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 3.19:

Two hundred RILs population derived from the cross of two sorghum lines contrasting for brown midrib bmr$_6$ developed to S3 generation

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 (Wild Type) 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 planted 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 individuals. 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 and varieties for biomass sorghum. An average of 5 heterozygous plants was recovered in each pot of 9 plants. Consequently, there is no need to develop the RILs.

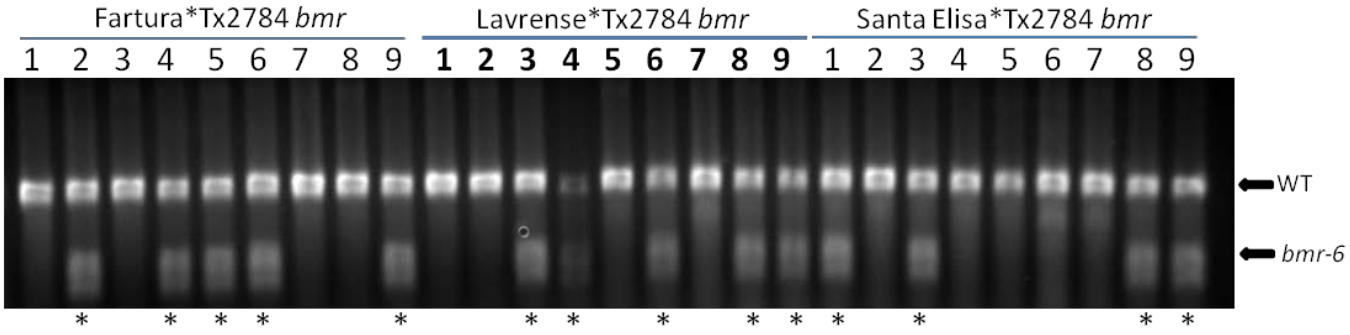

Figure 1. Screening of BC_1F_1 plants using *bmr*-6 CAPS marker. Three BC_1F_1 crosses were evaluated, which are shown at the top of the figure. The amplification products were digested with the restriction enzyme *BsaAI* and analyzed by agarose gel electrophoresis (1.2%, TAE 1X). Marker primers were 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.