Gene allelic diversity in the genomic region of the Stay-Green QTL-2 in sorghum

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Drought is the major constraint in Sorghum production worldwide. Sorghum stay green trait is an important secondary trait participating in yield improvement under terminal water deficit. Numerous QTL mapping studies have identified several QTLs for stay-green. We report here the bioanalysis of the stay green QTL-2 region based on synteny between rice chromosome 1 and sorghum chromosome 3 and assessment of the allelic diversity of chosen genes located under this QTL.

Step 1: Stay-Green QTL synthesis and projection on rice physical map.

A single Stay green QTL consensus map gathering the information of several QTL studies available in sorghum (Table 1) was constructed.
The map data and QTL positions were uploaded in a database. After marker and locus name curation, the QTL positions were projected on the Klein-2004 (Menz et al. 2002) map which shares the maximum number of common markers with individual QTL maps and is well anchored with the rice genome. QTL projections were performed using the MapDB software.
The resulting consensus QTL map was projected on rice TIGR V3 sequence map to identify the corresponding genomic regions.

Table1 : QTL studies involved in Stay-green consensus map
Crasta et al. 1999
Tao et al. 2000
Xu et al. 2000
Kebede et al. 2001
Haussmann et al. 2002
Sanchez et al. 2002

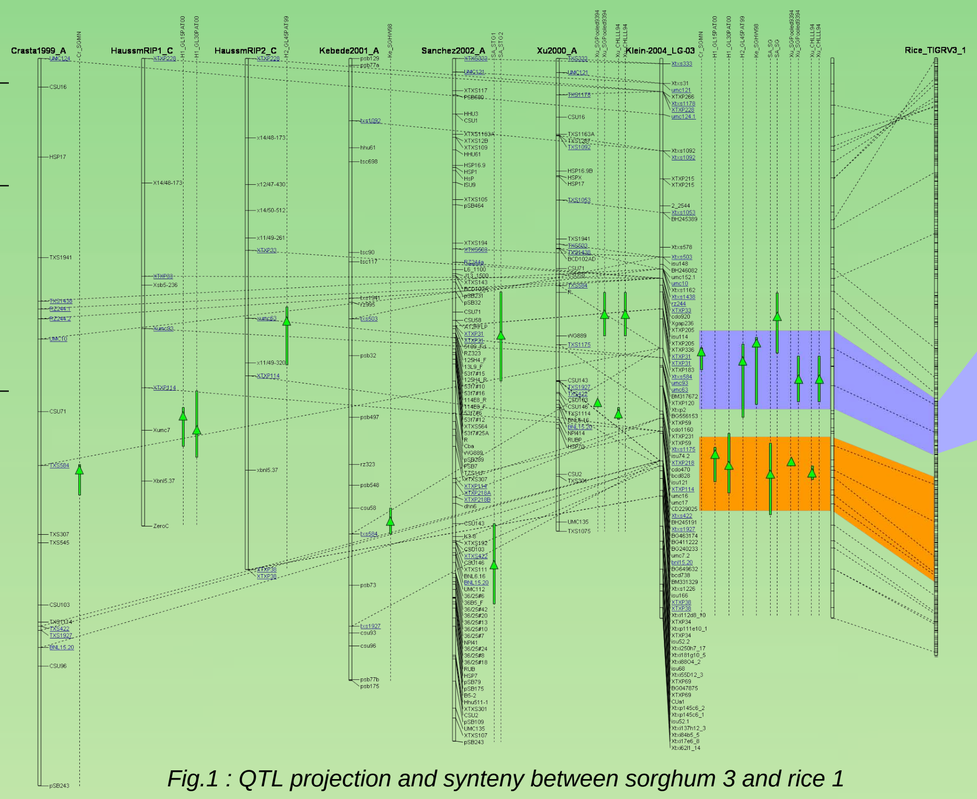
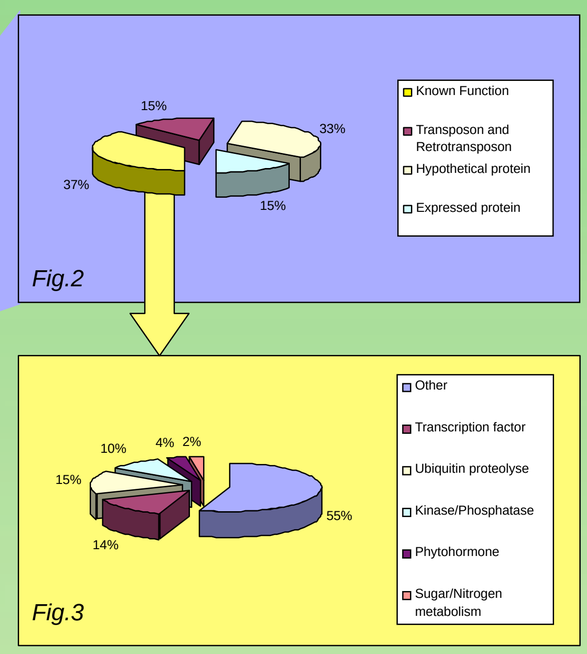


Fig.1 : QTL projection and synteny between sorghum 3 and rice 1

Step 2: Bioanalysis of the rice genomic region corresponding to the sorghum QTL-2

The QTL-2 region of sorghum corresponds to a 3.5Mb region on rice chromosome 1 TIGR V3. This region contains about 579 genes distributed as described in Figure 2.
A short functional classification of these genes shows groups with function related to transcriptional modification (transcription factor), ubiquitin proteolysis and autophagy, stress signal transduction (kinase, phosphatase), phytohormone biosynthesis and signaling, Krebs cycle and nitrogen remobilization,… (Figure 3).
An initial set of 10 genes was chosen: 5 transcription factors (2 Myb related protein, 2 WRKY related protein and Rav2-like protein), 2 genes involved in phytohormone action (an auxine efflux carrier and a cytokinine synthase), 1 gene involved in autophagy (beclin-1 like) and 1 in nitrogen remobilization (GOGAT).



Step 3: Sorghum orthologous genes amplification, genetic or *in silico* mapping

Two to six primers were designed per gene based on the Orion Genomics methyl filtered sequence or the Pratt's EST cluster sequence. A graphical outline of the expected amplified fragment was generated by SIM4 software. Both conserved (supposed to be exon) and non conserved (supposed to be intron or 5' and 3' UTR) regions between rice and sorghum were amplified (Fig 4).
To confirm the location of the genes in the sorghum QTL region, Bulk Segregant Analysis (BSA) mapping in three RILs population was performed (Fig 5). When no polymorphism was observed by BSA, mapping evidence of physical linkage between the gene and the closer syntenic marker was obtained *in silico*.

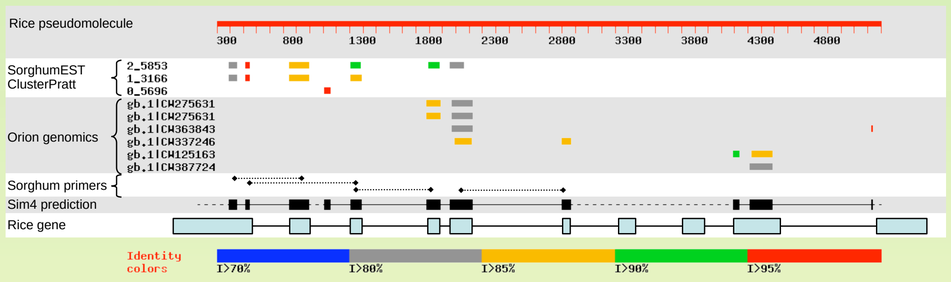


Fig.4 : Sim4 output and sorghum primers design for the beclin-1 gene

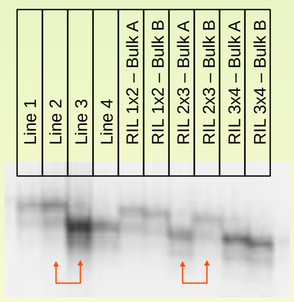


Fig.5 : Polymorphism and BSA mapping of Myb4-like gene amplification product in sorghum

Step 4: Ecotilling of selected genes in a panel of 64 sorghum varieties

Allelic diversity of the RAV-2 like gene and the auxine efflux carrier gene was assessed by EcoTilling on a panel of 64 sorghum accessions. These accessions were chosen to represent the global diversity observed in a working core collection of 205 accessions included in the 700 accessions genotyped in SP1. SSM249, a west African guinea sorghum, was chosen as reference DNA. 7 et 4 haplotypes were found for the RAV-2 like gene and auxine efflux carrier gene, respectively. The different haplotypes for the RAV-2 like gene are shown in Figure 6. The distribution of the different haplotypes among the NJ tree constructed with the 205 accessions based on RFLP data (Deu et al. *in press*) is represented on Figure 7.

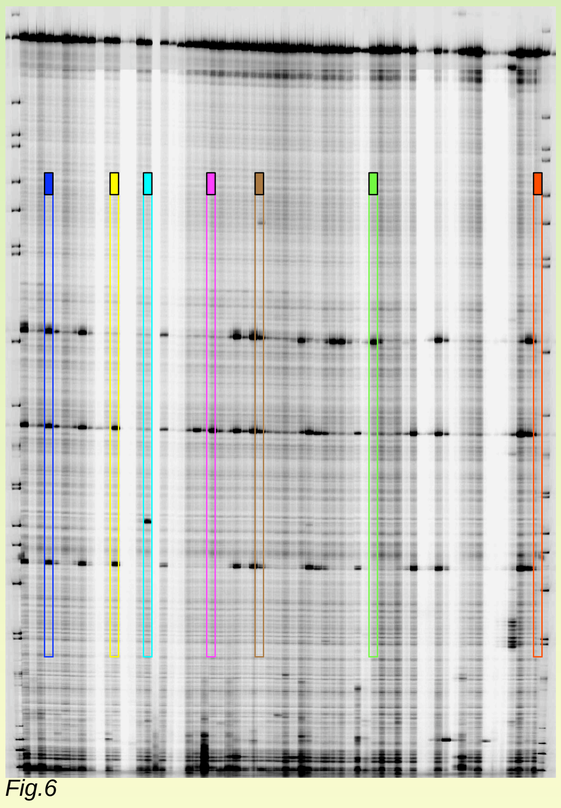


Fig.6

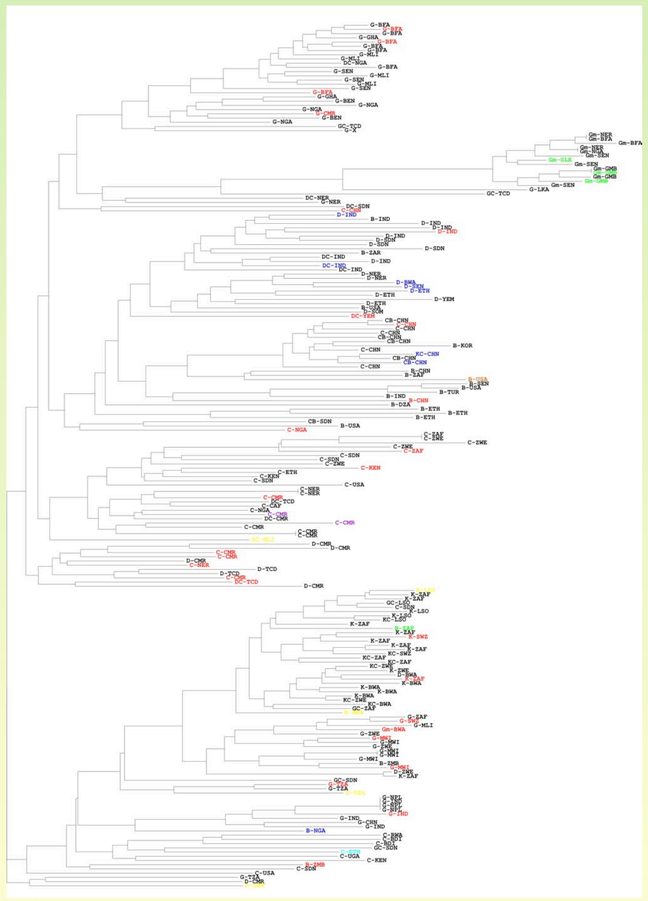


Fig.7

This work is a preliminary stage in the way towards studying the extent of local linkage disequilibrium that can be observed in the neighborhood of target genes under the stay green QTLs, and undertaking association studies. In this perspective, more gene based markers will be developed and mapped. Ecotilling will be extended to the core collection and pilot association studies will be performed on an adapted set of genotyped accessions.

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