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The sugarcane genome poses challenges that have not been addressed in any prior genome sequencing project. The main difficulties reside in the high polyploidy (2n ~ 12x ~ 120) and the high level of heterozygosity of cultivars, which make an assembly of the genome very challenging through classical whole genome shotgun sequencing approaches. We developed an alternative sequencing strategy that aims to produce the sequence of one monoploid genome. Our strategy is based on previous studies that demonstrated that sugarcane hom(e)ologous chromosomes share a very high level of micro-colinearity among themselves and show good micro-colinearity with sorghum. We used the Whole Genome Profiling technology (WGP™, KeyGene) to analyze a set of 20,736 BACs from cultivar R570. An average of 37.2 WGP sequence tags per BAC was generated that allowed the anchoring on the sorghum genome of 11,732 R570 BACs. A core set of 5,000 BACs representing the minimum number of BACs that best cover the gene rich part of the sorghum genome was selected. This set of 5,000 BACs has been sequenced using PacBio RSII technology through international collaborations. High quality sequences were obtained and almost all BACs could be assembled into single contigs. Genotyping by sequencing approaches are being used to anchor the BAC sequences onto the sugarcane chromosomes. A dense SNP genetic map has been built allowing us so far to anchor half of the BAC sequences. RNA from distinct tissues of R570 was also sequenced using Illumina Hiseq 2500 and these data were integrated in the annotation process to improve BAC sequence annotations. A sugarcane web portal is currently being developed together with user-friendly tools to make BAC sequences and gene annotations available in an exploitable form to the sugarcane community. This high quality reference sequence that corresponds to the gene rich part of a sugarcane monoploid genome will represent a very important resource for genetic, structural and functional genomic studies in sugarcane and also an essential framework to help assemble a whole genome sugarcane sequence.

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