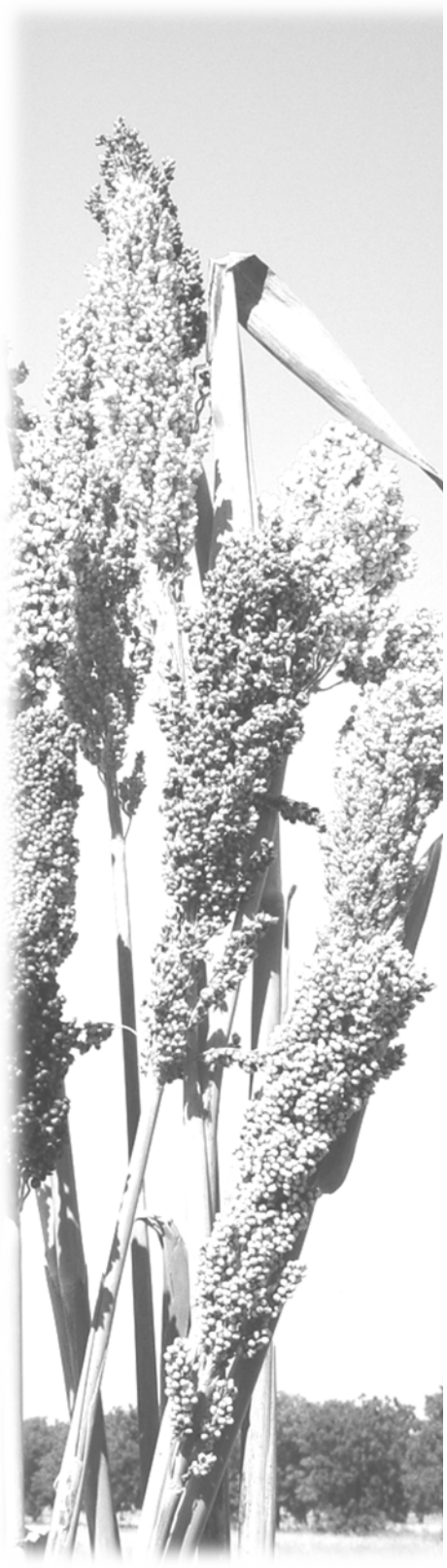
Seventh framework programme
Food, Agriculture and Fisheries, and Biotechnology

Specific International Co-operation Actions
Small or medium scale focused research project

Sweet Fuel

Sweet Sorghum
an alternative energy Crop



Grant Agreement n° 227422

WP3
Deliverable 3.20:

Two hundred RIL from bmr_{12} RIL population phenotyped brown midrib

Composition of the consortium
CIRAD
ICRISAT
EMBRAPA
KWS
IFEU
UniBO
UCSC
ARC-GCI
UANL
WIP

Protocols for marker assisted selection (MAS) and marker assisted backing have been published for the low lignin brown midrib mutant (*bmr*) after the development of this project proposal. Consequently it is not necessary to develop RILS for this activity. For *bmr*-6 mutants, a codominant Cleaved-Amplified Polymorphic Sequence (CAPS) marker reported by Sattler et al. (2009) was used to screen plants at early stages of backcrossing cycles in order to identify heterozygous BC_1F_1 plants. This marker was designed to verify the *bmr*-6 point mutation. Specific primers were designed to amplify a 613 bp fragment from the *Bmr*-6/*bmr*-6 alleles. The C-to-T transition mutation in *bmr*-6 allele created a *BsaAI* restriction site, so when the amplified fragment is digested with *BsaAI* and analyzed by agarose gel electrophoresis, it results in two visible fragments of 333 and 280 bp. On the other hand, the WT allele amplified fragment is not cleaved by *BsaAI*, allowing identification of heterozygous plants. BC_1F_1 plants (Expected ratio of 1:1 for desirable heterozygous plants and homozygous wild type) were originated from three crosses: Fartura*Tx2784 *bmr*, Lavrense*Tx2784 *bmr*, and Santa Elisa*Tx2784 *bmr*. BC_1F_1 plants were seeded in two pots (9 plants/pot) and genomic DNA from leaves of each plant was extracted using a Genogrinder when the plants were 2 weeks old. PCR amplification and BsaAI digestion of amplified products were carried as indicated by Sattler et al (2009). Figure 1 shows the results for the *bmr*-6 screening. The BC_1F_1 plants segregate 1:1 for dominant homozygous (wild type) and heterozygous. The identified heterozygous plants were selected to produce BC_1F_2 generation. The three BC_1F_2 populations were planted in the field and *bmr* progeny were selected with desirable agronomic traits. A composite was made of each BC_1F_3 progeny selected in each population. Each composite was planted in the field for seed increase and to make new selections among and within progenies to develop new R lines or varieties for biomass sorghum. An average of 5 heterozygous plants was recovered in each pot of 9 plants. ARMS (Amplification *Refractory* Mutation System) makers are under development for *bmr*-12 and *bmr*-18 mutants as was done for bmr-6. Consequently, there is no need to develop the RILs.

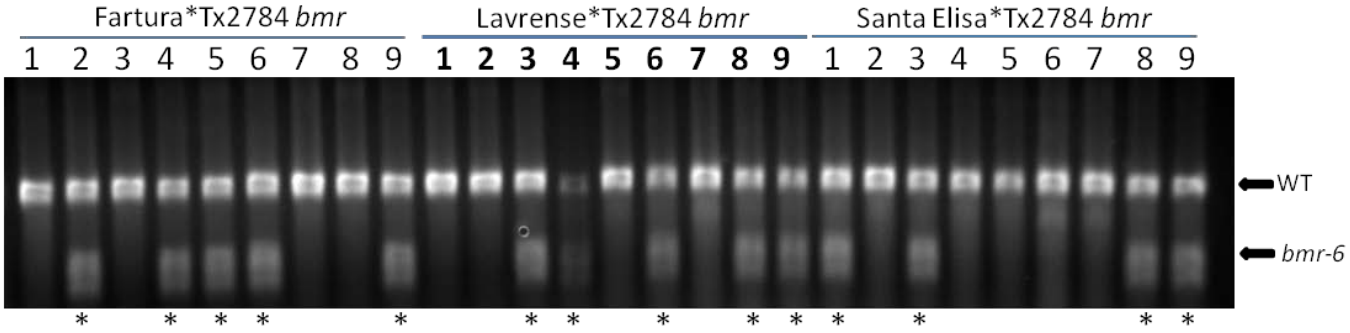


Figure 1: Screening of BC_1F_1 plants using *bmr*-6 CAPS marker. Three BC_1F_1 crosses were evaluated, which are shown at the top of the figure. The amplification products were digested with the restriction enzyme *BsaAI* and analyzed by agarose gel electrophoresis (1.2%, TAE 1X). Marker primers were used to amplify a 613-bp fragment of the *bmr*6 gene from sorghum genomic DNA. After digestion with BsaAI, the wild-type (WT) fragment remained a 613 bp , while the fragment from *bmr*-6 mutant allele resulted in two fragments of 333 and 280 bp. Heterozygous plants are marked by an asterisk.