# IOWA STATE UNIVERSITY

## Course: Plant Genetics

# Breeding in Apomictic Species

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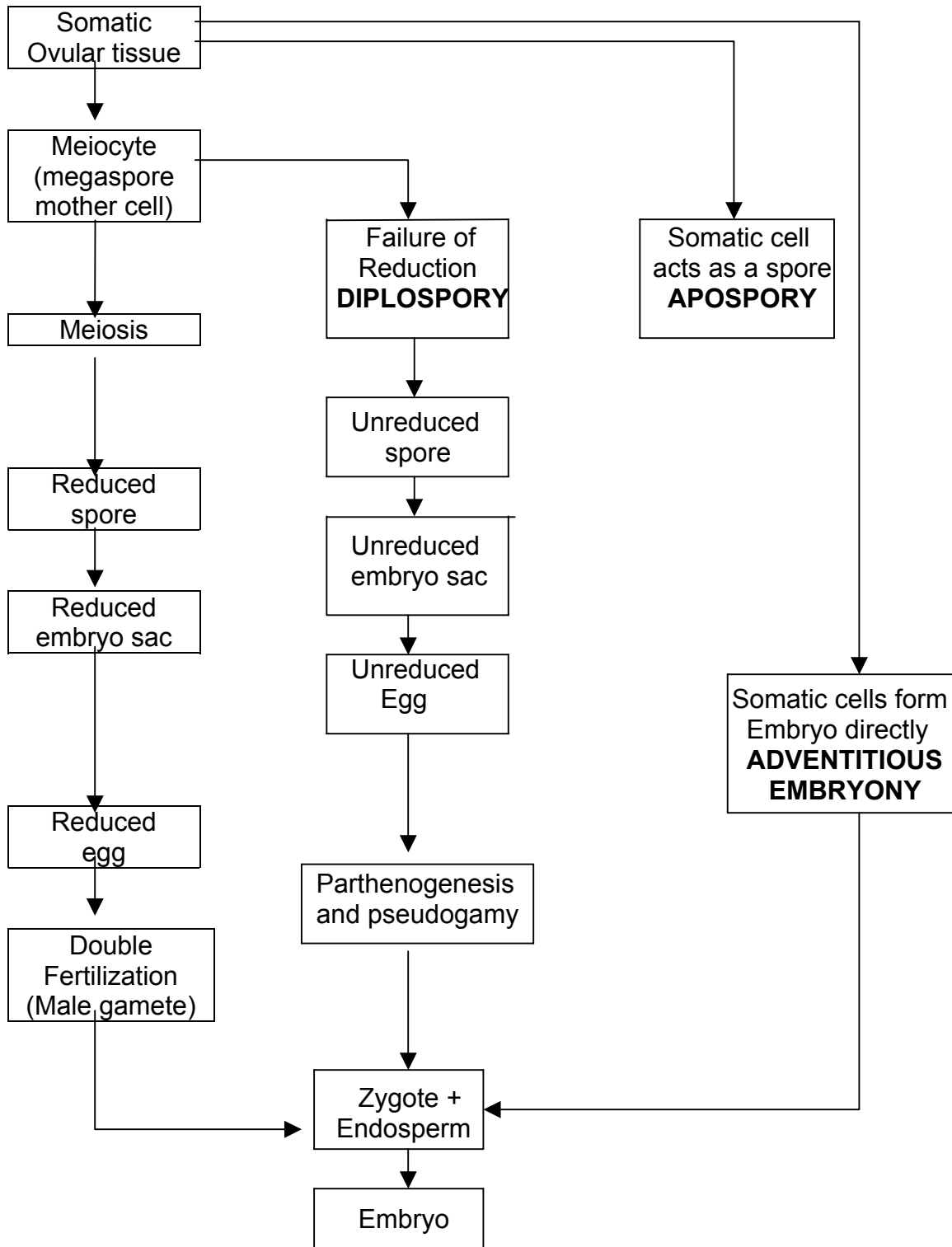Ames, IA.                              Spring Semester, 2002

## Introduction

In apomictic reproduction, an embryo is formed directly from a chromosomally unreduced megaspore mother cell or from a somatic cell of the nucellus or ovule. Apomixis makes vegetative reproduction or cloning through the seed possible. It fixes vigor by allowing a plant to clone itself indefinitely through seed.

Apomixis has received increased research emphasis in the past 20 years due to discoveries of partially apomictic (facultative) plants in cultivated species, discoveries of sexual plants in apomictic species, new information on genetic control and a broader awareness of the impact that apomixis could have on cultivar development.

Apomixis could have a major impact on seed-propagated food, forage and fiber production around the world. It would especially be beneficial in the major annual grain crops such as wheat, rice and soybean where hybrid vigor is present but systems for commercially producing hybrids may not be available and economical. In crops such as maize, sorghum and pearl millet, commercial hybrid production systems are available but apomixis could have a major impact by simplifying hybrid seed production and by making hybrids readily available and affordable in developing countries.

## Types of Apomixis

Many different classifications of apomixis types and pathways of development have been proposed over the years but the most widely accepted and easiest to use is that classification proposed by Nogler (1984). We are not going to describe each of the pathways in the present paper to avoid repetition of topics presented by the previous group. That could be resume in the following figure

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┌─────────────┐                                                    ┌──────────────────┐
│   Somatic   │───────────────────────────────────────────────────┤                  │
│Ovular tissue│──────────────────────────────┐                     │                  │
└──────┬──────┘                               │                     │                  │
       │                                      │                     │                  │
       ▼                                      │                     │                  │
┌─────────────┐                               │                     │                  │
│  Meiocyte   │──────────────────┐            │                     │                  │
│ (megaspore  │                  │            │                     │                  │
│mother cell) │                  │            │                     │                  │
└──────┬──────┘                  ▼            ▼                     │                  │
       │              ┌──────────────┐  ┌──────────────┐            │                  │
       ▼              │  Failure of  │  │ Somatic cell │            │                  │
┌─────────────┐       │  Reduction   │  │acts as a spore│           │                  │
│   Meiosis   │       │  DIPLOSPORY  │  │  APOSPORY    │            │                  │
└──────┬──────┘       └──────┬───────┘  └──────────────┘            │                  │
       │                     │                                      │                  │
       │                     ▼                                      │                  │
       │              ┌──────────────┐                              │                  │
       ▼              │  Unreduced   │                              │                  │
┌─────────────┐       │    spore     │                              │                  │
│   Reduced   │       └──────┬───────┘                              │                  │
│    spore    │              │                                      │                  │
└──────┬──────┘              ▼                                      │                  │
       │              ┌──────────────┐                              │                  │
       ▼              │  Unreduced   │                              │                  │
┌─────────────┐       │  embryo sac  │                              │                  │
│   Reduced   │       └──────┬───────┘                              │                  │
│ embryo sac  │              │                                      ▼                  │
└──────┬──────┘              ▼                          ┌──────────────────┐           │
       │              ┌──────────────┐                  │Somatic cells form│           │
       │              │  Unreduced   │                  │ Embryo directly  │           │
       ▼              │     Egg      │                  │  ADVENTITIOUS    │           │
┌─────────────┐       └──────┬───────┘                  │    EMBRYONY      │           │
│   Reduced   │              │                          └────────┬─────────┘           │
│     egg     │              │                                                         │
└──────┬──────┘              ▼                                                          │
       │              ┌──────────────┐                                                 │
       ▼              │Parthenogenesis│                                                │
┌─────────────┐       │and pseudogamy│                                                 │
│   Double    │       └──────┬───────┘                                                 │
│Fertilization│              │                                                         │
│(Male gamete)│              │                                                         │
└──────┬──────┘              ▼                                                          │
       │              ┌──────────────┐                                                 │
       └─────────────▶│   Zygote +   │◀────────────────────────────────────────────────┘
                      │  Endosperm   │
                      └──────┬───────┘
                             │
                             ▼
                      ┌──────────────┐
                      │    Embryo    │
                      └──────────────┘
```

Some examples of apomictic genera and their reproduction modes are given in the next table.

| Genera | Reproduction mode |
|---|---|
| Eragrostis | Diplospory |
| Chloris | Apospory |
| Brachiaria | Apospory |
| Cenchrus | Apospory |
| Panicum | Apospory |
| Paspalum | Apospory and Diplospory |
| Penisetum | Apospory |
| Tripsacum | Dipospory |
| Cytrus | Adventitious embryony |

**Characteristic features of Apomixis**

Apomixis affects both megasporegenesis and megagametogenesis, by usually does not alter pollen formation. Meiosis still occurs normally in the anthers, and viable, reduced pollen is usually produced in both aposporous and diplosporous apomicts.

a) Facultativeness. Apomixis is said to be facultative when some progeny also result from either a normal meiosis and/or a normal fertilization of the egg cell. Apomixis is said to be obligate when the progeny is 100% maternal. Some authors proposed that obligate apomixis may only be an artifact, due to several causes: The level of analysis, the size of flower/plant population analyzed and the screening tools.  The most striking success in apomictic forage grasses today, achieved with the genera *Brachiaria* and *Panicum* in Latin America, are related to tropical species that are facultative. For practical purposes, such as breeding, most apomicts are considered nearly obligate, i.e. the rate of offtype may be sufficiently low to be acceptable. Otherwise, it was shown in a few cases that facultativeness can be modified through selection.

b) Polyploidy: A common feature of wild apomicts is that except for a few academic exceptions, some of them possibly controversial, all apomicts are polyploids, and most of them are tetraploid. Polyhaploids found in the Dichanthium-Bothriochloa complex were usually very weak and totally male and female sterile. Two theories have been presented to explain the almost complete absence of apomixis at the diploid level. Nogler 1982 assumed that the dominant allele responsible for apomeiosis could not be transmitted through haploid gametes, meaning apomixis could not be recovered in wild diploids. The second hypothesis is that there is a dosage requirement in the number of dominant alleles present in any individual for apomixis to be expressed. Absence of

apomixis in wild diploids would, according with this theory, be due to lack of expression instead of non-transmission.

Another important question, with clear implications in breeding is whether their polyploidy is auto or allopolyploidy, for example, Paspalum apomictic tetraploid are mostly autotetraploid as well as Panicum. Most of the other apomictic genera are allopolyploids. The apparent controversy may result from the absence of any clear relation between the type of polyploidy and the presence of apomixis, which may essentially be trigged by a dominant allele at one single locus.


**Genetic control**

Genetic studies on the apomictic mechanisms have been difficult to conduct and many times are inconclusive because apomixis may not allow needed crosses and backcrosses to be made and segregating progenies to be observed. Understanding the inheritance of apomixis becomes more complex when plants reproduce by facultative apomixis. Unfortunately, genes controlling apomixis have not been discovered in most of our major cultivated species. However, genes controlling apomixis can probably be found in the wild species of the genus or related genera of most major cultivated crops in the world.

Progress is being made in understanding the genetic control of apomixis in various crops. It is generally concluded that apomixis is controlled by qualitative inheritance. The simple genetic control of apomixis improves the potencial for manipulating this reproductive mechanism and transferring it to other species. Efforts directed at determining the genetic basis of apomixis in several species have generally shown it to be under simple genetic control, for example in Bothriochloa, Panicum, Cenchrus, Paspalum, Brachiaria, Tripsacum, and possibly Eragrostis. Hence it should be possible to manipulate apomixis in a breeding program once cross-compatible sexual or highly sexual facultative apomicts are found.

Before genes controlling apomixis can be used, they have to be identified. Developments in molecular biological techniques should make it possible to clone desirable apomixis gene and transfer it into any crop. Although the previous statement sounds simple, much research is needed to locate molecular markers, clone the genes, insert the gene into a recipient specie, and get the gene to express itself phenotypically. Progress is being made in development molecular markers. Use of mutagens to produce mutant genes that cause plants to reproduce apomictically may be another source. Plants that reproduce by facultative apomixis have been induced in P. glaucum. Apomixis is expressed at a variable but generally low level in these mutants.

Phenotypic expression of a gene controlling apomixis is important in both, the originating species and in plants of the recipient species, genera, or family if the

gene is transferred. First, it should be noted that technically there are few obligate apomics because offtypes can usually be found if large enough population are observed in most apomictic species. In the other hand, apomictic reproduction in facultative types should be at a high enough level to provide the uniformity needed and to preserve the vigor of a particular genotype for its intended use. The amount of facultative reproduction that can be tolerate in a cultivar depend on its use. More offtype variation may be tolerated in a forage cultivar than in a grain crop where uniformity in height and maturity is critical for mechanical harvesting. Gene expression becomes even more critical when genes controlling apomixis are transferred from different species, genera and/or families. Within a species, apomixis may be almost obligate; however, in a different genetic background it may be expressed differently or with collateral effects. For example, sexual pearl millet x obligate apomictic *P. setacum* hybrids are obligate apomictic but are highly male and female sterile. High male sterility and facultative apomixis were observed in sexual maize x *Tripsacum dactyloides.* Environmental conditions are able to influence apomictic expression in some species. Some authors reported that application of inorganic salts to the growing media changed the frequencies of sexual and apomictic embryo sacs in Cenchrus ciliaris.


**Potential advantages of apomixis**

Introducing apomixis in a breeding program could have several advantages.
 When a gene(s) for apomixes is introduced in a sexual species, all germplasm within a species has potential as a parent of a new hybrid. The genotype of every apomictic is fixed in the $F_1$ generation and every apomictic genotype from a cross has the potential of being a cultivar. Gene combinations and vigor are not lost as in each segregating generation of sexual $F_1$ hybrids. The maintenance of elite genotypes is easy and efficient and there is no need of isolation to produce high quality pure seed.

Planting true breeding seeds from apomictic reproduction would have many advantages over tuber propagation in crops such as potato. Seeds would reduce the propagation and spread of diseases and virus, which are readily transmitted through the tubers. In addition, seed propagation by apomixes would greatly reduce the storage, shipping and planting costs and volume compared to tuber propagation.

In a commercial hybridization program where male sterility is used, apomixis eliminates the need to develop and maintain A-lines (male sterile), B-lines (male fertile maintainers) and R-lines (restorer lines). The A, B and R lines require time and testing for their development and space as well as isolation to maintain them. The development of A-lines rapidly narrows both nuclear and cytoplasmic gene pools that can be used to develop stable male sterility systems. Likewise, a search for R-lines to completely restore the A-lines again narrows the gene pool.

Apomixis would allow breeders to precisely engineer plants. It would allow one to develop genotypes with characteristics such as quality, responses to management and maturity that are highly reproducible from field to field and year to year. At the same time, a number of apomictic genotypes could be mixed together in various combinations to enhance genetic diversity to accomplish a specific goal.

## Breeding methods

Before beginning the description of some breeding methodologies, it is necessary to mention that the identification of apomictic plants is one of the most important points in any breeding program. This can be done by progeny testing open pollinated seed from selected plants. Morphologically variable progeny from a plant would indicate sexual origin. The frequency of uniform or maternal progeny from a plant would indicate the level of apomictic reproduction. At least 20 to 25 progenies are needed to obtain a reliable estimate of a plant's reproductive behavior, especially if it reproduces by facultative apomixis.

Cytological observations are more rapid than progeny testing for identifying the method of reproduction. New ovule-clearing techniques allow one to classify the reproductive behavior of a plant within 2 or 3 days after collecting the ovaries. In *Pennisetum, Paspalum* and *Panicum*, it is possible to collect a few flowers at the beginning of anthesis and to classify the reproductive behavior of the plant before it completes anthesis. Apospory and adventitious embryony are the apomictic mechanism easiest   to identify at anthesis; apospory can be identified by the presence of multiple embryo sacs, lack of antipodal development and shape and orientation of embryo sacs in the ovule; and adventitious embryony because the embryo develops as a bud-like structure through mitotic division of somatic cells of the ovule, integuments or ovary wall.

Diplospory is more difficult to identify and requires cytological observations at earlier ovule development than the two previous mechanisms. Lack of meiosis or a linear tetrad of megaspores is the best evidence for diplospory. It has been reported that the lack of fluorescing callose in the walls of dyads, tetrads and megaspore mother cells is also an indication for diplospory.
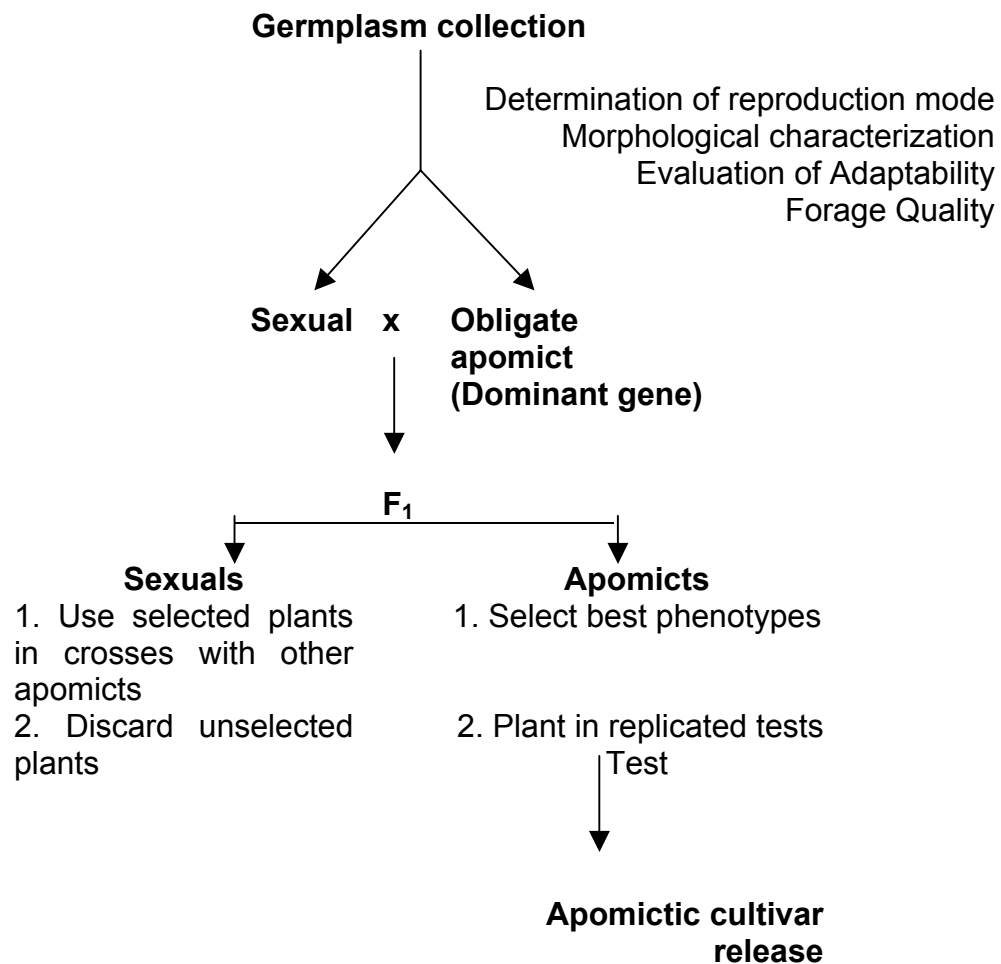
The ideal apomictic mechanism in a breeding program would be one that it is controlled by a dominant gene(s), is environmentally stable and reproduced only by obligate apomixis, especially when sexual counterparts are available for crossing with the apomictics. Indeed, obligate apomixis poses a formidable barrier to plant breeding: without new gene combinations that result from sexual cross breeding, genetic improvement cannot occur, except by rare, random and generally deleterious mutations. In truth, sexual or partially sexual plants have been found in native populations of most apomictic species, generating sufficient genetic variation to maintain the species under changing environments and

providing germplasm for plant improvement. Representative collections for most of the tropical apomictic grasses are limited; therefore, a key prerequisite for effective tropical forage breeding programs is to acquire diverse germplasm from the centers of origin of the target genus or species.

In a breeding program, it must be remembered that obligate apomictic plants can only be used as male parents in crosses. Microsporogenesis does function in apocmitics with resultant genetically recombine and chromosomally reduced male gametes. An apomictic plant must have some pollen fertility if it is to be used in a breeding program.

In order to simplify the following discussion and figures, it would be assume that apomixis is controlled by a single gene in a diploid plant. The results from various crosses would be modified and made more complex by modifiers genes, genetic background, ploidy, and if more than one gene controls apomictic reproduction.
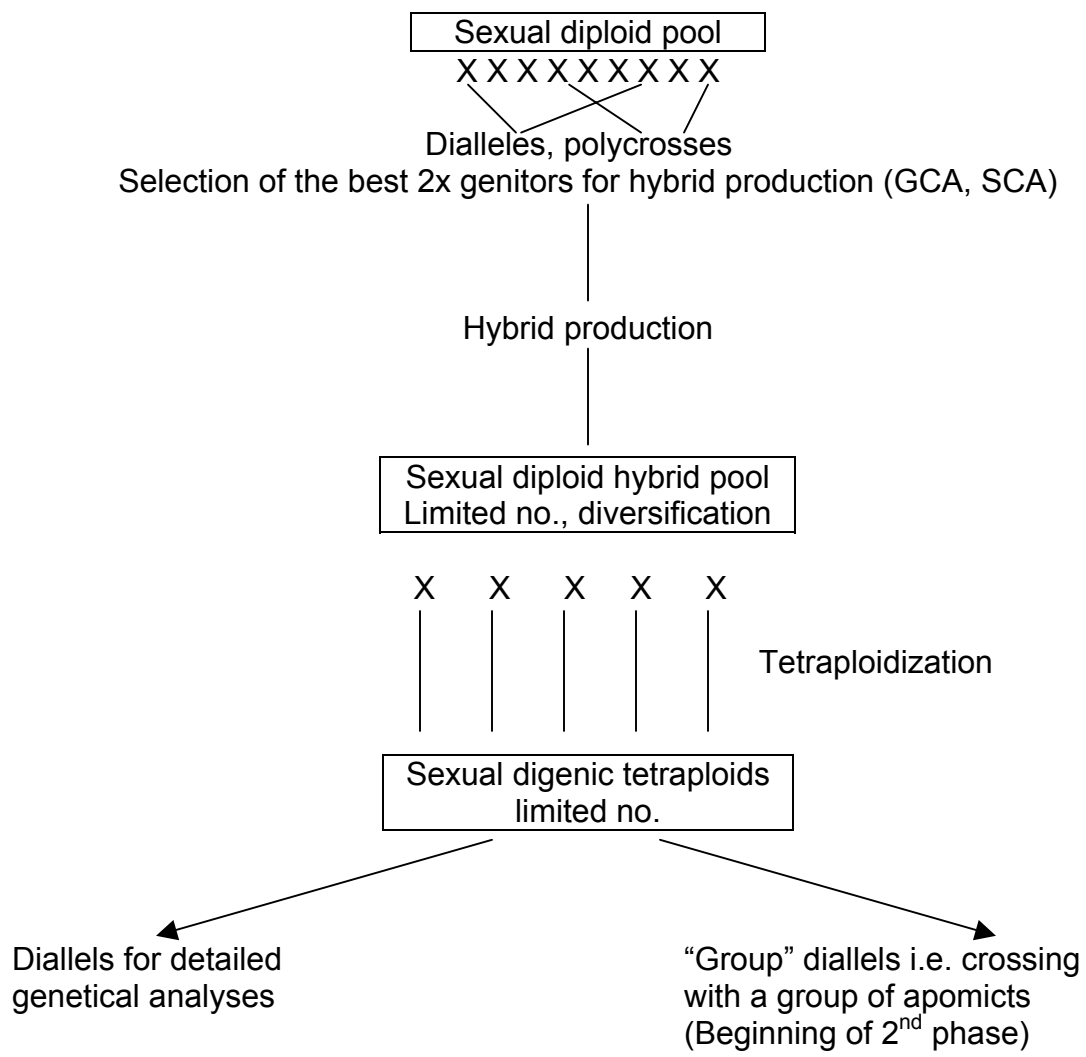
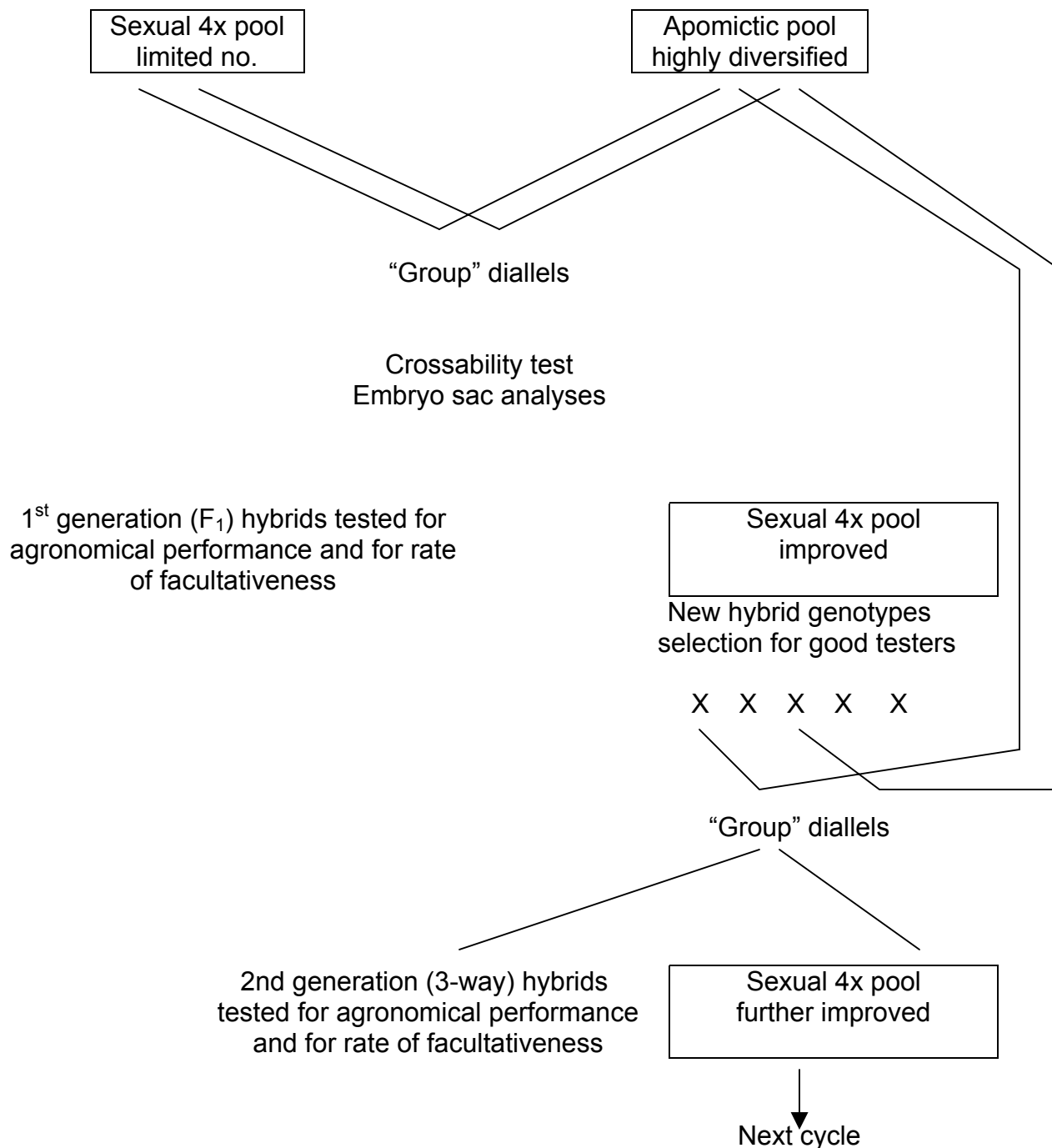A general selection and breeding scheme for apomictic forage species is presented in the following figure:

**Germplasm collection**

Determination of reproduction mode
Morphological characterization
Evaluation of Adaptability
Forage Quality

**Sexual  x  Obligate apomict (Dominant gene)**

**F$_1$**

**Sexuals**
1. Use selected plants in crosses with other apomicts
2. Discard unselected plants

**Apomicts**
1. Select best phenotypes

2. Plant in replicated tests
Test

**Apomictic cultivar release**

Apomixis controlled by a dominat gene would be the easiest to use in a breeding program because all apomictics would be heterozygous for the method of reproduction. Therefore, sexual x apomictic crosses result in both sexual and apomictic $F_1$ progenies. One would theoretically expect one half of the $F_1$ plants to be sexual and one half of the plants to be apomictic if apomixis is controlled by a single dominant gene. Sexual $F_1$ plants can be discarded or used in crosses with other apomictic plants to produce new apomictic hybrids and sexual plants with new gene combinantions. Using improved sexual plants in crosses with the improved apomictic plants from other crosses in each generation increases the likelihood of developing superior apomictic hybrids in succeeding generations. $F_1$ apomicts with desirable agronomic trait produced from the cross in the previous figure can be selected and immediately placed in replicated test to evaluate desired traits. Progeny tests for genotype stability are not necessary if the plants are obligate or at least highly apomictic. Superior genotypes can be released as cultivars.

The fundamental objective of any plant breeding program for an apomictic species in which genetic recombination can be achieved is the identification among segregating progenies of superior, true-breeding apomictic genotypes suitable for cultivar status. Breeding plans that are being implemented for Brachiaria assume simple (probably monogenic) control of apomixis and predominantly allogamous reproduction with high levels of self-incompatibility or strong inbreeding depression.

Sometimes publications on breeding procedures using apomictic species have been few and generally oversimplistic. Pernes et al. (1975) proposed a model for Guineagrass based in the one dominant allele hypothesis (see next 2 figures) The same unchanged model was presented again by Hanna in 1995 reflecting a stability that may be justified by the simplicity of the segregations generally observed among hybrids: sexual x apomictic crosses produce F1 hybrids that segregates for modes of reproductionin a 1:1 ratio. The sexual hybrids can then be either discarded or used to increase the diversity of the sexual pool (usually limited within the wild resources) and produce improved sexual progenitors for further crosses. Apomictic hybrids, on the other hand, from either the F1 or subsequent generations, are fixed and can directly enter trials for evaluation of desirable traits.
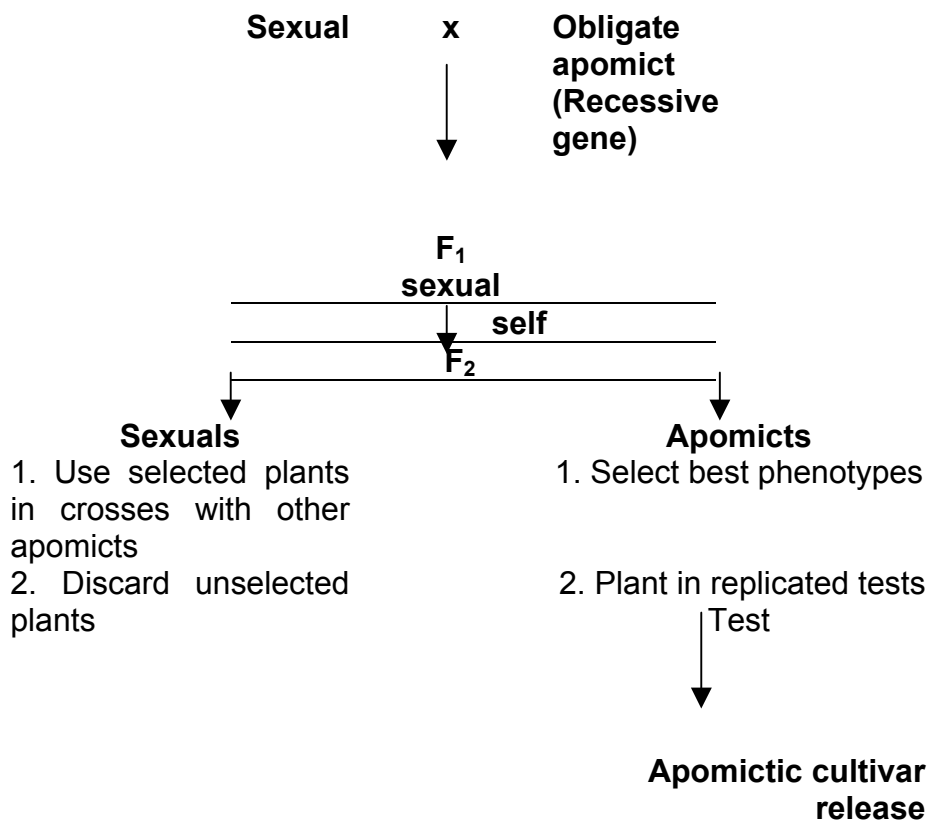
```
                    ┌─────────────────────┐
                    │  Sexual diploid pool │
                    └─────────────────────┘
                     X X X X X X X X X
```

Dialleles, polycrosses
Selection of the best 2x genitors for hybrid production (GCA, SCA)

Hybrid production

```
          ┌──────────────────────────────┐
          │  Sexual diploid hybrid pool   │
          │  Limited no., diversification │
          └──────────────────────────────┘

             X    X    X    X    X
                                           Tetraploidization

          ┌──────────────────────────────┐
          │   Sexual digenic tetraploids  │
          │         limited no.           │
          └──────────────────────────────┘
```

Diallels for detailed
genetical analyses

"Group" diallels i.e. crossing
with a group of apomicts
(Beginning of 2nd phase)

```
┌─────────────────┐                    ┌─────────────────┐
│  Sexual 4x pool │                    │  Apomictic pool │
│   limited no.   │                    │ highly diversified│
└─────────────────┘                    └─────────────────┘
```

"Group" diallels

Crossability test
Embryo sac analyses

1st generation (F$_1$) hybrids tested for
agronomical performance and for rate
of facultativeness

```
                          ┌─────────────────┐
                          │  Sexual 4x pool │
                          │     improved    │
                          └─────────────────┘
```
New hybrid genotypes
selection for good testers

X   X   X   X   X

"Group" diallels

```
2nd generation (3-way) hybrids      ┌─────────────────┐
tested for agronomical performance  │  Sexual 4x pool │
and for rate of facultativeness     │ further improved│
                                    └─────────────────┘
```

Next cycle

Most of the small number of plant breeding projects conducted for apomictic
species rely on large-scale hybridization between sexual and obligated apomictic
to produce large populations, from which superior apomictic hybrids are isolated.
Three-way or double crosses involving more than one apomictic male may be
required, depending on the distribution of desirable attributes among available
apomictic genotypes.  These schemes will require careful selection of parents

and the evaluation of large populations to find the desired combination of characteristics in a true breeding apomictic genotypes. Such approaches offer the opportunity to generate novel apomictic genotypes, however, they are essentially conservative in the longer term because the opportunity for genetic gain in eventually exhausted.

To continue genetic advances, a systematic scheme for recycling selected hybrids genotypes will be required (i.e. population improvement by recurrent selection). Any breeding population must obviously include sexual genotypes to ensure genetic recombination. An important consideration in the development of populations is whether to attempt to include and maintain apomictic genotypes in the populations.

If apomixis is controlled by a recessive gene then, all $F_1$ sexual plants are heterozygous for this recessive gene in crosses between sexual plants homozygous for method of reproduction and apomictic plants

**Sexual     x     Obligate apomict (Recessive gene)**

$F_1$
**sexual**

↓ **self**

$F_2$

**Sexuals**
1. Use selected plants in crosses with other apomicts
2. Discard unselected plants

**Apomicts**
1. Select best phenotypes

2. Plant in replicated tests
Test

**Apomictic cultivar release**

Compared to the cross in previous methods, this one requires selfing the $F_1$, a loss of vigor in progenies due to selfing results, and only one-fourth of the $F_2$ progenies are apomictic. However, it is possible to select superior apomictic transgressive segregates in the $F_2$ generation that are superior to the $F_1$.

Selection and testing of apomictic plants and release of apomictic cultivars would be similar to the procedure following for the cross in the previous method.
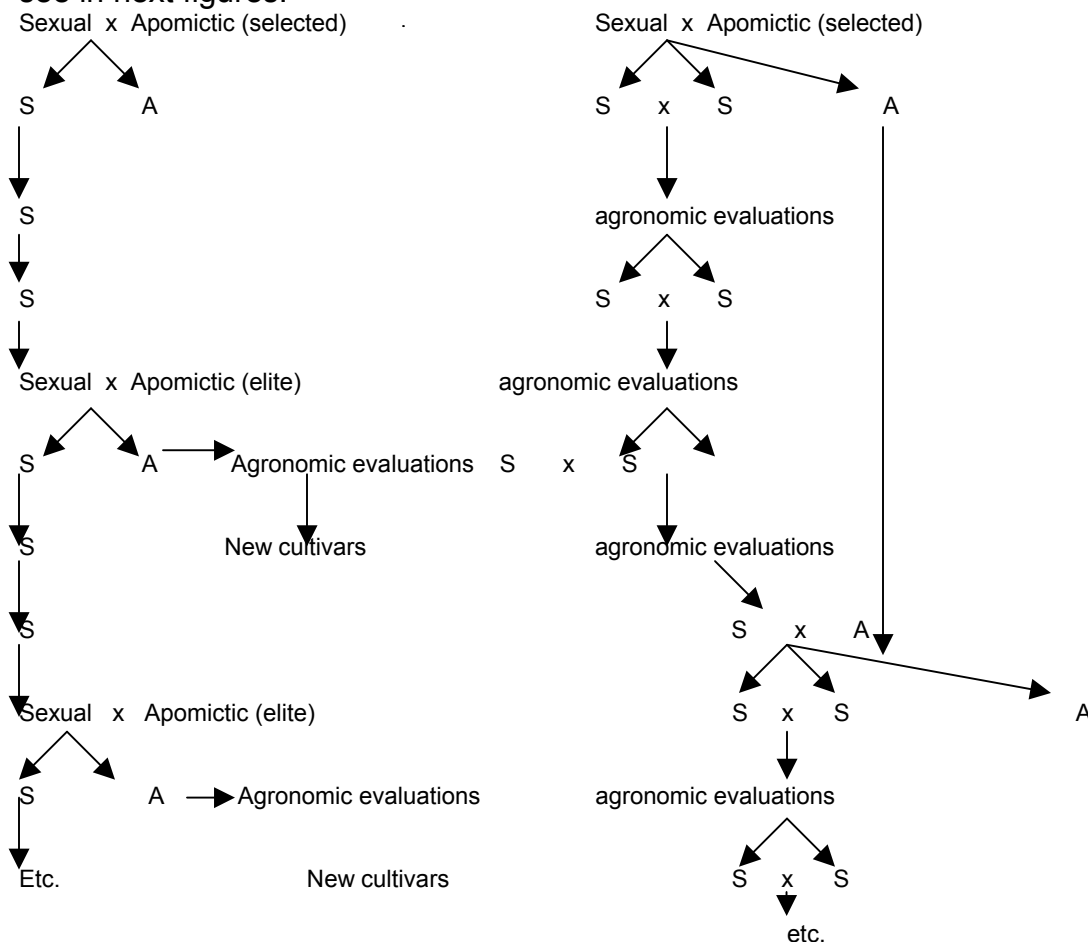
Other possibility would be the following scheme. Crosses between two sexual plants, both heterozygous for method of reproduction, results in $F_1$ plants that: 1) Breed true for sexuality, 2) Are sexual but heterozygous for apomixes and, 3) breed true for apomixis.

| **Sexual heterozygous, recessive gene** | **x** | **Sexual heterozygous, recessive gene** |
|---|---|---|

$F_1$

| **¼ Sexual Homozygous** | **½ Sexual heterozygous** | **¼ Apomictic** |
|---|---|---|
| 1. Use selected plants in crosses with other apomicts | 1. Follow procedure as $F_1$ Sexual plants in the previous figure | 1. Select best phenotypes |
| 2. Discard unselected plants | | 2. Plant in replicated tests |

**Test**

**Apomictic cultivar release**

This procedure can capture heterosis in apomictic plants in a similar way to the apomictic plants produced in the cross in the first method but only 25% of the $F_1$ plants can be apomictic in this cross. Sexual $F_1$ plants heterozygous for the gene controlling apomixis can be handled similarly to the $F_1$ plant and homozygous sexual plants should be handled similarly to the $F_2$ plants in the previous method. A sexual plant heterozygous for the genes controlling apomixis pollinated with an obligate apomictic is the most efficient way to develop superior apomictic cultivars when apomixis is controlled by a recessive gene. The outcome of the cross, the selection and testing of apomictic plants are similar to that for the cross in the first method, but sexual plants from this cross are heterozygous for genes controlling apomixis.

Considering again that apomixes is determined by one dominant gene, other possible breeding plan to mention is the Brachiaria model. The most important apomictic breeding programs are currently underway at CIAT (Colombia) and EMBRAPA (Brazil) focused on widely cultivated *B. decumbens* and *B. brizantha*. Another group has focused on breeding the sexual species of the complex (*B. ruziziensis*) planning to use apomictic hybrids among these three species. Valle and Miles (1992) referred to the simple model of Pernes et al. (1995) discussing some details about it. A fundamental concept in their consideration of the topic was that the original sexual pool is quite limited in most agamic complexes, and

nonexistent at the tetraploid level. Improving the sexual pool, therefore, is a preliminary requirement sometimes missed by some authors. As stated by Valle and Miles (1992), the improvement of the sexual population, when feasible, will strongly reflect in the resulting apomictic hybrids. The beauty of Pernes' system is that half of the superior individuals can be immediately transformed into cultivars through apomictic seed multiplication. The second phase of the model of Pernes et al. (1975) is a type of recurrent selection scheme, in which improvement is achieved by crossing selected sexual x apomictic genotypes and producing new populations segregating for apomixis and sexuality. This scheme relies on the capacity to simple recognize sexual from apomictic hybrids. Progeny testing is adequate at this level , even if it delays the information by one generation. As the frequency of favorable alleles is increased in the sexual population, hybridization with elite apomictic  genotypes will generate an array of improved apomictic and sexual segregants while the sexual population remains fully sexual, i.e., advanced from purely sexual clones selected from within the population. Superior apomicts in the hybrid populations would be candidates for cultivar release. They could also be used in the subsequent cycle of sexual x apomictic crosses, although it would not be expected that crossing back to the parental sexual pool would lead to maximum expression of heterosis. Variations to the Pernes' scheme have been proposed by Miles and Valle (1996) as we can see in next figures.
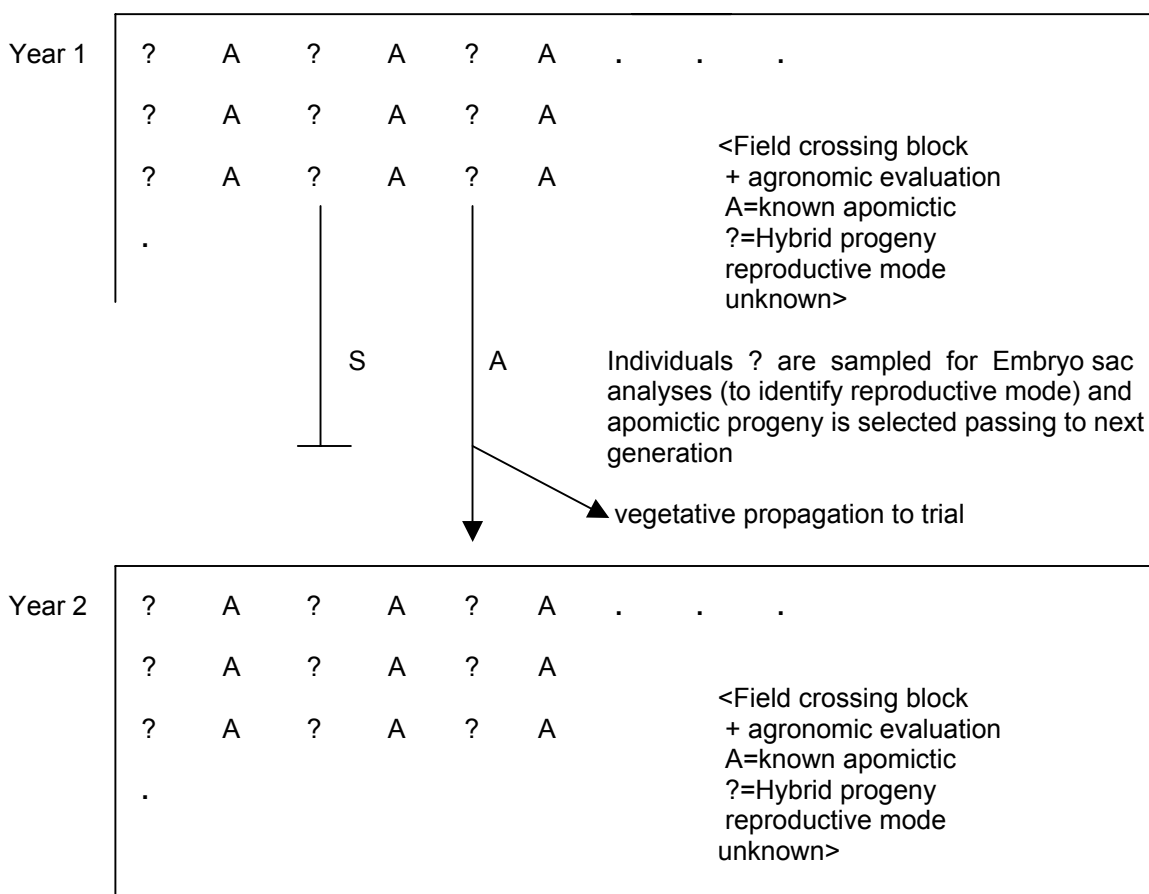
Sexual  x  Apomictic (selected)

S          A

S

S

Sexual  x  Apomictic (elite)

S          A  →  Agronomic evaluations

S          New cultivars

S

Sexual   x   Apomictic (elite)

S          A  →  Agronomic evaluations

Etc.          New cultivars

Sexual  x  Apomictic (selected)

S    x    S          A

agronomic evaluations

S    x    S

agronomic evaluations

S    x    S

agronomic evaluations

S    x    A

S    x    S          A

agronomic evaluations

S    x    S

etc.

They question whether cycles of "purely sexual" selection should be intercalated between cycles of sexual x apomictic hybridizations, in which modes of reproduction must be analized. This may reduce the number of screenings for modes of reproduction, but also may slow genetic gain. Such schemes still have to be tested before a reasonable compromise can be recommended.



Sexual (S)    x    Apomictic (A)
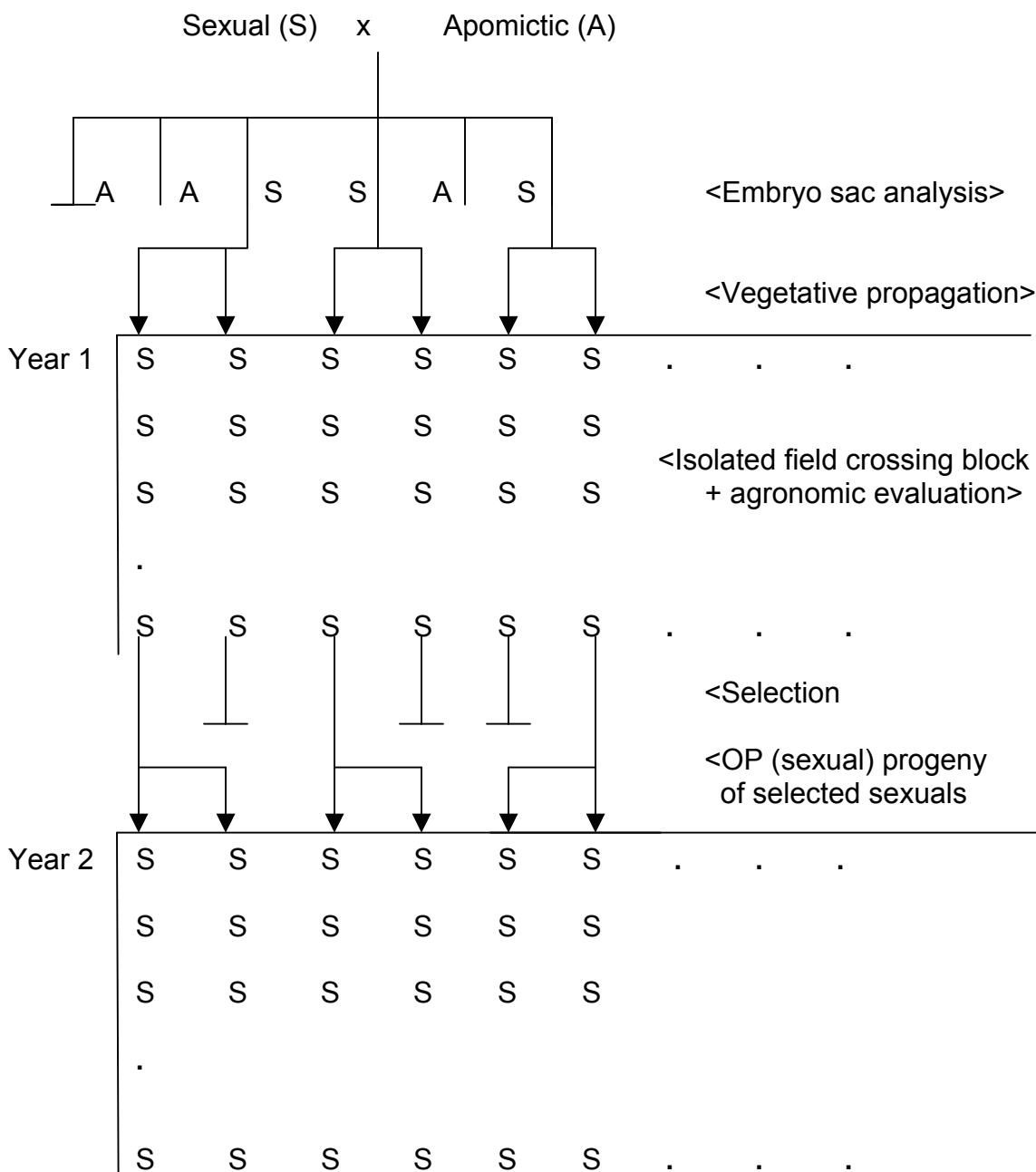
Another proposal for recurrent selection in apomictic species consider a second population containing both sexual and apomictic genotypes has been formed from sexual x apomictic hybrids (see next figure). In each generation, two types

of progenies are planted in alternating position in a square grid: apomictic progenies of selective apomictic (reproductive mode determined by embryo sac analysis) and open pollinated progeny of selected sexual segregants. The open pollinated progenies of sexuals will contain both, sexual and apomictic genotypes. Seed harvested from a sexual segregant will represent a new half sib family, the result of pollination by both sexual and apomictic plants in the crossing block. The "?" individuals are embryo sac analyzed determining if they are apomicts or sexual. If they are apomicts they are evaluated and selected according to their performance to obtain improved accessions. If "?" individuals are sexual their seed is harvested and planted in the next generation with elite apomicts.  In this scheme, both sexual and apomictic genotypes are being improved simultaneously, and new apomictic recombinants (candidates for new cultivars) will be identified in each cycle. However, the expense of determining reproductive mode with every generation is substantial. A critical assessment of the relative efficiency, genetic and economic , among alternative breeding schemes is needed.

More complex schemes might be envisioned. Since the desirable cultivar is essentially a hybrid, a recurrent selection scheme based on performance of hybrid genotypes, such as reciprocal recurrent selection or recurrent selection on specific combining ability may be appropriate.

| Year 1 | ? | A | ? | A | ? | A | . | . | . |
|--------|---|---|---|---|---|---|---|---|---|
|        | ? | A | ? | A | ? | A |   |   |   |
|        | ? | A | ? | A | ? | A |   |   |   |
|        | . |   |   |   |   |   |   |   |   |

<Field crossing block
 + agronomic evaluation
 A=known apomictic
 ?=Hybrid progeny
 reproductive mode
 unknown>

S              A              Individuals ? are sampled for Embryo sac analyses (to identify reproductive mode) and apomictic progeny is selected passing to next generation

vegetative propagation to trial

| Year 2 | ? | A | ? | A | ? | A | . | . | . |
|--------|---|---|---|---|---|---|---|---|---|
|        | ? | A | ? | A | ? | A |   |   |   |
|        | ? | A | ? | A | ? | A |   |   |   |
|        | . |   |   |   |   |   |   |   |   |

<Field crossing block
 + agronomic evaluation
 A=known apomictic
 ?=Hybrid progeny
 reproductive mode
 unknown>

## Apomixis transfer to sexual crops

The finding that apomixis can be easily transmitted into $F_1$ hybrids and further derivatives of sexual x apomictic crosses has led to a major challenge in modern plant breeding: the transfer of the trait to sexually reproducing crops.
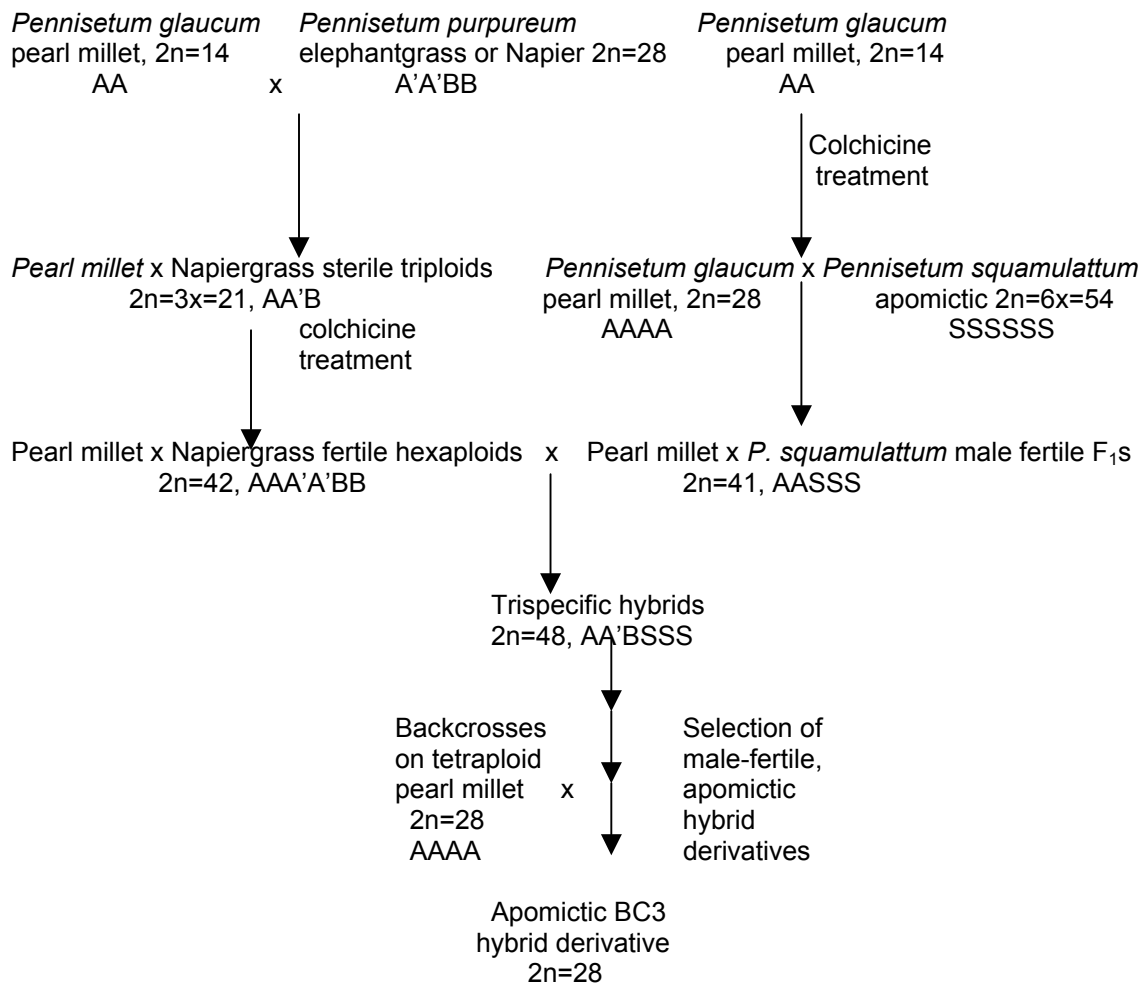
Whether the alleles conferring nearly obligate apomixis in wild grasses would normally be expressed after their transfer to a sexually reproducing relative such as wheat, maize, or pearl millet is as yet unanswered. Still, we know from pearl millet and from the ongoing maize-Tripsacum projects that hybrid derivatives can express apomixis as readily as the wild progenitor itself. In maize-Tripsacum BC1 hybrid derivatives, for example, 3% of the progeny are sexually-formed n+n off types, as found in the wild T. dactyloides accession used toproduce the $F_1$s.

Today, an important question regarding apomixis gene transfer and expression is wether we can induce recombination, i.e. allele exchanges at the loci involved in the control of the reproductive pattern, or whether we can only add foreign information through poorly controlled insertions (transformation) –a drawback that may not last very long due to technical progress underway.

### Pearl Millet

The genus *Pennisetum* includes many species that reproduce through apomixis. A few of them have been tested and successfully hybridized with pearl millet, *Pennisetum glaucum*. Such hybridizations, however, produced mostly male-sterile hybrids, which could not promote the transfer of a nearly obligate type of apomixis.

Dujardin and Hanna (1989b) described an obligate apomictic BC3 plant, resembling pearl millet, as recovered from the three-species hybrid made between *Pennisetum glaucum*, cultivated pearl millet, and apomictic *P. squamulatum, using napier, P. purpureum*, as a bridge species. The plant showed a high degree of sterility, and the backcross to introgress genetic background from P. glaucum conserving the apomictic genes could not be achieved.

*Pennisetum glaucum*　　*Pennisetum purpureum*　　　　　*Pennisetum glaucum*
pearl millet, 2n=14　　elephantgrass or Napier 2n=28　　pearl millet, 2n=14
　　　AA　　　　x　　　　A'A'BB　　　　　　　　　AA

　　　　　　　　　　　　　　　　　　　　　　　　　　│ Colchicine
　　　　　　　│　　　　　　　　　　　　　　　　　│ treatment
　　　　　　　▼　　　　　　　　　　　　　　　　　▼

*Pearl millet* x Napiergrass sterile triploids　　*Pennisetum glaucum* x *Pennisetum squamulattum*
　　2n=3x=21, AA'B　　　　　　　　　　　　pearl millet, 2n=28　　　apomictic 2n=6x=54
　　　　　　colchicine　　　　　　　　　　　　　AAAA　　　　　　　　SSSSSS
　　　　　　treatment

　　　　　│　　　　　　　　　　　　　　　　　　│
　　　　　▼　　　　　　　　　　　　　　　　　　▼

Pearl millet x Napiergrass fertile hexaploids　x　Pearl millet x *P. squamulattum* male fertile $F_1$s
　　　2n=42, AAA'A'BB　　　　　　　　　　　　　2n=41, AASSS

　　　　　　　　　　　　　　　│
　　　　　　　　　　　　　　　▼

　　　　　　　　Trispecific hybrids
　　　　　　　　2n=48, AA'BSSS

　　　　　　　　　　　　　│
Backcrosses　　　　　　　│　　Selection of
on tetraploid　　　　　　│　　male-fertile,
pearl millet　x　　　　　│　　apomictic
　2n=28　　　　　　　　 │　　hybrid
　AAAA　　　　　　　　　▼　　derivatives

　　　　　　Apomictic BC3
　　　　　　hybrid derivative
　　　　　　　　2n=28

## Maize

Different research groups have conducted studies regarding the inheritance and introgression of apomixis in maize through a wild relative Tripsacum dactyloides. The group leaded by Petrov in Russia crossed a tetraploid maize (2n=4x=40m) with an apomictic tetraploid Tripsacum (2n=4x=72t). Five male sterile F1 hybrid were obtained which had 2n=56=20m+36t chromosomes. Only one of the five $F_1$ hybrids was obviously facultative apomictic and was backcrossed using maize as pollinator. From 607 progeny plants (2n=38=20m+18t if they were sexual), 84.5% were maternal, 14% were 2n+n, and only 1.5% were n+n. This created a bottleneck for the Russian program because the low rate of sexually formed offtypes implied that a very large number of progeny would have to be produced and screened before sexually formed n+n offtype with low chromosome number and reproducing apomictically could be recovered.

After the disintegration of Petrov's group, the materials were transferred to Woodward, Oklahoma and Kindiger (1996) reconstructed the Russian Backcross

scheme. Two 39 chromosomes plants, which were 30m+9t, were obtained from a 58 chromosomes (40m+18t). This was interpreted as a result of sexuality, i.e. the fertilization by a n=10 maize sperm of the reduced female gamete with n=29=20m+9t. Studies on this material showed that the long arm of chromosome 16 of Tripsacum contained the gene (s) controlling apomixis. Cytological analysis of apomictic progeny from the 39 chromosomes plants revealed a translocation involving the long arm of chromosome 6 of maize and chromosome 16 of Tripsacum. Subsequent efforts are focusing on the isolation of the gene(s) controlling apomixis as a prelude to their modification and reintroduction and as the foundation of apomictic seed production. Actually, these results were the last recent published by this group which is still working on this project.

CIMMYT has a project in Mexico involving the same kind of research work, but again the problem that they have is with the model of reproduction that have impeded backcross-based introgression and recovering of the normal maize chromosome number.

**Wheat**

In an attempt to transfer apomixis to wheat, Carman et al. (1985) collected wild Elymus rectisetus genotypes from Australia and Tasmania finding some of them to be highly apomictic. Direct hybridization between wheat and E. rectisetus proved difficult, but a few hybrids were produced. Carman's group experienced the same problem with wheat as CIMMYT's group in maize.

# Biodiversity and Genetic vulnerability

A major concern for using apomixis in cultivar development is that a few superior cultivars would occupy most of the area planted to a particular crop. This could lead to several problems like the impact of corn blight in the 70's due to susceptibility of the major male sterility-inducing cytoplasm used to produce maize hybrids.

In the other hand use of apomixis in cultivar development could actually enhance genetic diversity. Each apomictic plants from a sexual x apomictic cross is potentially a unique cultivar regardless of the heterozygosity or homozygosity of its parents. Apomixis would allow breeders to build and fix unique genotypes that would be not possible or at least very difficult with sexual reproduction. Vulnerability due to cytoplasm would virtually be eliminated because a specific cytoplasmic-nuclear male sterility cytoplasmic would not be needed to commercially increase a hybrid. There could be as many different cytoplasms as there are commercial hybrids if apomixis is used in cultivar development.

## Impact on seed industry

Experts on apomixis have repeatedly mentioned the simplification of breeding procedures for grain crops as the major potential advantage of apomixis. It seems, however, that apomixis will first need to be transferred to a mjor crop before breeders begin to reexamine the field and how apomixis may be applied to their particular species. The advent of individual hybrid plant selection, in a world of sexual crops, where improvement has heretofore been on family based strategies, will enable scientists to create totally new breeding schemes, much more related to those used in clonal crops.

Apomixis would not doubt have an impact on the way commercial cultivar are produced and increased, production practices would be radically changed and at the same time greatly simplified. The need to maintain and increase parental lines and the need to be concerned about isolation to prevent outcrossing would be eliminated. The major concern in seed production would be to prevent mechanical mixtures. Outcrossing and offtypes would only be a problem when a cultivar is reproduced by some degree of facultative apomixis.

Some disadvantages could be that the farmers could save their own seed instead of purchasing new seed each year and another concern is the control of rights to germplasm. Documentation method would need to be refined and precise because of proliferation of cultivars in the markers, some with only small genetic differences.

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