

## Use of molecular markers in the sorghum breeding program at CIRAD

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### Introduction

The sorghum breeding program at CIRAD (ex IRAT) started in the 1960s. Initially, it was mostly based on mass selection among West African landraces, predominantly *guinea* forms which present adaptative traits to humid climate (lateness, photoperiod sensitivity, loose panicle,...). However, breeders could only succeed in limited progress because of the restricted variability exploited. This led to turn the breeding program on the use of crosses between local varieties and introduced elite materials, especially *caudatum* and *kafir* varieties from USA. It appeared difficult to obtain varieties combining both the productivity of exotic materials and the grain quality and adaptation traits of local ecotypes. The varieties showing a good agronomic performance were mostly susceptible to grain molds and various pests and diseases. Furthermore, they didn't present an acceptable grain quality. These observations got breeders to question about the existence of unfavorable genetic linkages which could explain the limited recombinations encountered in the progenies of *guinea* x *caudatum* crosses. They led to the recent works on the genetic diversity within cultivated sorghums, with a particular insight on the *guinea* race, and the QTL identification for grain quality, productivity, and adaptative traits like photoperiod sensitivity.

### Evaluation of genetic diversity

In sorghum (*Sorghum bicolor* ssp. *bicolor*), traditional cultivars were classified by Harlan and De Wet (1972) into five main races (*bicolor*, *caudatum*, *durra*, *guinea*, *kafir*) and 10

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intermediates (e.g. *bicolor-caudatum*, *durra-kafir*), mainly on the basis of spikelet and grain morphology.

A first study, using 25 agro-morphological traits (Chantereau et al., 1989), and performed on a representative sample of 157 traditional landraces, distinguished three groups with different cropping performances: 1) one corresponding to the *durra* race, hardy, and adapted to dry zones; 2) another which included two sub-groups corresponding to the *guinea* and *bicolor* races, hardy and adapted to wet zones; 3) and a third gathering the *kafir* and *caudatum* cultivars, high yielding, adapted to intermediate zones. This organization is in line with the racial classification, but it is not as discriminating.

Isozyme diversity provided a new insight into genetic diversity, showing a marked geographical structure. The 347 varieties, analyzed with 18 polymorphic loci that generated a total of 46 alleles, tended to cluster into a western African group, a southern African group and a central-eastern African group rather than into racial clusters (Ollitrault et al., 1989). An analysis, focused on the *guinea* race, whose grain characters are essential in West Africa, showed that this race can be split into three groups corresponding to sub-race *margaritifera*, whatever the geographic origin, to western African forms and to southern African forms, respectively (Dégremont, 1992).

RFLP markers provide the access to a potentially unlimited number of loci. They allow a thorough analysis of the organization of genetic diversity within cultivated sorghums. An RFLP analysis was performed on a sample of 94 accessions selected on the basis of their geographical origin and their racial classification (Deu et al., 1994). Thirty-five maize probes, well scattered over the maize genome, were used, each with at least one restriction enzyme. This gave 50 polymorphic probe-enzyme combinations which yielded 158 polymorphic individual bands. RFLPs offered a slightly different perception of the species genetic organization and inter-race relationships. The race *bicolor* appeared highly variable and included many rare markers. Its representatives do not form a specific group but they are scattered among the various clusters; some of them are strongly differentiated from the rest of the species. Race *guinea* appears with RFLPs as it appeared with isozymes, i.e. it is divided into three sub-groups, corresponding to the *margaritifera* subrace, the western African forms and the southern African forms. *Caudatum*, *durra* (mainly sampled from central-eastern Africa and Asia) and *kafir* accessions clustered into one group each.

As compared to isozymes, RFLPs highlighted the relationships between molecular variation and racial differentiation.

The congruence between the morphological classification and the classification based on

molecular markers raises the question of the evolution mechanisms affecting each type of characters. Morphological traits are obviously affected by human selection whereas molecular markers are most likely neutral. Initially, these correlations probably arose from the founder effect associated with domestication, followed by genetic drift. The persistence of such a correlation requires a restricted recombination between the genetic factors underlying these traits. This restriction can be due to the limited gene exchanges between races maintained by asynchronous flowering, ecological specialization, cultural practices or a contrasting microgeographic pattern related to ethnical preferences. Restricted recombination can also be the consequence of genetic linkage.

Analyses of mitochondrial DNA polymorphism were also undertaken to obtain further information on the relationships between and within wild and cultivated sorghum and to study the genetic origin of *guinea margaritifera* (Deu et al., 1995). This study confirmed the specificity of *guinea margaritifera* and demonstrated the presence of two genetic entities in this subrace. Furthermore, the diversity observed in cultivated forms was found to be encompassed within the wild pool, except for one of the two *guinea margaritifera* groups.

Traits and markers used in these studies have highlighted various structures and have provided fresh data that have helped in clarifying the genetic organization of the species *S. bicolor* and in increasing the efficiency of breeding efforts.

The differences observed in the genetic diversity structurations revealed by two types of markers, isozymes and nuclear RFLPs, may be due to a difference in the number of loci targeted, their genomic map localization or their specificity. The non concordance between the two structurations does not seem to be derived from a greater allelic richness for RFLP markers (Deu et al., in press). We are now engaged in a program for testing the validity of the above hypotheses. A novel investigation of the sorghum diversity is being performed with a greater number of RFLP probes, well scattered over the sorghum genome, and based on a more extended sample of cultivated sorghum accessions.

To reduce costs of genetic resources conservation and to improve their utilization, core collections, including maximum genetic diversity and best representing existing variation, must be developed. Different strategies of core collections constitution, such as random procedure and sampling in groups revealed by multivariate analysis, are being applied to the world collection of sorghum maintained at ICRISAT (Grenier et al, submitted). Subsets of accessions obtained with these methods were also screened with microsatellites in order to compare the allelic diversity retained in each subset. Data available for molecular markers (microsatellites and RFLPs) and agro-morphological traits are being used, in collaboration with ICRISAT and IRD, to test the efficiency of various strategies for sampling genetic

diversity and for constituting core collections.

Crosses between *guinea* accessions and accessions from other races often lead to a poor genetic complementarity with few promising progenies. Furthermore, crosses between local *guinea* landraces from West Africa produce a restricted variability. These observations, combined with specific characteristics of the *guinea* race, such as tallness, photoperiod-sensitivity, and high rate of cross pollinisation have limited the use of this race in breeding programs. The identification of three *guinea* sub-groups revealed by markers is promising regarding crosses management.

### **Quantitative trait loci mapping for grain quality and productivity**

In Africa, most sorghum breeding programs have been focused on agronomic performance in order to insure food security. However, grain quality is also an essential requirement for the development and the use of improved cultivars. Many quality criteria can be considered regarding the wide range of culinary dishes prepared with sorghum grains. This multiplicity of uses and the difficulty of designing rapid simple methods to evaluate complex parameters have delayed the development of improved cultivars with acceptable grain quality. Furthermore, it appears difficult to combine both, the productivity found in the *caudatum* race, and the grain quality, characteristic of the *guinea* race.

We developed a set of laboratory tests to predict the processing quality of sorghum varieties for tô, a thick porridge traditionally consumed in West Africa. The relationships between grain physico-chemical parameters and tô quality (firmness and good conservation capacity) have been established. Varieties with high amylose content, high starch solubility and good dehulling properties give a good tô quality (Fliedel, 1995). The dehulling behaviour of sorghum grains depends on grain hardness and vitreousness (Fliedel et al., 1989).

We have undertaken a QTL mapping experiment to better understand the genetic control of grain quality parameters and their relationships with the main components of sorghum productivity. The grain quality data and the RFLP diversity analysis have provided a basis for selecting parents for relevant crosses.

Two populations of recombinant inbred lines (RILs) were obtained by single seed descent from *IS 2807* (a genotype from the ICRISAT collection) used as female and *249 (IS 7680)* and *379 (IS 3833)* from the CIRAD collection, used as males. They were developed at the Saria experimental station (Burkina Faso) by INERA and CIRAD. The two populations comprised 90 and 110 individuals, respectively, and were called RIL249 and RIL379. *IS 2807* is a *caudatum* landrace from Zimbabwe with a medium productivity under Saria ecological

conditions and poor technological characteristics for West African traditional meal preparations. 249 is a *guinea* landrace from Burkina Faso, belonging to the West African *guinea* group as revealed by RFLP markers, 379 is a *guinea* landrace originated from Senegal but belonging to the South African *guinea* group (Chanterreau et al., 1994). 249 has good technological properties. 379 has a poorer quality and a lower productivity than 249.

Population RIL379 was grown in 1993 at the F5 generation, in a Fisher block experimental design with 3 replications. Population RIL 249 was grown in 1995 at the F7 generation, in a 9x10 lattice design with 3 replications.

Morphological traits were monitored in the field before harvest, recording plant height (PH), panicle compactness (PC) evaluated on a scale of 1 (loose) to 4 (compact) and panicle length (PL).

Yield components such as the number of kernels per panicle (NK), the kernel weight per panicle (KW), the thousand kernel weight (TK) and the threshing percent (TP, weight of grain as a percentage of total panicle weight) were measured after harvest.

For technological trait evaluation, several panicles of each RIL were bulked to represent the current generation. Flouriness (FL) was assessed by visual observation of the proportion of vitreous and floury endosperm on grain cross-sections. Grain samples were scored on a 1 to 5 scale. Dehulling yield (DY) was measured using a tangential abrasive dehulling device. Dehulling yield is the percentage of dehulled grain to whole grain weight.

For the RIL379 population, amylose content (AM) was measured by differential scanning calorimetry (Mestres et al., 1996). Protein content (PR) was evaluated by total nitrogen determination using the Kjeldahl method. For the RIL249 population, grain technological characteristics were evaluated by Near Infrared Reflectance Spectroscopy (NIRS). Kernel friability (KF) was determined by NIRS calibration based on the particle size index method (PSI) (Fliedel et al., 1989). PSI was determined as the proportion of milled grain passing through the sieve. It is inversely proportional to grain hardness. Kernel hardness (KH) was also estimated by the method of American Association of Cereal Chemists (1989).

Three additional traits were also recorded, the germination rate (GR), measured as the percentage of germinated grains in a petri dish and the mold sensitivity evaluated directly on threshed grains after harvest (MR), and the mold sensitivity after 6 days of germination in a petri dish (MG) as the percentage of molded grains.

The map of RIL249 was built using 115 RFLP maize probes, 8 sugarcane probes, 4 cloned

genes (ADH1, PEPC3, PEPC4 and PTA71) and 1 morphological marker (B2/b2) covering a total map distance of 878 cM. The map of RIL379 was constructed using 126 maize probes, 19 sugarcane probes, 4 cloned genes (ADH1, PEPC3, PEPC4 and PTA71), and 2 morphological markers (P/p, B2/b2) for a total map distance of 977 cM (Dufour et al., 1997). These maps correspond to about 90% of the saturated map of Pereira et al. (1994), and represent a large part of the sorghum genome. The average genetic distances between markers are 8 and 7 cM, respectively, for RIL249 and RIL379. Comparisons between these maps and those of Pereira et al. and Lin et al. (1995) show that linkage group K is a part of linkage group C (Dufour, 1996).

QTL detection was performed for the two populations using adjusted means for the three replications. A first analysis was conducted by simple interval mapping (SIM). The threshold for QTL declaration was fixed to a LOD value of 2.6 for RIL379 and 2.7 for RIL249. These values were determined by the permutation method described by Churchill and Doerge (1994). A second analysis was performed using composite interval mapping (CIM). CIM cofactors were chosen as the nearest marker of each QTL detected with SIM. CIM scans were performed with the same LOD threshold value as SIM. No CIM analysis was performed when no QTL was detected with SIM.

A major chromosomal region involved in yield component, germinative ability and morphological traits was found on the linkage group (LG) A for the RIL379 population (Figure1). On this LG, 4 QTLs with marked effects on germination rate, number of kernels/panicle, kernel weight/panicle and thousand kernel/weight were detected in association with 3 QTLs for plant height, panicle length and panicle compactness. Yield components, germinative ability, plant height and panicle length are also associated in the LG C for RIL379 while productivity traits and plant height/ panicle length are linked in the LG F for RIL249. In these genomic segments (A, C and F), the *guinea* alleles resulted in a high plant, producing good germinative ability, with a loose panicle, many large grains and thus good productivity. These results indicate that morphological traits, grain productivity and germinative ability are controlled by linked genes or genes with pleiotropic effects.

A chromosomal segment located on LG F was found to play a major role in grain quality (Rami et al., 1998). For RIL379, four QTLs for flouriness, dehulling yield, amylose content and mold resistance during germination were detected, very closely linked with each other. On the same linkage group, four important QTLs were detected on RIL249 for flouriness, kernel friability, kernel hardness and amylose content. These results are consistent with the close correlation found between amylose content and endosperm texture. The B2/b2 gene controlling the presence of a high-tannin testa layer in the grain has been phenotypically mapped in this region. This explains the visual quality criteria used by breeders (a quality

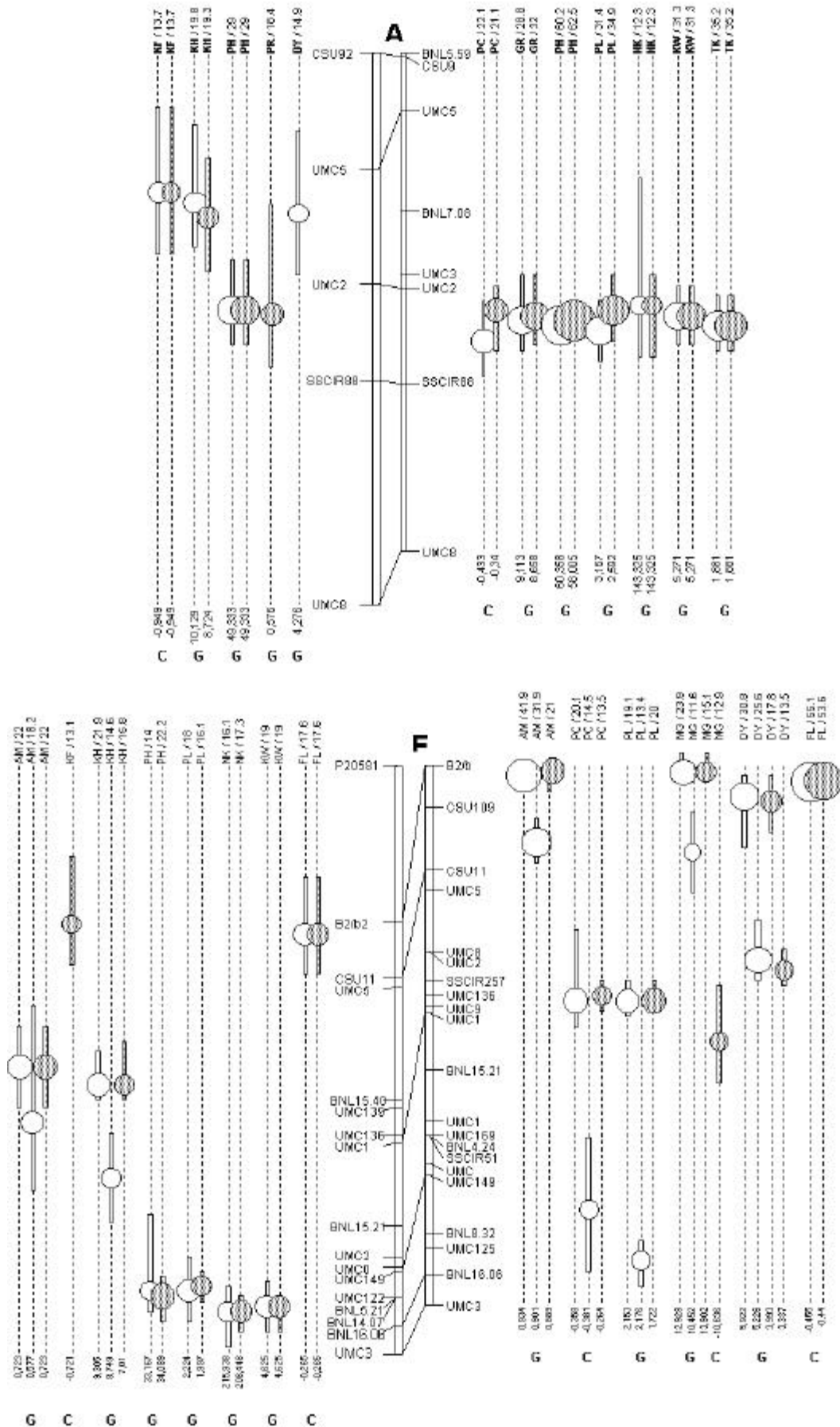
grain has a vitreous and hard endosperm and has no testa).

Other genomic regions are involved in grain quality, QTLs for kernel friability, kernel hardness, dehulling yield and protein content were detected on LG A for RIL249. This is consistent with the close correlations found between these traits; the *guinea* allele conferred a higher mechanical resistance to the kernel. Co-localizations of QTLs for protein content and kernel physical properties were also found in other segments of the genome : for example, QTLs for protein content and flouriness had a same map position on LG C.

Results obtained in both populations show that major QTLs for amylose content, dehulling yield, and kernel texture are not linked to productivity traits, while they are co-located with major QTLs for the mold resistance during germination and the tannin content. This means therefore that there is no genetic obstacle for recombination of genetic components of both productivity and grain quality in *caudatum* x *guinea* crosses.

The co-localizations of QTLs involved in protein content and grain hardness and vitreousness suggested the involvement of certain storage grain proteins in kernel physical properties. Further analyses focusing on different grain proteins families revealed that a great number of QTLs are involved in the regulation of the quantity of storage proteins in the grain, especially albumins and prolamins and that several QTLs for albumin quantity have a same map position as QTLs for grain hardness, flouriness and dehulling yield (Rami, 1999).

**Figure 1.** (Next page). QTLs detected on genetic linkage groups A and F for RIL249 (left) and RIL379 (right) populations. Traits names are given in the text. Each QTL is represented with a *circle* located on the LOD peak and with a *box* representing the confidence interval. *White* circles were QTLs detected with SIM and *grey* circles were QTLs detected with CIM. The *upper number* is the percentage of phenotypic variance explained by the QTL and the *lower number* is the additivity effect. *G* or *C* at the bottom of each QTL represents the parental origin (*guinea* or *caudatum*) of positive QTL effect.



## Quantitative trait loci mapping for photoperiod response in tropical sorghum

Daylength sensitivity in sorghum, originally classified as a short day species, has been systematically eliminated by breeders in order to enlarge the range of adaptability and extend the crop area to temperate environments. Nevertheless, photoperiod sensitivity remains an important trait for adaptation of African landraces to the weather resources of the environment. This weather resources is characterized by a greater degree of uncertainty of the occurrence of a rainfall event to commence than to end the rainy season. Photoperiod sensitivity appears a key feature matching flowering time to length of the rainy season. Moreover, in any one locality, photoperiodism, through timing, adjust flowering time to a short period. Parasites as midges have no time to build up. Short flowering period at the end of the rainy season when humidity declines also penalizes grain molds. Photoperiod sensitivity in West Africa plays an important role to secure the level and the quality of harvests (Curtis, 1968; Vaksman et al., 1996). Consequently, we think that a better understanding of the genetic bases will facilitate the transfer of photoperiod sensitivity from local to high yielding varieties developed for African farmers.

Results of classical genetic studies suggest that flowering is largely controlled by four genetic locus, *Ma1*, *Ma2*, *Ma3* and *Ma4*. (Quinby, 1973). Among them, *Ma1* is considered the most important. Recently, Aydin et al. (1997) showed that two other maturity loci designated *Ma5* and *Ma6* may also be involved in floral initiation of ultralate sorghum genotypes in the USA. Since the recent advent of molecular markers in sorghum genetics, QTL mapping for flowering time has already been documented. In this, using RFLP markers, Lin et al. (1995) identified one QTL located on linkage group (LG) B of Peirera et al. (1994) and assigned it to the *Ma1* gene. Childs et al (1997) mapped gene *Ma3* on LG C of Pereira et al (1994).

Photoperiod response in cereals has been shown to be determined by two main components : the basic vegetative phase (BVP) defined as the shortest possible time for floral initiation when the plants are not responsive to changes in photoperiod and the photoperiod sensitivity (PS) which expresses the varietal linear increase to flowering time as plants respond to daylength changes (Major, 1980). Similar phenotypes in flowering time can be produced by different combinations of BVP and PS. Consequently, flowering time can not be directly used to characterize each line for genetic studies of photoperiod response.

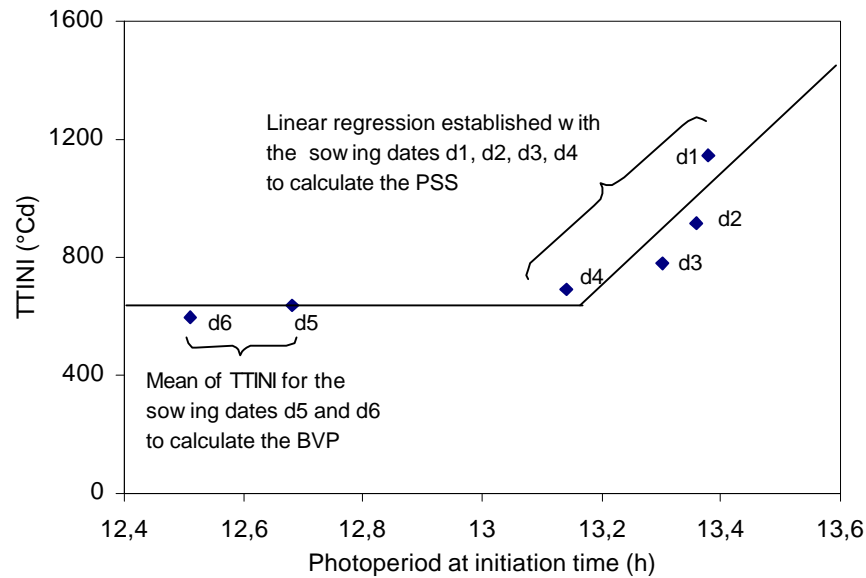
Several approaches can be implemented to investigate BVP and PS in sorghum. One of the less disturbing method is based on the observation of the plant development at different sowing dates in the field in a given natural location. To estimate the components of the photoperiod response of a given genotype, the duration of the vegetative phase is measured for each sowing date. These measures can either be used directly or they can feed a model

such as these based on the concepts developed by Major (1980) (Vaksmann et al., 1998).

Population RIL249 was used to perform the QTL mapping study of photoperiod response. For three years (1995-1997), the 85 RIL lines and their progenitors were sown at different dates during the rainy season at the Saria station, Burkina Faso (2° 09'W, 12° 16'N, altitude 300m). Sowing dates were 10 June 1997, 21 June 1995, 5 July 97, 21 July 1995, 4 September 1997, 22 September 1996. At each date, lines were arranged in a randomized complete-block design. Plot consisted of a single 5,1 m long row.

For each sowing date and each line, the number of leaves and the date of flag leaf emergence at the main stem of five random plants were scored two or three times a week from thinning. In each experiment, these data led to characterize genotypes for averaged durations of the vegetative phase from sowing to flag leave emergence. This was established in Vegetative Calendar Time (VCT, expressed in days), in Vegetative Biological Time (VBT, expressed in number of leaves), and in Vegetative Thermal Time (VTT, expressed in degree-days, °Cd ).

The varietal photoperiod response was also investigated following the CERES sorghum model using the concepts of Major (1980) (Alagarswamy and Ritchie, 1991). For each sowing date and each line, the vegetative thermal time from sowing to flag leaf emergence, VTT, was used to established the thermal time from sowing to panicle initiation, TTINI. For each line, TTINI for the six sowing dates was plotted against daylength at estimated panicle initiation date. This permitted to estimate the two main components of the photoperiod response according to Major (1980), BVP and PSS. BVP or intrinsic earliness was estimated as the averaged TTINI for the two September sowing dates. PSS characterizes the photoperiod sensitivity *sensus stricto*. It was estimated as the linear regression coefficient computed for the points related to the sowing dates of June and July. The traits are graphically illustrated by Figure 2.



**Figure 2.** Description of the components of photoperiod response according to Major (1980). From left to right, the spots corresponding to the sowing dates are in inverse order of their calendar position. Data from *IS7680*, one of the two parents of the RIL249 population, were used to establish the figure.

High correlations are observed between measures derived from the CERES model and direct measures of the photoperiod response. BVP is highly correlated with the measures of the vegetative phase length for the sowing date 4 September,  $VCT_5$ ,  $VBT_5$  and  $VTT_5$ . PSS is highly correlated with the variations of the vegetative phase between the first and fourth sowing dates (namely June 10 and July 21),  $\Delta VCT_{1-4}$  and  $\Delta VTT_{1-4}$ . According to these results,  $VCT_5$ ,  $VBT_5$  and  $VTT_5$  are related to the intrinsic earliness and  $\Delta VCT_{1-4}$  and  $\Delta VTT_{1-4}$  are related to the photoperiod sensitivity *sensus stricto*.

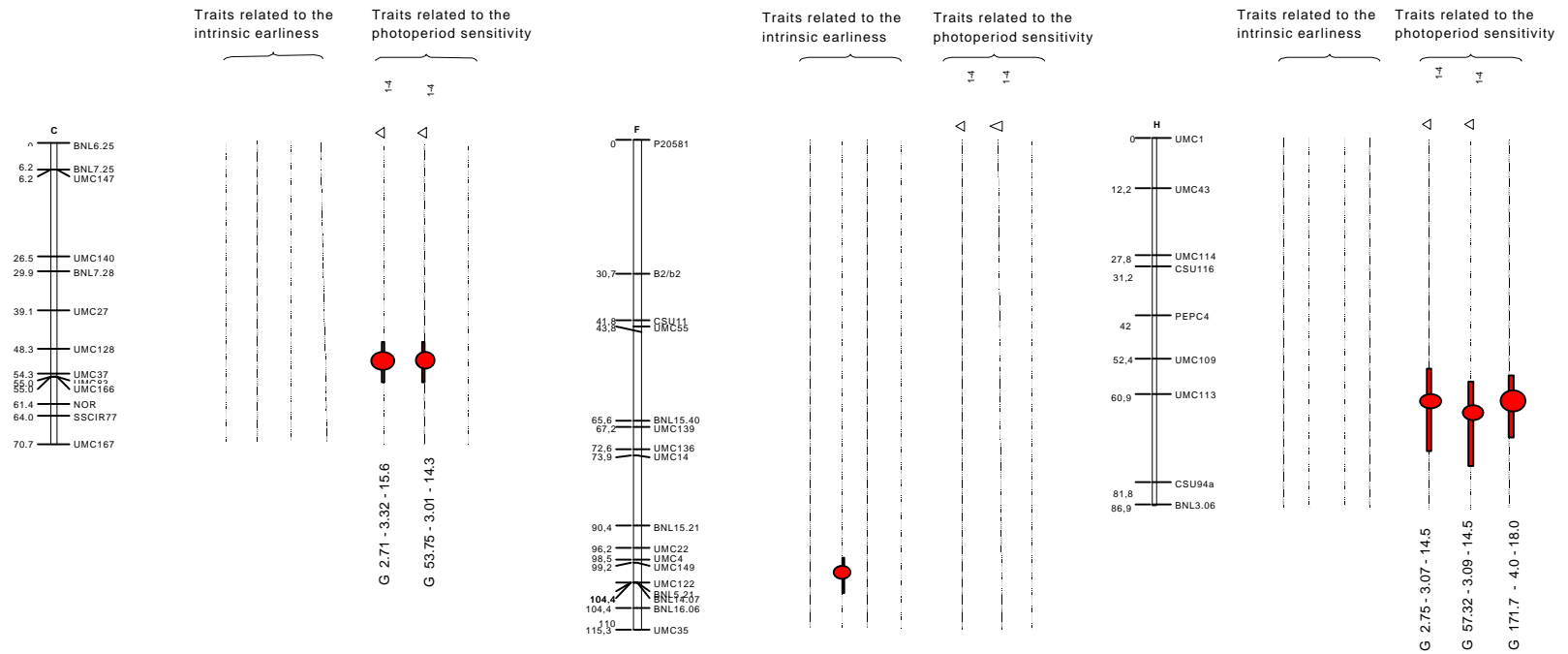
QTL detection was performed as described by Rami et al (1998). QTLs were detected on linkage groups (LG) C, F, and H, respectively (Trouche et al., 1998) (Figure 3). The contribution of QTLs to phenotypic variance ranges from 13.6 to 18.0 %. One QTL was detected for  $VBT_5$  and located on LG F. Two QTLs were detected for  $\Delta VCT_{1-4}$  and  $\Delta VTT_{1-4}$  on LGs C and H and had the same map position for both traits. On LG H, position was also congruent with a QTL detected for PSS with a major effect (18% of the phenotypic variance).

QTL detection was mainly successful for traits accounting for the photoperiod sensitivity *sensus stricto* (PSS,  $\Delta VCT_{1-4}$ ,  $\Delta VTT_{1-4}$ ). QTLs were located on LGs H and C. Among the traits accounting for intrinsic earliness (BVP,  $VCT_5$ ,  $VBT_5$ ,  $VTT_5$ ), only one QTL was detected for  $VBT_5$ . In this case, the small size of the RIL249 population could explain the

difficulty to detect QTLs with low effect. Laurie (1997) already noted in wheat that the map location of the genes implicated in earliness per se was poorly defined and that their effects were small.

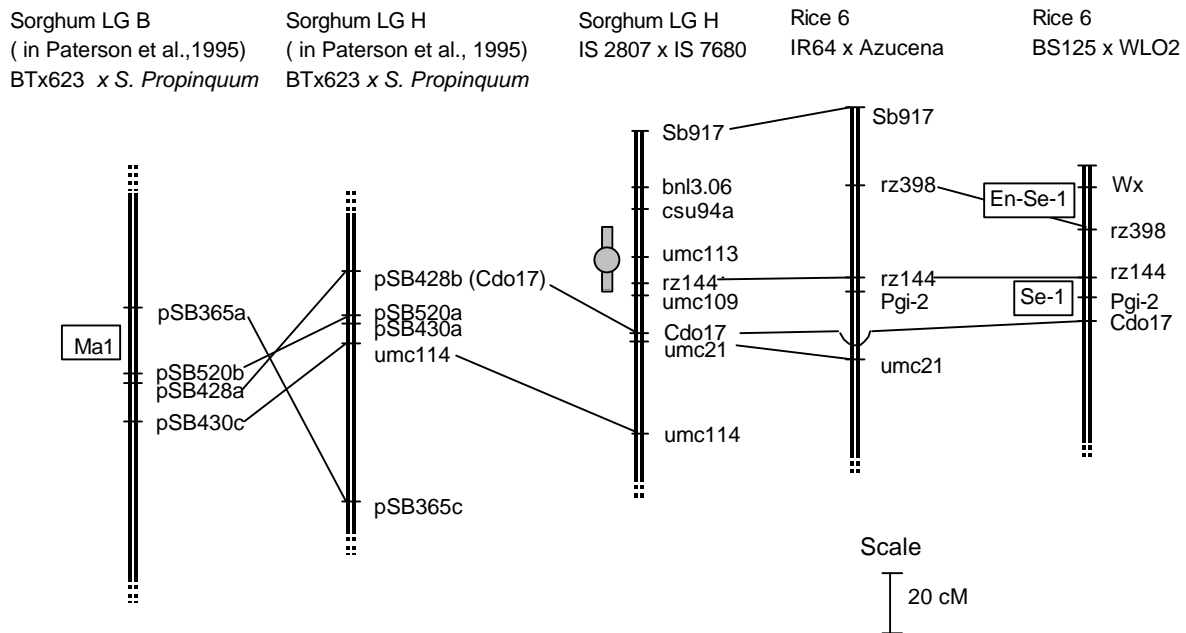
A same region on LG H carries QTLs for traits implied in photoperiod sensitivity *sensus stricto* (PSS,  $\Delta VCT_{1-4}$ ,  $\Delta VTT_{1-4}$ ). These QTLs probably tag a key region for photoperiod response in tropical sorghum. Paterson et al. (1995) established that several RFLP markers of LG B, located near *Mal*, were duplicated on LG H (Figure 4). This highlights a complex duplication of chromosome segments since the orders of markers and map distances are not conserved. *Mal* may have been involved in this duplication and give a paralogous gene on LG H that may correspond to the QTL detected on this LG in our study. It may also correspond to *Ma5* or *Ma6* that Aydin et al. (1997) have recently assumed to strongly inhibit floral initiation of some tropical genotypes in the USA.

The comparison of map location of QTLs and major genes for photoperiod response can also be extended to rice. The QTL region on sorghum LG H aligns with a region of rice chromosome 6 in the vicinity of probe *RZ144* and gene *Pgi-2* (Figure 4). *Pgi-2* is tightly linked to *Se-1*, a major photoperiod sensitivity gene (Mackill et al., 1993; Causse et al., 1994). This suggests that the QTL on sorghum LG H may be orthologous to *Se-1*.



**Figure 3.** QTLs detected for photoperiod response in RIL249 population. Each QTL whose LODscore is above the significant threshold is represented with a circle at the LODscore peak position. The line represents the one-LOD score confidence interval. From bottom to top, are given the additivity effect, the LODscore peak value and the percentage of phenotypic variance explained by the QTL. Letters G or C indicate the parental origin (C for caudatum IS 2807 and G for guinea IS 7680) of the allele increasing the value of the trait.





**Figure 4.** Comparative map position of the major genes and QTLs implied in flowering time or photoperiod sensitivity in sorghum and rice and located on LG H. For the map of sorghum RIL249 population, information from Boivin et al. (1999) has been added. Information for sorghum cross *BTx623 (Sorghum bicolor) x Sorghum propinquum* is taken from Lin et al. (1995) and Paterson et al. (1995). Information for rice cross *BS 125 (Oryza sativa) x WL02 (Oryza longistaminata)* is taken from Causse et al. 1994. Some unpublished information has also been used for the map of rice cross *IR64 x Azucena*.

The photoperiod response in tropical sorghum was here mainly investigated through two components : intrinsic earliness and photoperiod sensitivity. QTLs for these two components were mapped and this contributed to clarify relationships with the maturity genes. Further investigations with lines of various origins will be necessary to fully depict genetic control of photoperiod response in sorghum. In the future, markers may help transferring photoperiod sensitivity to elite cultivars for a better adaptation to tropical environments.

#### Mapping head bug resistance genes

The mirid panicle-feeding bugs, particularly *Eurystylus oldi* Poppius have recently become

key-pests of sorghum in West Africa. Feeding and oviposition of these bugs result in severe quantitative and qualitative losses, particularly on improved compact-headed cultivars. These pests are therefore a major threat to the increase of sorghum production through the extension of improved cultivars, which, although better yielding, are more susceptible to head bug damage than local loose-panicked guinea landraces (Ratnadass et al., 1998).

The availability of a reliable screening technique made it possible to identify sources of resistance to head bugs, and particularly to confirm high and stable resistance in compact-panicked sorghum cultivar *Malisor 84-7*. The major factor associated with this resistance seems to be a quicker endosperm hardening pattern in this cultivar, resulting in a shorter period during which head bugs can feed and lay their eggs in the maturing grains. Studies on the genetics of this resistance showed that it did not involve maternal effect, that it was highly heritable, and mainly under additive gene action. Using pedigree breeding selection, it has been possible to transfer head bug resistance from crosses between *Malisor 84-7* and high yielding cultivars and several advanced progenies which combine reasonable head bug tolerance and acceptable agronomic traits were obtained (Ratnadass et al., 1998).

The identification of useful molecular markers linked to head bug resistance genes should have a major impact on sorghum breeding across the West African region. A QTL mapping project aimed at identifying *Malisor 84-7* resistance genes was undertaken by CIRAD in Mali and France. It is based on a F<sub>2</sub>/F<sub>3</sub> progeny derived from a *Malisor 84-7* (resistant) x *S34* (susceptible) cross.

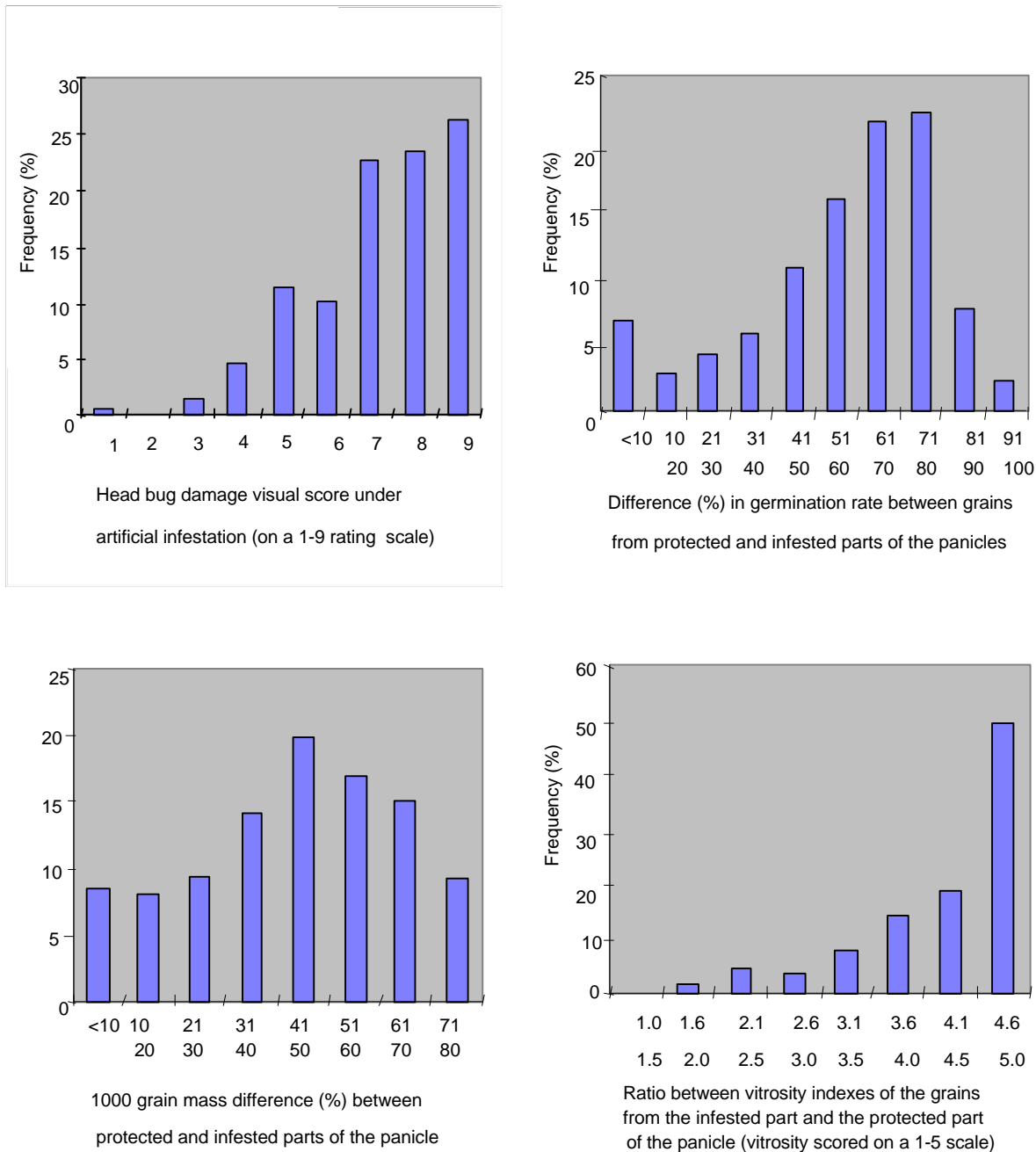
The F<sub>2</sub> evaluation trial was planted at Samanko (ICRISAT/CIRAD research station near Bamako, Mali) on 23 July 1997 in a plot consisting of ten 6-m rows, with an inter-row spacing of 0.75 m. In order to avoid selection, it was planted in continuous lines, and thinned 2 weeks after planting, so as to have an inter-plant spacing of 0.20 m, with one plant per hill. The F<sub>2</sub> plot was bordered with one row of each of the parents each side.

The head cage technique (Sharma et al., 1992) was slightly modified so as to allow the evaluation of individual panicles from F<sub>2</sub> and parental plants, for different parameters affected by head bugs. Just before the onset of anthesis, all sorghum heads were covered with paper pollination bags, which were then removed at the end of flowering, and replaced with infestation wire cages and blue muslin bags, so as to separate each panicle into two parts, an upper part, which was infested with 10 pairs of head bug adults, and a bottom part, which served as a protected control.

Cages were removed after 3 weeks, and the upper parts of the panicles were scored for head bug damage at grain maturity using a 1-9 visual rating scale where 1 = all grains fully

developed, of which less than 10% showing a few head bug feeding punctures and 9 = > 75% grains remaining undeveloped and barely visible outside the glumes. Each part of the panicle was then carefully threshed separately, and the following parameters measured on the grains: 1000 grain mass; grain vitosity (using a 1-5 scale) and germination rate.

More than 200 F<sub>2</sub> plants and 5 plants of each of the parents could be evaluated for each of the parameters. Good segregation for resistance was present in the cross, as reflected in Figure 5.



**Figure 5.** Frequency distribution of F<sub>2</sub> plants derived from a *Malisor 84-7* x *S34* cross for 4 characters measuring head bug attack.

As a first step to the construction of the genetic map, RFLP probes, selected according to their localization on our reference genetic map (Boivin et al., 1999), were screened for their ability to detect polymorphism between the two parental lines, *Malisor 84-7* and *S34*. The first probes were tested in combination with 5 restriction enzymes, *Bam*HI, *Dra*I, *Eco*RI, *Eco*RV and *Hind*III. Then, an additional restriction enzyme (*Sst*I) was chosen to improve the rate of polymorphism. Simultaneously, F3 seeds derived from the 210 F2 plants already characterized in the field at Samanko, were planted in a greenhouse. DNA was extracted from a bulk of leaves harvested on 5 to 7 F3 plants. DNA was then restricted, separately, with each of the six enzymes used for screening polymorphism. The level of polymorphism among the parents was about 20%, which is very low. Actually, 269 RFLP probes have been screened for polymorphism and 62 of them were polymorphic and have been scored on the whole progeny.

More RFLP markers, recently added on our reference map, remain to be screened for polymorphism between the two parental lines *Malisor 84-7* and *S34*.

Because of the low polymorphism rate that has been revealed until now, some linkage groups will be likely unequally and poorly covered by RFLP markers. Available microsatellites markers (Brown et al., 1996; Taramino et al., 1997) are being tested for their ability to detect polymorphism between the two parents. Among the 49 microsatellites screened actually, in agarose gels, 16 have detected polymorphism. The number of polymorphic microsatellites should be improved with the use of polyacrylamid gels, which enable a better resolution. Five microsatellites have been scored on the whole progeny.

We should have before long, sufficient number of markers, with adequate scattering on the genome, to construct the genetic map and perform the identification of QTLs involved in head bug resistance.

### **Synteny within the Poaceae**

Sorghum belongs to the Andropogoneae tribe of the Poaceae family, as two other important crops, maize and sugarcane. The genome colinearity with those two crops has been clearly established. Sorghum has the same basic chromosome number as maize,  $n = 10$ , but has a very different chromosomal organization. Map comparisons between the two crops reflect the complex duplicated nature of the maize genome, probably due to an ancient amphidiploid origin followed by numerous subsequent rearrangements (Hulbert et al., 1990; Whitkus et al., 1992; Melake-Berhan et al., 1993; Dufour et al., 1996; Glaszmann et al., 1997). Sugarcane cultivars are highly polyploid, aneuploid, interspecific hybrids. The genome colinearity is

however very well conserved with sorghum as it has been established in our laboratory based on more than 100 common mapped loci (Dufour et al., 1997 and unpublished results). Regarding genome organization, sorghum appears as the most simple crop of the Andropogoneae tribe. It could serve as a bridge for comparison with the other Poaceae species, especially rice which genome sequencing will be achieved in the next few years.

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