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Current status and future strategy in *Megathyrsus maximus* breeding program at the Alliance Bioversity - CIAT

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Key words: Reciprocal Recurrent selection (RRS); Recurrent Selection based on Specific Combining Ability (RS-SCA); Phenotyping; BNI potential.

Abstract

Megathyrsus maximus (Jacq.) B.K. Simon & S.W.L. Jacobs is one of the most important grass species cultivated for cattle production in cut and carry systems. There is a need to search for superior hybrids with high potential for mitigation and adaptation to climate change. A big proportion of the *M. maximus* germplasm, preserved in the CIAT GeneBank has been agronomically characterized and genotypes with desirable traits such as high drought tolerance, high BNI potential (Biological nitrification inhibition), high nutritional quality (biomass production, dry matter, crude protein, acid detergent fiber, neutral detergent fiber and dry matter digestibility) and high seed production have been identified.

The *Megathyrsus maximus* breeding program at the alliance Bioversity-CIAT (Alliance) started in 2016 following simultaneously two breeding schemes (Recurrent Selection based on Specific Combining Ability (RS-SCA) and Reciprocal Recurrent selection (RRS)). To start of RS-SCA scheme, a highly diverse, synthetic, sexual population was created, followed by the establishment of a factorial design using nine apomictic male testers and 30 sexual mothers. The aim of the factorial design was not only to estimate various genetic parameters, but also to identify the best possible apomictic tester with the best Combining Ability. Simultaneously, in order to start the breeding scheme of RRS, two open pollination blocks were established independently, following the heterotic pattern revealed by a population structure study using SSR markers.

In the future, we aim to apply simulation based R packages to predict the genetic gain in both scenarios, followed by the validation of the predictions with measurement of genetic gain based on field data, when considering the allocation of resources and logistics required in each scenario.

Introduction

Megathyrsus maximus (*Mm*) (Jacq.) B.K. Simon & S.W.L. Jacobs (Syn. *Panicum maximum* Jacq.), plays an essential role in livestock production systems in the tropics. This pasture is widely used in extensive production systems, silvopastoral systems (tropical America), and cut-and-carry systems (Africa). This perennial and apomictic grass has important characteristics such very leafy, high quality feed, high production potential, suited to grazing and cutting, drought tolerant, and early season growth in some lines (Cook et al. 2020).

The genebank of the Alliance preserves a large number (563 accessions) of *Mm* accessions, collected and introduced mostly from Africa (<https://genebank.ciat.cgiar.org/>; Koo et al. 2004). A big proportion of these accessions have been agronomically characterized and genotypes with desirable traits such as drought tolerance (Atlas, Centenario, Colônia, Mombasa, Sempre Verde, Tobiata; Cook et al., 2020), high BNI potential (Biological nitrification inhibition; Tobiata; Villegas et al. 2020), high nutritional value (CIAT 16035 c.v. BRS Zuri; Cook et al., 2020) and tolerance of low soil pH and high Al⁺⁺⁺ (CIAT/26900 c.v. Vencedor and hybrid Centenario; Usberti, 1986; Cook, 2020) have been identified. *Mm* commercially available genotypes exhibit desirable agronomic characteristics and adaptation mainly to the Brazilian market, however other markets like Africa and Asia have received far less attention. Cut and carry systems, predominant in Africa and Asia, are based on the use of Napier (*Pennisetum purpureum* Schum.) genotypes. *Mm* offers an alternative to Napier, as the attribute “plant height” is optimum for cut and carry systems, and additionally offers an excellent balance between forage production and nutritional value, without the disadvantage of pests and diseases currently faced by Napier. Despite being considered as an excellent alternative, some factors challenge the performance of *Mm* commercially: i) climate change puts at risk their potential production through worsening of drought periods and ii) evolution and adaptation of pests and diseases, which can become increasingly unpredictable (Chapman et al. 2012). Thus, there is a need to search for superior hybrids with higher potential for mitigation and adaptation to climate change.

The *Mm* breeding program at the Alliance pursues a product profile focused on pyramiding in one unique product, the good attributes of quality and biomass currently available in commercial varieties, but adding value through additional traits like adaptation to climate change with tolerance to drought and low fertility conditions. Furthermore, *Mm* has the potential to contribute to the mitigation of climate change in two ways. Firstly, by means of carbon dioxide fixation (Morgan et al. 1980) since *Mm* follows the C₄ photosynthetic pathway, which is considered more efficient in CO₂ fixation (Malaviya et al. 2020) due to higher photosynthetic rates (Ehleringer et al. 1993). Secondly, through biological nitrification inhibition, a phenomenon that enables a drastic reduction of nitrification through root exudation of biological inhibitors (Villegas et al. 2020).

Taking into account these considerations and adding that the Alliance's breeders have large experience in genetic improvement of grasses (*Urochloa*), we aim to describe the first steps followed by Forage Breeding Team at Alliance in their effort to build a new breeding program in *Megathyrsus maximus*.

Current status

Pursuing the exploitation of heterosis, the *Mm* breeding program at the Alliance started in 2016 following simultaneously two breeding schemes: i) Recurrent Selection based on Specific Combining Ability (RS-SCA), and ii) Reciprocal Recurrent selection (RRS). In both breeding schemes the aim is to accumulate heterotic effects over cycles having two separated heterotic pools (Worthington and Miles, 2015), however, the schemes differ in the number of populations for improvement. While RS-SCA aims to improve one unique breeding pool (females) maintaining the male tester always fixed, the RRS (widespread in Maize breeding) improves both female and male pools simultaneously. Based on quantitative genetics principles, the RS-SCA should deliver higher genetic gains in the short term but in the long term, RRS could double the genetic gains in comparison with RS-SCA. In order to test and profit from that assumption, the breeder at the Alliance decided to partition the investment and follow both strategies simultaneously as we will describe as follows.

Status and future of Recurrent Selection based on specific combining ability (RS-SCA).

The breeding cycle for RS-SCA in *Mm* consists in a three-step process. Firstly, genotypes from a recurrent selection population of sexual females are crossed to a genetically distant "male tester" genotype selected according to its combining ability. Secondly, superior parental females are chosen based on the performance of their testcross progeny. Thirdly, the selected parental females are recombined with the aim to form genotypes for the next cycle of selection (Worthington and Miles, 2015; Hull, 1945). Selected apomictic hybrids in Yr2 will be candidates for cultivar release. Thus, to start this breeding scheme some previous developments are required: i) to obtain a synthetic sexual breeding population (females) in *Mm*; and ii) To identify an appropriate apomictic "tester".

Step1: Obtaining a synthetic sexual breeding population in *Mm*.

In 2015 we identified a tetraploid ($2n=4X=32$) and sexual genotype (PM15), which opened the possibility and the promise of achieving effective genetic recombination in this important species. This discovery opened the way to develop a broad-based, synthetic sexual breeding population in *Mm*. For this, vegetative propagules of sexual PM15 were established in the field and were used as female parents exposing them to pollen from 137 apomictic and tetraploid genotypes from the Alliance genebank, in an isolated open pollination field. Seed was hand harvested on the PM15 plants and once dormancy stage was overcome, were germinated and reproduction mode of the progeny obtained (Pm16) was characterized using the molecular marker p779/p780, which allows identifying *ASGR-BBML* gene sequences present in apomictic genotypes in some Paniceae species (Worthington *et al.* 2016). Apomictic genotypes (118) were used in a progeny test to corroborate marker assertiveness and to identify promising hybrids. On the other hand, hybrids identified as sexual (156) were used to conform the first synthetic sexual tetraploid breeding population in *Mm* at the Alliance.

Step2: Identifying an appropriate apomictic "tester".

During 2019 and 2020, we formed the crosses required for the study of genetic parameters following a factorial genetic design with 30 putative sexual genotypes (mothers) and a group of 9 apomictic genotypes (fathers; CIAT/6893, CIAT/6986, CIAT/26936, Naturalizada, Agrosavia sabanera, CIAT/16031 (Tanzania1), CIAT/6890, CIAT/6992 (Mombasa), CIAT/6799). To achieve this, vegetative propagules of each sexual genotype were planted in a field plot alternated with clones of the apomictic genotype to be evaluated as male

tester, with the purpose that the pollen from all the putative-tester's clones could fertilize each and every sexual genotype. Each cross-block was isolated in order to avoid pollen contamination. Seed was hand harvested on the sexual genotypes. Seeds obtained from each plant constitute an independent family with half siblings. In total, we obtained 270 families.

In 2021, our aim is to evaluate the hybrid progeny obtained. Hence the harvested hybrid seed was stored in a cold room and will be sown in pots, characterize for its reproduction mode using the molecular marker p779 / p780, and apomictic genotypes will be evaluated in three different locations following a Federer's augmented design. This evaluation will have 15 apomictic hybrids per family, 30 genotypes used as mothers, 9 genotypes used as pollen donors, and three controls. Best linear unbiased predictors (BLUPs) obtained from data analysis will be used to correlate the genetic distance of the parents (previously genotyped) with hybrid performance. In addition, we would expect to obtain that the crosses from the parents with the greatest genetic distances are in turn the most productive, in other words, those that express the best heterotic effect.

Step3: Starting the first cycle of *RS-SCA*.

Once the best apomictic tester is identified and synthetic sexual breeding population has been obtained, the *RS-SCA* breeding scheme will be started. Considering i) the early flowering exhibited by *Mm* genotypes, and ii) the existence of a molecular marker which allows the fast identification of apomictic behavior in the genotypes, we have the expectation of adjusting the breeding cycle time to a total period shorter than three years.

Status and future of Reciprocal Recurrent selection (RRS)

The RRS breeding scheme allows the simultaneous improvement of both general and specific combining ability in two complementary populations that exhibit heterosis in their crosses (Worthington and Miles, 2015; Comstock et al. 1949). In comparison with the well-known RRS applied in other crops like Maize, RRS in apomictic forages imply an additional effort in logistics and population genetics, as both sexual and apomictic representatives should be generated for each breeding pool. This scheme also follows a three-step cycle, with some substantial modification in terms of logistics. The first step is the formation of hybrid progenies by crossing selected sexual plants from Population A to a random set of apomictic plants from Population B, and vice versa. Note that in order to avoid pollen-contamination a proper methodology for bi-parental crosses needs to be developed. The second step comprises the evaluation of half-sib families and checks at multiple field sites. Finally, the third step is the recombination of selected progenitors of superior families of Population A and B in separate nurseries (Worthington and Miles, 2015).

Step 1: Understanding population structure

A heterotic group is defined as a group of genotypes which display similar combining ability and heterotic response when crossed with genotypes from other genetically distinct germplasm groups (Melchinger and Gumber 1998). For an efficient RRS scheme, it is primordial to organize the germplasm into heterotic groups, in this sense, a large group of genotypes of *M. maximus* was previously genetically characterized and the study of population structure was done using SSR markers, as a result, genetic pattern for two heterotic groups were identified: K1 & K2 (Unpublished data).

Step 1: Forming heterotic groups

According to previous phenotypic characterization of germplasm, best representatives from each heterotic pool were planted in two isolated open pollination blocks to form two heterotic pools: K1 and K2 groups. Seed harvested on sexual and apomictic genotypes from each heterotic group are stored in cool room and will be used in a performance field test. Best apomictic and sexual representatives from both pools will be used to apply the RRS breeding scheme in the third step of recombination within each heterotic pool, and from this point, the cycle continues as described above.

Comparison of effectiveness among both schemes: RS-SCA and RRS scenario.

Different rates of genetic gain are expected for both schemes in time. In *RS-SCA* scheme, we hope to increase rapidly genetic gain given that it is logistically simpler and quicker, with the disadvantage of quickly depleting

genetic diversity. RRS scheme, despite being logistically more complex, genetic diversity is sustained in the long term. Then although RS-SCA can provide faster candidates for commercial release, RRS will guarantee the stability of the program in the long term. The strategy proposed in this document is to implement both scenarios simultaneously, doing an allocation of resources in the short term of 70% for RS-SCA / 30% for RRS, until high performing heterotic pools are achieved for RRS. Then, the main product pipeline will follow the RS-SCA scheme, and investments in RRS will be done for the next-generation pipeline. In the future, we aim to apply simulation-based R packages to predict the genetic gain in both scenarios, followed by the validation of the predictions with measurement of genetic gain based on field data.

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