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

Deliverable 3.5:

Ten to twenty varieties and hybrids evaluated for responses to P stress

Composition of the consortium

CIRAD
ICRISAT
EMBRAPA
KWS
IFEU
UniBO
UCSC
ARC-GCI
UANL
WIP

The major objective of this research component was to identify genotypes of sorghum for P acquisition efficiency. Considering the complexity of this objective, we pooled our resources in order to phenotype nearly a thousand sorghum genotypes for phosphorus acquisition parameters which have been genotyped by DNA sequencing (GBS) to identify genes in sorghum for P acquisition efficiency.

Background: One of the major mechanisms that plants have evolved to overcome low P availability is to maximize the ability of the roots to absorb P from the soil. P starved plants mobilize P through the exudation of organic acids, acid phosphatases and ribonucleases, resulting in enhanced P uptake (Hinsinger, 2001; Ryan et al., 2001; Dakora and Phillips, 2002; Hammond and White, 2008; Ma et al., 2009; Pang et al., 2009). Another strategy to cope with low P availability is to increase soil foraged area further by forming mycorrhizal symbiosis (Li et al., 2012; Smith and Smith, 2012; Rai et al., 2013). Due to low P mobility on tropical soils, changes in root architecture and morphology enhance P uptake by facilitating soil exploration (Williamson et al., 2001; Ho et al., 2005; Walk et al., 2006; Svistoonoff et al., 2007; Ingram et al., 2012; Niu et al., 2013). Root structural changes leading to higher P uptake include increased root hair growth (Yan et al., 2004; Haling et al., 2013; Lan et al., 2013) and length, and fostering lateral root growth over primary root growth (Williamson et al., 2001; Wang et al., 2013). In addition, larger root surface area is achieved by a combination of reduced mean root diameter and enhanced elongation of relatively thinner roots (Fitter et al., 2002). There is both intra- and interspecific genetic variation for P deficiency tolerance in crop species (Mudge et al., 2002; Paszkowski et al., 2002; Rausch and Bucher, 2002; Huang et al., 2011; Zhang et al., 2011) that can be explored to develop P efficient cultivars.

In rice, *phosphorus uptake 1* (*Pup1*), a major quantitative trait locus (QTL) for P deficiency tolerance donated by an *aus*-type Indian variety, Kasalath, was mapped to the long arm of chromosome 12 (Ni et al., 1998; Wissuwa et al., 1998; Wissuwa et al., 2002; Heuer et al., 2009). Near-isogenic lines (NILs) bearing the Kasalath allele at *Pup1* showed three-fold higher P uptake and grain yield in low P trials compared to the recurrent parent, Nipponbare, which is intolerant to P starvation (Wissuwa and Ae, 2001). Following high resolution mapping of *Pup1*, comparative sequence analyses of homologous Bacterial Artificial Chromosomes (BAC) showed that the Kasalath fragment contained several genes not present in Nipponbare, highlighting a ~90 Kb deletion in the Nipponbare reference genome that encompassed the *Pup1* locus (Heuer et al., 2009). Within this indel, *OsPupK46-2,* a gene encoding a serine/threonine kinase of the LRK10L-2 subfamily, was found to enhance grain yield and P uptake in rice lines overexpressing this gene, indicating that this protein kinase underlies the *Pup1* locus (Gamuyao et al., 2012). *OsPupK46-2*, which is now designated *phosphorus-starvation tolerance 1* (*OsPSTOL1*), was found to be upregulated in the root tissues of tolerant NILs under P-deficient conditions and was shown to increase P uptake by a physiological mechanism based on enhancement of early root growth and development. Furthermore, lines overexpressing *OsPupK46-2* showed a ~30% grain yield increase in comparison to the null lines, suggesting that *PSTOL1* has potential for molecular breeding applications to improve crop performance under low P conditions. Consistent with the proposed physiological mechanism underlying *OsPSTOL1*, the superior performance of the transgenic lines was related to enhanced root dry weight, root length and root

Results: About 60% of the genes in the sorghum genome are located in syntenic regions to rice (Paterson et al., 2009), emphasizing the potential for using comparative genomics for cross-species identification of genes underlying abiotic stress tolerance in the grass family. In this study, we undertook a comparative genomics strategy followed by association analysis to study the role of sorghum homologs of rice *PSTOL1* in tolerance to P starvation in sorghum. SNPs within *PSTOL1* homologs in sorghum, designated *SbPSTOL1*, were significantly associated with grain yield under low P conditions, and also root morphology and architecture traits in hydroponics. Data from different association panels indicated that *SbPSTOL1* increased biomass accumulation and P content in Africa in addition to grain yield under low P in Brazil, suggesting a stable effect which can be potentially used for molecular breeding applications. QTL mapping with a large sorghum recombinant inbred line population (400 RILs) was used to validate the association results, indicating that *SbPSTOL1* homologs colocalize with QTLs related to root morphology and performance under low P. Our results indicate that *SbPSTOL1* homologs have the ability to enhance P uptake and sorghum performance in low-P soils by a mechanism related not only to early root growth enhancement as was the case for rice *OsPSTOL1*, but also by modulating root system architecture.

These results were achieved through the efforts of several projects at Embrapa and collaborating institutions (Generation Challenge Programme projects G7010.03.06 and G7010.03.03, Embrapa Macroprogram among others) in addition to this SweetFuel project. The results of these combined efforts have been submitted for publication to Plant Physiology by the corresponding author from Embrapa, Dr. Jurandir V. Magalhães with the title “Duplicate and conquer: multiple homologs of *phosphorus-starvation tolerance 1* enhance phosphorus acquisition and sorghum performance in low-P soils. As a result of this collaborative research effort, we far exceeded our original goals; we actually identified genes for P acquisition efficiency that can be used in a molecular breeding program and not just a few genotypes of sweet sorghum. In a follow-on stage of this research, we will use the 275 member RIL population for presence of *SbPSTOL1* homologs, that was phenotyped (225 entries) for production and quality for two years in a dark red latosol (Al toxicity and low P in the subsoil) for two years and genotyped by sequencing (GBS) for validating this research development and in selecting superior genotypes in our breeding program.