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Apomixis in Farmers' Fields: Overview, Case Studies from Forage Grasses and Considerations for Future Apomictic Crops

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ABSTRACT

Apomixis occurs naturally in several commercially important species from diverse plant families. While in some of these species apomixis is yet to be exploited in breeding schemes aimed at fixing heterosis, genetic progress and cultivar development, in other species apomixis has been integrated at different stages of breeding. Some of the most relevant examples come from the subfamily Panicoideae, the second largest subfamily of the Poaceae, and are the main focus of this review. The subfamily encompasses many tropical and sub-tropical grasses and grains of worldwide economic importance. Apomictic tropical forages are prime examples of how apomixis can be used and exploited in the development of marketable cultivars, which are essential to the meat and milk production industries globally. The main commercial forages used as grass pastures covering millions of hectares in tropical and sub-tropical regions are polyploids exhibiting gametophytic apomixis that belong to the genus *Urochloa* spp. (brachiariagrasses) and to the species *Megathyrsus maximus* (guineagrass). Buffel grass (*Cenchrus ciliaris*) and *Paspalum* spp. are other important apomictic forages bred and used in these regions. Breeding involves large germplasm collections from the centers of origin of the species, and for most of them, sexually reproducing diploid plants have been found. Chromosomically duplicated plants that maintain sexual reproduction are used in crosses with apomictic genotypes for the development and selection of cultivars to be marketed or used as progenitors in subsequent breeding cycles. The peculiarities of each genus/species breeding programs, the cultivars obtained from these programs, and the impact of use of marker assisted selection in cultivar development are presented. In addition, the test or implementation of new technologies such as high throughput phenotyping, and the use of machine learning methods for trait prediction and genomic selection are positively impacting the selection and speed of development of new polyploid apomictic cultivars. Genetic transformation techniques, including genome editing, provide an additional layer for design of tailor-made, customer-oriented cultivars.

KEYWORDS
Cenchrus; clonal seeds; cultivar development; marker assisted selection; *Megathyrsus*; *Paspalum*; *Pennisetum*; *Urochloa*

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I. Introduction

Apomixis, an asexual mode of reproduction by seeds (Nogler 1984), is found in a wide range of plants globally. Apomictic plants bypass meiosis and fertilization to produce clonal embryos (Hand and Koltunow 2014). As a result, the seeds produced are genetically identical to the mother plant, shaping an effective method for cloning the maternal genotype (Mahlandt et al. 2023). Thus, apomixis is a useful tool in plant breeding, as it allows the fixation of a hybrid state and perpetuation of hybrid vigor (Grain 2001). Apomictic cultivars combine the advantages of clonal propagation and genetic uniformity with the benefits of seed propagation, particularly those involving higher multiplication rates, ease of mechanical seeding and ease of storage, among others (Kandemir and Saygili 2015). Additionally, apomictic crops offer two benefits to farmers: the ease of managing a uniform crop and the possibility to save their own seed year after year, without compromising productivity or the inherent attributes of the cultivar. This is particularly beneficial for low-income farmers in developing countries.

There are three primary types of apomixis depending on the origin and development of maternal embryos: apospory, diplospory, and adventitious embryony (Savidan 2000). The first two develop asexual embryos from unreduced embryo sacs (gametophytic apomixis), while the embryos in adventitious embryony arise directly from somatic cells in the ovular tissue (sporophytic apomixis). In aposporous plants, the female gametophyte is formed from a nucellar cell around the germline, whereas in diplosporous plants the gametophyte arises from the megaspore mother cell. The embryo develops from the unreduced egg-cell without fertilization through parthenogenesis, while the unreduced central cell may require fertilization or not for the development of the endosperm (pseudogamous or autonomous development, respectively) (Hand and Koltunow 2014).

In plants with sporophytic apomixis, somatic cells in the integuments of the nucellus acquire embryogenic capability and differentiate into embryos, producing seeds that contain both sexual and clonal embryos (Hand and Koltunow 2014). Knowledge about the type of apomixis and trait penetrance in a genotype is key for implementation of any breeding scheme. Despite apomixis being present in 1469 species, the developmental type of apomixis (based on embryological evidence) is known in only 67% of them (the rest is based mostly on flow cytometry seed screening and clonal offspring (Hojsgaard and Pullaiah 2022). The Asteraceae family contains the highest number of apomictic species (352), being diplospory the most prevalent type (78%). The Rosaceae family includes 298 apomictic species, with both diplosporous and aposporous types being frequently found. The third most apomictic-abundant family is Poaceae, with 242 species and a majority being aposporous (77%) (Hojsgaard and Pullaiah 2022).

While apomixis is generally absent in major crop plants, with some exceptions (such as citrus, see below), many crops have apomictic relatives (such as maize, sorghum, and pearl millet) and several minor and orphan crops are apomict. Species naturally lacking apomixis may become apomictic by introgression of the trait through wide crosses or by genetic modification. While the first strategy has shown little progress (see below; Savidan 1992), the second has demonstrated promising results through novel developments on site-directed mutagenesis (Mahlandt et al. 2023). Still, despite impressive advances, commercial synthetic apomictic crops are not yet available (see Reed et al. 2025 in this collection). In rice, synthetic hybrids capable of producing high rates of clonal seeds and maintaining the hybrid phenotype across successive generations have been developed (Vernet et al. 2022), and this is undoubtedly the crop that is about to reach the market.

This review highlights recent advancements in the breeding of natural apomictic species. Knowledge about how natural apomixis is exploited in breeding of major, minor and orphan crops is summarized. Tropical and sub-tropical forage grasses are used as a case study due to the level of advances in exploitation of apomixis and the integration of novel technologies into breeding pipelines for the release of new cultivars. Marker assisted selection (MAS) has become a powerful tool in the selection of desirable traits, accelerating the breeding process and improving the efficiency of developing high-performance apomictic cultivars. Novel technologies, such as genomic selection, high throughput phenotyping, gene editing, software development and use of artificial intelligence and machine learning are enhancing the precision of breeding programs, paving the way for more efficient breeding and fast development of apomictic forage grasses that cope with the current climatic challenges.

II. Apomixis breeding in nongrasses

Exploitation of apomixis has so far been limited to species carrying the trait or closely related species for which introgression is feasible. This excludes major crops for which apomixis is unknown, despite cases of major efforts to introgress the trait through wide crosses (e.g., Petrov 1986; Savidan and Berthaud 1994) and the generation of apomictic hybrids (e.g., maize-*Tripsacum* hybrids; Sokolov et al. 2024). Still, apomixis occurs naturally in several commercially important species from diverse plant families (reviewed in Hojsgaard and Pullaiah 2022). Apomixis is known in species of citrus (Rutaceae), mango (Anacardiaceae), mangosteen (Clusiaceae), hops (Cannabaceae), St. John's wort (Hypericaceae), *Beta* species (Amaranthaceae), crabapples and berries (Rosaceae), black nightshade (Solanaceae), guayule (Asteraceae), walnut (Juglandaceae), langsat (Meliaceae), Saba nut or Guinea peanut (Malvaceae) and in many grasses.

Most relevant challenges in breeding apomixis were already outlined by Asker and Jerling (1992) and many remain today. While in some of these species apomixis is yet to be exploited in breeding schemes aimed at fixing heterosis, genetic progress and cultivar development (e.g., *Beta lomatogona*, *B. trigyna* and *B. corolliflora*), in other species apomixis has been integrated at different stages of breeding (e.g., *Hypericum perforatum*, *Citrus* spp.) (Hojsgaard and Pullaiah 2022). Here we focus on some of the most relevant examples, and recommend the interested person to read species-specific research or review articles related to apomixis and breeding (e.g., Murthy et al. 2018).

In Rutaceae, apomixis has played an important role in shaping the biogeography and domestication of citrus species (Wu et al. 2021; Wang et al. 2022). Three ancestral species that reproduce only sexually (i.e. *Citrus reticulata*, *C. maxima* and *C. medica*) have generated many cultivars of mandarins, pummelos and citrons by sexual hybridization, thus maintaining higher levels of genetic diversity compared to most cultivars of orange (*C. aurantium*, *C. sinensis*), grapefruit (*C. paradisi*), lemon (*C. limon*) and some mandarin groups (satsumas and clementines) originated from nucellar seedlings or bud-sports (Khan 2007). Yet, the exploitation of apomixis in regular breeding programs is less evident. Scion and rootstock breeding is carried out either by sexual or somatic hybridization. The recovery of hybrids by sexual hybridization is challenged by high heterozygosity in citrus genomes (Wu et al. 2018), that produces wide segregation patterns of parental traits, as well as by the occurrence of apomixis in most species, which generates nucellar embryos that often outcompete zygotic ones (Koltunow 1993). The use of somatic hybridization by protoplast fusion permits to overcome such issues and possible sexual incompatibility between parents allowing germplasm expansion and ploidy manipulation, regenerating polyploid embryos and plants (Grosser and Gmitter 2011). Somatic hybrids rarely have direct utility as improved cultivars but as elite breeding parents of conventional crosses for scion and rootstock improvement. Selection is then imposed on zygotic progenies for the traits of interest, and nucellar (apomictic) seeds are used for standard (clonal) propagation of rootstocks (Grosser et al. 2000; Ruiz et al. 2018), granting rapid multiplication during cultivation in nurseries and stability of hybrid traits.

In the rose's family, apomixis is well known in important economic genera such as *Malus*, *Rubus* or *Fragaria* (Hojsgaard and Pullaiah 2022), and yet, it is currently not widely exploited as a breeding tool like it is in grasses. Perhaps the main reason is that many of those taxa do propagate vegetatively and therefore, the major advantage of apomixis (i.e. clonality) is at hand and used throughout breeding stages, for instance, to predict genetic potential using clonal replicates across multiple environments in multi-year trials (Iezzoni et al. 2020). An associated side challenge in clonally propagated crops is that clonal propagation captures all genetic effects, and therefore, adopting marker-assisted seedling selection suffers a lack of knowledge in genetic gain achievable from alternative strategies which requires model optimization of genetic gain from marker information (Ru et al. 2016). A second issue is facultativity, or the formation of both sexual and apomictic seeds. For instance, *Malus* and *Rubus* species holding gametophytic apomixis (apospory and/or diplospory) exhibit variable levels of trait expressivity (Olien 1987; Šarhanová et al. 2012, 2017) imposing challenges to breeding schemes. The same goes for conserved *Fragaria* cultivars (e.g. Zini 2025). Likewise, *Citrus* species holding sporophytic apomixis (integumentary and nucellar) must also deal with parallel development of sexual and apomictic embryos in single seeds, creating hurdles during identification and selection of segregant genotypes (see above). Another reason is the generation time, particularly among trees, that renders alternatives such as *in vitro* propagation more efficient when multiplying a targeted plant material.

Many species with apomixis or having closely related apomictic species have a relatively long history of domestication, starting with the selection of superior individuals from wild habitats (Janick and Moore 1996) and followed by breeding projects to generate elite cultivars. Despite apomixis has not systematically contributed to any stage of breeding selection, there are cases where apomixis present in a given variety is lost in successive crossing schemes of these crops.

In *Fragaria*, wide hybridizations within the genus and to the closely related genus *Potentilla* spp. were aimed at introducing exotic genes into strawberry and producing polyhaploid individuals which can be doubled to generate isogenic lines. Crosses between *F. vesca* and *P. anserina* generated matroclinous (putatively apomictic) seedlings that were chosen for traits that deviate from the maternal phenotype and the best apomictic clones were included in breeding and mass selection programs (Asker 1971; Galletta and Maas 1990).

In *Malus*, apomictic crab-apple species (such as *M. hupehensis*, *M. sargentii*, *M. sieboldii* or *M. sikkimensis*) have been involved in breeding programs to combine disease and insect resistance traits with other traits economically important for commercial rootstock and scion cultivars of apple (e.g., Ciccotti et al. 2008; Seemüller et al. 2008). The propagation of clonal rootstocks by apomictic seed is efficient and its use depends on compatibility with scion cultivars, including influence on tree size, productivity and stress tolerance (Sax 1949; Olien 1987; Schmidt 1988; Hu et al. 2021). Genomics-assisted breeding is using genomic and phenotypic information to increase the selection efficiency and accelerate apple breeding (e.g., Jung et al. 2020), and could facilitate the exploitation of apomixis to test phenotypic response in different environments.

In *Rubus* for example, domestication has reduced morphological and genetic diversity of the crop (Tanksley and McCouch 1997; Flint-Garcia 2013; Meng et al. 2022), with current cultivars bred from only three wild species, i.e., red raspberry (*R. ideaus* L.), black raspberry (*R. occidentalis* L.), and blackberry (*R. fruticosus* L.). In such context, initiatives like the RosBREED project (USA) are bridging genomics and genetics to make DNA-informed breeding, thus accelerating the improvement of Rosaceae crops by delivering cultivars with the disease resistance required by growers and the horticultural quality essential for the market (Iezzoni et al. 2020). In strawberry, marker-assisted selection is cost-effective not at the greenhouse stage but when applied at the end of the seedling trials, before clonal trials (Wannemuehler et al. 2020). Thus, lack of genetic segregation through apomixis could simplify and speed up selection stages.

As depicted above, apomixis breeding in nongrass species is advancing despite the obstacles and efforts required to introgress the trait into target genotypes, unlike grasses, whose bred genotypes are often apomictic. In nongrass species, elaborated crossing schemes and the use of novel technologies to make reproductive systems more permeable (e.g. by facilitating hybridization, circumventing crossing barriers, or breaking sexual incompatibility systems) can probe effective in harnessing this potential for apomixis breeding and clonal propagation (e.g., Taylor 2018). Meeting increasing demands on yield, quality, health benefits and visual appeal while keeping ecological plasticity will require widening wild germplasms resources which might benefit from naturally occurring apomixis, and use of advanced biotechnology in breeding.

III. Apomixis breeding in grasses

Apomictic tropical forages are prime examples of how apomixis can be used and exploited in the development of commercial cultivars, which are essential to the meat and milk production industries globally. Brazil stands as the leader in breeding apomictic forage species, with the highest number of released cultivars and the largest area planted with these cultivars. It is estimated that Brazil has over 100 million hectares of pastures covered by apomictic cultivars, predominantly of brachiariagrasses [*Urochloa* P. Beauv., syn. *Brachiaria* (Trin.) Griseb.] and guineagrass [*Megathyrsus maximus* (Jacq.) B.K. Simon & S.W.L. Jacobs, syn *Panicum maximum* Jacq.]. These cultivars are not only cultivated extensively in Brazil but are also exported to nearly all Latin America, and some African and Asian countries (Jank et al. 2014).

The success of the Brazilian breeding programs can largely be attributed to the significant investments made by the Brazilian Agricultural Research Corporation (Embrapa) since its establishment in 1973. Embrapa's investments have included funding for personnel, research agreements, human resource training, germplasm imports, and recent collaborations with the seed industry. For more than two decades, Embrapa has partnered with the Association for the Promotion of Research in Forage Breeding (Unipasto, Brasilia, DF, Brazil), an association of 31 seed producers who financially support Embrapa's breeding programs in exchange for exclusive commercialization rights of the developed cultivars (Jank et al. 2014).

Furthermore, the International Center for Tropical Agriculture (CIAT) in Colombia, a member of the Consultative Group on International Agricultural Research (CGIAR), also contributes to the breeding and development of cultivars from these species, providing cultivars to the Brazilian regional market, as well as to other countries in Central and S. America, USA, Australia, Sub-Saharan Africa and Southeast Asia (Burkart, 2024). Partnerships between international or national centers and the private sector play an important role in market penetration. As Embrapa and Unipasto, CIAT maintains a partnership with the Mexican seed company Papalotla, which assists the center in cultivar development and is responsible for their commercialization. Other than Embrapa and CIAT, some private companies have recently become engaged in the development of brachiariagrass and guineagrass cultivars in Brazil.

In addition to the breeding programs in Brazil and CIAT, breeding programs for apomictic *Paspalum* L. species have been ongoing in Argentina, USA, Brazil and Uruguay since many decades now. Cultivars from these breeding efforts are also commercially available. Meanwhile, breeding *Cenchrus ciliaris* L. (syn. *Pennisetum ciliare* (L.) Link.), commonly known as buffelgrass, is progressing in Argentina.

All these breeding programs are backed up by germplasm collections, constant survey of natural resources and search of new genotypes, and the characterization of such collections, particularly their reproductive system and morphological and genetic variability. In this context, the variability found in germplasm banks of a target species and related ones plays a central role in any breeding program. Although many cultivars result from direct selection of ecotypes guarded in gene banks, most are developed through crosses between accessions and exploitation of new genetic combinations in recombinant offspring.

A. Breeding systems: Ploidy levels and reproductive modes

Knowledge on the ploidy, reproductive mode and pollination system of a species is central to plan the most appropriate breeding methods for improvement, especially in grasses where a wide range of breeding systems occurs. Thus, evaluation of the breeding system of a genotype is a prerequisite before being incorporated in a breeding cycle.

Polyploidy is very common among species of the Poaceae family. According to Stebbins (1941), 70% of the grasses are natural polyploids and in a recent reassesment of apomixis in angiosperms, Hojsgaard and Pullaiah (2022) corroborated a tight association between gametophytic apomixis and polyploidy among grass species. Most of the important tropical forage plants utilized in pastures are polyploid apomicts (Savidan 2000). These include species of the genera *Urochloa*, *Megathyrsus*, *Paspalum*, and *Pennisetum/Cenchrus* which have become a central component of local agricultural systems, mainly in developing countries.

Thus, the determination of ploidy levels in apomictic genotypes with forage aptitude is essential to predict its reproductive mode (sexual or apomictic) and identify genotypes suitable for crossbreeding. Therefore, prospections of ploidy levels on grass germplasm accessions are a regular activity carried out by classic chromosome counts or flow cytometry, and provide additional information on basic chromosome numbers, the probable origin of polyploids and karyotype evolution. To mention a few examples, ploidy analyses are common in *Urochloa* (Darlington and Wylie 1955; Penteado et al. 2000; Valle and Pagliarini 2009), in *Cenchrus/Pennisetum* (Hignight et al. 1991; Visser et al. 1998; Burson et al. 2012; Carloni et al. 2018), in *Megathyrsus* (Savidan 1982; Tomaszewska et al. 2023), and in *Paspalum* (Honfi et al. 1990; Urbani et al. 2002; Hojsgaard et al. 2009). Such analyses are often combined with, or used as the basis for, more specific studies exploring the roles of hybridization and apomixis (Miles and Valle 1996; Worthington et al. 2016; Higgins et al. 2022), cytogenetics (Damasceno et al. 2023; Sales et al. 2024), phylogenetics (Tomaszewska et al. 2023; Masters et al. 2024), or reviews focused on breeding (Ortiz et al. 2013; Acuña et al. 2019).

Reproductive mode analyses in taxa with apomixis can be carried out with a variety of techniques (Hojsgaard and Pullaiah 2022). These include observation of phenotypic traits (e.g., multiple seedlings, seed fertility in otherwise sterile plants like wide hybrids or triploids), embryological evaluation, flow cytometry seed screenings, nonsegregating progenies and reproduction-specific molecular markers. However, embryological analysis of ovules at different developmental stages is the only methodology able to provide information on the reproductive pathway.

For instance, Brown and Emery (1958) evaluated the anatomy of mature embryo sacs among tropical and sub-tropical grasses to reveal that gametophytic apomixis is a characteristic of the subfamily Panicoideae. In grasses, the occurrence of mature apomictic embryo sacs lacking antipodals (such as *Panicum*-type or *Setaria*-type) is common and allows an easy differentiation from the sexual ones with antipodals (*Polygonum*-type) (reviewed in Hojsgaard and Pullaiah 2022). However, other cases in which the apomictic development produce an embryo sac with antipodals (such as *Hieracium*-type, *Antennaria*-type or *Taraxacum*-type) require detailed analysis of meiosis and embryo sac development for the identification of apomictic and sexual pathways. This must be done through sectioning and staining or clearing methods (e.g., Raposo et al. 2019; Sánchez et al. 2023). In grass species such as *Poa pratensis*, *Brachiaria decumbens* or *Elymus rectisetus*, lack or abnormal callose deposition around the megaspore cell was associated with apomictic development (Naumova et al. 1993; Peel et al. 1997; Dusi and Willemse 1999).

Nowadays, with advances in molecular techniques, the determination of the reproductive mode in individuals from different grass species using molecular markers and without the need of embryology has become a reality (see Section III. C). This allows the rapid evaluation and selection of progenies from experimental crosses, along with taxon-specific breeding activities.

B. Inheritance of apomixis, crossings and improvement strategies

Apomixis is dominant trait over sexuality and is inherited as a single gene or a group of closely linked genes (Savidan 2000). In monocot grasses, the main regulatory gene(s) controlling the trait behave as single dominant Mendelian factors (Ozias-Akins and van Dijk 2007). The genetic locus controlling apomixis has been originally identified as large chromosomal segments in both *Pennisetum/Cenchrus* species and *Paspalum* species and named the apospory-specific genomic region (ASGR) and the apomixis controlling region (ACR), respectively. Both are highly heterochromatic chromosomal regions and rich in repetitive elements and pseudogenes. Despite these attributes which make genetic mapping a challenge, both ASGR and ACR present a high degree of conservation among species which suggest a common evolutionary origin and has facilitated identification of conserved elements (Ozias-Akins et al. 1998; Roche et al. 2002; Pupilli et al. 2004; Goel et al. 2006; Hojsgaard et al. 2011; Worthington et al. 2019).

In terms of inheritance, hybrid progenies from crosses between a sexual mother and an apomictic father in brachiaria and guineagrasses reach expected levels for a dominant trait of around 50% but sometimes it is higher. However, in other grasses apomixis is often inherited to more than 50% reaching up to 90% or more of offspring. This pattern of genetic inheritance combined with the fact that apomixis shows inherently high penetrance (all individuals carrying either the ASGR or the ACL express apomixis) and that expressivity (within individuals) is high and can be improved facilitates the establishment of stable, high-performing hybrid cultivars. Thus, despite the molecular complexity of apomixis (see Schmidt 2025 in this collection), its pattern of segregation and expression makes its use in breeding tropical forage grasses straightforward (see details below). While apomixis facilitates cultivar development by providing a natural method to achieve uniformity, it also poses challenges for exploitation of standing variability due to lack of recombination. Thus, in order to maximize long-term genetic gain, a major focus of apomixis breeding is at securing the availability of homoploid sexual plants.

Since most polyploid apomicts form agamic complexes holding sexual diploids and polyploid apomicts (Karunarathne et al. 2024), the only way to have a sexual conspecific polyploid is through artificial chromosome doubling (see e.g., Simioni and Valle 2009; Weiler et al. 2015). These artificial sexual polyploids are then used as maternal parents in intraspecific crosses, such as in *P. maximum* (Savidan et al. 1989), or in interspecific crosses as in *brachiariagrasses*, where sexual tetraploized *U. ruziziensis* was crossed to apomictic *U. brizantha* and *U. decumbens* as male parents (Valle and Miles 2001; Valle and Pagliarini 2009; Worthington and Miles 2015). An alternative is the use of natural sexual polyploids from related species, but in such cases the formation of hybrid seed set is impaired to variable degrees.

Crossings can be done in the field or in the greenhouse, in the first case being facilitated by wind pollination where the sexual plant is planted in the center of a 25 m^2 plot of the apomictic male progenitor. The hybrid seeds are harvested from the sexual plant, grown and selected based on the positive attributes transmitted from the parents to the offspring and the inheritance of the apomictic trait (see further details below).

For crossings made in the greenhouse, an inflorescence of each sexual and apomictic parent can be bagged together during flowering and then the inflorescence of the male progenitor must be removed before seed shattering. Alternatively, a sexual inflorescence may be bagged alone and dusted every morning during the flowering time with pollen from the male progenitor, or the whole sexual plant can be placed in isolation from males and dusted daily with pollen from the apomictic progenitor. In cases where the sexual plant is self-compatible, self-pollination must be avoided by emasculation facilitated through fog chambers. These are the common approaches in breeding programs of apomictic tropical grasses.

In the frame of a breeding program in grasses, a comprehensive genetic improvement strategy aimed at enhancing multiple agronomic traits typically incorporates recurrent selection schemes: intra-population (RS-Intra), inter-population (RS-Inter), and for specific combining ability (RS-SCA) (Miles 2007; Worthington and Miles 2015). These strategies are designed to improve quantitative traits by harnessing different genetic effects: additive effects in RS-Intra, both additive and dominance effects in RS-Inter, and predominantly dominance effects in RS-SCA, thereby supporting long-term genetic gain.

The RS-Intra approach targets the genetic improvement of synthetic sexual populations (Miles 2007). This strategy entails the selection of sexual plants that are homozygous for the sexual mode of reproduction, which are then recombined to establish a population characterized by broad genetic variability. Following phenotypic evaluation and selection for target traits, the most promising individuals are crossed to initiate subsequent selection cycles. Moreover, selected sexual genotypes may be employed in directed biparental crosses with apomictic parents to produce segregating progenies.

A specific form of RS-Intra is recurrent selection for general combining ability (RS-GCA). In this approach, a polycross among diverse sexual genotypes is conducted to generate half-sib progenies. These progenies are evaluated for target traits, and the sexual parents contributing to the most superior progenies are recombined to form the next cycle. Similar to the conventional RS-Intra scheme, sexual genotypes exhibiting high general combining ability may also be used in biparental crosses to generate populations with high breeding potential.

The RS-Inter and RS-SCA schemes are particularly suited to exploit heterosis, which can be fixed in the first generation of crossing. Among these, RS-SCA is currently the primary recurrent selection strategy employed in the genetic improvement of apomictic grasses. This approach typically involves crossing a synthetic population of sexual genotypes with an apomictic tester parent. The resulting progeny is subjected to marker-assisted selection to determine the reproductive mode, with only apomictic individuals retained for further evaluation. Upon completion of phenotypic assessments, superior apomictic genotypes may be released as cultivars. Concurrently, the sexual genotypes that contributed to the most promising apomictic progeny are recombined to initiate a new RS-SCA cycle (Worthington and Miles 2015).

Directed biparental (DP) crosses are commonly used in short-term breeding programs to rapidly generate high-potential populations. In this scheme, sexual and apomictic parents with superior and complementary traits are selected (i.e., “good × good” crosses) to perform the crosses. Diallel or factorial crosses schemes are also employed to produce full-sib progenies between sexual and apomictic group of parents, enabling both intra- and inter-family selection and the estimation of general and specific combining abilities, thereby providing insights into trait inheritance.

The optimal selection scheme is determined by the breeding program’s objectives (short-term vs. long-term selection), the specific traits targeted for improvement, and the available human and infrastructure resources. Furthermore, all selection strategies can be significantly enhanced by the integration of modern tools such as marker-assisted selection, genomic selection, and high-throughput phenotyping. These advanced methodologies will be discussed in subsequent sections.

C. Molecular markers and marker-assisted selection for apomixis

Marker-assisted selection (MAS) is a technique used in plant breeding that allows indirect selection through molecular markers linked to genes or genomic regions associated with desirable traits (Collard and Mackill 2008; Das et al. 2017). The main approach of MAS in tropical grasses has been to identify molecular markers linked to genomic regions responsible for controlling the mode of reproduction. Molecular markers associated or linked with apomixis are used for the early identification of clonal progenies. This information is used to estimate rates of clonality and expected efficiencies in forage improvement programs.

The first report of molecular markers co-segregating with (aposporic) apomixis was in species of the genus *Pennisetum* (Ozias-Akins et al. 1993, 1998). Later studies targeted apomixis in other species using different types of markers (Barcaccia et al. 1998; Pessino et al. 2001; Dwivedi et al. 2007; Nakamanee et al. 2015; Kumar et al. 2017; Poblete-Vargas et al. 2018).

In *M. maximus*, Ebina et al. (2005) reported a linkage map for apospory using Amplified Fragment Length Polymorphisms (AFLP), and found nine markers that present strong co-segregation with the trait. Bluma-Marques et al. (2014) added four Random Amplified Polymorphic DNA (RAPD) markers linked to the apo-locus in the same species using segregant bulk analysis. For *P. notatum*, Martínez et al. (2001) identified a single dominant locus controlling apospory, with distorted segregation ratios suggesting pleiotropic effect and incomplete penetrance. The same markers linked to apospory were later used to select apomictic bahiagrass hybrids (e.g., Weiler et al. 2017). The development of molecular markers linked to apomixis has accelerated genetic analyses (Martínez et al. 2003) and facilitated marker-assisted selection in breeding programs (Zilli et al. 2015, 2018, 2019; Brugnoli et al. 2019, 2023; Marcón et al. 2019, 2020, 2024a). In *Urochloa*, the SCAR marker N14 was developed at CIAT (Pedraza Garcia 1995), however, it detects apomixis only when cv. Basilisk (*U. decumbens*) is the pollen donor.

Due to the relevant genetic mapping results in *Urochloa humidicola* that enabled the identification of apospory markers and their application in MAS, we utilize it here as a case study to describe the details of deploying MAS programs. Research on apomixis in this taxon has focused on the hexaploid cytotypes due to the unique existence of a sexual hexaploid genotype (Jungmann et al. 2010). Thus, using two different crosses between two apomictics pollen donors and the only sexual cytotype, two different F_1 mapping populations were obtained for genetic mapping and linkage groups development. Using the first F_1 population, Zorzatto et al. (2010) identified a RAPD marker mapped at 4.61 cM from the apospory locus and Vigna et al. (2016) identified the apo-locus in *U. humidicola* in linkage group 02, and positioned at 19.4 cM from the Single Sequence Repeat (SSR) marker Bh027.c.D2. Later, Moraes et al. (2023) developed a robust genetic map for *U. humidicola* using Single Nucleotide Polymorphisms (SNP) markers with allelic dosage information in the same population used by Vigna et al. (2016), and mapped the Apospory-Specific Genomic Region (ASGR) to a linkage group 3, in a region syntenic to chromosome 3 of *U. ruziziensis* and chromosome 1 of *Setaria italica*. In this study, two SNPs were found co-segregating with the apomictic phenotype and are now being used to develop Kompetitive allele specific PCR (KASP)-SNP markers for application in the breeding programs. Using a different mapping population, Worthington et al. (2019) mapped the ASGR to *U. humidicola* chromosome 1 and to *U. decumbens* chromosome 5, a region syntenic with chromosomes 1 and 7 of *S. italica*. Markers later associated with the ASGR-BBML (Baby Boom Like) gene (see below) co-segregated with the ASGR in both F_1 mapping populations (Worthington et al. 2019; Moraes et al. 2023).

Similarly, the primer pair p779/p780, originally developed from Bacterial Artificial Chromosome (BAC) clones in *Pennisetum squamulatum* (L.) R.Br., targeted exons 4 and 7 of the *PsASGR–BABY BOOM-like* (*psASGR-BBML*) gene, which controls parthenogenesis in *Pennisetum* and *Cenchrus* (Conner et al. 2008, 2013, 2015). This Sequence Characterized Amplified Region (SCAR) marker, which is linked to apospory, has been extensively applied in the CIAT's breeding programs (Worthington et al. 2016, 2019; Perea et al. 2023) and it was validated by Embrapa Beef Cattle researchers (Raposo et al. 2019) in *Urochloa* (Pedroso et al. 2024) and guineagrass (Santos et al. 2025) with reported accuracies of more than 90%. Consequently, the SCAR marker p779/p780 is now routinely used as a diagnostic tool for apomixis in molecular marker-assisted selection (MAS) programs for *Pennisetum/Cenchrus*, *Urochloa*, and *Megathyrsus*.

D. The use of molecular markers and MAS for other traits

Molecular markers have been used to assess and understand the genetic diversity and reproductive mechanisms of a species. In the case of brachiaria and guineagrasses, early studies using RAPD (Almeida et al. 2011) and SSRs (also called microsatellite) markers (Sousa et al. 2011a; 2011b) characterized the genetic variability of the populations available at the germplasm banks of Embrapa Beef Cattle. These markers were fundamental in evaluating the genetic relationships between different accessions, cultivars, and hybrids, and provided a robust basis for more targeted breeding strategies. Molecular makers have also been used to study the diversity of *Paspalum* germplasm in Brazil (Cidade et al. 2013; Garcia et al. 2013; Oliveira et al. 2016; Matta et al. 2023), and with ISSR (inter simple sequence repeats) molecular markers in Uruguay (Reyno et al. 2012). In Argentina, molecular markers are used to determine the hybrid origin of the progenies (Zilli et al. 2015; Novo et al. 2017; Urbani et al. 2017; Brugnoli et al. 2019, 2023). SSR, as codominant markers, were also used for the confirmation of inter- and intra-specific hybridizations in different *Paspalum* species (Lopes et al. 2018).

A practical example of the use of molecular (DNA-based) markers in breeding programs is through the analysis of genetic "fingerprints". Fingerprints are generated by different techniques that create unique genetic profiles for different plant genotypes and varieties, enabling researchers and breeders to identify, assess, and select between closely related genotypes for improved productivity and genetic traits, to detect contamination in seed lots and to ensure purity and plant proprietary rights. For brachiaria grasses, Embrapa is dedicating substantial efforts to developing fingerprint panels for all cultivars available in the market, as well as for new cultivars that are under development. Moreover, the development of SSR markers allowed the generation of multiplex panels (i.e. sets of markers that can be simultaneously analyzed) for molecular fingerprinting of cultivars and accessions, making genetic testing more efficient and cost-effective within the breeding programs.

Genetic mapping is fundamental for locating the genomic regions governing key agronomic traits. It establishes the foundation for MAS by requiring the initial development of high-resolution linkage maps and markers, leading to the subsequent mapping of commercially important loci (Ferreira et al. 2021). Today, different genetic maps are available for use in brachiaria grasses, such as the map developed comprising approximately 1,000 SNP markers evenly distributed across nine homology groups (Ferreira et al. 2019). This map enabled the identification of genomic regions (QTLs) associated with resistance to the spittlebug (*Notozulia entreriana* Berg), which is the main insect pest attacking *U. decumbens*. The phenotypic variation explained by QTLs ranged from 4.66% to 6.24%, reflecting the moderate heritability among clones estimated for spittlebug resistance within the population (Ferreira et al. 2019).

The advancement in sequencing technologies and the associated cost reduction had made it possible to produce reference genomes for nonmodel species such as *U. ruziziensis* ($2n = 2x = 18$) (Pessoa-Filho et al. 2019; Worthington et al. 2021), *U. decumbens* cv. Basilisk (Ryan et al. 2025), *M. maximus* (Jauregui et al. 2024), *C. purpureus* (Teshome et al. 2024), and the construction of a reference genome for *U. brizantha* cv. Marandu is ongoing (K. Meireles, personal communication). Similar genomic resources have boosted the generation of large-scale genotyping solutions in other crops and will have a similar impact on grass breeding, including the regular use of NGS methods and the development of panels for MAS.

Lara et al. (2019) evaluated different genomic prediction models applied to a recurrent selection population of autotetraploid guineagrass using Genotyping-by-Sequencing (GBS) data, considering allele dosage in the prediction. The authors found that tetraploid data provided higher predictive ability than diploid datasets for certain models, thus providing the first bioinformatic and modeling guidelines that could lead to additional gains in recurrent selection programs by considering tetraploid dosage in genomic selection (Lara et al. 2019). Deo et al. (2020) and Gesteira et al. (2025) developed linkage maps for guineagrass using SNP markers and allelic dosage information, allowing the identification of quantitative trait loci (QTLs) related to complex agronomic traits and apospory.

Despite these studies and the relevance of guineagrass for the market of tropical forages, the use of MAS for agronomic traits is not yet reported in the literature. However, the above-mentioned studies evaluating linkage maps, QTL associations (Deo et al. 2020; Gesteira 2021) and genomic selection models (Lara et al. 2019; Aono et al. 2022), combined with the availability of a reference genome (Jauregui et al. 2024), is paving the way for the use of new molecular tools in the breeding of guineagrass.

Ongoing studies primarily focused on *P. notatum* are utilizing GBS to identify SNP markers associated with seed production-related traits, such as seed shattering, seed set, apomixis, and resistance to Ergot (*Claviceps* spp.). These studies aim at the assisted selection of those traits.

IV. Case studies

A. Brachiariagrasses (Urochloa spp.)

Urochloa P. Beauv. comprises about 100 species native to tropical and sub-tropical regions worldwide, but the species most widely used as pasture originated in tropical and sub-tropical floodplains and savannahs from East Africa. These are *U. decumbens* (Stapf) R.D. Webster, *U. brizantha* (Hochst. ex A. Rich.) R.D. Webster, *U. humidicola* (Rendle) Morrone & Zuloaga and *U. ruziziensis* (R. Germ. and C.M. Evrard) Crins.

The first and most adopted *Urochloa* cultivars in Latin America, where the initial large-scale expansion happened, were *U. decumbens* cv. Basilisk, *U. brizantha* cv. Marandu and *U. ruziziensis* cv. Kennedy or ruzigrass. Both cultivars Basilisk and Marandu are tetraploid apomicts while Kennedy is diploid sexual.

As early as 1930 some ecotypes reached Australia including the apomictic accession of *U. decumbens* which became the cultivar Basilisk (CPI 1694) originally from Uganda (Oram 1990). Later in the 1950s, this cultivar was sent to Brazil where it was adopted because of its ease of establishment, rusticity and persistence. By the 1970s, it was widespread and opened the savanna areas in Central Brazil allowing the cultivation of many crops (Santos Filho 1996). The cultivar Basilisk has high biomass production in low fertility and acid soils, and tolerates direct grazing and animal trampling. However, it is susceptible to spittlebugs (*Hemiptera*, *Cercopidae*), a pest of economic importance in neotropics.

Urochloa brizantha cv. Marandu was released by Embrapa in 1984 and rapidly adopted due to its antibiotis resistance to spittlebugs, and similar palatability and quality as Basilisk. However, it is not as well adapted to low fertility and poorly drained soils. Still, Marandu is now the forage that occupies the largest pasture area in Brazil, estimated in over 50 million hectares (Jank et al. 2014).

Ruzigrass (*U. ruziziensis)*, is characterized by high seed production, high palatability and forage quality, higher nutrient requirements and soil drainage and it is highly susceptible to spittlebugs (*Hemiptera*, *Cercopidae*) (Miles et al. 2006). Due to its abundance of seed production (and consequently low commercial value), it is vastly used in integrated systems together with crops.

Urochloa humidicola has been extensively adopted in the neotropics, particularly in poorly drained soils, and it is characterized by its stoloniferous growth habit that allows it to evenly cover the soil, its tolerance to low fertility and acid soils, grazing and trampling tolerance, and its ability to resist spittlebugs. However, *U. humidicola* is very slow to establish and for this reason it is often planted mixed with *U. decumbens* that establishes faster, but it will be *U. humidicola* the species that will dominate the pasture in the long term. In addition, its nutritional quality is lower than the other commercial *Urochloa* species. Seed production can be low depending on environmental conditions, and the seed have long periods of dormancy.

Numerous studies have reported that certain genotypes of *U. humidicola* express biological nitrification inhibition (BNI), an important trait related to the reduction of N_2O emissions from pastures (Subbarao et al. 2013; Horrocks et al. 2019; Lombardi et al. 2022).

The increased demand for seed pastures brought about the development of an advanced tropical forage seed industry in Brazil, which is now the largest producer and exporter in the world. Thus, cultivars produced for Brazilian ecosystems ended up reaching animal production systems around the tropical world.

1. Germplasm

Urochloa germplasm was systematically collected by Dr. Bogdan (a researcher from Kitale, Kenya) during the 1950s (Keller-Grein et al. 1996), and it was exchanged among many institutions.

In the 1970s, important collections were made by both the Commonwealth Scientific and Industrial Research Organization (CSIRO, Australia) (Staples 1971; Strickland 1972) and the United States Department of Agriculture (USDA, USA) (USDA 1974, 1978) through missions not necessarily focused on *Urochloa*. A project of the Food and Agriculture Organization (FAO) based at Kitale, Kenya collected more than 200 accessions until 1984 (Ibrahim 1984; Keller-Grein et al. 1996).

The most thorough and important collection expedition was organized and conducted between 1984 and 1985 by CIAT's Tropical Pastures Program in collaboration with the International Livestock Center for Africa (ILCA), with support from the International Plant Genetic Resources Institute (IPGRI) and national agricultural research institutions in the six countries where the germplasm was collected (i.e., Kenya, Ethiopia, Tanzania, Zimbabwe, Rwanda and Burundi). Approximately 800 accessions, comprising a total of 23 *Urochloa* species, were collected (Keller-Grein et al. 1996). Over 400 accessions of 15 species were sent to Brazil and Colombia, where they have been extensively evaluated since 1986 (Pizarro 1992) and have become the basis for the breeding programs in both countries. Since 1988, agronomic evaluations of germplasm have also been conducted throughout Latin America, the results of which have been compiled in CIAT′s Annual Reports and in the reports presented and edited by Pizarro (1992).

Despite the large numbers of accessions collected in this and other expeditions, the *Brachiaria* germplasm currently does not yet represent the natural variability of the species, as some countries in Africa were never explored (Keller-Grein et al. 1996) (including Uganda, where the wild genotype from which the cultivar Basilisk derives was collected more than 70 years ago).

Embrapa Beef Cattle received the first sexual plant available to the *Brachiaria* breeding programs from the Catholic University of Louvain (Université Catholique de Louvain), Belgium, in the late 1980′s and it was later sent to CIAT. It was a diploid plant of *B. ruziziensis*, and its chromosome numbers were duplicated with colchicine (Swenne et al. 1981; Valle and Savidan 1996) for the production of a tetraploid sexual plant to be used in breeding with tetraploid accessions of *B. decumbens* and *B. brizantha*. This allowed the programs to combine the best characteristics of these species into a new line of interspecific cultivars (Miles 2006).

A detailed source of information about the biology, agronomy and improvement of *Urochloa* species can be found in Miles et al. (1996, 2004), and Valle and Pagliarini (2009).

2. Brachiariagrasses breeding program in Brazil

The breeding pipeline used in the *Urochloa* breeding program at Embrapa in Brazil (Figure 1) began with the availability of germplasm received from CIAT during the 80′s (see above) and its characterization for reproductive mode, ploidy level, genetic variability,

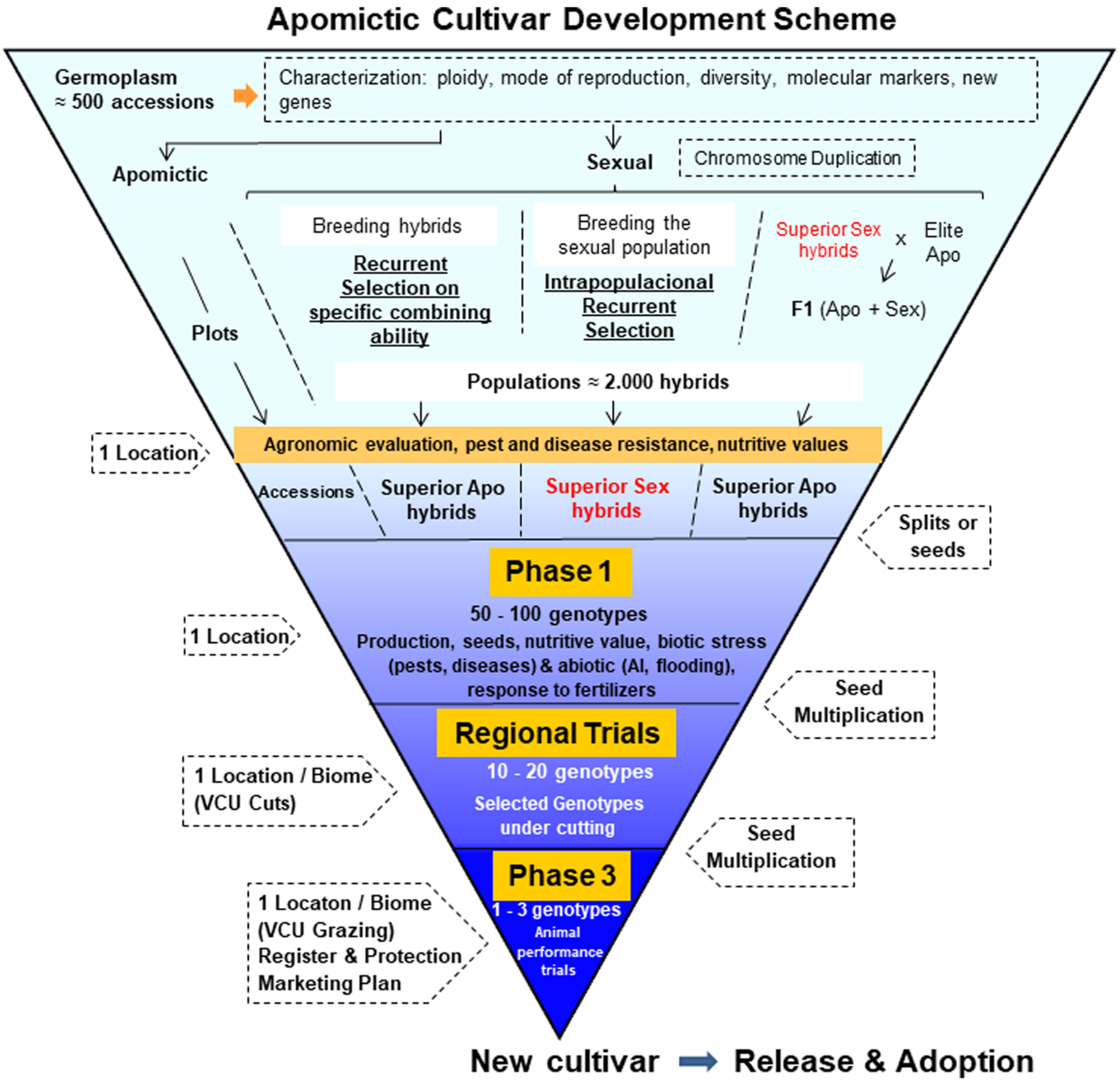

Figure 1. General breeding scheme of the stages and number of genotypes involved in the development of an apomictic cultivar of tropical forage grass (adapted from Jank et al. 2014).

and gene identification among others. Apomictic (tetraploid) accessions were evaluated in small plots, and sexual tetraploidized accessions were crossed with apomictic genotypes to obtain segregating progenies. The program was focused on developing apomictic tetraploid hybrid cultivars ($2n = 4x = 36$) through interspecific crosses between sexual tetraploidized *U. ruziziensis* genotypes, used as the female parent, with apomictic genotypes of *U. brizantha* and *U. decumbens*, used as male parents. Subsequently, two new programs for intraspecific hybrids were implemented based on a tetraploidized diploid accession of *U. decumbens* doubled with colchicine (Simioni and Valle 2011) and a sexual hexaploid *U. humidicola* which was found in the collection ($2n = 6x = 36$). In both cases, the use of these new sexual genotypes was aimed at the development of apomictic cultivars. Thus, the three programs are: one interspecific program *U. ruziziensis* × *U. brizantha* × *U. decumbens*, and two intraspecific programs for *U. humidicola* and *U. decumbens*.

In Phase 1, the most vigorous germplasm accessions and hybrids (50–100 genotypes) are evaluated under harvests, and new traits are quantified (biotic and abiotic stresses, seed production, response to nutrients, etc.). This phase is carried out in one location and the plants are evaluated for agronomic traits and nutritive value in plots of five clonal plants in the experimental field, and for seed yield in individual plants. Greenhouse evaluation is carried out for biotic stresses (Mateus et al. 2013, 2015; Oliveira et al. 2015; Figueiredo et al. 2019; Pandolfi Filho et al. 2016; Matias et al. 2016; Monteiro et al. 2016; Mendonça et al. 2013; de Assis et al. 2024; Dias Filho et al. 2020).

The selected genotypes follow to the next phase that involves regional trials, where they are evaluated under consecutive harvests to determine their performance under different environmental conditions. The goal is to identify genotypes with broad and narrow adaptation to the tested environmental conditions. The genotypes selected in this phase (often 1–3 genotypes) are then evaluated under grazing conditions (Phase 3) to determine animal performance (individual weight gain or milk yield and gains per area). Superior genotypes from phase 3 are registered, a marketing plan is made, and they are released as cultivars.

The efforts of the above breeding programs at Embrapa have resulted in the release of several *U. brizantha* cultivars such as Xaraés (2003), BRS Piatã (2007) and BRS Paiaguás (2013); *U. humidicola* cv. BRS Tupi (2012); and BRS Ipyporã (2017) (see details in Valle et al. 2022). The breeding pileline presented in Figure 1 takes about 8 to 10 years, considering the time lags due to seed multiplication requirements between stages, which is necessary to establish larger trials in more locations.

Following the three phases of the breeding program, from characterization of hundreds of genotypes and directed sexual × apomictic crosses, selection of tens of elite hybrids, and recommendation of 1 or few for cultivar release, the program goes from large numbers and broad genetic variability to a small number and a narrower genetic base (Figure 1). The breeding pipeline involves a multidisciplinary team of entomologists, geneticists, plant pathologists, seed technology experts, soil and pasture management scientists, and a technology transfer team to release a new cultivar.

The development of new forage cultivars is nowadays sped up by biotechnological advances that provide accuracy in the identification of hybrids, molecular markers linked to apomixis, resistance to biotic and abiotic stresses, etc. (see section IV).

For the three breeding programs detailed above, the selection schemes RS-Intra, RS–SCA and PD have been adopted (Figure 2).

The first selection scheme (RS-Intra) aims to improve the sexual population in the medium to long term, and provide improved sexual hybrids that can be used later in crosses with apomictic genotypes (Miles et al. 2006; Miles 2007). In this scheme, half-sib progenies are obtained through the recombination of superior sexual plants (Year 1) and these progenies are evaluated and selected in the following year (Year 2) for traits of interest. Using this approach, RS-Intra cycles were carried out in intraspecific *U. humidicola* programs (Berchembrock et al. 2015; Berchembrock 2017) and interspecific *U. ruziziensis* × *U. brizantha* × *U. decumbens* programs (Pandolfi Filho et al. 2016) aiming to increase resistance to spittlebugs (*Hemiptera: Cercopidae*), seed yield, and forage production. The improved sexual hybrids obtained were subsequently crossed with apomictic parents (PD strategy) aiming at the development of new cultivars.

The second selection scheme (RS–SCA) aims at promoting simultaneously population improvement

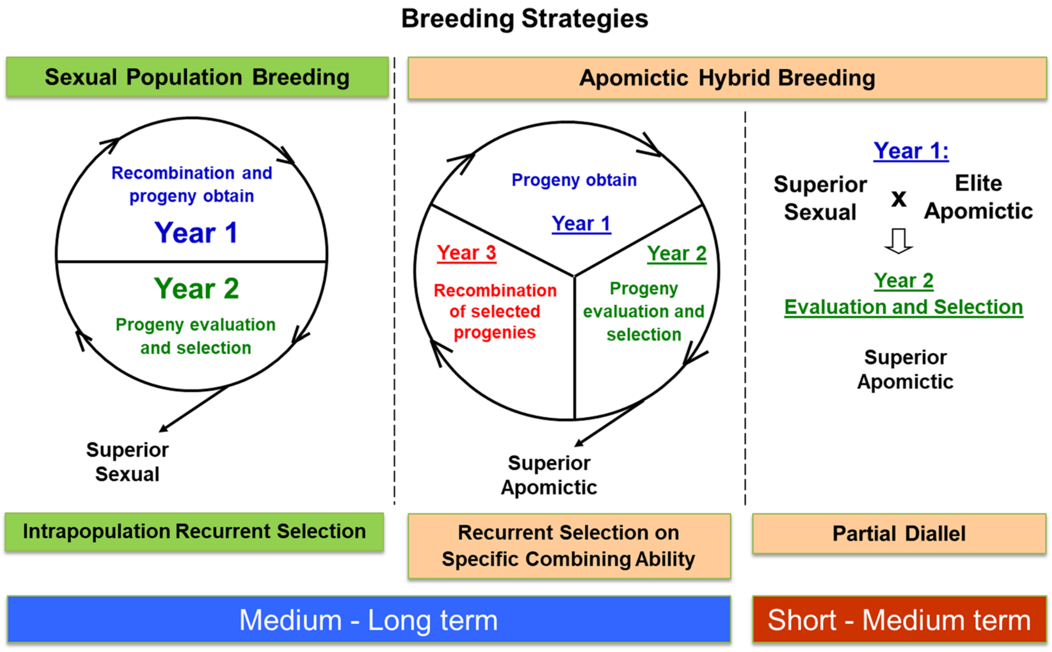

Figure 2. Breeding strategies used to develop new apomictic *Urochloa* cultivars in EMBRAPA.

and cultivar development in the medium to long term. In this scheme, full-sib progenies are obtained by crossing sexual plants with an apomictic tester (Year 1), are evaluated and selected for traits of interest (Year 2), and the sexual plants raising the selected progenies are recombined (Year 3) for the formation of a new selection cycle (Miles 2007). In the intraspecific program based on sexual colchiploid *U. decumbens*, one RS–SCA cycle was performed using *U. decumbens* cv. Basilisk as apomictic tester followed by selection of superior apomictic hybrids (Barrios et al. 2013; Matias et al. 2016).

The third selection scheme (PD), aimed at selecting superior apomictic hybrid progeny in a short-medium term, consists of directed crosses between superior sexual plants with elite apomictic genotypes, using a partial diallel scheme. Superior sexual plants selected in the RS-Intra or as sexual F1 hybrids from the PD can be used as female parents. The PD scheme has been used in Embrapa's three apomictic breeding programs, with new apomictic hybrids currently being in the final stages of evaluation and registration (Alves et al. 2014; Matias et al. 2018; Figueiredo et al. 2019; Gouveia et al. 2022).

3. Brachiariagrasses breeding program at CIAT

The International Center for Tropical Agriculture (CIAT), now together with the Alliance of Bioversity International, also leads breeding efforts in *Urochloa* for sustainable livestock production in tropical and sub-tropical regions, and more recently for temperate/mediterranean environments. CIAT's breeding program aims to develop improved cultivars with enhanced agronomic performance, nutritional quality, and tolerance to biotic and abiotic stresses that contribute to climate change mitigation, resilience and to food and nutrition security for the betterment of livelihoods.

To present date, the *Urochloa* breeding program prioritizes key traits to enhance productivity, resilience and environmental sustainability. Given the increasing frequency of extreme climate events, improving drought and waterlogging tolerance is essential, with a focus on water-use efficiency (Rao et al. 2007, 2011; Cardoso et al. 2015; Jiménez et al. 2017). Efforts to enhance pest resistance, particularly against spittlebugs (*Hemiptera*: *Cercopidae*), have successfully increased tolerance through recurrent selection with high rates of genetic gain (Miles et al. 2006; López et al. 2009; Cardona et al. 2010; Aguirre et al. 2013; Espitia et al. 2022). More recently, tolerance to spider mites *(Acari*: *Tetranychidae*), which can cause significant yield losses in *Urochloa* in East Africa, is being addressed (Espitia and Hernández 2021; Espitia et al. 2022; Espitia-Buitrago et al. 2023). Important agronomic traits such as biomass yield, seed yield, and nutritional quality have been increased over the breeding cycles (Espitia et al. 2024). The program screens for root architecture and nutrient acquisition efficiency, enhancing overall adaptation to low pH and low-fertility soils and high concentrations of aluminum and iron (Worthington et al. 2021). In addition, traits related to climate change mitigation are being assessed to develop cultivars with improved carbon sequestration ability and reduced enteric methane emissions from ruminant livestock (Gaviria-Uribe et al. 2020; Quintero-Anzueta et al. 2021; Arrechea-Castillo et al. 2024; Hernandez Alvarez et al. 2024; Molina-Botero et al. 2024).

3.1. Breeding strategies and approaches.

CIAT employs conventional breeding and modern biotechnological tools to accelerate genetic improvement in the development of interspecific brachiariagrass hybrids (Figure 3). Due to the monogenic segregation of the apomixis character, the apomixis marker p779/p780 (Akiyama et al. 2011) is used to characterize the F_1 progeny from the cross between sexual parents and an apomictic tester (Worthington et al. 2016). Progenies from these crosses undergo extensive testing. New technologies such as marker-assisted selection (MAS) and genomics, and high-throughput phenotyping are being used to improve selection accuracy through precise evaluation of forage traits under field conditions (Arrechea-Castillo et al. 2024).

After 11 cycles of RS-SCA scheme proposed by Miles (2007), the program has released 10 apomictic interspecific *Urochloa* hybrids. Hybrids are selected for their agronomic performance and improved characteristics in multi-environment trials (METs) across diverse agroecological zones to ensure broad adaptability and stability in different production systems (Table 1). Cultivar Mulato, released in 2001, was the first interspecific *Urochloa* apomictic hybrid cultivar (Miles 1999; Argel et al. 2005) followed by the most extensively grown *Urochloa* hybrid cv. Mulato II released in 2005 (Argel et al. 2007).

These cultivars are now widely adopted, and newer cultivars are complementing older releases throughout the tropics and subtropics in Latin America, Africa, Asia (Burkart 2024).

The *U. humidicola* breeding started in the early 2000s when a sexual accession of the species of the predominant ploidy number in the collection ($2n = 6x = 36$) was identified in the genebank collection at CIAT and EMBRAPA (Valle and Glienke 1991;

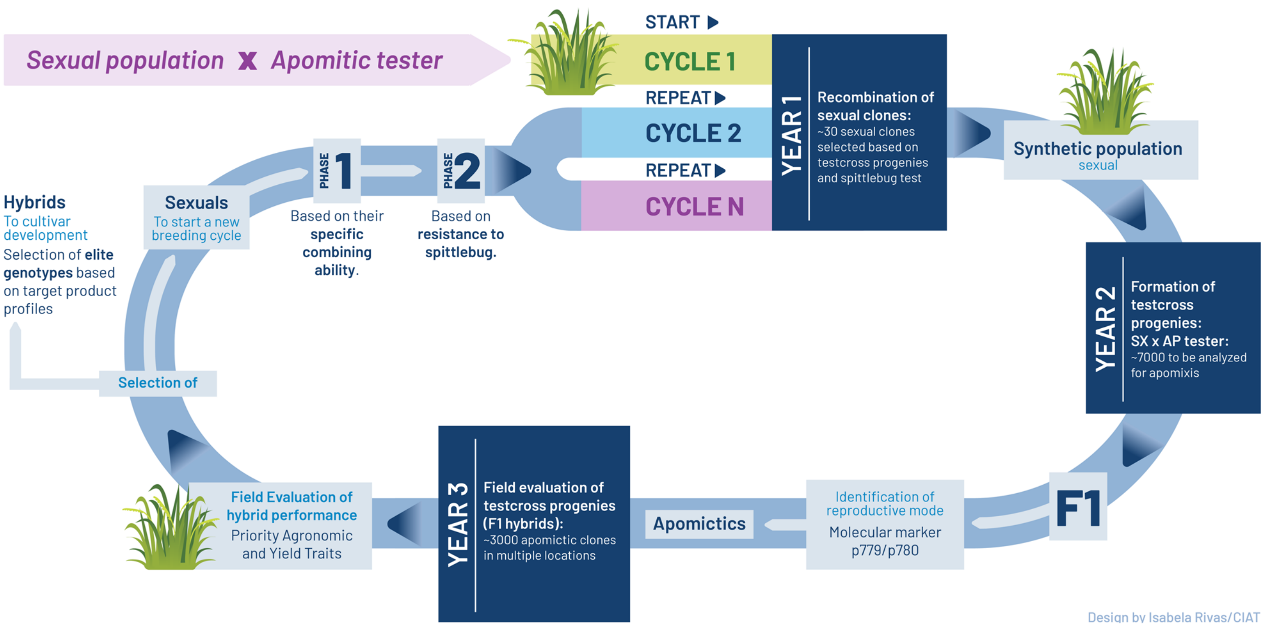

Figure 3. Current breeding scheme in interspecific *Urochloa* breeding in CIAT (from Miles 2007).

Table 1. Hybrid cultivars released from CIAT's interspecific *Urochloa* breeding program.

Cultivar Name	Hybrid	Product Line	Country of Registration
Mulato	CIAT 36061	High Productivity	Argentina; Australia; Bolivia; Brazil; Mexico; Nicaragua; Panama; South Africa; United States
Mulato II	CIAT 36087	High Productivity	Argentina; Australia; Bolivia; Brazil; Ethiopia; Kenya; Mexico; Nicaragua; Panama; South Africa; United States; Vietnam
Mestizo blend*	CIAT BR02/0465* (*component of the blend)	High Productivity	Australia; Mexico
Cayman	CIAT BR02/1752	Heavy Soils	Australia; Brazil; Colombia; Kenya; Mexico; Paraguay
Cobra	CIAT BR02/1794	Cut & Carry	Australia; Brazil; Colombia; Kenya; Mexico
Cayman	GP 0423	Heavy Soils	Australia; Brazil; Colombia; Kenya; Mexico; Paraguay
Camello	GP 3025	Drought	Australia; Brazil; Colombia; Mexico; Paraguay
Camello	GP 3207	Drought	Australia; Mexico; Paraguay
Cayman	CIATGP4009BR	Heavy Soils	Mexico
Okapi	CIATGP1005BR	Shaded environments	Brazil; Mexico

Miles 2006; Worthington et al. 2019). The main objective of CIAT's *U. humidicola* breeding program is to develop apomictic cultivars with improved forage quality, agronomic performance, seed yield, and ecosystem services adapted to the humid tropics and subtropics. The *U. humidicola* program follows the same breeding scheme described for interspecific *Urochloa* hybrids (Figure 3) and the apomixis diagnostic marker p779/p780 is being used (Worthington et al. 2019).

3.2. Future perspectives and research priorities. Future research will focus on:

- Developing low-methane-emitting forages to mitigate greenhouse gas emissions from livestock (Arango et al. 2024).
- Enhancing drought and waterlogging to withstand climate change-related stress.
- Integration of *Urochloa* cultivars into silvopastoral systems, crop rotation practices, and smallholder farming systems, with a focus on enhancing ecosystem services, improving soil fertility, and reducing the environmental impact of livestock systems (Notenbaert et al. 2021; Kaushal et al. 2025).
- Strengthening target product profiles, market segments, seed systems and impact assessments to increase adoption of improved forage technologies with a focus on gender equality and social inclusion (Burkart 2024; Junca et al. 2024).

CIAT's ongoing efforts in *Urochloa* breeding continue to play a crucial role in developing forages that support sustainable livestock production, food security, and environmental resilience. By improving forage productivity and adaptability, and corresponding seed supply systems (seed including vegetative propagation), these innovations strengthen rural livelihoods, boost income generation, and contribute to poverty alleviation and resilience, particularly among smallholder farmers in tropical and sub-tropical regions.

B. Guineagrass (*Megathyrsus maximus*)

Panicum is one of the largest genera in the Poaceae family, encompassing more than 400 species, both annual and perennial from the tropics and warm temperate areas (Wouw et al. 2008). *Panicum maximum* (guineagrass) was first described in 1781, later transferred to an unranked group in 1987 as *Urochloa maxima* (Jacq.) R.D. Webster, to be currently placed in the genus *Megathyrsus* with only one other species *Megathyrsus infestus* (Andersson) B.K.Simon & S.W.L.Jacobs (Simon and Jacobs 2003).

The migration of guineagrass to other countries likely occurred around the 17th century, from the west coast of Africa to the Caribbean Islands (Parsons 1972), then to Colombia (late 18th century) and Central America (mid-19th century). In Brazil, it was probably introduced with the slave ships from Africa (Chase 1944) wherever the boats were unloaded. The introduced cultivar in Brazil was called Colonião.

Guineagrass is one of the most productive high-quality tropical forage grass species propagated by seeds. It is found predominantly across tropical and sub-tropical regions worldwide (Bogdan 1977). It plays a significant role in livestock production, particularly cattle rearing and fattening, but also for milk cattle, sheep, goats and horses. It shows broad adaptation to the seasonally dry tropical biomes. In Brazil, it covers more than 20 million hectares and is recommended for the intensification of cattle production systems under rotational grazing management. However, it presents great production seasonality, with high productivity in the rainy season and very low in the dry season. It may be used as pasture, silage and hay and all its variations, as well as for cut–and-carry, very common in Asian countries. Several of these high biomass apomictic forage grasses, as guineagrass and braquiariagrasses, also have potential as bioenergy feedstock.

1. Germplasm

The high productivity and broad adaptability of guineagrass have triggered the collection of many accessions throughout the world (Jank 1995). However, collections conducted outside the species' center of origin often yielded limited genetic variability (Degras and Doussinault 1969; Millot 1972; Sidak et al. 1977; Savidan et al. 1984).

Studies on apomixis in guineagrass were initiated by French researchers Daniel Combes and Jean Pernès (ORSTOM/IRD - Institut de Recherche pour le Développement) and continued by their disciples, making it a modelspecies. The analysis of 154 accessions from diverse geographical origins (Africa, Australia, Latin America, and Asia) (Savidan et al. 1984), coupled with observations of 129 accessions collected in the Ivory Coast and from herbarium studies (e.g., Kew), indicated East Africa as the species' center of diversity (Combes and Pernès 1970; Savidan et al. 1984).

To further explore this, an expedition to Kenya and Tanzania in 1967 resulted in the collection of 249 samples, one of which (near Korogwe, Tanzania) was identified as diploid and sexual. This discovery prompted a second expedition in 1969 around Korogwe, where 22 more sexual plants were collected (Combes and Pernès 1970). These expeditions marked the first organized efforts to collect a tropical forage species in its center of origin.

Significant research conducted in the Ivory Coast included efforts to duplicate diploid sexuals, crossbreed, assess ploidy levels, and investigate reproductive modes and variability (Combes and Pernès 1970; Combes 1975; Savidan 1975, 1982, 1983; Chaume 1985). Notably, Yves Hervé Savidan's thesis demonstrated that apomictic reproduction in guineagrass is controlled by a single gene or a closely linked gene cluster (Savidan 1975, 1982, 1983).

After completing his thesis, Dr. Savidan was granted authorization by ORSTOM to offer the *P. maximum* collection to the broader tropical world. Among the various proposals received, Embrapa Beef Cattle was selected to receive the entire collection including the sexual tetraploid plants (Savidan et al. 1989), as well as receiving Dr. Savidan in Brazil as a consultant during the first five years. Later, between 1984 and 1987, also due to Dr. Savidan's efforts, part of this ORSTOM apomictic germplasm was sent to research institutions in Colombia (CIAT), Mexico (Faculty of Veterinary Medicine and Animal Husbandry, National Autonomous University of Mexico), and Cuba (Indio Hatuey Experimental Station) (Savidan et al. 1989).

In addition to the French research efforts, Japanese researchers also conducted collection trips to East Africa between 1971 and 1973. Hojito and Horibata (1982) collected 140 accessions, including one sexual, diploid plant (Nakajima et al. 1979). Chromosome doubling of this plant using colchicine (Nakagawa and Hanna 1992) led to genetic improvement efforts in

Japan. Similarly, American researchers, including Smith (1972), Hanna et al. (1973), and Burton et al. (1973), selected highly sexual tetraploid accessions from South African apomictic materials.

2. Guineagrass breeding program in Brazil

Through an agreement-cooperation signed in 1982 between EMBRAPA and ORSTOM, Embrapa Beef Cattle in Campo Grande, MS, Brazil, received its entire guineagrass collection, consisting of 426 apomictic accessions and 417 tetraploidized sexual plants, considered representative of the natural variability of the species (Savidan et al. 1989).

The apomictic accessions were subjected to agronomic evaluation in field plots and underwent morphological characterization over a two-year period at Embrapa Beef Cattle. The objectives of these evaluations were threefold: (1) to identify superior accessions for direct release; (2) to characterize the phenotypic variability within the collection for utilization in genetic improvement programs; and (3) to identify suitable male parents for the initiation of a breeding program (Jank 1995).

The sexual plants were subjected to preliminary visual assessment for vigor and flowering intensity, and the most promising individuals were subsequently evaluated agronomically alongside the apomictic accessions, with the aim of selecting appropriate female parents for the breeding program (Jank 1995).

Selection for yield proved to be relatively straightforward, as 40% of the evaluated accessions exhibited higher dry matter and leaf dry matter yields than the cultivar 'Colonião', which was widely cultivated in Brazil at the time (Jank et al. 1994). Notably, in comparison to 'Colonião', over 76% of the accessions had greater yield during the dry season, 70% of the accessions had superior regrowth following defoliation, but only 20% exhibited greater seed yield and leaf protein content (Jank 1995). Substantial phenotypic variation was observed across all morphological traits examined, including plant height, leaf width, panicle morphology, growth habit, inflorescence characteristics, and pubescence of leaves and stems, among others (Jank 1995).

For the selection of the germplasm accessions to be used in the breeding strategy, divergent accessions were selected based on agronomic evaluation, morphological characterization (Jank 1995) and genetic divergence obtained with molecular markers (Sousa et al. 2011a, 2011b).

The selection and breeding at Embrapa Beef Cattle follows a scheme applicable to both *P. maximum* and apomictic *Brachiaria* breeding programs (Jank et al. 2014), as illustrated in in Figure 1. This scheme outlines that accessions or hybrids resulting from crosses between sexual plants and apomictic genotypes are initially evaluated in small plots over a two-year period (see Figure 1, phase 1) (Jank et al. 1994). The best-performing individuals are subsequently tested in regional trials (five to seven locations) for an additional two years (Lédo et al. 2008; Fernandes et al. 2014), with the top one or two selections undergoing evaluation under grazing conditions (Andrade et al. 2013; Maciel et al. 2018; Braga et al. 2019). Concurrently, experiments assessing tolerance to biotic and abiotic stresses are conducted in controlled greenhouse environments or field settings. These experiments provide critical data for selection of the best materials and, particularly, for cultivar release.

Controlled crosses are conducted in the field, as described in section *III. B. "Inheritance of apomixis, crossings and improvement strategies"*. The current breeding strategy used at Embrapa Beef Cattle is an adaptation from Chaume (1985). This selection scheme has two main objectives: 1) increase the genetic variability of the sexual pool with high breeding value hybrids; and 2) continuously improve and release highly apomictic hybrids. In this scheme, sexual plants selected from the sexual pool are crossed with apomictic accessions or improved hybrids from the apomictic gene pool in a factorial mating design. The program initiated with a small sexual pool composed of the introduced tetraploidized sexual plants and the large germplasm pool of apomictic accessions. In each crossing cycle, the progenitors are new apomictic from the apomictic gene pool and sexual plants selected from the previous cycle. Thus, the sexual pool is increased with the sexual hybrids obtained and the apomictic hybrids follow agronomic evaluations.

In order to meet specific market demands (e.g., low-growing cultivars tolerant to water-logged conditions or cultivars tolerant to periods of drought), biparental crosses can be performed between high-performance sexual and apomictic progenitors that exhibit genetic divergence and high complementarity for key traits.

In Phase 1 (Figure 1), hybrid seed obtained from the sexual plants are germinated in the greenhouse and are transplanted to the fields as: a) for a biparental scheme, individual plants spaced 1 – 1.5 m where they are visually scored for vigor, diseases and overall performance (Jank 1995). Later, cuttings of the selected hybrids are planted in 10-plant replicated plots (two lines of 5 plants each) for agronomic evaluation (Jank et al. 1994); b) for factorial crosses scheme, 10-plant plots with replication, in which each plant is a distinct hybrid (Pereira 2019). The advantage of this method is that both general and specific combining abilities are evaluated at the same time and selection of hybrids is based on best plants within best families as well as within families.

Strategies of spatial corrections and inclusion of additive kinship matrixes are also being applied to increase the accuracy of selection, especially in spaced plant evaluations (Ragalzi et al. 2023). Novel methods, such as high-throughput phenotyping (Rodrigues et al. 2023) and marker assisted and genomic selection (Lara et al. 2019) are potential tools to be used in this stage to accelerate cultivar development.

2.1. Released cultivars. The breeding program in Brazil has successfully released six *P. maximum* cultivars (Jank et al. 2022). The initial four cultivars were directly selected from the germplasm collection, whereas the subsequent two are F1 hybrids derived from crosses between sexual and apomictic genotypes. The cultivars selected from the germplasm bank are: 'Tanzânia-1′ (released in 1990), 'Mombaça' (1993), 'Massai' (2001), and 'BRS Zuri' (2014) (Table 2). The hybrid cultivars are 'BRS Tamani' (2015) and 'BRS Quênia' (2017) (see details in Jank et al. 2022). It is estimated that these six cultivars collectively occupy over 20 million hectares in Brazil and are exported to nearly all Latin American countries (Jank et al. 2014). Furthermore, the earlier releases have been exported to some African and Asian countries.

Table 2. Guineagrass cultivars released by the Embrapa Beef Cattle program in Brazil.

Cultivar	Year released	Plant Size	Main characteristics
Tanzânia-1	1990	Medium	80% > productive than traditional cv. Colonião and easier to manage
Mombaça	1993	Tall	130% > productive than 'Colonião'. It is the most planted, around 15 million hectares in Brazil
Massai	2001	Short	Thrives in conditions where other forages fail in dry areas and low-fertility soils.
BRS Zuri	2014	Medium/tall	Higher weight gain per animal and per hectare than other cultivars
BRS Tamani	2015	Short	For pregnant cows and weaning calves and other categories, has high forage quality and tillering
BRS Quênia	2017	Medium	High quality and weight gain per animal and per hectare, easier to manage in the pasture

3. Guineagrass breeding program at CIAT

Megathyrsus maximus is a species with broad adaptation to the seasonally dry tropical biome. Extensive characterization of the germplasm collections showed that its natural agronomic characteristics make it suitable for forage production in tropical and sub-tropical livestock systems, both for cut and carry and direct grazing (Toledo et al. 1986; Thomas and Lapointe 1989; Wouw et al. 2008). A need has been established to increase the productivity of pastures in the tropics to sustain the increased demand for livestock products and environmental co-benefits of pastures (Horrocks et al. 2019; Philip et al. 2019; Paul et al. 2020; Notenbaert et al. 2021; Dey et al. 2022). Studies by Villegas et al. (2020), and Carvajal-Tapia et al. (2021, 2023) have shown vast variability in *M. maximus* collection at CIAT's genebank for important productivity traits such as biomass and nutritional quality and for environmental traits such as biological nitrification inhibition and more recently the germplasm collection and elite genotypes from the breeding program are being characterized for traits involved in carbon sequestration and reduction of enteric methane emissions from livestock (Mayorga et al. 2024; Wenzl 2023; LMF (Low Metane Forages) Project communication 2024).

However, *M. maximus* is a predominantly apomictic species (Warmke 1954; Savidan et al. 1989). The discovery in the late 2000s of a tetraploid sexual accession made it possible for the breeding program at CIAT to start (Hernandez and Castiblanco 2021). The creation of a synthetic sexual population has allowed the program to implement the RS-SCA breeding scheme (Figure 3) proposed by Miles (2007) for *Urochloa*. In this scheme, the apomixis marker p779/p780 is central to the identification of apomictic genotypes that are advanced to cultivar development and sexual genotypes that are selected for crossing in the next generation (Worthington et al. 2016; Hernandez et al. 2021). Since then, utilizing the most advanced phenotyping techniques, the program has developed elite germplasm with superior traits to be released as apomictic cultivars (Mazabel et al. 2020; Hanley et al. 2021; Ruiz-Hurtado et al. 2024).

C. Paspalum spp.

Paspalum L. is one of the largest grass genera, comprising approximately 310 to 350 species native to the Americas, predominantly in tropical and sub-tropical regions (Zuloaga and Morrone 2005; Morrone et al. 2012), with its center of diversity in South America (Chase 1939). The genus exhibits remarkable variability in species and habitats, supported by their diverse genetic systems (ploidy levels, phylogenetic origins, reproductive modes, and mating systems). Such variability underpins its wide range of applications (Ortiz et al. 2013). *Paspalum* is exploited as a minor cereal crop, forage and turf grass, for soil stabilization, and as ornamental plants (Acuña et al. 2019). Moreover, it is an attractive biological model for studying apomixis, providing a dual advantage as a source for candidate gene discovery and as an important target in breeding programs (Ortiz et al. 2013).

There are several *Paspalum* breeding programs worldwide. This review, however, will focus on the five *Paspalum* breeding programs for forage in tropical America: one located in Argentina, one in Uruguay, one in the USA and two in Brazil. All share long-term collaborations and are based at the Institute of Botany of the Northeast (IBONE, Corrientes, Argentina), the Faculty of Agricultural Sciences of the National University of the Northeast (FCA-UNNE, Corrientes, Argentina), the Agricultural Sciences Research Institute of Rosario (IICAR, Santa Fe, Argentina), the Faculty of Agronomy (UBA, Buenos Aires, Argentina), the Agronomy Department at the University of Florida (Gainesville, FL, USA), the Federal University of Rio Grande do Sul (UFRGS, Porto Alegre, RS, Brazil) and Embrapa Southeast Livestock (São Carlos, SP, Brazil) and the National Institute of Research in Agriculture (INIA, Tacuarembó, Uruguay).

In Argentina, the focus of the breeding program is on developing forage cultivars, from species including *P. notatum* Flügge, *P. simplex* Morong, species within the Plicatula group and *P. dilatatum* Poir. The focus of the Uruguayan breeding program is similar, and involves *P. notatum*, *P. dilatatum* and *P. plicatulum* Michx., with minor efforts in *P. urvillei* Steud. In the USA, breeding for forage involves *P. notatum* and in Brazil, Embrapa breeds *Paspalum regnellii* Mez and species from the Plicatula group, while at UFRGS, *P. notatum*, *P. guenoarum* Arechav., *P. lepton* Schult. syn. *P. nicorae* Parodi and *P. urvillei* are bred.

1. Germplasm

Many germplasm collecting expeditions have been carried out in Brazil (Barreto 1963; Valls 1994), Argentina, and Uruguay (Reyno et al. 2012). In Brazil, collections led by the Embrapa Genetic Resources and Biotechnology (CENARGEN) with the participation of Dr. Byron Burson (USDA) have gathered many *Paspalum* accessions. Part of this material is duplicated and conserved at ARS-GRIN in the USA (J.F.M. Valls, personal communication). The National Plant Germplasm System from the United States Department of Agriculture maintains a germplasm collection with 154 accessions collected in various countries, primarily from South America. The Brazilian germplasm collection consists of 1091 accessions from 76 different species (https://av.cenargen.embrapa.br/avconsulta/Passaporte/buscaNc.do#).

In Uruguay, 417 individuals were collected in 2006 (Reyno et al. 2012). Besides this, the germplasm bank from the Agronomy College of the University of the Republic maintains 1012 accessions from more than 30 *Paspalum* species (R. Vidal, personal communication).

The IBONE and FCA-UNNE maintains an *in-vivo* germplasm bank that comprises 571 accessions from 73 *Paspalum* species (Acuña et al. 2019; Brugnoli, pers. comm.). This collection encompasses accessions spanning a broad ecological spectrum, with diverse genetic systems.

2. Paspalum breeding programs in Brazil

2.1. Breeding program at Embrapa.

The forage breeding program at Embrapa is focused on the evaluation and selection of apomictic ecotypes, and intra- and inter-specific hybridizations aiming at the release of superior apomictic cultivars.

The *Paspalum* germplasm was initially characterized for number of chromosomes, chromosome behavior, ploidy, and mode of reproduction (e.g. Adamowski et al. 2000; Pagliarini et al. 2001; Gonçalves et al. 2021; Matta et al. 2023). The majority of the genotypes are tetraploid and apomictic, but sexual diploids and apomictic hexaploids were also found.

Molecular characterization of the germplasm was also done to estimate the genetic diversity (Cidade et al. 2013; Matta et al. 2023) and to indicate the best parents for hybridization (Matta et al. 2023).

Many accessions of the Brazilian gene bank were evaluated throughout Brazil (e.g. Pizarro et al. 1997; Ramos 2002; Meirelles et al. 2013). The species *P. guenoarum*, *P. notatum* and *P. secans* were found to be promising in the Amazon biome (Souza-Filho et al. 1992), as well as *P. atratum* (Meirelles et al. 2011). This one stood out under grazing at Embrapa Southeast Livestock (Provazi et al. 2008). Due to the low quality of many *Paspalum* accessions, shear strength of leaves was found to be negatively associated with forage quality (de Lucena et al. 2017) and may be used for indirect assisted selection (Barros et al. 2022).

In the state of São Paulo, Brazil, of 215 accessions evaluated, 72% produced viable seeds, and 58 accessions (27%) were selected for yield (Batista and Godoy 1998, 2000). One of these selected accessions was

Paspalum regnellii BRA-019186, which was later registered as cv. BRS Guará. It is a high-quality productive cultivar, demonstrating its potential as alternative forage to *U. brizantha* cv. Marandu monoculture in Amazonian forage-based livestock systems (Tesk et al. 2020). It exhibited greater herbage accumulation and nutritional value compared to cv. Marandu (Meirelles et al. 2013).

Cultivar BRS Guará is used as sexual parent in this and other breeding programs due to its natural tetraploid sexual nature, in contrast to most species which are tetraploid apomictic, thus eliminating the need for chromosome duplication of the common diploid sexual forms (Acuña et al. 2019; Matta et al. 2023).

The breeding program at Embrapa Southeast Livestock began in 2015 and other species from the Virgata group are also used as female parents (Lopes et al. 2018), due to their natural sexual tetraploidy. Problems of flowering asynchrony in the program are overcome by using a protocol developed by Dinato et al. (2018) for pollen cryopreservation of *P. notatum*, which was later adapted to *P. atratum*, *P. mallacophyllum* and *P. regnellii* (Dinato et al. 2024).

For the Plicatula group, tetraploidization through colchicine of sexual diploid accessions resulted in the development of sexual tetraploids (Fávero et al. 2020). Following a polycross among the diploid sexual accessions from the Plicatula group identified in the germplasm bank (Gonçalves et al. 2021), seeds were collected, germinated and the paternity test were performed using different SSR markers to confirm hybridizations and identify the pollen donor. Subsequently, seeds and plantlets were exposed to different colchicine treatments and some plants were obtained (Fávero et al. 2020). These plants are now being evaluated for viability, seed production and the presence of chimeras (A. P. Fávero, personal communication).

The breeding program at Embrapa is still at its initial, thus no hybrids are yet available for release as cultivar.

2.2. Breeding program at the Federal University of Rio Grande do Sul (UFRGS).

In 2009, UFRGS initiated a breeding program for *P. notatum* and species within the Plicatula group. The selection schemes and strategies used for cultivar development were based on phenotypic selection of ecotypes and F_1 hybrids.

2.2.1. P. notatum. The argentinian IBONE provided UFRGS three sexual tetraploid genotypes: C4-4x (Quarin et al. 2001), Q4188, and Q4205 (Quarin et al. 2003), which were used to perform the first crosses with two elite native apomictic tetraploid ecotypes 'Bagual' and 'André da Rocha'. From the seeds obtained in these crosses, a progeny of 198 hybrid plants was generated, which was subsequently evaluated for mode of reproduction (Weiler et al. 2017) and agronomic potential (Weiler et al. 2018). Several traits were evaluated, but mainly forage and seed yield, tolerance to frost, and forage seasonal distribution and persistence. The progenies were extensively tested in three locations in southern Brazil and many apomictic hybrids showed excellent performance and advanced for future evaluations. On the other hand, many sexual progenies also showed good performance, and these were again used in new crosses, initiating new cycles of selection.

Weiler et al. (2015) induced polyploidy in the diploid sexual cultivar Pensacola through colchicine treatment, resulting in the development of three sexual tetraploid plants. This approach expanded the genetic base and enhanced the search for superior genotypes with desirable agronomic traits for forage production. Consequently, new crosses have been conducted, and the resulting hybrids have been evaluated for their mode of reproduction (Krycki et al. 2020; Machado et al. 2021) and agronomic potential (Machado et al. 2017; Ghellar Junior et al. 2022; Krycki et al. 2022; Souza et al. 2023).

Currently, the program has several apomictic hybrids with superior agronomic performance, exceeding cv. Pensacola in forage and seed yield and persistence, and one of these hybrids is in the process of registration within the MAPA.

2.2.2. P. guenoarum. The development of the first interspecific hybrids of *Paspalum guenoarum* was possible using the sexual tetraploid *Paspalum plicatulum* plant known as '4PT' (Aguilera et al. 2011). The breeding methodology was very similar to the described above for *P. notatum*, using two *P. guenoarum* elite ecotypes as pollen donors, "Baio" and "Azulão" (Steiner et al. 2017). These hybrids were later evaluated in field trials in three locations, and their reproductive mode was determined. The agronomic performance studies demonstrated that the hybrids exhibited high genetic potential for forage production, with dry matter yields reaching 19,074 kg ha^{-1}, cold tolerance (Motta et al. 2017, 2020a, 2020b; Saraiva et al. 2021; Scienza et al. 2024), and seed yields of up to 2380 kg ha^{-1} (Scienza et al. 2024). The most promising hybrids advanced to the mandatory trials required by the MAPA for cultivar registration and protection, and one of them is now under the process of registration (M. Dall'Agnol, personal communication).

For both species, *P. notatum* and *P. guenoarum*, studies have shown that when used in mixtures with temperate legumes, the yield obtained is similar to that achieved with nitrogen doses between 60 and 120 kg/ha/year. This finding is very important because it reduces costs and decreases environmental impacts while producing forage with better quality than that produced by the grasses alone (Motta et al. 2017, 2020b).

2.2.3. P. lepton (syn. P. nicorae). Although the *P. lepton* breeding program at UFRGS is at an earlier stage, the species has been extensively studied over the past three decades. Studies with *P. lepton* accessions have determined chromosome numbers, meiotic behavior, and pollen fertility (Reis et al. 2008), as well as morphological variation, forage yield and nutritional value (Reis et al. 2010), genetic variability for forage traits (Pereira et al. 2012; Motta et al. 2013), adaptability and stability, variability and progeny selection, and genetic gain (Pereira et al. 2017).

In 2017, 159 *P. lepton* accessions collected from four regions of Rio Grande do Sul, Brazil, were incorporated into the UFRGS germplasm bank. Of these, 84 accessions were evaluated for forage and seed production (Silveira 2023). The most promising germplasm, in terms of forage and seed yield is under further agronomic evaluations, aiming to use this species to recover degraded areas, since it has a good adaptation to sandy soils (M. Dall′Agnol, personal communication).

2.3. Cultivars released on the market. Fourteen cultivars of seven different species of *Paspalum* are registered at the MAPA - CultivarWeb platform. (https://sistemas.agricultura.gov.br/snpc/cultivarweb/cultivares_registradas.php). The cultivars registered as forage are *P. atratum* cv. Pojuca and *P. regnellii* cv. BRS Guará, both released by Embrapa but currently not available in the market. Two cultivars, one from *P. dilatatum* and the other from *P. notatum* are registered but no further details are included. The cultivar of *P. notatum* probably corresponds to cv. Pensacola.

3. Paspalum breeding program in Argentina

The development of cultivars has been constrained by the scarcity of sexual tetraploid germplasm. A breakthrough was achieved with the creation of sexual tetraploid lines via chromosome doubling of sexual diploids (Quarin et al. 2001). This innovation enabled hybridization between sexual and apomictic genotypes, unlocking the genetic variability within apomictic ecotypes and facilitating the development of synthetic sexual tetraploid populations (Zilli et al. 2018, 2019; Novo et al. 2020).

The IBONE and FCA-UNNE breeding program prioritizes several key traits to develop superior forage cultivars. A crucial objective is to extend the period of active growth—thereby increasing forage availability during early spring, late fall, and even winter in milder sub-tropical climates—by selecting for genotypes with reduced photoperiod sensitivity and tolerant to low temperatures (Acuña et al. 2009, 2011; Marcón et al. 2024a).

Seed production remains essential for both establishing new pastures and commercializing improved cultivars. Consequently, breeding efforts have focused on seed yield and seed quality (Acuña et al. 2009; Chamorro et al. 2024). In *Paspalum*, significant seed shattering has been observed, making improved seed retention imperative to minimize losses (Chamorro et al. 2024).

Forage nutritive value has also been a focus for improvement. Apomictic hybrids showed increased levels of omega-3 fatty acids in their leaves (Marino et al. 2025); and forage palatability is a fundamental trait in the development of forage cultivars for grazing (Marcón et al. 2023).

The main methods used for breeding apomictic *Paspalum* species in Argentina are ecotype selection, hybridization, and population improvement. In the ecotype selection scheme, genotypes are evaluated across multiple environments and years for forage production, quality, and resistance to relevant biotic and abiotic stresses. The superior selected genotype can be registered and released as a new cultivar. In Argentina, several *Paspalum* cultivars, such as Cambá FCA (*P. atratum*), Chané FCA and Ramirez INTA (*P. guenoarum*), and Relincho FAUBA (*P. dilatatum*), have been released using this method.

Seeds from various collections of *Paspalum atratum* were incorporated into the IBONE Germplasm Bank in Corrientes, Argentina. After several years of evaluation and selection, a collection originally obtained from a waterlogged field in Campo Grande, MS, Brazil, was registered in 1997 as the cultivar Cambá-FCA by FCA-UNNE. It is possible that other cultivars of the species, such as, Pojuca (Brazil), Suerte (USA), and Ubon (Thailand), belong to the same genotype, as they all originate from the same geographical source (Acuña et al. 2019). Similarly, multiple accessions of *Paspalum guenoarum* were evaluated across different environments, leading to the registration of the cultivar Chané-FCA in 2022 by FCA-UNNE. This cultivar is derived from an accession collected in Santa Cruz Department, Ñuflo de Chávez Province, Bolivia.

Among ongoing work, Marcón et al. (2018) evaluated five ecotypes of *P. atratum* and six of *Paspalum lenticulare*. Their findings highlighted the accession U44 of *P. atratum* from Cáceres, Mato Grosso do Sul, Brazil, which exhibited superior forage characteristics and high animal preference. Marcón et al. (2023) reported superior forage characteristics and animal preference of accession U44, which makes it a promising candidate for cultivar registration.

Researchers from the Faculty of Agronomy Sciences of Buenos Aires (FAUBA) conducted evaluations and selection in *P. dilatatum* (dallisgrass) collected in the temperate region of Argentina, leading to the registration of the cultivar Relincho-FAUBA in 2003. This cultivar is a pentaploid apomictic genotype (Schrauf 2003).

Hybridization has been used as a mechanism in breeding programs for *P. notatum*, *P. simplex*, and the Plicatula group. Figure 4 illustrates the phases of the breeding program carried out in Argentina. This program involved generating apomictic hybrids by crossing artificially induced tetraploid sexual genotypes with tetraploid apomictic genotypes from a broad geographic range within the natural distribution of the genus (Zilli et al. 2015; Novo et al. 2017; Urbani et al. 2017; Brugnoli et al. 2019). Corroborating the hybrid origin of the progeny in such crosses is crucial; therefore, progeny tests using molecular markers are performed in all programs (Zilli et al. 2015; Novo et al. 2017; Urbani et al. 2017; Brugnoli et al. 2019, 2023). Segregation of progeny based on the mode of reproduction results from this type of cross. Several tools were used for the early and cost-effective identification of apomictic hybrids. In *P. notatum*, hybrids were classified by their mode of reproduction using a RAPD marker 100% linked to apomixis (Martínez et al. 2001), while in *P. simplex,* identification was performed using a SCAR marker (Cáceres et al. 2001). Besides, identification can also be performed by flow cytometry seed screening.

Apomixis expressivity can vary throughout the season and across genotypes. Apospory expressivity was determined through embryo sac observation in *P. notatum*, and by flow cytometry seed screening in *P. simplex* and the Plicatula group. Hybrids with a high apomixis expressivity and superior performance are selected for plot evaluations. At this stage, hybrids are assessed alongside other cultivars for seasonal forage production, seed yield, and other agronomic traits (Urbani et al. 2017; Brugnoli et al. 2023; Schulz et al. 2023; Chamorro et al. 2024).

Currently, these hybrids are being evaluated across multiple environments and for their response to grazing. Upon completion of these trials, one genotype will be selected for commercialization. An example of product created through the hybridization scheme was the development of *P. notatum* cv. Boyero-UNNE (Urbani et al. 2017).

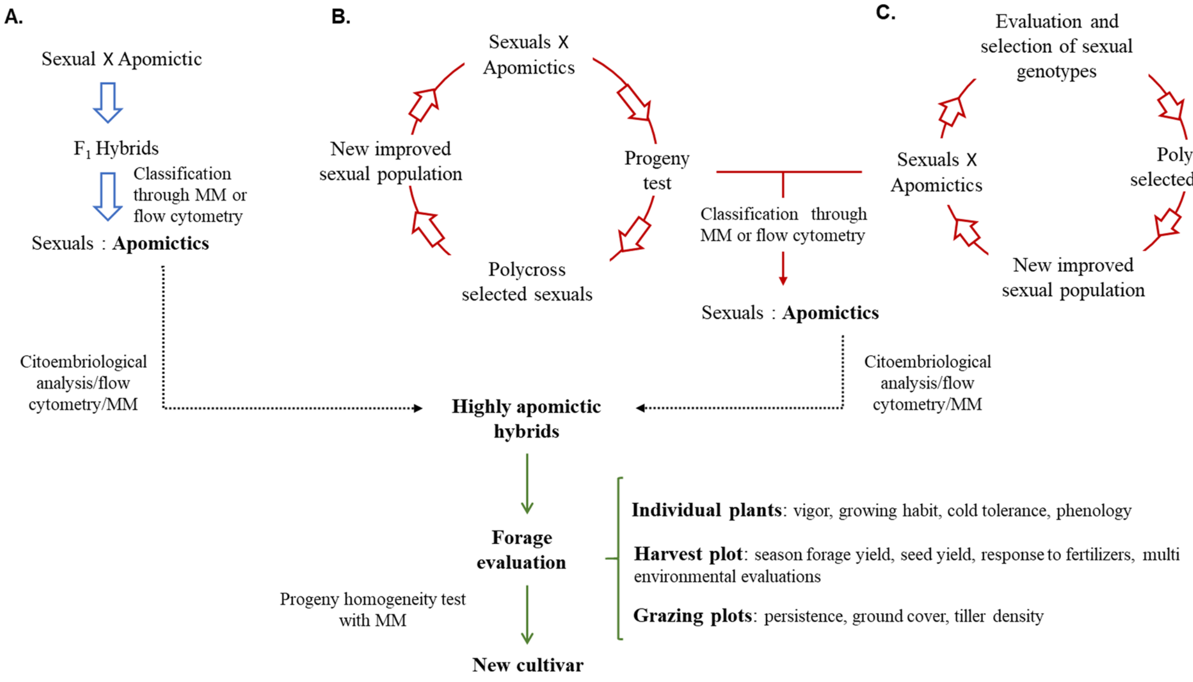

Figure 4. Scheme of breeding methods used for apomictic *Paspalum* species. A. Hybridization scheme, B. Recurrent selection based on progeny tests, and C. Phenotypic recurrent selection. MM: molecular markers.

Hybridization is the most useful breeding method for the *Paspalum* species, but low genetic diversity in the sexual germplasm poses limitations. In response, two sexual synthetic tetraploid populations have been generated, one for *P. notatum* (Zilli et al. 2018, 2019) and the other for the Plicatula group (Novo et al. 2020). The availability of sexually diverse synthetic tetraploid populations creates new opportunities for breeding by accumulating favorable alleles using recurrent selection cycles (Figure 4b and 4c). Recurrent selection based on combining ability (RS-GCA) and recurrent phenotypic selection (RPS) have been used to enhance the new sexually diverse germplasm of *P. notatum* (Marcón et al. 2020). Both methods are schematically illustrated in Figure 4b and 4c, respectively. New sexual genotypes improved by both methods were crossed by a group of superior apomictic hybrids, and progenies were evaluated in the field. RPS was equal to or more efficient than RS-GCA, as RPS generated greater or similar proportion of families exhibiting heterosis and greater or similar genetic progress (Marcón et al. 2020).

The mode of reproduction and apospory expression in the new hybrids showed asegregation ratio favorable for sexual hybrids, and most of the apomictic hybrids exhibited high apospory expression (Marcón et al. 2024a). When comparing both schemes, no differences in segregation ratio for mode or reproduction, proportion of highly apomictic hybrids, and hybrid field performance were observed. Therefore, the RPS is the most appropriate breeding method for *P. notatum*.

In the Plicatula group, the new sexual germplasm was evaluated for plant vigor, seed set, ergot tolerance, regrowth after flowering, and cold tolerance. Subsequently, a group of outstanding plants were selected and crossed by two apomictic testers belonging to *P. guenoarum*. The newly obtained progeny was evaluated, and a group of 10 sexual genotypes was selected. These new sexual genotypes contained genes recombined from six species that could be used to obtain improved apomictic hybrids (Novo et al. 2020).

4. *Paspalum* breeding program in the USA

Bahiagrass has naturalized in the southeastern USA, and it is one of the most important cultivated warm-season grasses in Florida (Chambliss 2002). The species is popular because of its adaptation to low soil fertility and low input management. Although bahiagrass is mostly used as forage for grazing cattle, additional uses include low maintenance turfgrass, groundcover in crop rotations and seed production (Rios et al. 2017). Seed production is a very important aspect in bahiagrass plantings because both diploid and tetraploid races are primarily established by sowing seed (Rios et al. 2015, 2020).

Bahiagrass is a highly diverse species with different ploidy levels and linked reproductive modes (Quarin 1992). The species is divided into two botanical varieties: the diploid races known as *P. notatum* var. *saurae* Parodi (Parodi 1948), with long, narrow leaves, smaller spikelets, longer internodes and taller than the common form (Rios et al. 2017). The common form is tetraploid ($2n = 2x = 40$). Compared to diploids, tetraploids have broader leaves, fewer inflorescences, and shorter internodes (Rios et al. 2017).

The center of origin of diploid bahiagrass is the northeast region of Argentina, corresponding to Corrientes, Entre Rios, and the eastern edge of Santa Fe (Daurelio et al. 2004). This race was introduced to Florida and has spread throughout the southeastern region. It was first discovered growing near the docks in Pensacola, Florida (Finlayson 1941; Burton 1967), and it was introduced by the Bureau of Plant Industry and grown by the Florida Agricultural Experiment Station in 1913. In contrast, the tetraploid races have a wider range of natural distribution and are found in pastures from central Mexico to Argentina and throughout the West Indies (Chase 1929). Hanson (1972) reported five major tetraploid bahiagrass types in the USA: common, Argentine, Paraguay, Paraguay 22, and Wilmington. Burton (1955) and Acuña et al. (2007) reported that diploid genotypes produce their progeny through sexual events, which imply the successful completion of meiosis and double fertilization and are primarily cross-pollinated. The cultivars Pensacola, 'Tifton 9′, 'TifQuick' and 'UF-Riata' are diploids and reproduce sexually (Blount and Acuña 2009), whereas tetraploid genotypes reproduce by apomixis (Burton 1948; Acuña et al. 2007). In apomictic bahiagrass, pollination and fertilization of the polar nuclei are both essential for endosperm development and seed formation; therefore, they are considered pseudogamous apomicts (Quarin 1999). Obligate apomictic ecotypes that have been domesticated in the US include Argentine, Paraguay, Paraguay 22 and Wilmington (Blount and Acuña 2009; Rios et al. 2017). However, plants that reproduce by apomixis also retain the potential to reproduce sexually and are known as facultative apomicts (Rios et al. 2013b). Additionally, sexual tetraploid bahiagrass genotypes have been experimentally created by duplicating the chromosome number of diploid plants (Quesenberry et al. 2010), and were successfully used in crosses with obligate apomictic lines as pollen donors (Acuña et al. 2009, 2011).

Breeding strategies differ for diploid and tetraploid bahiagrass due to their associated modes of reproduction (Blount and Acuña 2009). In diploids, traditional breeding methods such as recurrent phenotypic selection were applied to improve forage production and other traits (Burton 1982). However, apomixis constitutes a barrier for traditional plant breeding in tetraploid obligate apomicts. Although their occurrence is rare in nature, obligate apomictic ecotypes have been introduced into the US, including the cultivars Argentine, Paraguay, and Paraguay 22 (Blount and Acuña 2009). In order to overcome the barrier to recombination imposed by apomixis, the University of Florida Forage Breeding program developed sexual tetraploid plants by chromosome doubling (Quesenberry et al. 2010) and used these plants as females in crosses to produce tetraploid hybrids (Acuña et al. 2009, 2011). These progenies were evaluated under different conditions and years for multiple traits. Acuña et al. (2011) reported broad genetic variability for growth habit, freeze tolerance, and cool-season growth in studies conducted in Florida. Some tetraploid hybrids were also included in the study conducted in two locations in Florida by Rios et al. (2017), and Hybrids 3 and 93 exhibited improved traits for cool-season growth and turfgrass applications, in addition to acceptable seed yield and quality (Rios et al. 2017). More recently, Hayes et al. (2023) evaluated advanced breeding lines under different grazing management. Authors reported Hybrids 3 and 93 were 50% more productive in early and late season than most entries, including Argentine bahiagrass, and the higher production did not compromise stand persistence or nutritive value. These tetraploid hybrids are currently under consideration for cultivar release(s) by the University of Florida.

Mutagenesis is another method to create genetic variants, and has proven useful for breeding. In bahiagrass, Burton (1974, 1992) did not identify novel morphological traits; whereas Kannan et al. (2015) selected Argentine-mutant plants with improved turf traits following chemical mutagenesis of seeds with sodium azide, callogenesis and plant regeneration. In an attempt to increase the morphological variability in tetraploid apomictc bahiagrass, several mutagenic plants were generated at the University of Florida by exposing either seedlings or portions of rhizomes from two tetraploid cultivars, Argentine and Wilmington, to different mutagenic treatments (X-rays, Gamma rays and ethyl methane sulfonate) or exposure of cells to sodium azide while in tissue culture (Rios et al. 2013a). The mutant plants were planted in the field at the UF Plant Science Research and Education Unit (PSREU), Citra, FL in 2008 and were evaluated for turf performance during three growing seasons. Phenotypic selection based on leaf color, canopy density, spread and seed head production resulted in mutant genotypes being selected for exhibiting superior turfgrass quality compared to the parental lines and other industry standard bahiagrass cultivars (Rios et al. 2017). Several of these advanced mutant lines were also tested for grazing tolerance by Hayes et al. (2023), with the goal of developing multi-purpose varieties (forage, seed, and sod production). Several mutant plants are being evaluated in replicated field trials on-site and on-farm in Florida for their consideration for cultivar release(s).

4.1. Cultivars on the market. In Florida, bahiagrass has a century-long history as "common" bahiagrass. It was first introduced by the Bureau of Plant Industry and grown by the Florida Agricultural Experiment Station in 1913. Since the initial release, new cultivars have been adopted. The most popular cultivars are:

Diploids

- Pensacola: originally from the northeast of Argentina, seed arrived in Pensacola, Florida as ship ballast in the 1920s. This grass was identified in 1938 by E. H. Finlayson, Escambia County Extension Agent and he collected, distributed, and promoted this grass for pastures and land conservation. Pensacola bahiagrass has narrow leaves, good seed production, and high persistence under grazing. It is more cold-tolerant than most tetraploid types. Most bahiagrass pastures in the Southeastern U.S. today are Pensacola bahiagrass.
- Tifton 9: this cultivar was the first cultivar to be developed using recurrent selection. It was developed and released by the University of Georgia and the U.S. Department of Agriculture (USDA) in 1989 by Dr. Glenn Burton. Tifton 9 is a selection from Pensacola that was found to have superior yields in early spring and late fall.
- TifQuik: it was released by the USDA and the University of Georgia, has fewer hard seeds and results in a more rapid stand establishment. In the spring, it grows faster than Tifton 9 or Pensacola because of increased seedling emergence.
- UF-Riata—The University of Florida released this cultivar in 2008. UF-Riata was selected from Pensacola for further improvement in early spring and late fall production, improved frost tolerance, and improved disease resistance.

Tetraploids

- Common: it has a prostrate growth habit, is cold sensitive, good at spreading, and is persistent under grazing.
- Paraguay and Paraguay 22: these ecotypes from Paraguay are more upright than common bahiagrass but are not routinely used in pastures. Very few acres planted to Paraguay remain in Florida.
- Argentine—This ecotype from Argentina is highly productive with excellent spreading, lower seedhead production, but it has less frost and cold tolerance than Pensacola. It is estimated that Argentine is the second most grown variety in Florida.
- Wilmington: originally collected in coastal North Carolina, this cultivar has a finer leaf texture than typical tetraploid bahiagrass introductions, darker green color, and is adapted to higher latitudes. This is a cultivar with potential as a turfgrass for low maintenance.

5. Paspalum breeding program in Uruguay

5.1. Paspalum notatum collection, characterization and clonal selection. The tetraploid cytotype of *Paspalum notatum* is one of the main constituents of the natural grasslands in Uruguay. In Uruguay, Rosengurtt et al. (1970) described the presence of two varieties (*P. notatum* var. *latiflorum* Döll and *P. notatum* var. *notatum*), based on the panicle size and other morphological traits.

Individuals showing different phenotypes within a population were prioritized at the collection time to maximize the collection's diversity. To assess the genetic variability, a subset of 210 individuals from the collection were genotyped with ISSR (inter simple sequence repeats) molecular markers (Reyno et al. 2012). Cytometric analyses and chromosome counts were used to determine the ploidy level of the individuals, all of which were found to be tetraploids. Intra- and inter-population variability was found both in genomic DNA content and at the genotypic level. Several multilocus markers were shared among individuals within populations and among populations over moderate geographical ranges, while very dissimilar genotypes were found within the same population. Part of the genetic variance among populations can be explained by a broader scale geographic structure which partly coincides with the traditionally recognized grassland management ecoregions. Despite the apparently high degree of genetic admixture within populations, groups of related genotypes seem to follow a broader geographical structure in the area under study (Reyno et al. 2012).

The collection was evaluated several years and in different locations for morphological and agronomical characterization. Traits as dry matter production, inflorescence size, growth capacity, seed yield, ergot incidence and severity, among others were assessed (Do Canto et al. 2010; Reyno et al. 2012; Catanzaro et al. 2015; Do Canto et al. 2023). Several traits showed significant genotypic effect and large broad-sense heritability suggesting that clonal selection within a diverse panel of genotypes could be an effective way to develop superior varieties (Do Canto et al. 2023). From these evaluations, a single clone coded TB 42 was selected. This clone belongs to *P. notatum* var. *latiflorum*, and was commercially named as INIA Sepé, registered at the National Institute of Seed of Uruguay (Reg. No. 2019210), and released by the National Institute of Agricultural Research (INIA, Uruguay), in December of 2019. INIA Sepé is an apomictic autotetraploid cytotype ($2n = 4x = 40$) evaluated from 2007 to 2013 in several locations. The selection process was focused on dry matter and seed production, ergot incidence, colonizing capacity and adaptation to a broad range of soils. INIA Sepé showed higher dry matter and seed productivity than the tetraploid apomictic cultivars Argentine, Paraguay, and Paraguay 22. Furthermore, a lesser ergot incidence was observed in INIA Sepé (Do Canto et al. 2023). When compared to the diploid cultivars Pensacola, Tifton 9 and Bellaca (a Pensacola derived cultivar), INIA Sepé showed broader and shorter leaf blades, higher LAI (leaf area index), and heavier tillers (Giorello et al. 2021). Since its identification as a promising genotype, this material has been studied across multiple disciplines such as ecophysiology, plant nutrition, seed production and physiology, and animal nutrition, seeking to understand the mechanisms that will optimize its management, seed formation and quality, and its suitability as forage (Bertoncelli 2018; Bartaburu and Otegui 2021; Giorello et al. 2021; Tafernaberry et al. 2022; Giorello et al. 2023).

5.2. Unlocking genetic variability through interspecific hybridization: Breeding for ergot resistance. Efforts to generate *Paspalum dilatatum* cultivars in Uruguay, began in the 1960s with the prospection of ecotypes collected in Uruguay, which eventually totaled 280 accessions. Selection between and within ecotypes resulted in the generation of four cultivars: Estanzuela Chirú, Estanzuela Yasú, Estanzuela Caracé, and Estanzuela Tabobá (Millot 1969; Carámbula et al.

1978). Of these, the cultivar Estanzuela Chirú was prioritized due to its long cycle and productive behavior (Formoso and Allegri 1984; Formoso 2003). However, seed production and especially susceptibility to Claviceps have limited commercial seed production and therefore its adoption by breeders (García 1971; Formoso 2003). Disease severity varies widely depending on environmental conditions with high humidity and moderate temperatures up to 25 °C being particularly favorable for infection (Do Canto et al. 2023; Oberti et al. 2024).

In the genus *Paspalum*, differences in ploidy and genomic composition have been frequently reported, pointing out the occurrence of autopolyploids (*P. notatum*, *P. malacophyllum*, etc.) but also the natural occurrence of allopolyploids (*P. flavescens*, *P. dilatatum*, *P. dilatatum* var. Chirú, etc.). Bashaw and Funk (1987) mentioned that for the successful breeding of apomictic species, the availability of compatible sexual and apomictic materials, efficient crossing techniques and the possibility of recovering apomictic hybrids or progenies must be considered, and *P. dilatatum* biotype Estanzuela Chirú meets these three conditions.

Paspalum malacophyllum ($2n = 2x = 40$) is resistant to ergot and therefore, is being used as a male donor in crosses with *P. dilatatum* cv. Estanzuela Chirú ($2n = 2x = 60$) and the sexual biotype LE 69.12 derived from autopollination of Chirú to incorporate ergot immunity and improve seed production (fruit set and germination) in a single line (Reyno et al. 2023). From more than 20,000 crosses, pentaploid ($n + n$) and octoploid ($2n + n$) hybrids have been generated, determined by molecular markers, cytometry and chromosome counting (Reyno et al. 2023). As reported by Koltunow and Grossniklaus (2003), sexuality and apomixis are not mutually exclusive modes of reproduction and through early crosses it was possible to fertilize reduced (n) and nonreduced (2n) gametes. These hybrids showed a high percentage of seed germination (approx. 70% in the different generations of pentaploids and 50% in the octoploids). As observed by Bennett and Bashaw (1960) in *Paspalum* and reported by Roy et al. (2022) in *Pennisetum*, genomic addition is a pathway for the expression of variability in apomictic species. In our work, notable differences were observed in plant size and habit, leaf tenderness, floral induction and panicle size, with the presence of yellow anthers (Figure 5 - all parents have purple-violet anthers). These hybrids are being evaluated for ergot reaction in the field under naturally high inoculum pressure. Evaluations in fields highly infested with *C. paspali* and environments with high predisposition to disease progression are crucial for efficient selection.

D. Buffelgrass (*Cenchrus ciliaris*)

Cenchrus ciliaris L. is a warm-season perennial C4 grass (Hanselka et al. 2004) that has proved useful in

Figure 5. *P. dilatatum* cv. Chirú. A. Floral opening of the glumes (1–3), emergence of the feathery stigma (4 to 7) and purplish anthers (5 to 9). Between 4 and 5, pollination takes place for the generation of hybrids. In 8 and 9, the release of pollen from the anthers is visible. B: Panicle of the hybrid 23.797.1.6 with yellow anthers, from a cross between Chirú × *P. malacophyllum*. Photos in A: I. Rodríguez, composition C. Goñi/S. Murchio/M. Dalla Rizza.; in B: F. Pérez Viglione).

a wide range of environments due to its high biomass yield, drought tolerance, resistance to overgrazing, high efficiency in the use of nutrient and water resources, and capacity for carbon sequestration and soil erosion control (Al-Dakheel and Hussain 2016). It is an important genetic source for abiotic stress tolerance in the current climate change scenario, especially in arid and semi-arid regions worldwide. Buffelgrass covers more than 50 million hectares worldwide (Hanselka et al. 2004) and is one of the most widely used forage species in Africa, India, Asia and America (Griffa et al. 2006; Burson et al. 2015; Negawo et al. 2020). Therefore, this species is particularly suitable for the semi-arid Northwestern Argentinean (NOA) region where cattle production is predominantly pastoral. Nevertheless, only a few genotypes are used in breeding, and the cv. Texas is the most widespread (Gómez Martínez 2009; Griffa et al. 2017).

1. Germplasm

Buffelgrass accessions were introduced in Argentina between 1980 and 1990 (Ayerza 1981). Currently, the Institute of Physiology and Plant Genetic Resources (IFRGV) of the National Institute of Agricultural Technology (Instituto Nacional de Tecnología Agropecuaria, INTA) maintains a germplasm collection with twenty-six accessions. which includes tetraploid ($2n = 4x = 36$) and aneuploid ($2n = 43$ and 44) individuals (Carloni et al. 2018). The collection has been characterized morphologically (Griffa et al. 1999; Griffa 2002; Grunberg and Griffa 2021) using descriptors established for the identification of germplasm accessions and conservation purposes (Griffa et al. 2017). Moreover, the collection was characterized for forage quality (Ribotta et al. 2005; Carrizo et al. 2021), tolerance to salinity, to high temperatures and to drought (Tommasino et al. 2018; Carrizo et al. 2021) and was profiled by molecular markers (Griffa et al. 2006; Griffa 2010). This information is used during selection of the best parentals in the current breeding program focused on generating apomictic cultivars adapted to the harsh climatic conditions of the region. These include low seasonal rainfall (averaging 350 mm), high temperatures, intense solar radiation during the summer months, and soils with varying levels of salinity (Quiroga et al. 2013).

2. Buffelgrass breeding program in Argentina

Aposporous apomixis is the main reproductive mode in buffelgrass (Quiroga et al. 2013; Sharma et al. 2014), followed by pseudogamy (Shafer et al. 2000), with sexually reproducing genotypes being absent or very rare (Bashaw 1962; Kumar et al. 2010).

The species was considered an obligate apomict for many years, and breeding was limited to ecotype selection until Bashaw (1962) discovered an off-type mutant plant in south Texas which resulted to be sexual. This plant was used as the female parent in crosses to superior apomictic accessions (Taliaferro and Bashaw 1966). In 1997, seeds derived from the sexual plant discovered by Bashaw (1962) were kindly provided by Texas A&M University, College Station, to INTA to cope with ongoing breeding plans in Argentina.

Cenchrus ciliaris is a protogynous, cross-pollinated allotetraploid with variable levels of self-compatibility (Hanselka et al. 2004; Sánchez et al. 2023). Hence, controlled crosses using the sexual tetraploid genotype mentioned above were carried out by bagging inflorescences and pollinating pistils before and during protogyny with pollen from Biloela, a selected apomictic genotype (Griffa et al. 2010). The objectives of the breeding are to obtain productive abiotic stress-tolerant cultivars.

The putative F_1 hybrid plants were evaluated and one abiotic stress-tolerant genotype that exhibited superior forage characteristics was selected and later registered (see details below; INASE 2010; Griffa et al. 2011). The other hybrids showed poor forage productivity and significant susceptibility to salt stress (Griffa et al. 2005; Lanza Castelli et al. 2010), very similar to the mother parent (Griffa et al. 2010; Quiroga et al. 2013).

In this allotetraploid, the formation of F1 hybrid genotypes with promising agronomic features largely depend on favorable combinations of polygenes controlling quantitative characteristics with additive effects, and/or the occurrence of heterosis in the component traits of biomass production (Griffa et al. 2006). To benefit from this, the development of new sexual genotypes for use in the buffelgrass breeding program is recommended. Alternatively, recombinant offspring from facultative apomictic buffelgrass genotypes can be identified and selected for improvement.

Seasonal changes and climatic variables have been identified as modulatros of the relative proportions of apomixis and sexuality in several *Paspalum* species (Rios et al. 2013b; Karunarathne et al. 2020; Soliman et al. 2021; Reutemann et al. 2022). Likewise, the expression of sexuality in facultative apomictic genotypes of buffelgrass is influenced by bioclimatic variables during the flowering season (Sánchez et al. 2023). As the highest potential for sexual reproduction occurs at the beginning of the flowering season promoted by a prolonged photoperiod, is the preferable time for selection of recombinant offspring.

2.1. Cultivars on the market. Controlled crosses between the introduced sexual genotype and the apomictic cultivar Biloela were focused on generating recombinant plants tolerant to high temperatures, drought and salinity. From these crosses, one F_1 plant identified as hybrid through morphology, molecular markers and cleared ovules was selected due to its excellent dry matter production and forage quality, seed production and high tolerance to drought and salinity (Griffa et al. 2010, 2011, 2012). This genotype was named Lucero INTA PEIMAN and registered in the National Cultivar Register (RNC) and the National Cultivar Property Register (RNPC) - National Seed Institute (INASE 2010). A transfer agreement was made with a seed company for its commercial distribution.

2.2. Nonconventional strategies for Cenchrus ciliaris breeding. With the goal of increasing genetic variability for its use in breeding buffelgrass in Argentina, nonconventional methods such as tissue culture and mutagenesis are used (Ma et al. 2021; Bekalu et al. 2023; Chen et al. 2023). *In vitro* regeneration of whole buffelgrass plants through somatic embryogenesis has been used for agrobacterium-mediated transformation (e.g., Laishram et al. 2020; Goyal et al. 2023).

Induced mutagenesis techniques using physical, chemical and biological mutagens are regularly used to improve numerous agricultural crops, such as wheat, rice, cotton, barley, banana, sunflower, some legumes, and ornamental plants (Chen et al. 2023). Mutants of buffelgrass as have been developed by irradiation of seeds of the cv. Biloela and *in vitro* selection of mutated plant regenerants (López Colomba et al. 2006; 2011, 2013). One of such genotypes showed greater tolerance to water stress than the commercial cultivars Biloela and Texas 4464. It was called Orión INTA and was registered in the RNC and RNCP in 2018. In 2019, a transfer agreement was made with a seed company for its commercial distribution.

V. Novel techniques for use in cultivar development

A. Use of high-throughput phenotyping and artificial intelligence

A primary objective of apomictic forage breeding programs is to continuously enhance forage yield and quality, as well as resilience to both biotic and abiotic stresses. Traditionally, breeders have relied on methods like visual scoring, sample clipping, and plot mowing to select for these traits (Gebremedhin et al. 2019). However, traditional phenotyping methods are not only expensive and time-intensive but also susceptible to human error and limited in the number of plants or plots that can be assessed (Cudjoe et al. 2023). Moreover, they often fail to capture the dynamic nature of plant traits and their responses to environmental changes over time (Moreira et al. 2020).

High-throughput phenotyping (HTP) has emerged as a promising solution to these limitations, offering a way to rapidly, nondestructively, and accurately assess plant traits on a large scale (Gebremedhin et al. 2019). HTP provides several key advantages over traditional methods, such as: efficiency increase by enabling rapid evaluation of numerous plants or plots, which reduces phenotyping time and costs (Ayankojo et al. 2023). HTP techniques are nondestructive, allowing for repeated measurements on the same plants over time, thus providing valuable insights into plant growth and development in perennial species (Biswas et al. 2021; Kim et al. 2021). It also improves accuracy and precision through the use of sensors and imaging technologies that capture detailed information about plant traits, leading to more accurate measurements compared to visual scoring (Shakoor et al. 2017). The use of HTP promotes objective data collection by reducing the subjectivity associated with manual and visual phenotyping, thereby providing more reliable data for breeding decisions (Koltes et al. 2019), while it also provides high-resolution temporal data, enabling data collection at multiple time points (Moreira et al. 2020).

HTP technologies can be applied across a range of environments, from controlled laboratory settings to open-field conditions (Li et al. 2020). It enables the evaluation of specific phenotypic traits through the integration of various sensing platforms, including proximal systems (e.g., cameras, smartphones, phenomobiles), aerial platforms (e.g., unmanned aerial vehicles (UAVs) manned aircraft), and even satellite-based space platforms. These platforms support a diverse array of sensors - such as RGB, multispectral, hyperspectral, thermal, fluorescence, LiDAR/radar, and X-ray - capable of detecting electromagnetic radiation reflected or emitted by biological structures ranging from tissues and organs to whole plants and plant communities (e.g., pastures). Feature extraction from sensor data may involve straightforward yet effective methodologies, such as vegetation indices (Araus et al. 2022), or leverage advanced machine learning algorithms for more complex pattern recognition and trait prediction (Gill et al. 2022). Regardless of the analytical approach, the workflow typically involves the segmentation of target regions (e.g., individual plants or plots), quantification of relevant features, and subsequent association of these features with the phenotypic traits of interest.

HTP is increasingly being implemented to improve the efficiency of breeding programs for apomictic forage species (see Supplementary Material). Several studies have combined advanced deep learning algorithms with data from affordable platforms and sensors, such as UAV-RGB and mobile-RGB, in guineagrass to predict traits related to forage mass (Castro et al. 2020; Oliveira et al. 2021; Santos et al. 2022; Rodrigues et al. 2023). These studies explored various convolutional neural network algorithms, pretrained models, multi-view model fusion, and the impact of image resolution. The results demonstrate the potential of these methods, achieving correlations between 0.76 and 0.94, between the target characteristics and the image features, particularly with the model fusion approach. This model fusion strategy improved the performance of individual models by 20–33%. Oliveira et al. (2021) utilized both real and predicted data in genetic models for direct and indirect selection, using the predicted data as a secondary trait. Their findings indicated that larger population sizes increase the effectiveness of indirect selection, highlighting the value of HTP techniques in breeding.

Vegetation indices, derived from spectral band images captured by RGB and multispectral sensors, have also been linked to forage mass. In *Urochloa* spp., the Normalized Difference Vegetation Index (NDVI) showed correlations between 0.62 and 0.85 with shoot biomass, green leaf biomass, and dead leaf biomass under waterlogged conditions (Jiménez et al. 2017). In another study with *Urochloa* hybrids, Jiménez et al. (2020) predicted forage dry weight with high accuracy using vegetation indices calculated from RGB images in a partial least square regression model. Similarly, Paes et al. (2023) found high correlations (0.75 to 0.85) between the soil adjusted vegetation index (SAVI) and green and dry matter weight in *Paspalum* spp. These results, obtained with manually operated cameras or devices, offer advantages over traditional assessment methods by being less time-consuming, costly, and applicable on a larger scale.

The use of HTP to predict nutritional value is still limited. However, Jiménez et al. (2020) have shown that vegetation indices can predict crude protein with high accuracy using partial least square regression.

The damage caused by spittlebugs is a significant biotic stress for apomictic forage species. Traditional visual assessments of this trait are often subjective and inaccurate. To address this, Hernandez et al. (2020) developed a digital damage index based on RGB images for evaluating *U. humidicola* genotypes. They found high correlations (0.90) between the digital damage index and visual assessments. The authors suggest that using digital damage indices can improve the efficiency of phenotypic assessments in breeding programs due to higher heritability values and faster phenotyping.

Drought is a major abiotic stress that reduces pasture production. Drought-stressed plants often exhibit altered physiological functions, including reduced photosynthetic efficiency. Itam et al. (2024) evaluated drought tolerance in Kentucky bluegrass by assessing various genotypes in a growth chamber with a fluorescent light capture sensor. Using principal component analysis, they identified parameters related to photoprotection (Non-Protochemical-Quenching) as the most effective traits for estimating drought tolerance. These traits are expressed early in plants, which facilitates early selection and precise management.

Medeiros et al. (2020) explored the use of X-ray tomography to improve phenotyping for physical and seed quality attributes, which are key traits for breeding programs. They found correlations of 0.66 to 0.99 and above 0.94 between digital traits obtained from digital images and physical and seed quality attributes, respectively. The authors concluded that this approach is simple, fast, robust, and low-cost, making it suitable for breeding programs.

Despite the studies mentioned above, the research and practical application of HTP in apomictic species remains at an early stage of development compared to its implementation in other crops, including those cultivated for forage purposes (Cheng et al. 2025). This highlights a significant opportunity to leverage HTP for accelerating the genetic improvement of forage cultivars. The integration of HTP with genomic and environmental data represents a promising frontier in modern plant breeding, enabling more precise and data-driven selection strategies (Crossa et al. 2021). However, investment in breeding programs for apomictic species remains limited, thereby constraining both the development and large-scale adoption of these transformative technologies in plant improvement efforts.

B. Use of machine learning (ML) for reproduction mode prediction

Mixed linear models (MLMs) have been the standard in genomic prediction models. However, the emergence of machine learning (ML) methods has introduced a powerful alternative that leverages complex patterns in genomic data to improve prediction accuracy (Guo 2021). Unlike MLMs, which rely on statistical assumptions about genetic relationships, ML models are highly flexible and capable of capturing nonlinear interactions between genetic markers and traits (Lourenço et al. 2022).

GBLUP and related MLMs methods are well-established due to their interpretability and efficiency, but they are limited when traits are influenced by nonadditive genetic interactions or complex epistatic effects (Weissbrod et al. 2016). In contrast, ML classification models do not rely on predefined genetic assumptions but instead learn patterns directly from training data. This allows them to automatically select the most informative markers and improve predictive performance. However, their effectiveness depends on dataset size, feature selection, and computational resources (Guo 2021; Li et al. 2024). To address concerns about interpretability, ML models use explainability techniques such as SHAP (SHapley Additive exPlanations) values (Tsui et al. 2024), which quantify the impact of individual genomic features on model predictions. In genomic prediction, where features are single nucleotide polymorphisms (SNPs) mapped to specific genomic positions, SHAP values highlight which genetic markers are most strongly associated with a given trait. SNPs with high SHAP values play a key role in distinguishing between trait categories, such as sexual and apomictic reproduction. However, while SHAP identifies predictive markers, it does not directly test for statistical association in a population-based framework like traditional genome-wide association studies (GWAS). Therefore, additional validation through GWAS or functional annotation is necessary to confirm the causal relationship between SNPs and the trait.

Comparisons between ML and MLMs for predicting trait-marker associations have shown that ML methods often outperform MLMs models in classification tasks (Jones et al. 2023; Xiang et al. 2023; Fernandes et al. 2024). However, for regression tasks, such as predicting days to flowering, linear models have remained competitive. Since apomixis is a qualitative trait, its presence or absence in plants can be effectively predicted using ML classification models. A model comparison using an *Urochloa* pseudo-nested population consisting of four families with 301 genotypes phenotyped for apomixis across 11,744 SNP markers demonstrated that ML and GWAS approaches yielded similarly strong results. The results from 5-fold cross-validation further highlight the superior performance of ML models in classification tasks. F_1 scores indicate that models such as XGBoost, AdaBoost, and Random Forest classifiers achieved the highest classification accuracy. Model loss plots show that these high-performing models also had lower training and validation loss, reflecting better generalization compared to models such as SVC and Gaussian Process classifiers, which exhibited higher variance and lower prediction accuracy. Confusion matrices reinforce that XGBoost and Random Forest classifiers provided the best balance between true positive and true negative classifications. Furthermore, XGBoost's SHAP analysis identified key markers in chromosome 17 of *U. decumbens*, so matching the results from GWAS, further supporting the utility of ML models in genomic prediction. However, while these markers are highly predictive, their biological significance and direct causal relationship to the trait require further validation through GWAS and functional genomic analyses.

C. Genomic selection

Breeding is resource-intensive, requiring schemes that balance time and expenses. Bernardo (2002) found that for high-heritability traits, phenotypic selection approaches the maximum genetic gain possible, limiting benefits from molecular markers. However, for low-heritability traits, such as complex abiotic responses, the use of molecular markers through a genomic selection (GS) approach can triple genetic gains compared to phenotypic selection alone. Therefore, the efficient identification of the best crosses at early stages would allow for the identification of the best and most cytogenetically stable parents and progenies. Consequently, all subsequent stages of the breeding program will certainly benefit from genomic prediction and the unbiased estimation of marker effects.

The use of genotyping data can enhance genetic improvement in perennial apomictic species by fine mapping quantitative traits and developing markers to be exploited under MAS (Deo et al. 2020), and/or by performing selection of candidates based on prediction of breeding potential based on genomic information, also known as GS (Lara et al. 2019; Matias et al. 2019). MAS and GS can accelerate selection and optimize breeding decisions, such as reducing trial sparsity or minimizing the number of environments while maintaining genetic gains. A common MAS application involves selecting plants with more recurrent parent markers while retaining beneficial alleles from the donor parent, such as in breeding for disease resistance. Another common application of MAS in breeding across tropical grasses involves the characterization of the mode of reproduction, as described in previous sections.

Advances in sequencing technologies have resulted in different strategies to retrieve genotypic data in apomictic crops (Ferreira et al. 2021). While genotyping costs continue to decline, phenotyping costs remain stable or increased due to rising labor, trial establishment and maintenance, etc. GBS based on reduced genome representation by restriction enzymes, although having relatively high missing data rates, has been successfully used to genotype *Urochloa* spp. and guineagrass (Deo et al. 2020; Matias et al. 2019; Lara et al. 2019; Ferreira et al. 2021). Skim sequencing was used to develop markers in *Urochloa* in CIAT's breeding program. This program uses recurrent selection from 10 founders, primarily *U. brizantha*. These founders were sequenced at ~20X coverage.This depth corresponds to approximately 5X coverage per haploid genome complement, enabling adequate marker development and genotyping, while a subset of the 2019 selection cycle's offspring underwent 1X coverage (skim-seq). Using *in silico* imputation, missing SNPs were inferred from the founders, achieving an imputation accuracy of 0.95. Various imputation tools were tested and Glimpse resulted to be the most accurate, while Beagle offered the best tradeoff between accuracy and usability.

GS employs genome-wide markers to predict breeding potential, relying on statistical modeling rather than direct marker-trait associations. It requires a genotyped and phenotyped training population to build prediction models (Meuwissen et al. 2001). In a typical 5-fold cross-validation, 20% of trait values are hidden while the remaining 80% train the model. In practice, GS complements rather than replaces phenotypic selection. GS also enhances sparse testing, predicting trait values across environments to reduce trial costs.

CIAT's *Urochloa* interspecific breeding program, for instance, evaluates breeding lines across seven environments. In a recent trial, nearly all lines were tested across all sites, producing a robust dataset for GS validation. Focusing on plant height (h^2=0.57), cover area (h^2=0.48), and biomass (h^2=0.41), a 5-fold cross-validation with 20 repetitions yielded the highest accuracy for cover area (0.85), followed by height (0.68) and biomass (0.5). Notably, cover area outperformed height despite its lower heritability and reliance on high-throughput imaging (J. Vega, personal communication). These results support a transition to sparse testing, where GS substantially reduces costs (Jarquin et al. 2020).

The availability of high-fidelity genomic references has revolutionized breeding programs for diploid *P. notatum* (Yan et al. 2022; Vega et al. 2024; Vigna et al. personal communication) and *P. vaginatum* (Sun et al. 2022; NCBI GCA_030272315.1). Reference genomes are also available for *C. purpureus* (Teshome et al. 2024), *M. maximus* (Jauregui et al. 2024), and *U. decumbens* (Ryan et al. 2025) and diploid *U. ruziziensis* (Worthington et al. 2021). In addition, assemblies are being prepared for diploid *P. plicatulum* and tetraploid *P. malacophyllum* (Oberti et al. 2022). Leveraging these resources will empower breeders to pinpoint SNP markers crucial for MAS and GS approaches.

Genomic studies have produced a wealth of information on sequences and genetics, and have generated numerous genetic markers, which are essential for the genomic (Hayes et al. 2013; Grattapaglia 2022), and genome wide selection of apomictic tropical forage grasses (Pessoa-Filho et al. 2019; Ferreira et al. 2021; Simeão et al. 2021; Chen 2024). Such studies have fostered statistical and analytical advancements to overcome the challenges of analyzing these apomict polyploid plants. Gold-standard SNP discovery methods typically involve resequencing individual samples at sufficient coverage and mapping reads to a reference genome (McKenna et al. 2010), including low-coverage GBS approaches (Elshire et al. 2011).

For GS, marker utility is maximized when they are uniformly distributed across the genome, rather than being linked solely to QTLs. After the skim-sequencing data from CIAT was imputed, approximately 45,000 high-quality markers were generated. To assess different panel efficiencies, marker subsets (ranging from 300 to 5,000 markers) were selected using two methodologies: random selection for uniform distribution or targeted selection based on significant GWAS -values. These established panels were then tested to determine their predictive abilities. GWAS-derived markers required recalibration in each training fold to avoid data leakage. Reducing markers to 1,000 (one per 2 Mbps) had minimal accuracy loss, but skewing selection toward QTLs negatively impacted performance. Ensuring even distribution—either randomly or systematically—was the best approach.

Linkage disequilibrium (LD) between markers and QTLs is crucial for genomic selection utilizing genomic information (Bourke et al. 2018a). It is imperative to clarify that QTL denotes solely the statistical association between a genomic region and a trait. Association analysis based on population-level LD is employed for fine mapping (Resende et al. 2013). Linkage can arise either when a gene directly influences a trait (functional association) or when LD exists between a marker and the gene controlling the trait (nonfunctional association). In the former case, the gene's effect is directly assessed, and the marker is considered functional, often termed a quantitative trait nucleotide (QTN). In the latter, the linkage test depends on LD between the marker and a quantitative trait locus (QTL). A mutation arising on a chromosome generates a haplotype encompassing adjacent loci. This mutation tends to be inherited within the same haplotype across subsequent generations, unless recombination occurs, which disrupts LD. This LD is the basis for association mapping (Resende et al. 2013).

1. Genetic and quantitative genetics in apomictic forages

Most apomictic species are facultative apomicts, meaning that some level of residual sexuality is observed and therefore a small fraction of the seeds are produced by sexual reproduction (Karunarathne et al. 2024). Consequently, the reproductive mode of an accession is often ambiguous without a proper evaluation (see Hojsgaard et al. 2016). Depending upon the developmental stage analyzed (flower buds, seeds or offspring), the reproductive assesment might range from fully sexual to partially sexual, or completely apomictic (Acuña et al. 2007; Rios et al. 2013b; Hojsgaard et al. 2016). This reproductive ambiguity has potential implications for microsporogenesis of the apomictic parent in controlled crosses with obligately sexual plants (female parent). It may also affect the expected reproductive mode composition of the progeny in breeding programs. Moreover, this can lead to errors in linkage mapping (Deo et al. 2020), association mapping (Laucou et al. 2018), haplotype assignment, and the estimation of marker effects, all of which are critical in genomic prediction. Nevertheless, a recent tool based on SNP molecular markers that aids the off-types' identification within biparental progenies of polyploid species was developed (polyCID Shiny app - Martins et al. 2021).

In many accessions of polyploid tropical forage grasses, the mode of polyploidy (allopolyploidy, autopolyploidy, or segmental polyploidy) remains undetermined (Karunarathne et al. 2024). According to Bourke et al. (2018a), understanding the meiotic behavior of a species is essential for accurate analysis of polyploid data using allele dosage calling, which depends on the expected segregation ratios within the F_1 autotetraploid population. Autotetraploids, such as the apomictic *M. maximus* and the sexually reproducing *U. ruziziensis*, differ from allotetraploids in number of multivalent chromosomal associations during meiosis. This distinction highlights the need to use specialized methodologies and tools in genetic research and linkage mapping (Gallais 2003). Autotetraploid plants display polysomic inheritance, which can be identified through cytogenetic analysis by observing tetravalent formation and segmental pairing among partially homologous chromosomes (Stebbins 1947), as well as through molecular inference (Worthington et al. 2021). Tetravalent chromosome pairing can lead to the production of unbalanced gametes and non-Mendelian inheritance patterns. However, in colchicine-induced autotetraploids, chromosome pairing does not exclusively form tetravalents, nor does it exhibit other meiotic abnormalities (Pagliarini et al. 2008). Regardless of the rate of tetravalent formation, the issues of defining allele dosage in tetraploids still remain unresolved (Gallais 2003; Phillips 2024). Thus, in tropical apomictic forage grasses, polyploidy and the segregation of the reproductive mode among the progeny introduce further analytical complexities for the estimation of marker effects and genomic values, thereby impacting efforts to increase selection accuracy and reduce the duration of breeding cycle.

2. Application of genomics in apomictic forages

GS emphasizes the simultaneous prediction (without the use of significance tests for individual markers) of the genetic effects of thousands of SNP markers dispersed throughout the genome of an organism to capture the effects of all loci (both small and large effects) and identify the overall genetic variation of a quantitative trait (Meuwissen et al. 2001; Resende and Alves 2020).

In cases of complete or near-complete LD, estimated marker effects remain consistent across families and generations within a given environment. The number of markers employed is directly related to genome size, the extent of LD, and population structure (Ballesta et al. 2020). Larger genomes, rapid LD decay, and larger effective population sizes necessitate higher SNP marker densities.

Given that SNP markers are biallelic, the inference of allelic dosage effects is contingent upon the genetic effects of interactions (Gallais 2003; Mackay et al. 2019). Exclusive additivity can result in a greater number of genotypic value classes compared to other first-degree allelic interactions, necessitating the study of allele dosage in GS. In autotetraploids or populations derived from their "allotetraploids," such as those observed in *M. maximus* and *U. ruziziensis*, the additive genetic variance and narrow-sense heritability (NSH) cannot be accurately estimated solely through half-sib progeny testing or regression of offspring on progenitors. This is because half-sib families may contain fractions of dominant genetic variance (Gallais 1989).

Genomic Best Linear Unbiased Prediction (G-BLUP) analysis of half-sib polyploid data enables the estimation of broad-sense heritability (BSH) by leveraging all genetic relationships available in the kinship marker matrix. This approach accounts for "identity-by-state" dominance relationships, which allow for the estimation of dominance effects. When combined with estimated additive genetic effects, these dominance effects facilitate the calculation of the total genotypic value, thereby enabling the estimation of BSH. This methodology has been successfully applied in studies of *M. maximus* hybrids (Lara et al. 2019) using a limited number of parents, as well as in *Urochloa* interspecific hybrids (Matias et al. 2019).

D. Software development for polyploids

Apomictic grass species are polyploid and many have a hybrid origin (See Section III for details). Thus, multiple genome copies must pair, recombine and segregate during meiosis, any bias having direct consequences in the transmittion of the genetic material across generations, and consequently, in the breeding strategies. In this section, we present advances on software development for genetic analyses of polyploid organisms, with a focus on platforms that address relevant polyploid attributes, like genome copies and composition, number of alleles, occurrence of polysomic inheritance, and its implications to segregation and inheritance.

1. Bioinformatics and genotype calling

The first differences in analytical procedures and software tools appear at the genotyping step. Since most genotyping platforms rely on DNA sequencing, a diverse range of challenges arise when dealing with polyploid sequence data. The vast majority of datasets come from next-generation sequencing technologies, like GBS (Elshire et al. 2011) and targeted sequencing (Hardwick et al. 2017), which are mainly based on sampling DNA sequences from chromosomes. These are heavily dependent on sample sizes for correct genotype estimation, and optimal sample sizes increase significantly with the increase in ploidy level. In addition, polyploid genomes tend to be highly repetitive and heterozygous (Soltis and Soltis 2012), which makes bioinformatics processes even harder due to highly homologous regions and bring extra difficulties in assembling long, repetitive, and heterozygous reference genomes.

Resolving genomic complexities usually involves combining multiple sources of data, like long and short read sequences, and other technologies, like Single Molecule Real-Time (SMRT) sequencing and Hi-C, with multiple algorithms for genome assembly. A few examples of software tools that can be used to assemble polyploid reference genomes are: *Canu* (Koren et al. 2017), a genome assembler designed to deal with long-range sequence data; *polyHap* (Su et al. 2008), a software for phasing and imputation on polyploid genotypes; *HapCUT2* (Edge et al. 2017), a haplotype assembler for multiple sequencing technologies; *Ranbow* (Moeinzadeh et al. 2020), another haplotype assembler that uses short sequence data; and *TriPoly* (Motazedi et al. 2018), a tool that performs haplotype estimation using sequencing data from related individuals.

The second step is finding points of variation along the genome. Examples of software tools often used to perform variant calling in polyploids are the SAMtools toolkit (Danecek et al. 2021); *TASSEL4Poly* (Glaubitz et al. 2014; Pereira et al. 2018), a modified version of *TASSEL* that handles high read depths; *Stacks* (Catchen et al. 2011), a de-novo-based tool to identify variants in short-sequence, restriction enzyme-based datasets; *FreeBayes* (Garrison and Marth 2012), a Bayesian haplotype-based genetic polymorphism discovery and genotyping tool; and *GATK* (McKenna et al. 2010), a complete toolkit to perform variant discovery in high-throughput sequencing data. The last two software, *FreeBayes*, and *GATK*, are capable of considering the ploidy level of the organism under study and exporting the estimated genotypes in the polyploid format.

When polyploid genotypes are not exported or available, it is often necessary to utilize the sequence read count information to properly estimate the genotypes within the species' ploidy range. *FitPoly* (Voorrips et al. 2011) implements a classification mixture model weighted by expected frequencies of the genotypic classes in the population and provides the probability distribution of the dosage-based genotypic classes for the individuals as the output. *ClusterCall* (Carley et al. 2017) does a similar strategy but is restricted to tetraploid organisms. *SuperMASSA* (Serang et al. 2012) uses the ratio of the two allele channels weighted by genotypic frequency in the studied population, classifying individual genotypes in terms of their dosages by using a Bayesian Network. The software supports F_1 and Hardy-Weinberg Equilibrium (HWE) models as *a priori* known population structures. *PolyRAD* (Clark et al. 2019) is also based on a Bayesian approach and includes both F_1 and HWE structures, with a focus on low-read-depth datasets and the ability to perform variant calling along the pipeline. More recently, the R package *updog* (Gerard et al. 2018) was proposed to handle overdispersion, sequence errors, and genotype biases, characteristics that are often found in sequence-based datasets. The package supports *a priori* population structures and can export genotype probabilities in addition to the estimated dosage genotypes.

2. Linkage analysis and QTL mapping

With properly estimated dosage genotypes in hand, the next step in most analytical pipelines is often utilizing the genetic data to extract meaningful information about the population under study. In many cases, this involves the utilization of genotypes to study the genetic structure, architecture, and behavior of a given population, including relatedness studies, linkage analysis, QTL mapping, genome-wide association studies, and genomic prediction. Similarly, software tools for such analyses need to consider relevant aspects when dealing with polyploid species.

Linkage analysis studies in polyploid organisms have relied for a long time on diploid-based methods or their adaptations, like pseudo-test crosses (Grattapaglia and Sederoff 1994). Starting in the 2000s, research and software were developed to overcome the main challenges and limitations often observed when studying outcrossing and polyploid species. The software *TetraploidMap* (Hackett and Luo 2003) was the first tool specifically designed to construct linkage maps in autotetraploid species. The software considered marker genotypes in the polyploid dosage format and was later updated with more robust models and features as *TetraploidSNPMap* (Hackett et al. 2017). It was a breakthrough for linkage analysis in polyploids, with advanced features and support for several markers and individuals. However, it had support for autotetraploids only. In the late 2010s, *polymapR* (Bourke et al. 2018b) was released with an implementation that could handle higher ploidy levels, supporting polyploid organisms up to autohexaploids. This was an important milesone for linkage analysis in polyploids, however, the software was still heavily dependent on two-point information and lacked support for multipoint estimation and higher ploidy levels. Given these limitations, more refined approaches have been developed and released, such as the R package *MAPpoly* (Mollinari and Garcia 2019). The software is able to overcome previous limitations with improved performance, with support for a multipoint procedure to jointly estimate linkage phases and recombination fractions in the final map. The method is also able to handle higher ploidy levels and all levels of information at the same time, thus improving the informativeness of the linkage analysis. More recently, further developments on more complex scenarios, like multi-parental populations, have been proposed. The package *PolyOrigin* (Zheng et al. 2021) is able to reconstruct haplotypes in populations of autotetraploids derived from crosses between multiple parents based on a known sequence order and previously estimated recombination fractions.

As with linkage analysis, methods and tools for QTL mapping have evolved significantly over time, from early approaches in diploid species to more sophisticated methods adapted to polyploid organisms. Early methods, such as single-marker regression, provided a foundational understanding but suffered limitations, including confounded effects and restricted genomic coverage. Advancements led to the development of interval mapping (IM), which improved QTL localization by leveraging genotype probabilities across marker intervals (Lander and Botstein 1989). This method was later refined into composite interval mapping (CIM) to control for background genetic variation (Zeng 1994). The introduction of multiple interval mapping (MIM) represented a further enhancement, allowing for the simultaneous detection of multiple QTL and their interactions (Kao et al. 1999). Most of these methods were initially designed for diploid species, posing challenges for polyploid organisms due to their complex inheritance patterns. Early adaptations for polyploids relied on single-dosage markers, limiting their applicability to broader polyploid scenarios (Hackett et al. 2001). Significant breakthroughs in polyploid QTL mapping include the extension of IM for autotetraploids (Hackett et al. 2014) and the development of *polyqtlR*, which supports higher ploidy levels (Bourke et al. 2021). Subsequent methodologies leveraged the power of MIM and support to higher densities and ploidy levels to perform trait-marker associations in polyploids, like the R package *QTLpoly* (Pereira et al. 2020). Recent advancements allowed overcoming more complex scenarios, like multi-parental populations. The R package *diaQTL* (Amadeu et al. 2021) allows for QTL mapping in outbred diploid and autotetraploid populations that derive from crosses between multiple parents by using the haplotypes calculated with the software *PolyOrigin*.

In the absence of a predefined population structure, marker-trait associations can still be performed by using population-agnostic methods, like multiple regression, GWAS, and genomic prediction. The R package *GWASpoly* (Rosyara et al. 2016) aims to extend and provide GWAS capabilities to autopolyploid species, with an implementation of the Q + K mixed model. While there are R packages that offer extended support for single marker analysis and genomic prediction in polyploids, the major challenges still rely on correctly constructing meaningful models and estimating relationship matrices.

3. Miscellaneous

Other tools are available to provide solutions to different aspects of polyploid data analysis. The determination of the ploidy level stands as a very important aspect that needs to be considered when the species' ploidy level is unknown, the taxa exhibit a complex history with multiple base chromosome numbers, or in presence of irregularities such as aneuploidies. The software *SuperMASSA* (Serang et al. 2012), can estimate marker ploidy levels based on F_1 or HWE scenarios, using allele intensities or read counts information under a Bayesian graphical network. More recently, the software *Qploidy* (available at: https://github.com/Cristianetaniguti/Qploidy) was proposed to estimate ploidy levels and aneuploidies using genotyping platform data, such as targeted sequencing and SNP arrays. The R package *VIEWpoly* (Taniguti et al. 2022) provides complete integration of outputs to visualize and explore results from several software hereby mentioned, including linkage analysis, QTL mapping, and GWAS studies, together with genomic and gene annotation resources, in an interactive and user-friendly framework. The R package *AGHmatrix* (Amadeu et al. 2023) provides an efficient and reliable framework for estimating additive and dominance relationship matrices based on various methods available for polyploids, either using pedigree, genomic information, or both sources of information.

While there have been substantial advancements in software development for polyploids and the polyploid community, polyploid species still lag behind advancements for diploid species due to their increased complexity and subsequent challenges in data analysis and genetic studies. Thus, more efforts, resources, engagement, and collaboration between members of the polyploid community are necessary to put these developments in pair with the diploid scientific community.

E. Genetic transformation and editing

CRISPR/Cas-mediated gene editing is revolutionizing crop improvement. The system consists of a guide RNA that directs the Cas protein (typically Cas9) to target specific DNA sequences, where it creates double-strand breaks that allow researchers to either anull unwanted genes, insert new genetic material, or re-write existing sequences. Unlike traditional genetic modification techniques, CRISPR can make precise edits that are often indistinguishable from natural mutations, making it a powerful tool for crop improvement that can accelerate breeding programs and address agricultural challenges more efficiently than conventional methods. The intricate genome structure of polyploid apomictic crops pose significant obstacles to genetic manipulation. Polyploidy introduces genetic redundancy, typically necessitating the modification of all gene copies to achieve desired loss-off function phenotypes with gene editing but also offering the ability to fine-tune phenotypes depending on the number of co-edited copies (Eid et al. 2021; Brant et al. 2024a). This complexity complicates precise modifications at target loci, making genome editing more challenging (May et al. 2023). Recalcitrance to tissue culture and genetic transformation and the need for high mutagenesis efficiency to co-target multiple copies/alleles are also significant bottlenecks (Altpeter et al. 2016; Ghogare et al. 2021; Lee and Wang 2023). Off-target effects of gene editing, unintended transgene silencing and tissue culture derived somaclonal variation can result in undesired genetic alterations (Senthil-Kumar and Mysore 2011; Guo et al. 2023; Linacero and Ballesteros 2024). Regulatory complexities associated with genetically modified organisms (GMOs) pose significant barriers, with high costs and public skepticism further complicating adoption (Beker et al. 2016; Bhajan et al. 2022). The development of transgene-free editing methods is critical for reducing regulatory burdens and enhancing public acceptance (Menz et al. 2020). However, apomicts have a very low level of sexual reproduction, making it very difficult to remove transgenes through Mendelian segregation (Brant et al. 2021). Therefore, nonintegrative gene editing approaches delivering the editing tools transiently or in form of nonintegrative protein and RNA complexes are being developed (Cai et al. 2025).

Various genetic transformation approaches have been employed to enhance the traits in many tropical forages. Apomixis can be an advantage, as it enables us to maintain agronomically superior genotypes through seeds. However, transferring new traits in apomictic species by crossing can be challenging. Plant transformation and genome editing can therefore be a useful strategy for introducing genetic variability in apomictic cultivars. Furthermore, creation of new traits or stacking of favorable alleles can be achieved by these breeding techniques.

Smith et al. (2002) pioneered the genetic transformation of apomictic bahiagrass, introducing herbicide resistance by biolistic transfer of the *bar* gene. The high escape rate, highlighted the need for improved selection strategies. Altpeter and James (2005) developed an efficient transformation protocol for the turf and forage cultivar 'Argentine', using the selectable marker *npt*II and paromomycin as selective agent following biolistic gene transfer into mature seed derived callus. Mancini et al. (2014) and Narancio et al. (2024) compared callus induction and selection methods for apomictic genotype Q4117 and cultivar 'INIA Sepé' from *P. notatum* and cultivar 'Marandu' from *U. brizantha*, confirming the effectiveness of mature seeds as explants and the use of dicamba for callus induction and paromomycin for selection, as reported earlier by Altpeter and James (2005). Himuro et al. (2009) and Narancio et al. (2024) validated GFP as a transformation reporter. Sandhu and Altpeter (2008) achieved high co-transformation and co-expression of unlinked transgenes in bahiagrass via biolistic transfer, ensuring stable inheritance in apomictic progeny. They reported that DNA quantity during particle coating corresponds to transgene copy number, offering a means to control transgene performance.

Sandhu et al. (2007) reported resistance to glufosinate ammonium herbicide application in 'Argentine' bahiagrass expressing the *bar* gene under field conditions. For pest resistance, Luciani et al. (2007) successfully expressed *cry1Fa* from *Bacillus thuringiensis* in bahiagrass, achieving 83% fall in armyworm mortality enhancing resistance to fall armyworm. In abiotic stress tolerance, James et al. (2008) introduced *DREB1A* under the *HVA1* promoter, enhancing drought and salt tolerance, while Xiong et al. (2010) confirmed *HvWRKY38*'s role in drought tolerance.

Sandhu et al. (2009, 2010) assessed gene flow risk in transgenic bahiagrass, confirming low pollen-mediated transfer due to apomixis and ploidy barriers. Mancini et al. (2018) identified *QGJ* as crucial for aposporous embryo sac formation, advancing apomixis research.

While CRISPR/Cas9-mediated mutagenesis was first achieved in an apomict by Henderson et al. (2020, in *Hieracium*), May et al. (2023) achieved the first CRISPR/Cas9-mediated mutagenesis in an apomictic forage grass (bahiagrass) by targeting the *MgCh* gene with high co-editing efficiency of the different copies/alleles. Evidence of homology-directed repair suggests potential for enhanced genome editing in polyploid species (May et al. 2023; Brant et al. 2024b).

In conclusion, genetic transformation has improved bahiagrass for stress tolerance. Efficient transformation protocols, transgene containment strategies, and CRISPR-based editing provide promising avenues for crop improvement and apomixis research.

VI. Considerations for future apomictic cultivars

Todays breeders are aware of the need to create climate-smart cultivars that meet the SDG requirements (The United Nations Sustainable Development Goals, SDG -https://sdgs.un.org/goals) and, for instance, contribute to decreasing methane emissions.

As pastures are converted into agriculture and integrated crop-pasture systems, grazing is pushed to marginal lands, areas with poor soil, limited water, waterlogging or harsh conditions. This phenomenon is especially important in South America, and results in the need of forage cultivars better adapted to such marginal environments with productive limitations. Therefore, germplasm collections and the availability of grass species with diverse ecological requirements are central for the initiation of breeding programs aimed at covering market needs. In this framework, for instance, Marcón et al. (2024b) pointed out the breeding potential of unexplored species for developing cultivars adapted to areas experiencing alternating periods of drought and waterlogging throughout the year. The association between endophytes and temperate forage grasses is another critical factor affecting cultivar performance. However, the presence and interaction with endophytes are often overlooked in tropical species. Recent reports indicated that various fungal and bacterial endophytes are associated with *Paspalum* species (Depetris et al. 2020; Chen et al. 2022; Paula et al. 2022), and some endophytes positively impact growth and seed yield (Depetris et al. 2020, 2023). Further research is needed to determine how to transfer the endophyte technology to the seed market.

This review has summarized advances in apomixis breeding and cultivar development in nongrasses and apomictic grasses, presenting the state of the art for those used as pastures in tropical and sub-tropical regions of the world and the cultivars released by the breeding programs that contribute to the meat and milk industries worldwide. The review focussed on apomictic cultivars of forage grasses developed by earlier and current breeding programs in Brazil, Colombia, USA, Argentina and Uruguay, denoting the crucial role of institutional collaborations to address common challenges through collective effort.

Relevant to these programs are the development of molecular tools to accelerate the breeding process. Identifying QTLs for traits that are difficult to measure, such as reduced cool-season dormancy in the subtropics, seed retention and ergot tolerance is one of the primary focuses.

Despite emerging technologies like genome editing and genetic modification approaches face significant challenges for application to polyploid apomictic plants, they present a promising perspective. Advanced genome editing tools, the use of drones and artificial intelligence are expected to aid breeding programs in the near future, increasing the efficiency and reducing costs of these programs, as well as contributing to the release of new improved cultivars to the market.

In summary, apomictic breeding of tropical and sub-tropical forage grasses has become a cornerstone of agricultural development in several countries. With continued research, investment, and technological innovation, the future of apomictic forages looks promising, offering sustainable solutions for pasture-based livestock systems globally.

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Data availability statement (DAS)

This paper is a review and was prepared based on published information, thus, there is no unpublished data being presented.

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