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# Congresso Brasileiro de Melhoramento de Plantas

**tema** MELHORAMENTO  
DE PLANTAS:  
Projetando o Futuro

**14 a 17**  
de Agosto de 2017

Rafain Palace Hotel  
& Convention Center  
Foz do Iguaçu - PR

**E-Book**

Volume 1  
2017



## **E-BOOK do 9º CBMP**

‘Melhoramento de plantas: Projetando O Futuro’

[ ISBN 978-85-94437-00-6 ]

Agosto/2017

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# DEVELOPMENT OF SINGLE NUCLEOTIDE POLYMORPHISM (SNP) MARKERS FOR GENETIC MAP SATURATION OF *UROCHLOA HUMIDICOLA*

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Large areas of Brazil are destined for the cultivation of pasture, where the purpose is to feed cattle. Among these, it stands out *Urochloa humidicola* (syn. *Brachiaria humidicola*), a hexaploid specie that reproduces by apomixis. In the breeding program developed by Embrapa Beef Cattle, the commercial cultivar BRS Tupi was crossed with the accession H031, unique sexual genotype of the Germplasm Bank, generating 279 hybrids F<sub>1</sub>. These hybrids were used in construction of a genetic map based on microsatellite markers, developed exclusively for the species. This current map consists of 294 markers, distributed in 49 linkage groups. In order to increase the density of markers on map, the main objective of this work was identify and development SNP markers from the foliar transcriptome of BRS Tupi and H031 genotypes. For this, the reads of each genotype were mapped in transcriptome assembly and prospection of SNP was made using CLC Workbench software. Contigs sequences containing these loci were grouped, creating a reference bank used for a new mapping and prospection of SNP, allowing the selection of common SNP in both genotypes. In these, 15,215 loci were identified. According to the technique that will be used in genotyping, a series of filtrations was performed: i) elimination of markers that did not have a region of at least 150 bp on each flank and/or had another SNP at 50 bp or less distance; ii) identification of introns in the flanking portions of SNP through BLAST using *Setaria italica* data from Phytozome v9.1; iii) BLAST with the genome of *S. italica* as a query and also as subject, considering the genes with a single hit as a unigene; iv) then, contigs of *U. humidicola* were aligned with these genes via BLAST, and only those with similarity greater than 70% were selected. At the end of all filtering steps, we obtained 251 candidates SNP. Of these, 76 SNP are associated with nitrogen fixation pathways, cellulose and lignin metabolism, C4 metabolism, and flood tolerance. These were submitted to primer design in Assay Design Suite from Agena Bioscience® software, for genotyping on MassARRAY® System Agena Bioscience™. The SNP markers developed will be used to saturate the genetic map of the *U. Humidicola* and represent a significant set of tools that will benefit breeding programs for this species.

**Keywords:** koronivia grass; polyploidy; RNASeq

**Acknowledgment:** FAPESP, CNPq and Embrapa Beef Cattle.