

Quantitative trait loci mapping at Cirad for the sorghum improvement

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Abstract — Techniques in the use of molecular genetic markers have been developed to evaluate the genetic resources and understand the organisation and expression of the sorghum genome. At Cirad, Dna (Rflp) markers has been used to establish one genetic linkage map in sorghum. This map provides an opportunity to identify and map genes contributing to yield components and grain quality. The detection of Qtls was undertaken using recombinant inbred lines derived from a cross between a guinea landrace and a caudatum landrace. The results have enabled two major and independent genomic regions contributing to yield components and grain quality to be located. Strong Qtls associations have been observed i) between the weight and number of seeds per panicle, the weight of one thousand seeds, the panicle form and the germinative ability, and ii) between the amylose content, the grain vitreousness, the grain hardness, and one locus coding for the absence of a testa in the grain. This preliminary study did not permit explanation of the observed antagonism between grain productivity and grain quality in sorghum breeding. However, it should be continued. Already, it has opened new paths for thinking in sorghum breeding programs.

Résumé — Identification de locus intervenant dans l'expression de caractères quantitatifs au Cirad pour l'amélioration du sorgho. Des techniques de marquage moléculaire ont été développées pour évaluer les ressources génétiques et comprendre l'organisation et l'expression du génome du sorgho. Au Cirad, des marqueurs de l'Adn (Rflp) ont ainsi été utilisés pour établir une carte génétique du sorgho. Cette carte permet d'identifier et de cartographier des locus intervenant dans l'expression de la qualité technologique du grain et des composantes du rendement. La recherche de Qtl (Quantitative trait loci) a été réalisée à partir d'une population de lignées recombinantes issue d'un croisement guinea X caudatum. Les résultats ont permis de localiser deux zones génomiques majeures et indépendantes pour les composantes de la productivité et de la qualité chez le sorgho. Des associations fortes de Qtl ont été observées entre (i) le poids et le nombre de grains par panicule, le poids de mille grains, la forme de la panicule et la faculté germinative et (ii) la teneur en amylose du grain, la vitrosité du grain, la

dureté du grain et un locus codant pour l'absence d'une couche brune dans le grain. Cette étude préliminaire n'a pas permis d'expliquer d'une façon simple l'antagonisme observé en sélection entre les composantes de la productivité et de la qualité des grains. Cependant elle devrait se poursuivre par de nouvelles expérimentations. D'ores et déjà, elle a ouvert de nouvelles pistes de réflexion quant aux programmes d'amélioration du sorgho.

Sorghum (*Sorghum bicolor* L. Moench) is staple diet of people living in much of the semi-arid tropics in Africa and Asia. In terms of annual global production in Africa, sorghum was the second most important cereal crop in 1995 (Fao, 1995).

Cirad (Centre de coopération internationale en recherche agronomique pour le développement) has conducted cooperative research since the 1960's to evaluate the germplasm and to increase yield potential of cultivated ecotypes in western Africa.

However sorghum production is subject to various biotic and abiotic stresses, and acceptable grain quality is essential for traditional food like the thick porridge or 'tô' in western Africa. Sorghum breeding programmes at Cirad have never completely been successful in combining productivity, quality and adaptation. This is probably due to the polygenic nature of these traits.

The development of Dna markers and saturated genetic maps has enabled genes contributing to complex traits to be mapped using quantitative trait locus (Qtl) analysis (Paterson *et al.*, 1988). In sorghum several studies have permitted the dissection of complex polygenic traits into simple Mendelian factors and a better understanding of genetic corre-

lations between associated traits (Pereira and Lee, 1995 ; Paterson *et al.*, 1995 ; Lin *et al.*, 1995).

The present report describes a part of the Qtl mapping programme conducted at Cirad to (i) identify the map location and genetic effect of Qtls associated with grain quality and productivity in sorghum; and (ii) study the genetic basis for the negative phenotypic correlations found between these two important traits.

Qtl analysis

The sorghum mapping population consisted of 110 $F_{5,6}$ recombinant inbred lines (Ril). It was developed at Inera (Institut d'études et de recherches agricoles) in Burkina Faso and was developed from an intra specific cross between ecotypes Is 2807 and Ssm 379, a caudatum from Zimbabwe and a guinea from Republic of South Africa, respectively. A sorghum linkage map was constructed for this population with heterologous maize and sugarcane probes. The protocol for the mapping and the resulting Rflp map (restriction fragment length polymorphism) are described in Dufour *et al.* (1996 a and b).

The F_6 families were evaluated in 1993 at the Saria breeding station (Inera, Burkina Faso) for yield compo-

nent traits (number and the weight of seed per panicle, weight of one thousand seeds). The primary components of 'tô' quality as defined by Fliedel (1994) were evaluated at the Cirad laboratory of cereals technology, (grain sucrose and protein content, and a physical measurement of the grain hardness related to the decortication). The endosperm texture (visual estimation of the grain hardness), the germination percent and panicle length and density were also mesured.

We used single factor analysis of variance (Edwards *et al.*, 1987) in this investigation, for the detection of Qtls. Thus for each locus a regression analysis was done with the F_6 variable mean and the classes were the two genotypes scores. The threshold level of significance for a Qtl was a probability of $< 0.5\%$. The resulting significant F values (Qtl detection) were plotted against the positions of the loci on the Rflp map. A two factor analysis of variance with a F-tests at 0.01% level of significance was used to test for possible digenic epistasis among Qtls identified in the first stage. Figure 1 shows the Qtl-markers associated with grain and panicle traits across all the sorghum linkage groups. Putative Qtls were located to the markers in that region with the highest R^2 values, R^2 values represent the percentage of the total variation accounted for by each marker, vertical lines represent those markers that are significantly associated with each trait.

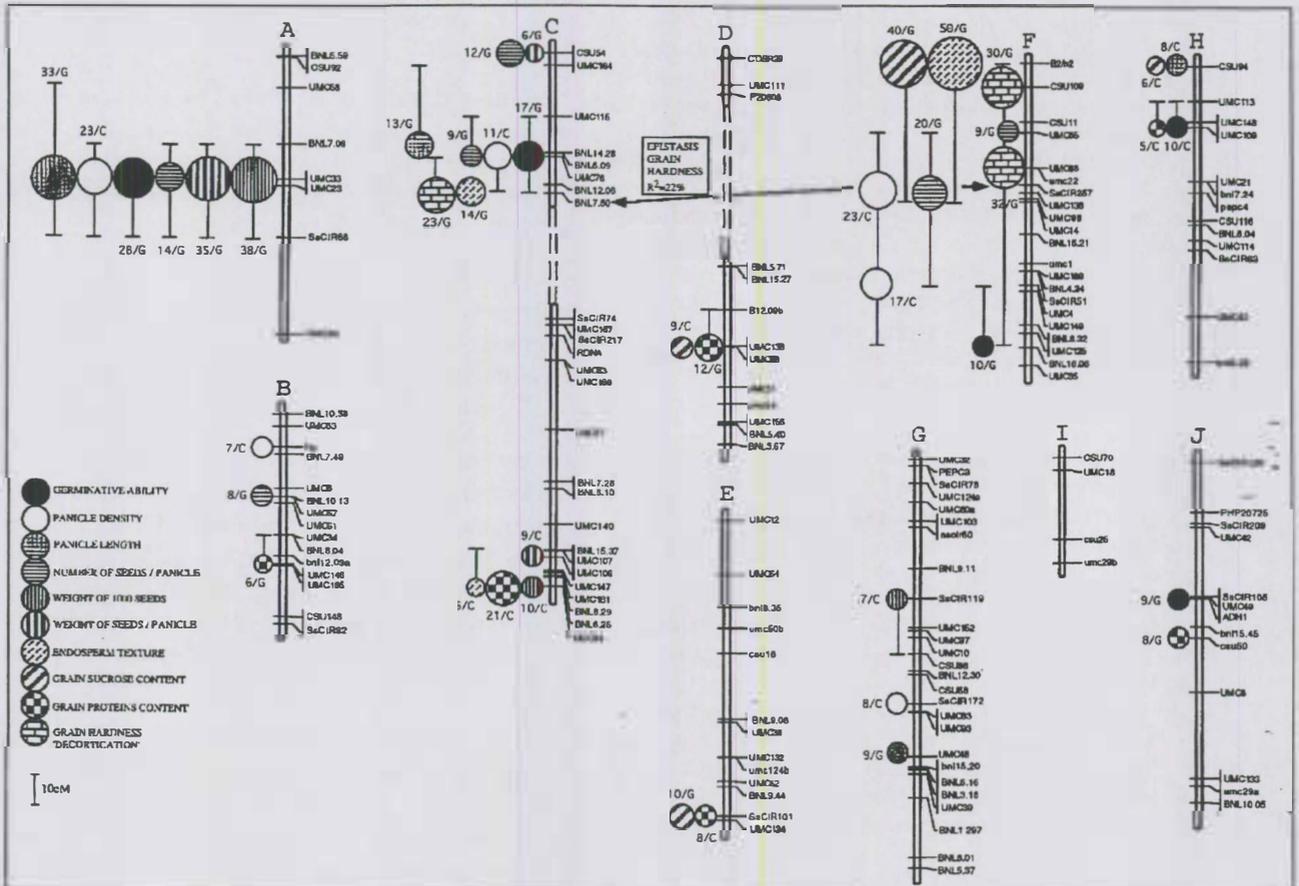


Figure 1. Qtls mapped.

Qtls mapped

In this population, single factor analysis of variance identified four main genomic regions in linkage groups A, C and F with significant effects on the studied traits.

In the linkage group A, the loci Umc 23 and Umc 33 have a strong association with the yield components, the germinative ability and the panicle form. In the study of Pereira and Lee (1995), this location corresponds to a Qtl with major effects on several traits (tiller number, stem diameter, panicle dimensions, number of seeds-branches and plant height). Qtl studies in different species and/or populations strongly indicate that a relatively few genes account for very large portions of phenotypic variation, with additional genes accounting for progressively smaller portions of variance (Lande and Thompson, 1990). Thus in sorghum, this Qtl could correspond to a single key dwarfing gene with pleiotropic effects, dw_3 (Schertz, 1973). Comparative genome analysis indicates that this major sorghum Qtl may correspond to orthologous dwarfing mutant genes in maize, br_1 and/or an_1 (Beavis *et al.*, 1991).

The yield components, the germinative ability and the panicle form are also associated in the linkage group C close to the segment delimited by Umc 115 and Bnl 7.50. In both genomic segments (A and C), the favourable alleles for the increase of yield, good germinative ability and largest panicle, are brought by the guinea parent. It reinforces the idea that these traits are strongly associated in sorghum races and encoded by major genes that are linked or have pleiotropic action, and could be explained partly by the weak recombination observed between them in this guinea X caudatum progeny.

It is possible that difference for germinative ability contributed indirectly to selection of guinea panicle and yield traits during the development of the Rils. Sorghum seeds are sown in hills. The more vigorous plants are more likely to survive and result in indirect selection for the guinea genes.

The genomic segment defined by the loci $B_2.b_2$ and Umc 114 in the linkage group F is particularly important for the eating quality in sorghum. We observed a strong association between the allele b_2 coding for the absence of a testa in the grain (Doggett, 1988) and major Qtls for the grain hardness, the sucrose content and the structure of endosperm. It confirms visual quality defined by breeders (without testa and with a corneous endosperm).

Secondary genomic regions also influence quality. The negative relation generally observed between protein and sucrose contents is shown by two Qtl associations in linkage groups D and E with opposite effects to those predicted by the parental phenotypes.

Concerning Qtls in linkage groups D and H for protein and sucrose content the favourable alleles for the quality came from the caudatum Is 2807. These Qtls may be responsible for the transgressive segregation observed for the eating quality in this progeny. We also found evidence of epistasis between Qtls in genetic variation of grain hardness, mapped in linkage groups C and F. But this interaction should be confirmed because analysis of epistasis is more subject to the problem of false-positive detections than is analysis of individual Qtls.

Comparative genome analysis with maize indicates that the major sorghum Qtl for sucrose content on linkage group F may correspond to orthologous genes in maize, se_1 and/or o_2 (Matz *et al.*, 1994 ; Tadmor *et al.*, 1995). Moreover, the minor Qtl for sucrose content on linkage group H corresponds certainly to the waxy locus, mapped in a homologous location in sorghum by Melake-Berhan *et al.* (1993).

Finally in our study, there is only one significant and negative Qtls association for the grain productivity and quality in the linkage group C between the loci Bnl 15.37 and Umc 84. Consequently it does not help to explain why it is so difficult to obtain favourable recombinants for both traits.

Prospects

This Qtl experiment probably does not reflect the real complexity of quantitative inheritance for the grain productivity and quality in sorghum, but simply has detected a subset of Qtls. Especially in sorghum, where we observed a strong sub-structuring of genetic diversity (Deu *et al.*, 1994, 1995), strict additivity of gene action is unlikely to describe individual effects of Qtls. Dominance (inaccessible in Rils) and epistasis effects should be investigated in new progenies, experimental designs and new environments to understand inheritance of individual genes contributing to these traits at the molecular level.

However, the sorghum Rflp map made at Cirad, will provide the opportunity to accelerate the breeding programmes via a marker-assisted selection (Mas). For example, Mas could enable mapped Qtls for yield from caudatum genetic background to be incorporated into guinea cultivars, obtaining more productive selected lines and maintaining the guinea characteristics of eating quality and adaptation to the cultural environment of western Africa. In practical sorghum breeding programmes, it would be helpful to have Pcr markers (polymerase chain reaction) that are technically simple and rapid, by a conversion of mapped Rflp markers into Sts markers (sequence tagged sites) and/or the development of microsatellites markers (Brown *et al.*, 1996).

The mapping of Rflp heterologous probes has demonstrated that gene content and order (synteny) is highly conserved between genomes of grass species (Moore *et al.*, 1995). Indeed, sorghum and foxtail millet maps show extreme similarity with rice (Paterson *et al.*, 1995 ; Devos pers. Com.). Like in the present report, synteny has made it possible to compare locations of genes and Qtls affecting common phenotypes between reproductively isolated species. Synteny will increase our knowledge on physiological and biochemical pathways and lays the foundation for new strategies for gene and genome studies in sorghum. Comparative Qtl mapping for the grain quality between maize and sorghum will continue at Cirad (J.F. Rami, Ph.D).

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References

BEAVIS W.D., GRAND D., 1991. A linkage map based on information from for F2 populations of maize (*Zea mays* L.). *Theor. Appl. Genet.* 82 : 636-644.

BROWN S.M., HOPKINS M.S., MITCHELL S.E. SENIOR M.L., WANG T.Y., DUNCAN R.R. GONZALES-CANDELAS F., KRESOVICH S., 1996. Multiple methos for the identification of polymorphic simple sequence repeats (SSRs) in sorghum (*Sorghum bicolor* (L.) Moench), *Theor. Appl. Genet.*, 93 : 190-198.

DEU M., GONZALEZ-DE-LEON D., GLASZMANN J.C., DEGREMONT I., CHANTEREAU J., LANAUD C., HAMON P., 1994. RFLP diversity in cultivated sorghum in relation to racial differentiation. *Theor. Appl. Genet.* 88 : 838-844.

DEU M., HAMON P., CHANTEREAU J., DUFOUR P., D'HONT A., LANAUD C., 1995. Mitochondrial DNA diversity in cultivated sorghum. *Genome* 38 : 635-645.

DOGGET H., 1998. Sorghum. JOHN WILEY and sons, Inc., New-York, NY, USA.

DUFOUR P., 1996a. Cartographie moléculaire du génome du sorgho (*Sorghum bicolor* L. Moench) : Application en sélection variétale ; cartographie comparée chez les andropognées. Thèse. Université Paris-Sud. Paris 11 Orsay, 103 p.

DUFOUR P., GRIVET L., D'HONT A., DEU M., TROUCHE G., GLASZMANN J.C., HAMON P.,

1996b. Comparative genetic mapping between duplicated segments on maize chromosomes 3 and 8 and homoeologous regions in sorghum and sugarcane. *Theor. Appl. Genet.* 92 : 1024-1030.

EDOUARDS M.D., STUBER C.W., WENDEL J.F., 1987. Molecular marker-facilitated investigations of quantitative-traits loci in maize. I. Numbers, genomic distribution and types of gene action. *Genetics* 116 : 113-125.

FLIEDEL G., 1994. Evaluation de la qualité du sorgho pour la fabrication du tô. *Agriculture et développement* 4 : 12-21.

LANDE, RUSSELL, R. THOMPSON, 1990. Efficiency of marker-assisted selection in the improvement of quantitative traits, *Genetics*, 124 : 743-756.

LIN Y.R., SCHERTZ K.F., PATERSON A.H., 1995. Comparative analysis of QTLs affecting plant height and maturity across the Poaceae, in reference to an interspecific sorghum population. *Genetics* 141 : 391-411.

MATZ E.C., BURR F.A., BURR B., 1994. The BNL Map. *Maize Genetics Cooperation News Letter.* 68 : 198-208.

MELAKE-BERHAN A., HULBERT S.H., BULTER L.G., BENNETZEN J.L., 1993. Structure and evolution of the genomes of *Sorghum Bicolor* and *Zea mays*. *Theor. Appl. Genet.* 86 : 598-604.

MOORE G., DEVOS K.M., WANG Z., GALE M.D., 1995a. Grasses, line up and form a circle. *Curr. Biol.* 5 (7) : 737-739.

PATERSON A.H. LANDER E.S., HEWITT J.D. PETERSON S., LINCOLN S.E., TANKSLEY S.D., 1988. Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. *Nature* 335 : 721-726.

PATERSON A.H., LIN Y.R., LI Z., Schertz K.F., DOEBLEY J.F., PINSON S.R.M., LIU S.C., Stansel J.W., IRVINE J.E., 1995. Convergent domestication of cereal crops by independent mutations at corresponding genetic loci. *Science* 269 : 1714-1718.

PEREIRA M.G., LEE M., 1995. Identification of genomic regions affecting plant height in sorghum and maize. *Theor. Appl. Genet.* 90 : 380-388.

SCHERTZ K.F., 1973. Single Height-Gene Effects in Hybrids of doubled haploid *Sorghum bicolor* (L.) Moench. *Crop Science*, 13 : 421-423.

TANDMOR Y., AZANZA F., HAN T., ROCHEFORD T.R., JUVIK J.A., 1995. RFLP mapping of the sugary enhancer 1 gene in maize. *Theor. Appl. Genet.*, 91 : 489-494.