Developing a bioinformatics pipeline for *urochloa* spp. 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 ploidy, the lack of a tetraploid *Urochloa* reference genome and the consequent drawback of using only simplex markers to construct linkage maps. 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.

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.

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).

Previous studies in our group aimed to identify the apospory-specific genomic region (ASGR) in a biparental population and QTL related to aluminum tolerance (Worthington et al., 2016, 2021). The linkage maps were constructed using two different diploid reference genomes of *Setaria viridis* and *U. ruziziensis,* CIAT 26162. Consequently, the markers did not have allele dosages to distinguish between the heterozygote genotypes and the genotype calling step was performed with discrete data.

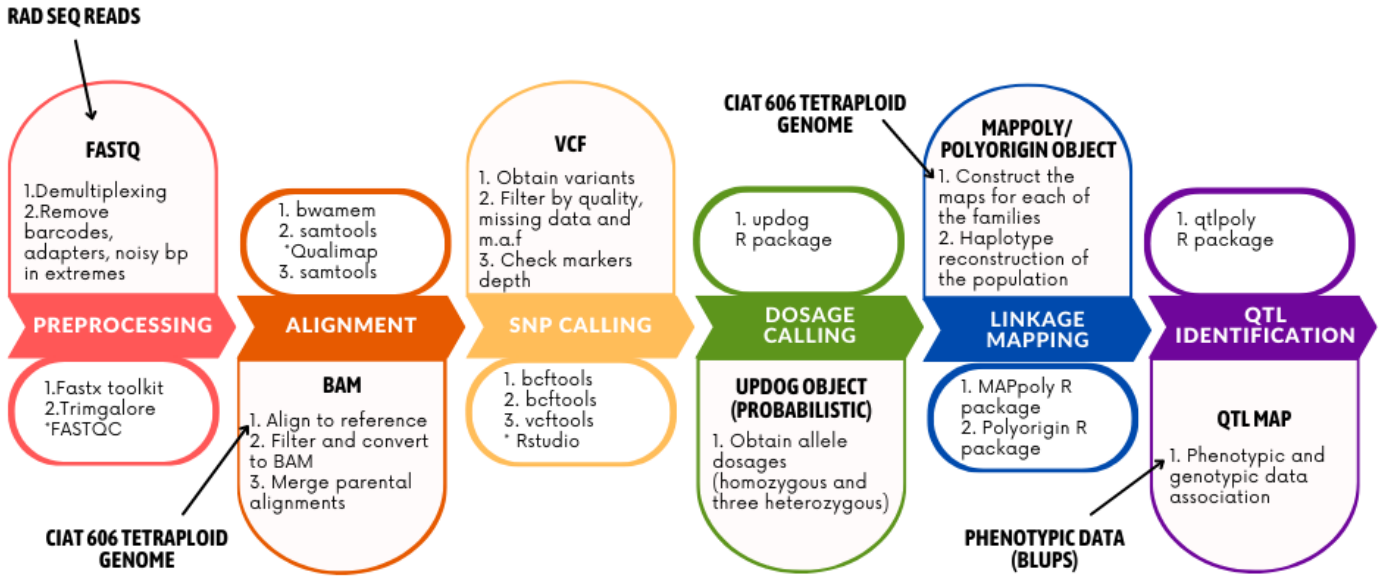
Data

Considering this, we constructed an adapted Nested Associated Mapping (NAM) 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, 1x118). To increase the depth of the parental reads, we sequenced each individual four times.

Pipeline

Based on previous studies (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 tools:



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 multiparenting population

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