Apomixis: Occurrence, Applications and Improvements

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ABSTRACT

Apomixis is an asexual mode of plant reproduction through seeds. A common feature of all apomicts is the autonomous development of embryos and the generation of progenies that are exact genetic replicas of the mother plant. The aims of studying apomixis are to unlock the diversity of apomictic plants and to make it feasible to transfer apomixis to agriculturally important genotypes, therefore conferring them the ability of cloning through seeds. In this chapter we present a short review on the incidence of apomixis in plants of floricultural interest and the description of the recent advances in the molecular and genetic characterization of natural apomicts.

1. INTRODUCTION

The term apomixis was formerly used as a synonym of vegetative propagation. At present, it is preferentially restricted to the sense of agamospermy, i.e. asexual reproduction by seeds (Asker and Jerling 1992). This mode of reproduction occurs in about 35 families of angiosperms (Hanna and Bashaw 1987), including Asteraceae, Rosaceae, Poaceae, Orchidaceae and Liliaceae, in which ornamental species can be found.

In sexual reproduction meiosis reduces the chromosome number of the megaspore mother cell to form reduced megaspores, one of which develops into an embryo sac containing the female gamete (the egg cell). In most plants these embryo sacs have eight nuclei and are described as *Polygonum* type (Willemse and van Went 1984). The fusion of two unique haploid gametes, derived from the random assortment of the genetic material occurring during meiosis, results in the generation of diploid and genetically diverse progenies. In contrast, in apomictic reproduction, the embryo develops autonomously from an unreduced cell having the same set of maternal chromosomes and giving rise to plants that are clones of the mother plant.

Apomictic plants are the result of either one of two types of development, sporophytic or gametophytic (Fig. 1). In sporophytic apomixis or adventitious embryony, embryos are formed directly from unreduced cells of the nucellus, or inner integument, while the developmental pathway of meiotic embryo sac is maintained (Lakshmanan and Ambegaokar 1984). Gametophytic apomixis is characterized by apomeiosis. Meiosis is either altered or totally bypassed, and as a consequence, an unreduced female gametophyte, or embryo sac, is formed (Asker and Jerling 1992, Nogler 1994). There is no fusion of male and female gametes and the egg cell develops autonomously, by parthenogenesis, generating an embryo that keeps the same set of maternal chromosomes. Apospory and diplospory are different types of gametophytic apomixis. In diplospory, the megaspore mother cell bypasses or fails to achieve meiosis, but through mitoses forms an embryo sac with all unreduced cells distributed as in the meiotic embryo sac of the *Polygonum* type. In this case, the sexual process is completely compromised. In apospory some nucellar cells, called aposporous initials enter in mitosis directly and unreduced embryo sacs are formed. Sexuality and apospory can occur simultaneously in the same ovule (Harlan *et al.* 1964). If fertilization of the central cell is necessary to form the endosperm, the system is regarded as pseudogamous, if not, it is autonomous.

Association between apomixis, polyploidy and polyembriony is recorded for many species. Apomicts are generally polyploids, tetraploidy being the commonest level and very few diploid apomicts existing in nature (Asker and Jerling 1992, Carman 1997). The reason for this association is still not understood (Bicknell and Koltunow 2004). Besides apomixis, anomalies involving ovule development can result in polyspory (bispory and tetraspory) and polyembryony. In polyspory ovule development is disrupted, but reduced egg cells are formed and require fertilisation (Carman 1997). Polyembryony refers to the formation of multiple embryos in one ovule and some authors consider adventitious embryony as a form of polyembryony. Asker and Jerling (1992) point out that these are distinct phenomenon. In polyembryony embryos may result from - the presence of multiple embryo sacs in an ovule, - synergids or - the cleavage of the cells produced by the zygote

and not from unreduced cells of the nucellus as defined for adventitious embryony. In contrast, in Carman's (1997) review on polyembryony and apomixis, the term apomixis was restricted to gametophytic apomixis and adventitious embryony was regarded as polyembryony. Based on cytological, phylogenetic and genomic data, he suggested that apomixis; polyspory and polyembryony have a common origin that is the asynchronous expression of duplicate genes in polyploids. Until now, induction of polyploidy in sexual plants by application of suppressors of cell division could not elucidate the association between apomixis and polyploidy. In *Paspalum*, facultative apomicts were generated by duplicating the chromosome number of previously sexual diploids after colchicine treatment (Quarin *et al.* 2001), while in *Brachiaria brizantha* (Pinheiro *et al.* 2000, Araújo *et al.* 2005) and *B. ruziziensis* (Gobbe *et al.* 1981 1982), and *Tripsacum* (Leblanc *et al.* 1995a) tetraploids derived from duplicating sexual diploids maintained their sexuality (Lutts *et al.* 1994).

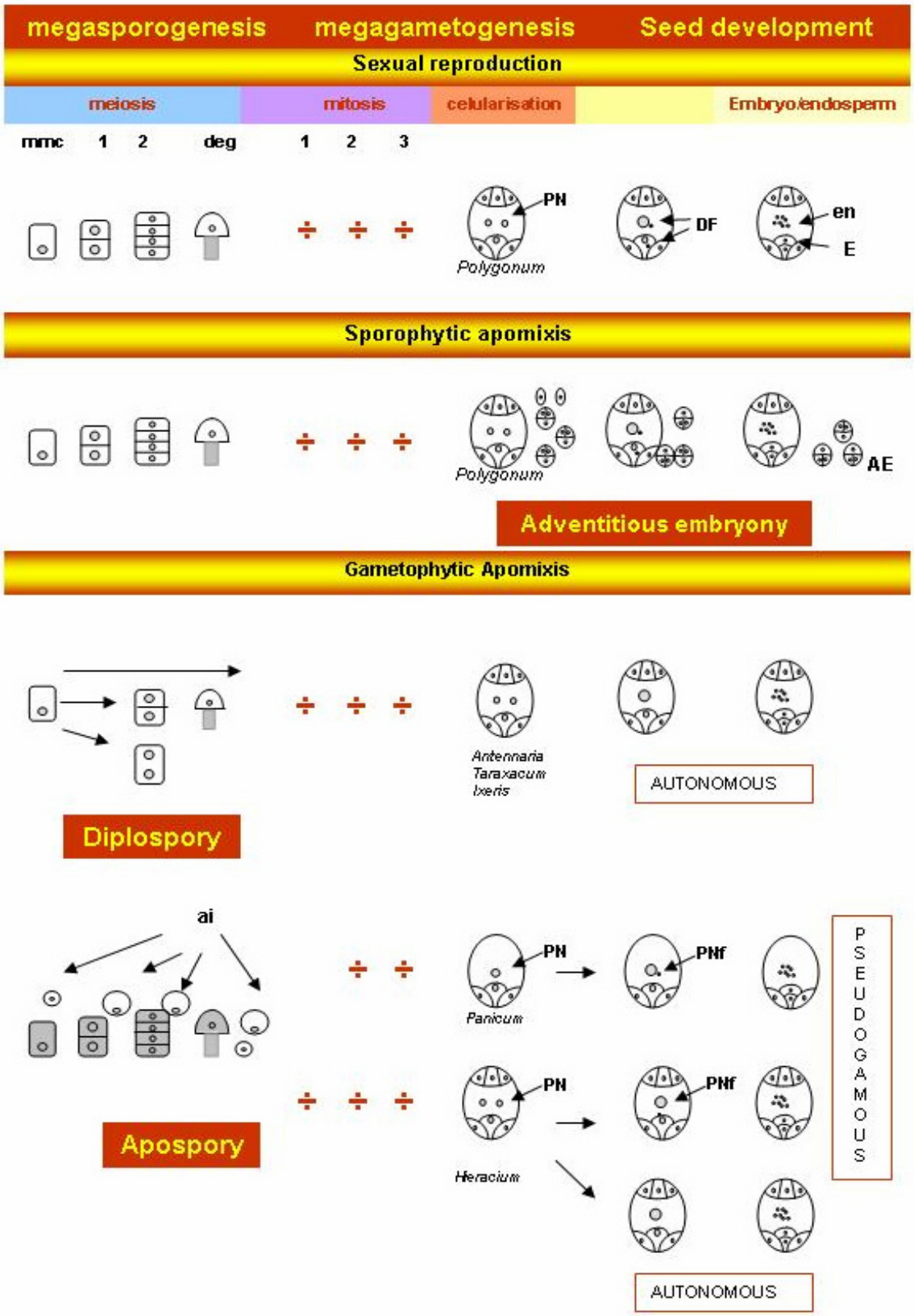

Fig. 1 Schematic representation of the mechanisms of sexual and apomictic reproduction and their respective types of embryo sac, based in Nogler (1984), Asker and Jerling (1992), Koltunow (1993). ai = aposporous initials; AE= adventitious embryos; DF = double fertilisation (fertilisation of egg cell and polar nucleus); E= embryo; en = endosperm; PN = polar nucleus; PNf = polar nucleus fertilisation. The cells in grey indicate that they can degenerate in that stage of development.

In apomicts, pollen formation often occurs normally in anthers generating viable reduced pollen (Nogler 1984a, Czapik 1994). An apomict able to generate at least part of its progeny by sexuality is regarded as facultative. Progenies from facultative apomicts segregate into maternal (apomictic) and non-maternal or aberrant (derived from sexuality) classes.

Apomictic plants bearing fertile pollen can be used to pollinate sexual or facultative apomictic plants to transfer genetic characters to their offspring. Even so, a frequent limitation of crosses between sexuals and apomicts is ploidy number. Apomictic species are organised in agamic complexes, where individuals of different ploidy levels exhibit characteristic reproductive modes. While diploids are sexual, polyploids express apomixis. Considering that new apomictic polyploids can eventually be originated from diploids by sequential steps of 2n + n hybridisation, it is clear that this peculiar strategy allows the maintenance of a certain degree of variability among polyploids, even when they are apomictic (Daurelio *et al*. 2004).

The interest in studying apomixis came long after its first reports. Asker and Jerling (1992) and Nogler (1994) cite some of Gregory Mendel's studies carried out on *Hieracium* sp., nowadays known as a genus with apomictic species. Mendel obtained homogenous offspring from crosses among these plants, which did not match with his hypothesis about segregation and were incorrectly attributed to an unusual self-fertilization. Evolutionarily, apomixis was considered a dead end and for breeding purposes, an obstacle. With the exception of the grasses, *Citrus*, mango, roses, orchids and berries, apomixis is not found in cultivated plants.

With the advances of biotechnology an interest in apomixis increased. Gene transfer between plants, independently from sexual compatibility, is already a reality and the control of apomixis by genetic engineering is a challenge to different research groups around the world.

In floriculture, vegetative propagation of individuals is desirable as it is a way to keep low genetic variability in the propagated culture. There are several methods of vegetative propagation such as: rooting of cuttings, induction of adventitious buds, grafting and *in vitro* techniques (Grunewaldt 1988). Micropropagation brings great commercial advantages and success stories about the utilization of tissue culture technology are numerous. In fact, micropropagation and the production of clonally uniform plants has become an industry all over the world involving axillary branching and somatic embryogenesis. There are still some ornamental plants in which a vegetative propagation method is not fully established, and with a limited multiplication rate. One can imagine the impact of transferring apomixis to commercial plants (Bashaw 1980, Hanna and Bashaw 1987, Asker and Jerling 1992, Koltunow 1993, Jefferson 1994, Savidan 2000, Bicknell and Koltunow 2004, Spillane *et al.* 2004). Its controlled use can bring a direct advantage, such as fixing and cloning through seeds elite genotypes and hybrids. Cloning by seed cultures that are actually vegetatively propagated is of floricultural interest since it can be used to restrain viral diseases, once viruses can accumulate in the plants diminishing yield and quality. Another advantage of bringing apomixis to elite genotypes and hybrids is to allow small farmers to propagate their own seeds. As a result, commercial production will be simplified, with a subsequent decrease in cost. Another expected benefit of apomixis is the possibility of fixing locally adapted varieties. Their ability to survive under local stresses like extreme climate conditions or under pathogen pressure would be fixed and used in propagation (Jefferson 1994). In addition, breeding of natural apomicts is very difficult because of the impossibility of making crosses and incorporating a new trait as a consequence of this mode of reproduction. Study of apomixis can be envisaged to find a way to control the expression of genes responsible for apomixis. This would allow the transmission of the male progenitor characteristics to the progeny. In this way, at least in theory, interfering in the expression of one or a few genes, apomixis could be silenced, allowing recombination, and re-expressed in improved hybrids.

2. IDENTIFICATION OF APOMIXIS IN PLANTS

Czapik (1994) discussed the parameters on how to detect apomixis in angiosperms. From geographic distribution and morphological features in the field to cytological and embryological observation, there are many ways to identify and to confirm the occurrence of apomixis.

Plants that reproduce strictly by apomixis do not segregate; the progeny is identical to the mother plant. Therefore, in the field, the observation of non-segregation of maternal phenotypic characteristics is an indication of apomixis. In the case of autonomous apomixis, one can observe the occurrence of seed set in plants that had their female flowers isolated or, that had their stigmas and anthers excised from the flowers. This is not possible for pseudogamous apomicts because, although there is no need for fertilization to trigger embryo development, it is still necessary to form the endosperm and a viable seed. Reproduction by apomixis has been reported in many species of the *Asteraceae*, a family very important as an ornamental, because of their showy flower-heads (Cronquist 1982). To investigate its occurrence in some subtropical species, autopollinization was avoided by mechanical methods such as excision of anthers and stigmas. Although fruits were observed in 5 among 22 species (parthenocarpy), just two of them were able to produce viable seeds suggesting an apomictic mode of reproduction (Werpachowski *et al*. 2004). Apomixis can be evidenced more accurately by analysis of the segregation of biochemical and molecular markers. Isozymes and RAPD (Random amplified polymorphic DNA) markers are useful for detecting precociously the mode of reproduction in hybrids, as shown in *Poa pratensis* (Mazzucato *et al.* 1995). In *Rosa,* polyploidy and unbalanced meiosis are present in all members of the section *Caninae,* dog rose, corroborating the hypothesis of apomixis previously built by the observation of a pronounced morphological influence of the maternal parent. Indeed, analysis of RAPD markers showed no inheritance from the male parent (Werlemark 2000). To our knowledge a definitive study of the embryology of this plant has not yet been performed. RAPDs were also successfully applied to the analysis of apomixis in different segregant population of *Paspalum notatum*, an important grass for turf and forage (Ortiz *et al.* 1997). In tetraploid populations of Aronia, the mother parent and the offspring showed identical RAPD profiles (Persson Hovmalm *et al.* 2004). In the savanna grass, *Hyparrhenia diplandra*, highly variable micro satellites were used to show the occurrence of facultative apomixis with rare events of sexual reproduction (Durand *et al.* 2000). In *Lilium*, GISH (genome *in situ* hybridization) and FISH (fluorescent *in situ* hybridization) probes were used to distinguish maternal and paternal chromosomes for hybrid verification and a suspicion of apomixis was discarded (Marasek *et al.* 2004).

Many cytoembryological characteristics of apomictic plants can be used as circumstantial indicatives of apomixis. Gametophytic apomicts are generally polyploids, while related sexuals are diploids (Carman 1997). Chromosomes counting in root tips (Pozzobon and Valls 1997) and estimation of DNA content by flow cytometry (Galbraith 1989) are largely employed to determine ploidy. Though a direct correlation of apomixis and polyploidy is not established, polyploidy can indicate a possible presence of apomixis.

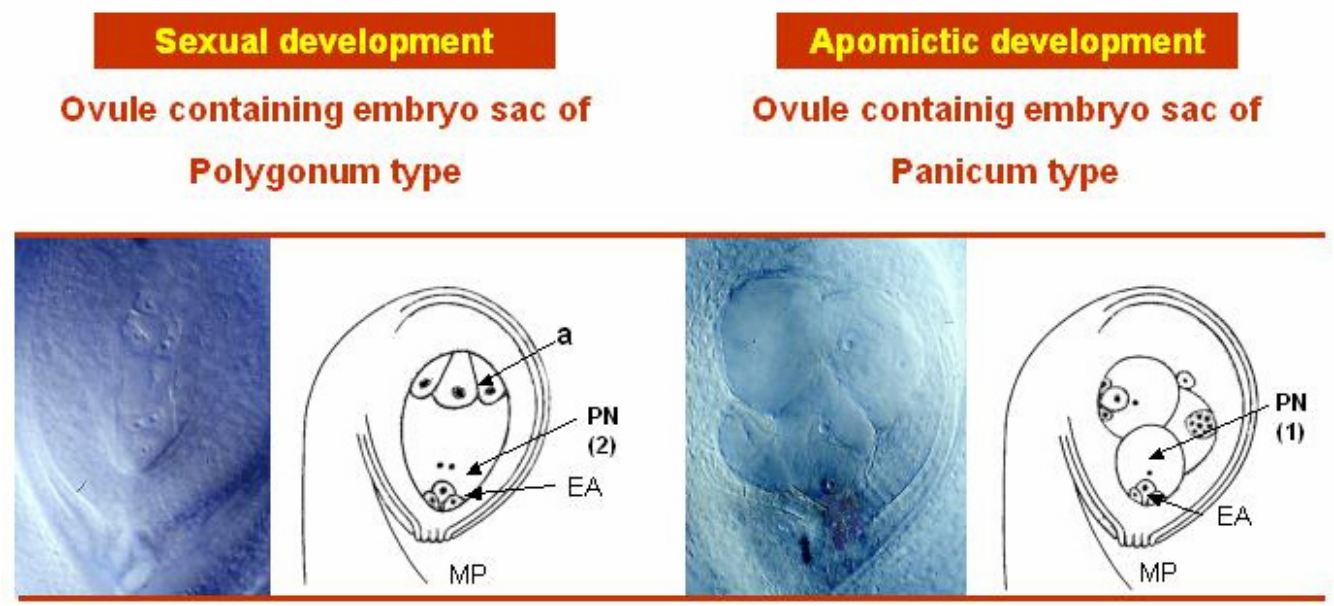

Fig. 2 Cleared ovules of *Brachiaria decumbens* and schematic representation. Left, an ovule bearing only one embryo sac of the *Polygonum* type that is present in sexual plants. At the right aposporous embryo sacs of *Panicum* type in the same ovule. a = antipodals; EA = egg apparatus; MP = micropyle; PN = polar nucleus.

Apomixis occurs in the female part of the flower, the ovary and more specifically in the ovule. The morphological characterization of ovule development is a traditional way to predict apomixis by detecting meiosis failures, abnormal pattern of callose deposition, presence of aposporous initials, presence of more than one embryo sac in an ovule (Fig. 2), formation of typical or irregular embryo sacs, polyembryony, presence of embryos in ovules before fertilization, etc. These characteristics can be observed by light microscopy using microtome sections or clearing methods. Both procedures can be used to establish the development of ovules, embryo sacs and embryos and to detect signs of apomixis (Young *et al.* 1979). The nucellar or integumentary origin of the adventitious embryos can only be claimed after embryological studies. It was established, besides adventitious embryony, three main types of diplosporic embryo sac development - *Taraxacum*, *Ixeris* and *Antennaria*, all of them with 8 nuclei, and two types of aposporic development - *Hieracium* with 8 nuclei and *Panicum*, with 4 nuclei (Fig. 1) with some exceptions (Asker and Jerling 1992, Koltunow 1993). Although variation in the number of nuclei have been reported for some species of the Poaceae (Quarin 1992, Bonilla and Quarin 1997, Acuña *et al.* 2005); the *Panicum* type is easily distinguishable from the meiotic embryo sac because its nuclei are distributed only in one pole of the embryo sac (Fig. 2). In contrast, to confirm the occurrence of any other type of apomeiosis it is necessary to make a more precise observation during early megasporogenesis once they have 8 nuclei resembling a meiotic embryo sac of the *Polygonum* type. Sometimes, it should be accompanied by cytogenetical analysis during embryo sac mitotic divisions. Ultra structural observations, rather then used to identify the type of apomixis, add clues about precise points of cellular development as shown in *Sarcococca humilis* (Naumova and Willemse 1982) and *Panicum* (Naumova and Willemse 1995).

Clearing methods, such us described by Herr (1971) or Young *et al.* (1979) are very useful for rapid screening of numerous samples. They are faster and easier than sectioning and staining because nuclei and walls of reproductive cells can be observed in intact pistils and ovules, previously incubated in methylsalicylate, with interference contrast microscopy. For example, they have been employed to analyze natural apomicts and their hybrids with sexuals in diplosporous autonomous apomictic *Taraxacum officinale*, dandelions (van Baarlen *et al.* 2002) and large segregant F_1 populations of *Paspalum* sp. (Martínez *et al*. 1999 2001). Clearing methods can also be used in combination with aniline blue to detect callose deposition during megasporogenesis (Peel *et al.* 1997), which can to some extent be related to apomixis or sexuality. Abnormal callose deposition was found during apomictic megasporocyte development in *Poa pratensis* (Naumova *et al*. 1993), in aposporous *Panicum maximum* (Naumova *et al.* 1993), *Pennisetum squamulatum* (Peel *et al.* 1997) and *Brachiaria decumbens* (Dusi and Willemse 1999). The cell wall morphology during megasporogenesis showed deficiency in callose deposition in facultative diplosporous apomictic *Elymus rectisetus* (Crane and Carman 1987) and it was used to classify the hybrids between *Elymus rectisetus* and *Triticum aestivum* L. (Peel *et al.* 1997).

Occurrence of polyembryony can be an indicative of apospory or adventitious embryony (Mendes-Rodrigues *et al*. 2004) and has to be determined by embryological methods or during seed germination. But, polyembryony does not necessarily imply apomixis. Alternatively, diploid plants can result from zygote cleavage or haploid plants can result from development of one or more synergids or antipodals and from more than one embryo sac in the ovule (Bewley and Black 1983, Lakshmanan and Ambegaokar 1984). Isozymes or molecular analysis of seedlings can determine their zygotic or adventitious origin. Nucellar polyembryony is found in *Rutaceae*, *Anacardiaceae*, *Myrtaceae*, *Cactaceae* and Orchidaceae (Lakshmanan and Ambegaokar 1984). Seed morphology was used to quantify sexual and asexual reproduction in a population of *Spiranthes cernua*, Orchidaceae (Schmidt and Antlfinger 1992) that has integumentary polyembryony (Lakshmanan and Ambegaokar 1984).

2.1. Incidence of Apomixis

Asker and Jerling (1992) showed a very detailed description of the distribution of apomixis in angiosperms. They added data to a precedent list of species and genera from Nygren (cited by Asker and Jerling 1992). According to these authors apomixis has been observed in about 15% of angiosperm families, 75% of them being *Poaceae*, *Asteraceae* and *Rosaceae*.

In certain taxa, a particular apomictic mechanism is dominant, as apospory in *Poaceae* and *Rosaceae* and diplospory in *Asteraceae*. Adventitious embryony is mainly found in tropical or subtropical woody plants with multiseeded fruits.

Among the genera of floricultural interest in which apomixis was reported, there are some from the Asteraceae family such as diplosporic *Taraxacum*, dandelion, and *Erigeron* and aposporic *Achillea* and *Hieracium* (Terziiski *et al*. 1995). In Rosaceae, apospory was detected in the genera *Alchemilla*, *Amelanchier*, *Cotoneaster*, *Crataegus*, *Malus*, *Potentilla*, *Rubus* and *Sorbus* (Asker and Jerling 1992, Amsellem *et al.* 2002), apospory and diplospory in *Crataegus* (Dickinson *et al*. 1996). Adventitious embryony was reported in Liliaceae, *Tulipa* and *Lilium* (Marasek *et al.* 2004), Orchidaceae in *Nigritella*, *Zeuxine* and *Zygopetalum* (Asker and Jerling 1992) and in Cactaceae, *Opuntia* (Asker and Jerling 1992, Negron-Ortiz 1998).

Grasses from the Poaceae family are largely used as ornamental plants in parks and gardens. They maintain soil fertility, create a habitat for wildlife and provide recreational space for sport and leisure while contributing to the general landscape. Grasses cover 26% of the world's total land area (Humphreys 2005) and because of their economical importance, apomixis in grasses has been far more studied than in any other group of species. Several grass genera of ornamental importance contain apomictic species such as: *Calamagrostis*, *Eragrostis*, *Panicum*, *Paspalum*, *Pennisetum* and *Poa*. Breeding such species is restricted in many cases to the selection of superior genotypes from the natural populations. Because of this, collection of germplasm is one of the most important points to take in account. After that, determination of the reproductive mode of each accession as well as the chromosome number is needed. After field evaluation, cultivars are obtained directly by multiplying the desired genotypes by seed. For instance, in *Poa pratensis* (Kentucky bluegrass), a pseudogamous facultative aposporous apomictic is used as a turf grass; cultivars can be originated by a single plant selection and multiplication (Douglas Brede and Willard 1993) or selection in F1 progeny (Douglas Brede *et al*. 1993).

2.2. Genetic Bases of Apomixis

Genetic diversity, a consequence of sexual reproduction, in a population can guarantee its survival to biotic or abiotic stress conditions. Although apomicts can be found in 33 of 460 families of angiosperms (Carman 1997), until now their role in evolution is not completely understood. Here, we will concentrate on the genetic bases of gametophytic apomixis, in which the embryo originates from an unreduced gamete, because it is the type of apomixis better studied so far. Information about sporophytic apomixis can be found on reviews by Nogler (1984), Koltunow (1993) and Bicknell and Koltunow (2004).

Nogler (1984a) considered that probably the basic determinants of apomixis could have been originated by mutation and most of the other genes involved in the process would probably be similar to those implicated in sexuality. More recently, apomixis has been accepted as a result of a rearrangement of the developmental programs that constitute the normal sexual pathway (Grimanelli *et al*. 2001, Koltunow and Grossniklaus 2003, Tucker *et al*. 2003). It is a heritable trait whose genetic control in many species still remains unclear.

Despite its broad distribution within the angiosperms, apomixis is not very common in the major crops. This condition forced studies in the field to be performed in wild species that are polyploids, highly heterozygous and genetically poorly characterised. In grasses, inheritance analyses are carried out normally on segregating F_1 progenies originated from crosses between sexual and apomictic progenitors. Families of this type are generated using natural sexual diploids or, if available, sexual polyploids (natural or artificially generated) as mother plants and apomicts as pollen donors. Backcrosses (F_1 sexual x sexual progenitor or F_1 apomictic x sexual progenitor) and F_2 (F_1 sexual x F_1 sexual or F_1 sexual x F_1 apomictic) are also recommended to confirm the results (Martínez *et al.* 2001). Progenies are then classified as apomictic or sexual by cytoembryological methods or progeny tests.

Detailed descriptions about the genetic control of apomixis in several species can be found in previous works (Nogler 1984a, Asker and Jerling 1992, Koltunow 1993, Pessino *et al.* 1999, Savidan 2000). Genetic analysis in *Panicum maximum* (Savidan 1981) and *Hieracium aurantiacum* (Bicknell *et al.* 2000) indicated the trait was under the control of a simple dominant factor. In these works, apomeiosis and parthenogenesis were shown to co-segregate strictly, suggesting that these two components rely on the same genetic control, or that parthenogenesis is a pleiotropic consequence of apomeiosis. However, the complexity of the apomictic process has lead to often conflicting interpretation of results in different species (Pessino *et al.* 1999).

In a way to simplify the analysis, most studies concentrated in the inheritance of apomeiosis (apospory or diplospory). In the grasses, after the pioneer work in *Panicum* (Savidan 1981) studies in *Pennisetum* (Sherwood *et al.* 1994), *Brachiaria* (Valle *et al.* 1994), *maize-Tripsacum* hybrids (Leblanc *et al.* 1995a) and *Paspalum* (Martínez *et al.* 2001) agreed in indicate that both apospory and diplospory were controlled by one dominant locus. Although in *Paspalum* and *Pennisetum* a strong distortion in the transmission of apospory was observed (Martínez *et al.* 2001, Roche *et al.* 2001).

A fine characterisation of the inheritance of apomixis in tetraploid *Tripsacum dactyloides*, a wild relative of maize, showed that diplospory segregated in a non-mendelian fashion (Grimanelli *et al.* 1998a). Linkage maps for the segment controlling diplospory in tetraploid apomicts were used to compare the same linkage group in diploid sexual plants. The data show that recombination is strongly suppressed in the segment that controls apomeiosis, which therefore behaves as a single genetic unit. This unit represents 40 cM on the map developed for sexual *Tripsacum*; this block probably contains several hundred genes, of which an unknown number would participate in apomixis (Grimanelli *et al.* 1998b).

A similar structure was found in *Pennisetum* where several markers belonging to an apospory specific genomic region (ASGR) co segregate strictly linked to apospory. This ASGR resulted partially hemizygous and showed a strong restriction in recombination (Ozias-Akins *et al.* 1998, Roche *et al.* 2001). Experiments using *in situ* hybridisation with markers completely linked to apospory in the species confirmed that a single chromosome is sufficient for the transmission of apomixis (and molecular markers linked to it) and showed that ASGR is located at the end of a single metacentric *P. squamulatum* chromosome (Goel *et al.* 2003).

In *Paspalum notatum* (Martínez *et al.* 2003, Stein *et al.* 2004) and *Panicum maximum* (Ebina *et al.* 2005) numerous molecular markers segregating completely linked to apospory were also detected. These particular distribution of markers in both species suggested a strong restriction in recombination around the apospory locus because it is unlikely that a small genomic region became saturated with markers while the rest of the genome do not. In *P. notatum* the chromosome block carrying the locus for apospory (in which numerous markers are completely linked) contains repetitive elements and could actually span for a segment of 25-30 cM (about 40 Mpb for the species) (Stein 2006). This particular chromosome segment showed also preferential chromosome pairing (disomic inheritance), while the rests of the genomic complement have a polisomic behaviour (Stein *et al.* 2004).

The lack of recombination associated with the control of apomeiosis can be associated with a strategy for avoiding dispersion of factors that need to co-segregate strictly if apomixis is to be determined (Grimanelli *et al.* 1998a).

Attempts to map apomeiosis with common molecular probes in several species, including *Tripsacum* (Grimanelli *et al.* 1998b), *Brachiaria* (Pessino *et al.* 1997 1998) and *Paspalum* (Pupilli *et al.* 2001, Martínez *et al.* 2003), all forage grasses, have shown the genomic regions that controls the trait are distinct (i.e. non-homologous) among those species. However, the region is conserved within the three species of the genus *Paspalum* (Pupilli *et al.* 2004). This means that apomixis, in its various forms, probably arose in different grass species through the action of different genetic loci (Grimanelli *et al.* 2001). Moreover, in particular cases, this gene(s) appeared to be associated with some lethal factor affecting part of the female or male gametes (Grimanelli *et al.* 1998b, Martínez *et al.* 2001, Pupilli *et al.* 2001).

Although it is the case for most apomicts studied, there are some exceptions. In the triploid *Erigeron annus*, diplospory and parthenogenesis are controlled by independent loci (Noyes and Rieseberg 2000). In *Poa pratensis* a recent analysis involving several segregating populations from inter-crossing and selfing of obligated and sexual and facultative apomictic plants proposed that five major genes control apomixis in the species. Differences in expressivities and interactions among them would be responsible for the inheritance and the wide variation of the mode of reproduction. In this system also apomeiosis and parthenogenesis segregated independently (Matzk *et al*. 2005).

2.3. Apomixis and Biotechnology improvements

Basically, two groups of procedures have been considered for transferring gametophytic apomixis into sexual species: i) wide hybridisation between a sexual plant and a wild apomictic relative; ii) genetic transformation of sexual cultivars with genes considered to control the onset of the character. The first approach has already been attempted in several species, generating apomictic backcross plants with additional chromosomes and a high degree of seed abortion (Spillane *et al.* 2004). The second one still remains hypothetical.

The first proposal aiming to introduce apomixis from wide crosses was applied nearly 40 years ago, by hybridisation of tetraploid maize with tetraploid *Tripsacum dactyloides* (Petrov *et al.* 1979). Similar attempts produced intra-specific hybrids of maize-*Tripsacum* that reproduce by apomixis (Kindiger *et al*. 1996a, Savidan 2000 2001). However, as hybrids obtained after a series of backcrosses are completely male sterile, progress in recovering the maize genome is strongly associated with the degree of facultativeness and hence to some expression of sexuality. The lack of such plants is at present challenging this strategy. An additional difficulty observed is the strict requirement of a 2 maternal: 1 paternal genome ratio necessary to develop the endosperm and set viable seeds in maize. In spite of these problems, significant advances have been achieved as a result of these works (Savidan 2000). The *Tripsacum* chromosome carrying the genes for apomixis was identified and compared with its syntenic region in maize (Kindiger *et al.* 1996b, Grimanelli *et al.* 1998b). Moreover, a segregation-distorter factor promoting the elimination of the apomictic alleles when transmitted by haploid gametes was proposed to explain the relationship between apomixis and polyploidy (Grimanelli *et al.* 1998b). Likewise, a program initiated at the end of the seventies (Hanna *et al*. 1993) attempting the introgression of apomixis into pearl millet (*Pennisetum glaucum*) from *P. squamulatum* is still being carried out. The transference of apomixis gene(s) in a breeding program was successfully performed in guinea grass (*Panicum maximum* Jacq.). "Natsukaze" was the first apomictic hybrid cultivar generated from crosses between a sexual plant and an unidentified tetraploid apomictic line through open pollination (Sato *et al*. 1990).

Genetic transformation of sexual cultivars with genes controlling the expression of the apomictic character is considered as a technical alternative. It opens future perspectives dependent on the identification of this kind of genes. In the last few years molecular marker technologies and molecular biology approaches have produced a considerable amount of new knowledge that could be useful for the future isolation of genes related to the trait. Several markers co-segregating with apomixis in grasses such as hybrids maize-*Tripsacum* (Leblanc *et al.* 1995, Kindiger *et al.* 1996b); *Pennisetum* (Gustine *et al.* 1997, Ozias-Akins *et al.* 1998), *Brachiaria* (Pessino *et al*. 1997 1998), *Paspalum* (Pupilli *et al.* 2001, Martínez *et al.* 2003, Stein *et al.* 2004) and *Panicum* (Ebina *et al.* 2005) have been reported. Markers linked to the trait are useful for studying the transmission of apomixis and for attempting any of the map-based cloning strategies. Efforts to disclose the component of the genomic region associated with apomixis using this approach have revealed its complex nature. In *Pennisetum squamulantum,* SCAR markers tightly liked to apospory were used for isolating the corresponding BACs clones (Roche *et al.* 2002). Classes of BAC clones grouped by apomixis-linked SCAR markers did not overlap, indicating that building a contig spanning the apomixis locus likely will require multiple walking steps (Roche *et al.* 2002). Thus, because the characteristic of the region involved (long chromosome segment with restriction in recombination and the presence of repetitive elements) cloning the critical genes involved in apomixis could represent an enormous effort.

The complexity of the genetic control of the trait is challenging the strategies for transferring apomixis to sexual crops by genetic engineering methodologies, since the gene responsible still has to be cloned and validated and probably it would be necessary to manipulate several factors that segregate independently.

Recent works focused on gene expression studies have reported the isolation of mRNA transcripts specific to flowers or ovaries at different developmental stages in several apomictic grasses such as *Pennisetum ciliare* (Vielle-Calzada *et al.* 1996), *Brachiaria* (Leblanc *et al.* 1997, Dusi 2001, Rodrigues *et al*. 2003), *Paspalum notatum* (Pessino *et al.* 2001), *Panicum maximum* (Chen *et al.* 1999) and *Poa pratensis* (Albertini *et al.* 2004 2005). Several cDNA sequences showed homologies with genes of known functions as *asg-1*: a gene similar to *rd22* of *Arabidopsis thaliana* which is induced in seed by drought (Chen *et al.*1999); *arp1*: from *P. notatum* similar to kinesin KatD of *Arabidopsis thaliana* (Pessino *et al.* 2001); *arp2* y *arp3* homologous to a SGT protein and an aldehyde dehydrogenase, respectively (Pessino S, pers. comm.); *PpSERK*: from *P. pratensis* similar to SERK (Albertini *et al.* 2004 2005). *ASG-1* expression in *Panicum maximum* and *Paspalum notatum* seems to be restricted to immature pollen grains and embryos of sexual and apomicts but specific to aposporous initial cells in ovules (Chen *et al.* 2005). In *Brachiaria brizantha*, a pseudogamous apomictic (Alves *et al.* 2001), cDNA specific from the developmental steps of aposporous and sexual ovaries showed homologies with myosin, exonucleases, a super-family of RecB, kinase MAP, aquaporin, a protein translocator factor and the ribosomal protein 60S (Rodrigues *et al*. 2003). *In situ* localization of the differential cDNA points to specific moments of the apomictic ovary development (unpublished results). Expression analysis of this type followed by characterisation of the possible biological role of the detected RNA transcripts can lead to the identification and cloning of genes involved in the early steps of apomictic development and may also contribute to isolate the trigger of apomixis itself.

Other procedures such as fine mapping around the apo-locus (Stein *et al*. 2004), transposon tagging and mutagenesis (Bicknell *et al*. 2001)

are currently being developed and can have an important role in detecting apomixis-related genes. For instance, approaches to generate apomictic mutants from sexual plants have not succeeded in recreating the character but allowed the identification of genes involved in the control of particular developmental steps that are characteristic of apomixis, such as the proliferation of endosperm in the absence of fertilisation (Grossniklaus 2001, Koltunow and Grossniklaus 2003).

3. CONCLUSION

Much work is being done in the world towards developing new improved ornamental plants from seeds. Many of the new varieties are hybrids. Cloning by seeds can be a tool for their breeding, eliminating segregation in the progeny. This practice can become available as a result of advances in apomixis research. Incorporating genes for apomixis can also contribute to developing new cultivars from varieties that are locally adapted, increasing the diversity of ornamental plants. This chapter aimed to introduce the many ways to identify apomixis, its incidence in flowering plants and the present state of knowledge in the field. The characterisation of the phenomenon will be improved with the advent of large-scale genomic analysis and high throughput gene discovery. Though numerous questions about gene action, function and regulation of apomixis still remain to be answered, a promising scenario is open for the near future. Our understanding of the molecular bases of apomixis has greatly increased in recent years, supported by the development of powerful molecular biology technologies and the interest in apomixis showed by research institutions and scientists. Many of the results obtained so far have already contributed to the breeding of apomictic species, many of which are important natural resources, and will contribute to programs aiming at the generation of improved and asexually seed-propagating crops.

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REFERENCES

Acuña CA, Martínez EJ, Quarin CL (2005) Sexual diploid and apomictic tetraploid races in *Thrasya petrosa* (Gramineae). *Australian Journal of Botany* 53, 479-484
Albertini E, Marconi G, Barcaccia G, Raggi L, Falcinelli M (2004) Isolation of candidate genes for apomixis in *Poa pratensis* L. *Plant Molecular Biology* 56, 879-894
Albertini E, Marconi G, Reale L, Barcaccia G, Porceddu A, Ferranti F, Falcinelli M (2005) *SERK* and *APOSTART*. Candidate genes for apomixis in *Poa pratensis Plant Physiology* 138, 2185-2199
Alves ER, Carneiro VTC, Araujo ACG (2001) Direct evidence of pseudogamy in an apomictic *Brachiaria brizantha* (Poaceae) *Sexual Plant Reproduction* 14, 207-212
Amsellem L, Pailler T, Noyer J-L, Hossaert-McKey M (2002) Characterization of pseudogamous apospory in the reproductive biology of the invasive weed *Rubus alceifolius* (Rosaceae) in its area of introduction. *Acta Botanica Gallica* 149, 217-224
Araujo ACG, Nobrega JM, Pozzobon MT, Carneiro VTC (2005) Evidence of sexuality in *Brachiaria brizantha* (Poaceae) artificial tetraploid plants and progeny. *Euphytica* 144, 39-50
Asker SE, Jerling L (1992) *Apomixis in Plants*, CRC Press Inc Boca-Raton, Florida, USA
Bashaw EC (1980) Apomixis and its application in crop improvement. In: Fehr WR, Hadley HH (eds) *Hybridization of Crop Plants*, ASA and CSSA, Madison, Wisconsin, pp 45-63
Bewley JD, Black M (1983) *Physiology and Biochemistry of Seeds, in Relation to Germination*, Springer-Verlag Berlin Heidelberg New York
Bicknell RA, Borst NK, Koltunow AM (2000). Monogenic inheritance of apomixis in two *Hieracium* species with distinct developmental mechanisms. *Heredity* 84, 228-237
Bicknell RA, Podivinsky E, Catanach A, Erasmuson S, Lambie S (2001) Strategies for isolating mutants in *Hieracium* with dysfunctional apomixis. *Sexual Plant Reproduction* 14, 227-232
Bicknell RA, Koltunow AM (2004) Understanding apomixis: recent advances and remaining conundrums *The Plant Cell* 16, S228-S245
Bonilla JR, Quarin CL (1997) Diplosporous and aposporous apomixis in a pentaploid race of *Paspalum minus Plant Science* 127, 97-104
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
Chen L, Miyazaki C, Kojima A, Saito A, Adachi T (1999) Isolation and characterization of a gene expressed during early embryo sac development in apomictic guinea grass (*Panicum maximum*). *Journal of Plant Physiology* 154, 55-62
Chen L, Guan L, Seo M, Hoffmann F, Adachi T (2005) Developmental expression of ASG-1 during gametogenesis in apomictic guinea grass (*Panicum maximum*). *Journal of Plant Physiology* 162, 1141-1148
Crane CF, Carman JG (1987) Mechanisms of apomixis in *Elymus rectisetus* from eastern Australia and New Zealand. *American Journal of Botany* 74, 477-496
Cronquist A (1982) *Basic Botany*, Harper and Row Publishers, NY
Czapik R (1994) How to detect apomixis in Angiospermae. *Polish Botanical Studies* 8,13-21
Daurelio DL, Espinoza F, Quarin CL, Pessino SC (2004) Genetic diversity in sexual diploid and apomictic tetraploid populations of *Paspalum notatum* situated in sympatry or allopatry. *Plant Systematics and Evolution* 244, 189-199
Dickinson TA, Belaoussoff S, Love RM, Muniyamma M (1996) North American black-fruited hawthorns. I. Variation in floral construction, breeding system correlates, and their possible evolutionary significance in Crataegus sect. Douglasii Loudon. *Folia Geobotanica et Phytotaxonomica* 31, 355-371
Douglas Brede A, Huff DR, Funk CR (1993) Registration of "Nublue"Kentucky Blegrass *Crop Science* 33, 1407-1408
Douglas Brede A, Willard WE (1993) Registration of "NuStar" Kentucky Blegrass *Crop Science* 33, 1414-1415
Durand J, Garnier L, Dajoz I, Mousset S, Veuille M (2000) Gene flow in a facultative apomictic Poacea, the savana grass *Hyparrhenia diplandra. Genetics* 156, 823-831
Dusi DMA (2001) Apomixis in *Brachiaria decumbens* Stapf. PhD thesis, University of Wageningen, Wageningen, 167 pp
Dusi DMA, Willemse MTM (1999) Apomixis in B*rachiaria decumbens* Stapf.: gametophytic development and reproductive calendar *Acta Biologica Cracoviensia Series Botanica* 41, 151-162
Ebina M, Nakagawa H, Yamamoto T, Araya H, Tsuruta S, Takahara M, Nakajima K (2005) Co-segregation of AFLP and RAPD markers to apospory in guineagrass (*Panicum maximum* Jacq.). *Grassland-Science* 51, 71-78
Galbraith DW (1989) Analysis of nuclear DNA content in plant cell by flow cytometry. ***Biologia Plantarum*** 31**, 113-120**
Gobbe J, Longly B, Louant BP (1982) Calendrier des sporogénèse et gametogénèse femelles chez le diploïde et le tetraploïde induit de *Brachiaria rhuziziensis* (Graminée). *Canadian Journal of Botany* **60**, 2032-2037
Gobbe J, Swenne A, Louant BP (1981) Diploïdes naturels et autotetraploïdes induits chez *Brachiaria ruziziensis* Germain et Evrard: critères d'identification. *Agronomie Tropicale* 36, 339-346
Goel S, Chen Z, Conner JA, Akiyama Y, Hanna WW, Ozias-Akins P (2003) Delineation by fluorescence *in situ* hybridization of a single hemizygous chromosomal region associated with aposporous embryo sac formation in *Pennisetum squamulatum* and *Cenchrus ciliaris*. *Genetics* 163, 1069-1082
Grimanelli D, Leblanc O, Espinosa E, Perotti E, Gonzales de Leon D, Savidan Y (1998a) Non-Mendelian transmission of apomixis in maize-*Tripsacum* hybrids caused by a transmission ratio distortion. *Heredity* **80**, 40-47
Grimanelli D, Leblanc O, Espinosa E, Perotti E, Gonzales de Leon D, Savidan Y (1998b) Mapping diplosporous apomixis in tetraploid *Tripsacum:* one gene or several genes? *Heredity* **80**, 33-39
Grimanelli D, Leblanc O, Perotti E, Grossniklaus U (2001) Developmental genetics of gametophytic apomixis. *Trends in Genetics* 17, 597-604
Grossniklaus U (2001) From sexuality to apomixis: molecular and genetic approaches, p. 168-211. In: Savidan Y, Carman JG, Dresselhaus T (eds) *The Flowering of Apomixis: From Mechanisms to Genetic Engineering*, CIMMYT, IRD, European Commission DG VI (FAIR), Mexico, DF
Grunewaldt J (1988) General aspects of genetics in plant propagation. *Acta Horticulturae* 266, 277-282
Gustine DL, Sherwood RT, Huff DR (1997) Apospory-linked molecular markers in buffelgrass *Crop Science* 37, 947-951
Hanna WW, Bashaw EC (1987) Apomixis: its identification and use in plant breeding. *Crop Science* 27, 1136-1139
Hanna WW, Dujardin M, Ozias-Akins P, Lubbers E, Arthur L (1993) Reproduction, cytology and fertility of pearl millet x *Pennisetum squamulatum* BC4 plants. *Journal of Heredity* 84, 213-216
Harlan JR, Brooks MH, Borgaonkar DS, de Wet JMJ (1964) Nature and inheritance of apomixis in *Bothriochloa* and *Dichanthium*. *Botanical Gazette* 125, 41-46

Herr JM (1971) A new clearing-squash technique for the study of ovule development in angiosperms. *American Journal of Botany* 58, 785-790
Humphreys MO (2005) Molecular breeding for the genetic improvement of forage crops and turf. *Proc. 4th Intl. Symp. Mol. Breed. Forage Turf*, a Sat. Workshop of the XXth Intl. Grassland Cong. July 2005, Aberystwyth, Wales, UK
Jefferson RA (1994) Apomixis: A social revolution for agriculture? *Biotechnology and Development Monitor* 19, 14-16
Kindiger B, Bai D, Sokolov V (1996a) Assignment of a gene(s) conferring apomixis in *Tripsacum* to a chromosome arm: cytological and molecular evidence. *Genome* 39, 1133-1141
Kindiger B, Sokolov V, Dewald CL (1996b) A comparison of apomictic reproduction in eastern gamagrass (*Tripsacum dactyloides* (L.) L) and apomictic maize-*Tripsacum* hybrids. *Genetika* 97, 103-110
Koltunow AM (1993) Apomixis: Embryo sacs and embryos formed without meiosis or fertilization in ovules. *The Plant Cell* 5, 1425-1437
Koltunow AM, Grossniklaus U (2003) Apomixis: a developmental perspective. *Annual Review of Plant Biology* 54, 547-574
Lakshmanan KK, Ambegaokar KB (1984) Polyembryony. In: Johri BM (ed) *Embryology of Angiosperms* Springer-Verlag, Berlin, pp 445-474
Leblanc O, Armstead I, Pessino S, Ortiz JPA, Evans E, Valle CB do, Hayward MD (1997) Non-radioactive mRNA fingerprinting to visualize gene expression in mature ovaries of *Brachiaria* hybrids derived from *B. brizantha*, an apomictic tropical forage. *Plant Science* 126, 49-58
Leblanc O, Grimanelli D, González-de-León D, Savidan Y (1995b) Detection of the apomictic mode of reproduction in maize-*Tripsacum* hybrids using maize RFLP markers. *Theoretical and Applied Genetics* **90**, 1198-1203
Leblanc O, Hernández M, Bello S, Garcia V, Bertaud J, Savidan Y (1995a) Chromosome doubling in *Tripsacum*: the production of artificial, sexual tetraploid plants. *Plant Breeding* 114, 226-230
Lutts S, Ndikumana J, Louant BP (1994) Male and female sporogenesis and gametogenesis in apomitic *Brachiaria brizantha*, *Brachiaria decumbens* and F1 hybrids with sexual colchicine induced tetraploid *Brachiaria ruziziensis*. *Euphytica* 78, 19-25
Marasek A, Hasterok R, Wiejacha K, Orlikowska T (2004) Determination by GISH and FISH of hybrids status in *Lilium*. *Hereditas* **140**, 1-7
Martínez EJ, Quarín CL, Hayward MD (1999) Genetic control of apospory in apomictic *Paspalum* species. *Cytología* 64, 452-433
Martínez EJ, Hopp HE, Stein J, Ortiz J-PA, Quarin CL (2003) Genetic charcterization of apospory in tetraploid *Paspalum notatum* based on the identification of linked molecular markers. *Molecular Breeding* 12, 319-327
Martínez EJ, Urbani MH, Quarin CL, Ortiz J-PA (2001) Inheritance of apomixis in bahiagrass, *Paspalum notatum. Hereditas* 135, 19-25
Matzk F, Prodanovic S, Baumlein H, Schubert I (2005) The inheritance of apomixis in *Poa pratensis* confirms a five locus model with differences in gene expressivity and penetrance *Plant Cell* 17, 13-24
Mazzucato A, Barcaccia G, Pezzotti M, Falcinelli M (1995) Biochemical and molecular markers for investigating the mode of reproduction in the facultative apomict *Poa pratensis*. *Sexual Plant Reproduction* 8, 133-138
Mendes-**Rodrigues C, Carmo-Oliveira R, Oliveira PE** (2004) Embrionia adventícia e apomixia em Bombacaceae. In: Carneiro VTC, Dusi DMA (eds) *Clonagem de Plantas por Sementes: Estratégias de Estudo da Apomixia*, Brasília-DF Brazil Embrapa, v.1 pp 35-46
Naumova T, Den Nijs APM, Willemse MTM (1993) Quantitative analysis of aposporous parthenogenesis in *Poa pratensis* genotypes. *Acta Botanica Neerlandica* 42, 299-312
Naumova T, Willemse MTM (1982) Nucellar polyembryony in *Sarcococca humilis* - ultrastructural aspects. *Phytomorphology* 32, 94-108
Naumova T, Willemse MTM (1995) Ultrastructural characterization of apospory in *Panicum maximum*. *Sexual Plant Reproduction* 8, 197-204
Negron-Ortiz V (1998) Reproductive biology of a rare cactus, *Opuntia spinosissima* (Cactaceae), in the Florida keys: why is seed set very low? *Sexual Plant Reproduction* 11, 47-55
Nogler GA (1984a) Gametophytic apomixis. In: Johri BM (ed) *Embryology of Angiosperms*, Springer-Verlag, Berlin, pp 475-518
Nogler GA (1984b) Genetics of apospory in apomictic *Ranunculus auricomus* V. Conclusions *Botanica Helvetica* 94, 411-422
Nogler GA (1994) Genetics of gametophytic apomixis - A historical sketch. *Polish Botanical Studies* 8, 5-11
Noyes RD, Rieseberg LH (2000) Two independent loci control agamospermy (apomixis) in the triploid flowering plant *Erigeron annuus*. *Genetics* 155, 379-390
Ortiz J-PA, Pessino SC, Leblanc O, Hayward MD, Quarin CL (1997) Genetic fingerprinting for determining the mode of reproduction in *Paspalum notatum,* a subtropical apomictic forage grass. *Theoretical and Applied Genetics* 95, 850-856
Ozias-Akins P, Roche D, Hanna WW (1998) Tight clustering and hemzygosity of apomixis-linked molecular markers in *Pennisetum squamulatum* implies genetic control of apospory by a divergent locus that may have no allelic form in sexual genotypes. *Proceedings of the National Academy of Sciences USA* 95, 5127-5132
Pozzobon MT, Valls JM (1997) Chromosome number in germplasm accessions of *Paspalum notatum* (Gramineae). *Brazilian Journal of Genetics* **20**, 29-34
Peel MD, Carman JG, Wu L-Z, Wang RRC (1997) Meiotic anomalies in hybrids between wheat and apomictic *Elymus rectisetus* (Ness in Lehm.) A. Love & Connor. *Crop Science* 37, 717-723
Persson Hovmalm HA, Jeppsson N, Bartish IV (2004). RAPD analysis of diploid and tetraploid populations of Aronia points to different reproductive strategies within the genus. *Hereditas* 141, 301-312
Pessino SC, Ortiz JPA, Leblanc O, Valle CB do, Evans C, Hayward MD (1997) Identification of a maize linkage group related to apomixis in *Brachiaria*. *Theoretical and Applied Genetics* 94, 439-444
Pessino SC, Evans C, Ortiz JPA, Armstead I, Valle CB do, Hayward MD (1998) A genetic map of the apospory-region in *Brachiaria* hybrids: identification of two markers closely associated with the trait. *Hereditas* 128, 153-158
Pessino SC, Ortiz JPA, Hayward MD, Quarin CL (1999) The molecular genetics of gametophytic apomixis*. Hereditas* **130**, 1-11
Pessino SC, Espinoza F, Martínez EJ, Ortiz J-P, Valle EM, Quarin CL (2001) Isolation of cDNA clones differentially expressed in flowers of apomictic and sexual. *Paspalum notatum Hereditas* 134, 35-42
Petrov DF, Belousova NI, Fokina ES (1979) Inheritance of apomixis and its elements in corn-*Tripsacum* hybrids. *Genetika* 15, 827-836
Pinheiro AA, Pozzobon MT, Valle CB do, Penteado MIO, Carneiro VTC (2000) Duplication of the chromosome number of diploid *Brachiaria brizantha* plants, using colchicine. *Plant Cell Reports* 19, 274-278
Pupilli F, Lambobarda P, Quarin CL, Arcioni S (2001) The chromosome segment related to apomixis in *Paspalum* simplex is homologous to the telomeric region of the long arm of rice chromosome. *Molecular Breeding* 8, 53-61
Pupilli F, Martinez EJ, Busti A, Calderini O, Quarin CL, Arcioni S (2004) Comparative mapping reveals partial conservation of synteny at the apomixis locus in *Paspalum* spp. *Molecular Genetics & Genomics* **270**, 539-548
Quarin CL (1992) The nature of apomixis and its origin in Panicoid grasses. *Apomixis Newsletter* 5, 8-15
Quarin CL, Espinoza F, Martínez EJ, Pessino SC, Bovo OA (2001) A rise of ploidy level induces the expression of apomixis in *Paspalum notatum*. *Sexual Plant Reproduction* 13, 243-249
Roche D, Chen Z, Hanna WW, Ozias-Akins P (2001) Non-mendelian transmission of an apospory-specific genomic region in a reciprocal cross between sexual pearl millet (*Pennisetum glaucum*) and an apomictic F1 (P. *glaucum x P. squamulatum*). *Sexual Plant Reproduction* 13, 217-223
Rodrigues JCM, Cabral GB, Dusi DMA, Mello LV, Rigden D, Carneiro VTC (2003) Identification of differentially expressed cDNA sequences in ovaries of sexual and apomictic plants of *Brachiaria brizantha. Plant Molecular Biology* 53, 745-757
Sato H, Shimizu N, Nakagawa H, Nakajima K (1990) A new registered cultivar "Natsukase" of guineagrass. *Japan Agricultural Research Quarterly* 23, 196-201
Savidan Y (1981) Genetics and utilization of apomixis for the improvement of Guineagrass (*Pannicum maximum*). Proc. XIV Int. Grass Cong, Lexington, KY, USA, pp 182-184
Savidan Y (2000) Apomixis: Genetics and breeding. *Plant Breeding Reviews* 18, 13-86
Savidan Y (2001) Transfer of apomixis through wide crosses. In: Savidan Y, Carman JG, Dresselhaus T (eds) The flowering of apomixis: from mechanisms to genetic engineering. CIMMYT, IRD, European Commission DG VI (FAIR), Mexico, DF, pp 153-166
Schmidt JM, Antlfinger AE (1992) The level of agamospermy in a nebraska population of *Spiranthes cernua* (Orchidaceae). *American Journal of Botany* 79, 501-507
Sherwood RT, Berg CC, Young BA (1994) Inheritance of apospory in buffalo grass. *Crop Science* 34, 1490-1494
Spillane C, Curtis MD, Grossniklaus U (2004) Apomixis technology development-virgin births in farmers' fields? *Nature Biotechnology* 22, 687-691
Stein J, Quarin CL, Martínez EJ, Pessino SC, Ortiz J-P A (2004) Tetraploid races of *Paspalum notatum* show polysomic inheritance and preferential chromosome pairing around the apospory-controlling locus. *Theoretical and. Applied Genetics* **109,** 186-191
Stein J (2006) Caracterización del grupo de ligamiento donde se localiza el locus responsable de la aposporía. In: Estudios de localización y herencia de genes relacionados con la apomixis en razas tetraploides de *P. notatum*. PhD Tesis, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario (UNR), pp 158
Terziiski D, Jurukova-Grancharova P, Robeva P, Daskalova T (1995) Contribution to the embryology of genus Achillea L. (Asteraceae). *Nauka-za-Gorata* 32, 36-42
Tucker MR, Araujo ACG, Paech N, Hecht V, Schmidt EDL, Rossel J-B, De Vries SC, Koltunow AMG (2003) Sexual and apomictic reproduction in *Hieracium* subgenus Pilosella are closely interrelated developmental pathways *Plant Cell* 15, 1524-1537
Valle CB do, Glienke C, Leguisamon GOC (1994) Inheritance of apomixis in *Brachiaria*, a tropical forage grass. *Apomixis Newsletter* 7, 42-43
van Baarlen P, de Jong HJ, van Dijk PJ (2002) Comparative cyto-embryological investigations of sexual and apomictic dandelions (*Taraxacum*) and their apomictic hybrids. *Sexual Plant Reproduction* 15, 31-38
Vielle-Calzada J-P, Nuccio ML, Budiman MA, Thomas TL, Burson BL, Hussey MA, Wing RA (1996) Comparative gene expression in sexual and apomitic ovaries of *Pennisetum ciliari* (L.) Link. *Plant Molecular Biology* 32, 1085-1092
Werlemark G (2000) Evidence of apomixis in hemisexual dogroses, Rosa section Caninae. *Sexual Plant Reproduction* 12, 353-359
Werpachowski JS, Varassin IG, Goldenberg R (2004) Ocorrência de apomixia e partenocarpia em algumas espécies subtropicais de *Asteraceae. Revista Brasileira de Botânica* 27, 607-613
Willemse MTM, van Went JL (1984) The female gametophyte. In: Johri BM (ed) *Embryology of Angiosperms*, Springer-Verlag, Berlin, pp 159-191
Young BA, Sherwood BT, Bashaw EC (1979) Cleared-pistil and thick-sectionning techniques for detecting aposporous apomixis in grasses. *Canadian Jounal of Botany* 57, 1668-1672