described are based on genes involved in sexual reproduction that are asynchronously deregulated through a change in expression due to genome collision or polyploidization. Although speculative, these models offer the most plausible explanation to how a complex trait such as apomixis could have appeared.

Conclusions

Although apomixis is seemingly inherited in a simple way, recent data suggests that the phenomenon is more complex. Apomixis is derived from an alteration in the normal sexual reproduction pathway. Heterochronic or asynchronous deregulation of gene expression due to polyploidization is an attractive model explaining this alteration of sexuality. The failures to produce apomictic crops through conventional wide hybridization approaches could be a consequence of the composite nature of apomixis.

Plant reproductive development is a plastic phenomenon, which is demonstrated in nature by the diverse pathways that produce apomixis. On this basis, a better understanding of sexual reproduction in model plants such as *Arabidopsis*, rice or maize should permit the identification of genes that would potentially allow engineering of apomixis in crops. Numerous genes controlling the different reproductive processes are available (reviewed in Spillane *et al.* 2001). Mutants producing functional unreduced eggs cells have been isolated in barley (*triploid inducer*) and maize (*elongate*). The *elongate* locus has been isolated and presents opportunities to engineer apomeiosis in crops (Grimanelli *et al.* 2004).

Several genes involved in triggering embryogenesis have been identified in *Arabidopsis* (Spillane *et al.* 2001). Tissue and time specific expression of these genes could offer ways to engineer parthenogenesis.

Fertilization independent seed mutants allowing a partial development of the endosperm in absence of fertilization have been isolated in *Arabidopsis* (Grossniklaus *et al.*, 1998 Luo *et al.* 1999). Although seed development is defective in these mutants, understanding the mode of action of the genes responsible presents a potential for the production of autonomous endosperm.

In the near future, the increase in knowledge of the genetic and molecular control of plant reproductive development will provide better insights into the regulation of apomixis.

The improvements in seed production, plant performance and yield that can certainly come from engineering apomixis are now more urgently needed than ever. Real progress towards the creation of apomixis in crops has come in the form of increased understanding that the complex developmental modifications cannot be obtained by simple genetic changes. Nevertheless the repeated evolution of apomixis in wild plants must sustain our efforts to understand and harness its molecular basis.

References

Carman JG (1997). Asynchronous expression of duplicate genes in angiosperms may cause apomixis, bispory, tetraspory, and polyembryony. Biological Journal of the Linnean Society 61, 51-94.

Crane CF (2001). Classification of apomictic mechanisms. In: The flowering of apomixis: From mechanisms to genetic engineering [edited by Savidan Y; Carman JG; Dresselhaus T]. Mexico DF, Mexico: CIMMYT, IRD, European Commission DG VI (FAIR), pp.

Ernst A (1918). Die Bastardierung als Ursache der Apogamie im Pflanzenreiche. Jena, Ger.: Fischer

Grimanelli D; Leblanc O; Perotti E and Grossniklaus U (2001). Developmental genetics of gametophytic apomixis. Trends in Genetics 17, 597-604.

Grimanelli D; Garcia M; Kaszas E; Perotti E and Leblanc O. (2003) Heterochronic expression of sexual reproductive programs during apomictic development in *Tripsacum*. Genetics 165, 1521-31.

Grimanelli D; Perotti E and Leblanc O (2004) Characterization of the *elongate1* mutant in maize. 46Th Maize Genetics Conference, Camino Real, Mexico City.

Grossniklaus U; Vielle-Calzada JP; Hoeppner MA and Gagliano WB (1998). Maternal control of embryogenesis by MEDEA, a Polycomb-group gene in *Arabidopsis*. Science 280: 446-50.

He P; Friebe BR; Gill BS and Zhou JM (2003). Allopolyploidy alters gene expression in the highly stable hexaploid wheat. Plant Mol. Biol. 2003 52, 401-14

Koltunow AM and Grossniklaus U (2003). Apomixis: A developmental perspective. Annu. Rev. Plant Biol. 54, 547-74.

Luo M; Bilodeau P; Koltunow AM; Dennis ES; Peacock WJ and Chaudhury AM (1999). Genes controlling fertilization independent seed development in *Arabidopsis thaliana*. Proc.Natl.Acad. Sci USA 96, 296-301.

Mogie M (1992). The evolution of asexual reproduction in plants. London: Chapman & Hall

Nogler GA (1984). Gametophytic apomixis. In Embryology of Angiosperms [edited by Johri, B.M.]. Berlin: Springer-Verlag, pp. 475-518.

Quarin CL; Espinoza F; Martinez EJ; Pessino SC and Bovo OA (2001). A rise of ploidy levels on meiosis and mode of reproduction in *Paspalum notatum*. Sex. Plant Reprod. 13, 243-49.

Richards AJ (1986) Plant breeding systems. London, UK: Chapman & Hall, 529 p.

Spillane C; Steimer A and Grossniklaus U (2001). Apomixis in agriculture: the quest for clonal seeds. Sex. Plant Reprod. 14, 179-87.

Toniessen G (2001). Feeding the world in the 21st century: Plant breeding, biotechnology, and the potential role of apomixis. In: The flowering of apomixis: From mechanisms to genetic engineering [edited by Savidan, Y; Carman JG; Dresselhaus T]. Mexico DF, Mexico: CIMMYT, IRD, European Commission DG VI (FAIR), pp. 1-7.

Wakana A and Uemoto S (1988). Adventive embryogenesis in *Citrus* (*Rutaceae*). II. Postfertilization development. American Journal of Botany 75, 1033-1047.