

DEVELOPING A BIOINFORMATICS PIPELINE FOR AN *Urochloa* INTERSPECIFIC MAPPING POPULATION: FROM RAD SEQUENCING DATA TO IDENTIFICATION OF QTL FOR SPITTLEBUG RESISTANCE

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Report

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Summary

The development of molecular technologies for breeding tetraploid brachiaria interspecific hybrids (*Urochloa* spp.) was limited by its high ploidy, the lack of an *Urochloa* reference genome and the apomictic nature of the tetraploid materials in the *U. ruziziensis*/*U. brizantha*/*U. decumbens* agamic complex. However, the current increasing availability of open-source tools for genomic data in polyploid species has broadened the genetic resources that are available for the breeding of these forages. Using RAD sequencing data from a multiparenting mapping population, a new fully resolved genome of *U. decumbens* and phenotypical scoring for tolerance and antibiosis to *Aeneolamia varia*, we designed a bioinformatics pipeline to construct genetic maps that allow us to identify QTL associated with resistance to this spittlebug species. The next steps are to test different software and techniques for each phase in the pipeline, control the quality of the outputs and deliver accurate results from the phenotypic and genotypic data association.

Background

Breeding for resistance to the spittlebug (Hemiptera: Cercopidae), a key pest of brachiaria grasses in America, has been one of the main objectives of CIAT since the interspecific breeding scheme was established more than 30 years ago. Studying the genetic architecture of complex traits like this, is crucial for understanding and advancing the breeding progress in polyploids. A major challenge, however, is that QTL discovered in single biparental populations, derived from highly heterozygous outbred individuals can lose their predictive ability when applied to the wider breeding populations (Zheng et al., 2021).

The most economically relevant species of *Urochloa* are polyploid apomicts, meaning that the offspring are genetically identical to the parents, which imposes a limitation in the development of new cultivars as sexual apomicts are not available for the production of hybrids (Worthington and Miles 2015; Worthington et al., 2016). The polyploidization through colchicine doubling of a sexual diploid *U. ruziziensis*, allowed the crossing between plants of the agamic complex *U. ruziziensis*/*U. brizantha*/*U. decumbens* resulting in hybrid progenies that have been used in a recurrent selection breeding scheme (Miles et al., 2007).

Marker-assisted selection has contributed to optimize, shorten and improve the accuracy of the selection process for quantitative, mono or oligogenic traits (Hasan et al., 2021), being essential for constructing genetic maps to identify the loci associated to a determined trait. Previous studies in our group aimed to identify the apospory-specific genomic region (ASGR) and QTL related to aluminum tolerance in an interspecific biparental population from the BRX 44-02 (sexual autotetraploid) and CIAT 606 (apomictic segmental allopolyploid) (Worthington et al., 2016, 2021). The linkage maps were constructed using two

different diploid reference genomes of *Setaria viridis* and *U. ruziziensis*, CIAT 26162 and consequently single dose markers. These genetic map's analysis also provided evidence of preferential pairing of homologs in the *U. decumbens* parental CIAT 606.

The recent advances in next generation sequencing technologies and the available software tools for polyploids make it possible to improve the maps' quality by increasing the number of markers. We aim to test new software in an interspecific multiparental *Urochloa* population to create a new and improved genetic map for *U. decumbens* (Scott et al., 2020, Nielsen et al., 2011).

Objective

Develop a bioinformatics pipeline for the analysis of RAD sequencing data of an *Urochloa* interspecific mapping population for identifying QTLs associated with spittlebug resistance.

Data

We constructed an adapted Nested Associated Mapping (NAM) F1 population of *Urochloa* spp. using contrasting parents for the spittlebug resistance trait (Fig. 1). During 2020-2022, the population was phenotyped in no-choice tests to the nymphal attack of *Aeneolamia varia* (Hemiptera: Cercopidae) and sequenced with RADseq technique using PstI enzyme (single end, 118bp). To increase the depth of the parental reads, we sequenced each individual four times.



Figure 1. Br15b mapping population pedigree

Pipeline

Based on previous studies (Bourke et al., 2016; Ferreira et al., 2019; Mollinari et al., 2020; Taniguti et al., 2022.; Worthington et al., 2016), we designed a workflow from the preprocessing of the raw RADseq reads to the QTL identification using different software. Considering that the meiotic behavior of the parents and the progeny is not fully elucidated, we propose testing multiple tools and approaches (Fig. 2).

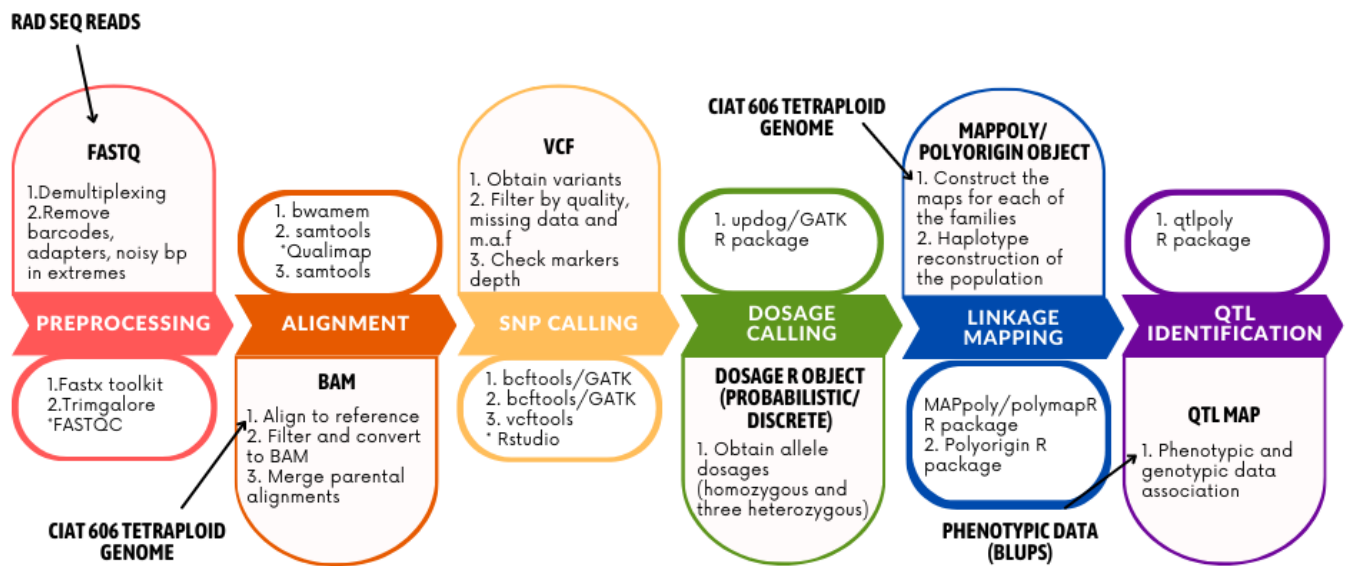


Figure 2. Bioinformatics workflow for QTL identification in Br15b mapping population

Future actions – Expected outcomes

1. Use the different software through the pipeline with quality control measures at each step
2. Test discrete and probabilistic data of genotype calling to build the linkage genetic maps
3. Identify QTL and quantify the genetic effects for antibiosis and tolerance traits in the multiparental population

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