Research priorities for next-generation breeding of tropical forages in Brazil

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Abstract: *Pasture is the main food source for more than 200 million cattle heads in Brazil. Although Brazilian forage breeding programs have successfully released well-adapted, high-yielding cultivars over the years, the use of genomic tools in these programs is currently limited. These tools are required to tackle the main challenges for tropical forage breeding in Brazil. In this context, this note lists the main research priorities raised at the workshop "Breeding Forages in the Genomic Era", which are necessary to accelerate the use of genomic tools for next-generation breeding of tropical forages and allow breeders to increase genetic gains. Additionally, an online discussion forum (hosted at http://www.cnpgl.embrapa.br/genfor) has been launched to strengthen collaborations among research groups. The research priorities and more synergistic collaborations will assist researchers and decision-makers in delivering a sustainable increase in production of animal products, especially beef and milk, which are required to feed a rising world population.*

Key words: *Discussion forum, genomic tools, molecular breeding, molecular markers, pasture.*

INTRODUCTION

A revolution in plant breeding has been led by the availability of genomic information that is crucial to answer relevant biological questions and to pave new ways for basic and applied research. The higher throughput and lower costs brought about by next-generation sequencing technologies have allowed plant breeding programs to obtain important genomic information. For instance, a large number of markers, such as simple sequence repeats (SSRs) and single-nucleotide polymorphisms (SNPs), can be identified. Arrays with thousands of highly reproducible SNP markers can be produced. To circumvent high costs of SNP arrays and ascertainment bias, genotyping-by-sequencing can be performed. Polymorphisms underlying important traits can be detected by QTL mapping, genome-wide association studies, and genome resequencing. Also, genomic selection for traits controlled by several genes with small effects has been shown to increase genetic gains (Bhat et al. 2016).

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However, application of genomic tools in important tropical forages for Brazilian breeding programs is still limited. For instance, marker-assisted selection has not yet been routinely implemented in these breeding programs, and even though more than 100 plant genomes have been sequenced, genome sequences are available for only two tropical forages (*Cajanus cajan* and *Cenchrus americanus* syn. *Pennisetum glaucum*) and a few species closely related to important tropical forages in Brazil (e.g. *Arachis duranensis*, *Arachis ipaensis* and *Setaria italica*) (Michael and VanBuren 2015, Bertioli et al. 2016, Varshney et al. 2017). Reasons for the low use of genomic tools in tropical forages may probably rely on (i) the intrinsic characteristics of tropical forage breeding, such as research efforts distributed among many forage species, which in many cases have different ploidy levels and reproductive modes, including apomixis; (ii) the limited budgets for breeding; and (iii) the fact that most of the breeding programs have been relatively recently created.

In order to foster discussion and consider research opportunities for the application of genomic tools in tropical forage breeding, researchers from different Brazilian institutions attended the workshop "Breeding Forages in the Genomic Era" held at Embrapa Gado de Leite (Embrapa Dairy Cattle) in November 2016. During the workshop, attendees discussed the main challenges for tropical forage research in Brazil and raised the top priorities to accelerate the use of genomic tools in breeding programs. In this context, this note aims to draw the attention of the scientific community for the requirements argued during the workshop that are needed to implement next-generation breeding of tropical forages. The goal also extends to the evaluation of the status of genomic tools application in tropical forages and the announcement of an online discussion forum that has been created to stimulate and increase cooperative actions among research institutions.

TROPICAL FORAGES IN BRAZIL: IMPORTANCE AND MAIN CHALLENGES FOR BREEDING

Brazil is among the top beef and milk producing countries in the world. Meat and dairy industries are highly important for the Brazilian economy with total domestic sales for both industries reaching, in 2016, approximately US$ 60 billion (R$ 200 billion) (ABIA 2017). Brazilian beef and dairy production is mainly based on pasture systems. Although numbers may vary in different studies due to the hard task of land use mapping, current estimates claim that approximately 60 million hectares (M ha) of the Brazilian territory consists of native pastures and about 100 M ha is covered with cultivated pastures, which compose the main food source for 209 million cattle heads.

Changes in land use are expected due to the eventual expansion of the areas used for crops and planted forest to meet future demands for food, fiber, and energy. This expansion will probably occur on pasturelands and, indeed, these areas have been replaced by cropping and forestry in the past few years (IBGE 2016). The reduction in Brazilian pasturelands has been followed by an increase in cattle herds, resulting in a higher proportion of cattle heads per hectare (Figure 1). This fact implies that major efforts to increase forage productivity are required. Moreover, the current productivity of Brazilian pasturelands is lower than their estimated carrying capacity (274-293 million animal units), revealing a potential for increments (Strassburg et al. 2014). Productivity increase of pasturelands is a multifaceted problem dependent on the pasture management, animal performance, and technology level adopted by farmers,
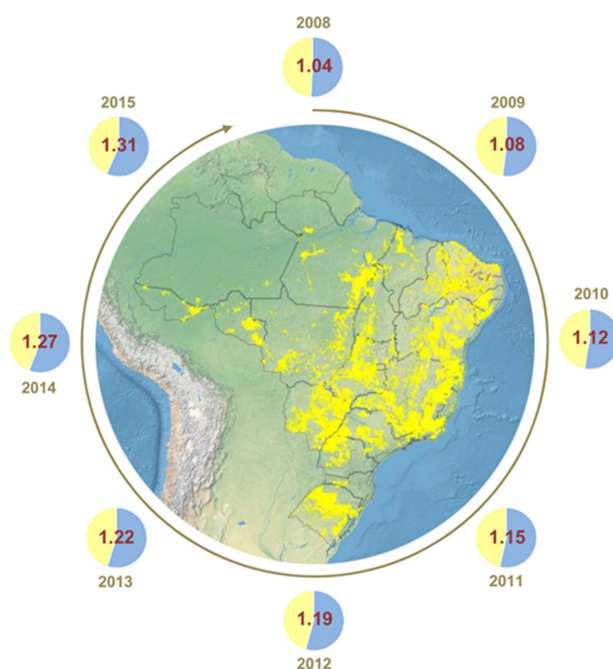

Figure 1. Evolution of pasture area and cattle herd in Brazil. The map represents the area with pastures in Brazil (in yellow) and it was obtained from https://pastagem.org/ (accessed on May 29, 2017) available at the Laboratório de Processamento de Imagens e Geoprocessamento da Universidade Federal de Goiás (LAPIG/UFG). The graphics indicate the proportion of the pasture area (in million hectares represented in yellow) versus the size of the cattle herd (in millions of cattle heads represented by blue). The numbers (in red) show the calculated cattle heads per hectare.

among other factors. However, forage productivity and adaptation are definitely important factors.

Thus, to increase productivity in pasturelands, Brazilian tropical forage breeding programs will have to address challenges such as (i) reduction of losses due to biotic stresses, especially spittlebug attacks, (ii) increase of adaptation, based on forecasted climate changes (especially heat, drought, and flood tolerance), and (iii) improvement of nutritive value for better animal performance (more beef and milk per kg of pasture). An association of these challenges is also concerning since climate change can impact forage nutritive value and spittlebug development (Wheeler and Reynolds 2013, Fonseca et al. 2016).

LACK OF GENOMIC INFORMATION FOR TROPICAL FORAGES

Worldwide, hundreds of plant species can be used for animal feeding. More than 20 forage grasses and legumes with different levels of economic importance have been grown in Brazil, not considering temperate pasturelands (Fonseca and Martuscello 2010). Brazilian tropical forage breeding programs have mainly targeted species such as *Andropogon gayanus*, *Arachis* spp., *Cenchrus* spp. (especially *C. ciliaris* syn. *Pennisetum ciliare* and *C. purpureus* syn. *Pennisetum purpureum*), *Cynodon* spp., *Megathyrsus maximus* (syn. *Panicum maximum*), *Paspalum* spp., *Setaria sphacelata*, *Stylosanthes* spp., and *Urochloa* spp. (*U. brizantha* syn. *Brachiaria brizantha*, *U. decumbens* syn. *Brachiaria decumbens*, *U. humidicola* syn. *Brachiaria humidicola*, and *U. ruziziensis* syn. *Brachiaria ruziziensis*), each one with a specific purpose or niche. Still, only the four species of the genus *Urochloa* represent approximately 85% of the Brazilian cultivated pasture area with one cultivar, the tetraploid apomictic *U. brizantha* cv. Marandu, being estimated to cover around 50 M ha (Jank et al. 2014). The importance of *Urochloa* spp. is demonstrated by its presence in near half of the studies using genomic tools in tropical forages (this information is based on studies published in the last five years). A great number of these studies have focused on (i) the development of molecular markers to assess germplasm diversity and structure and (ii) the molecular aspects of reproduction with an emphasis on apomixis, in part stimulated by the demand of breeding programs for marker-assisted selection of apomictic hybrids. Clearly, the importance of this information is indisputable. However, the application of genomic tools for purposes such as QTL mapping, genes isolation, genome sequencing and assembly, or marker-assisted selection is rare or, in most cases, non-existent.

RESEARCH PRIORITIES AND AN ONLINE DISCUSSION FORUM TO HELP ACHIEVE NEXT-GENERATION BREEDING OF TROPICAL FORAGES

Several well-adapted, high-yielding cultivars have been released by Brazilian tropical forage breeding programs. However, to tackle the main challenges for tropical forage breeding in Brazil, the genetic gains for important traits need to be increased, and the timeframe required for developing cultivars needs to be shortened. In this way, genomic information in tropical forages is required to pave the way for next-generation breeding, which refers to the use of different tools (such as sequencing, bioinformatics, and automatic phenotyping) that allow breeders to achieve increased genetic gains (Barabaschi et al. 2016). To accelerate the use of genomic tools in tropical forages breeding in Brazil, the following research themes must be prioritized:

Genome sequencing, high-throughput genotyping, and germplasm characterization

Few reports using next-generation sequencing for tropical forages are available (Silva et al. 2013; Toledo-Silva et al. 2013; Vigna et al. 2016a; Pessoa-Filho et al. 2017). Based on the low cost and high throughput of sequencing, the time is ripe for efforts of genome sequencing and assembly in tropical forages. In addition to sequencing, chromosome conformation capture methods allow chromosome-length scaffolding of large genomes (Phillippy 2017). With a good reference genome in hand, germplasm can be deeply characterized by re-sequencing pools of diverse accessions. This would lead to the discovery of thousands of SNPs that could be used in genotyping platforms for germplasm characterization, genome-wide association studies (GWAS), and genomic selection. Studies focused on gene function or molecular control of phenotypes of interest will benefit from the availability of reference genomes.

Identification of genes, QTL, and pathways associated with heat/drought/flood tolerance, spittlebug resistance, and nutritive value

Most of the few mapping studies have focused on *Urochloa* spp. (Thaikua et al. 2016; Vigna et al. 2016b; Worthington

et al. 2016). To identify QTL associated with important traits, not only higher number and a broader range of molecular markers are required, but also suitable mapping populations need to be developed. Depending on the genetic architecture of the trait, efforts to identify potential contrasting parents for bi-parental crosses or to design diverse germplasm panels for GWAS are required. The development and availability of SNP markers in affordable genotyping platforms are needed. Moreover, few transcriptomic studies in tropical forages have been published (Silva et al. 2013; Toledo-Silva et al. 2013; Vigna et al. 2016a). The availability of transcriptomic, proteomic, and metabolomic data would foster studies on key pathways associated with important traits in tropical forages. The identification of trait-associated polymorphisms would lead to opportunities for genome editing.

Implementation of genomic selection

Increased genetic gain due to reduced cycle time is the main advantage of genomic selection in forage breeding (Resende et al. 2014). Current studies on cool season forages are limited to the evaluation of genomic prediction accuracies, mostly with simulated data. Initial results are promising, with notable improvements over phenotypic selection alone. The development of adequately sized training populations is essential to start the validation and evaluation of selection based on estimated breeding values (Resende et al. 2014). Under the condition that a genome-wide set of SNP markers is available for a given species, genotyping platforms with low costs per marker and good reproducibility should be carefully chosen.

Development and use of large-scale phenotyping tools

Automated or semi-automated high throughput, non-invasive, and robust phenotyping techniques are important to correctly evaluate plant traits and allow faster association with genotypic data. Progress in different areas has allowed high-capacity data recording for an affordable price (Araus and Cairns 2014). However, bottlenecks still exist when associating external phenotypes with specific physiological mechanisms. This fact results in the need for the adoption of high-dimensional physiological phenotyping, leading to a precise characterization of internal phenotypes. In this way, complex traits can be broken down into individual components of physiological traits. Moreover, for perennial plants, as most tropical forage grasses, phenotyping in glasshouses may not be feasible due to large space requirements. High-throughput phenotyping under field conditions would be useful for tropical forage breeding. Nevertheless, up to now, studies with this purpose are scarce (Jiménez et al 2017).

In addition to these well-defined research priorities, the development and application of genomic knowledge in tropical forage breeding will depend on adequate financial support and cooperative actions among research institutions. The investments and the economic viability required to move towards next-generation breeding of tropical forages, including marker-assisted breeding, should be examined on a case-by-case basis, considering a trade-off between cost and breeding efficiency. This efficiency is expected to increase with genomic selection (Bhat et al. 2016), although the cost analysis of this approach indicates that gains in efficiency of forage breeding programs will compensate only with costs value of US$0.001 per marker (Resende et al. 2014). The investments needed for developing genomic tools in tropical forage breeding will eventually require greater public research funding since tropical forage breeding in Brazil is mainly performed by public institutions (Jank et al. 2014). Additional sources of funding can rely on an ongoing partnership with a Brazilian consortium of forage seed producers as well as on potential new partnerships. Whether at a public or private institution, strategic decision-makers need to take into account the research priorities detailed in this note, stimulating calls for proposals that target the application of genomic tools in the routine of tropical forage breeding programs. Besides greater research funding, strategies to simplify procedures and reduce costs should be considered, such as bulked sample analysis (Zou et al. 2016). Regarding the cooperative actions among institutions, the synergy will greatly benefit from an online discussion forum (hosted at http://www.cnpgl.embrapa.br/genfor) created to improve communication and information sharing among research scientists interested in applying genomic tools to tropical forage breeding in Brazil. All researchers interested in using these tools for the research priorities highlighted in this note are welcome to join this discussion forum.

FINAL CONSIDERATIONS

The use of genomic tools in tropical forage breeding programs must be prioritized to increase genetic gains and

reduce the time required to obtain new tropical forages cultivars. Clearly, conventional and genomic-based breeding should not be viewed as contrasting approaches as they both have common goals. Nevertheless, genomic information will certainly contribute to achieving a sustainable increase in the production of animal products, especially beef and milk, required to feed a world population of 9.6 billion people predicted by 2050 (Gerland et al. 2014). The use of genomic tools in tropical forage breeding is challenging; however, a lot can be learned from other polyploid plant species with large genomes, which have already been used for genome sequencing and editing (Wang et al. 2014, Michael and VanBuren 2015). The main challenges for breeding tropical forages in Brazil are the improvement of productivity, spittlebug resistance, nutritive value, climate change adaptation, as well as the circumvention of intrinsic issues, such as apomixis, and the tools to face these challenges exist and are becoming more accurate and accessible. The capacity to work synergistically and the online discussion forum announced here are good allies that can help Brazilian scientists strengthen collaborations. Besides the tools and the synergistic collaboration, greater research funding will also be required. The funding distribution must not result in unnecessary competition between ongoing breeding programs and new initiatives.

ACKNOWLEDGMENTS

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