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ORIGINAL PAPER

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Use of trial clustering to study QTL × environment effects for grain yield and related traits in maize

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Abstract A population of 300 $F_{3:4}$ lines derived from the cross between maize inbred lines F2 and F252 was evaluated for testcross value in a large range of environmental conditions (11 different locations in 2 years: 1995 and 1996) in order to study (1) the magnitude of genotype × environment and (2) the stability of quantitative trait loci (QTL) effects. Several agronomic traits were measured: dry grain yield (DGY), kernel weight, average number of kernels per plant, silking date (SD) and grain moisture at harvest. A large genotype × environment interaction was found, particularly for DGY. A hierarchical classification of trials and an additive main effects and multiplicative interaction (AMMI) model were carried out. Both methods led to the conclusion that trials could be partitioned into three groups consistent with (1) the year of experiment and (2) the water availability (irrigated vs non-irrigated) for the trials sown in 1995. QTL detection was carried out for all the traits in the different groups of trials. Between 9 and 15 QTL were detected for each trait. QTL × group and QTL × trial effects were tested and proved significant for a large proportion of QTL. QTL detection was also performed on coordinates on the first two principal components (PC) of the AMMI model. PC QTL were generally detected in areas where QTL × group and QTL × trial interactions were significant. A region located on chromosome 8 near an SD QTL seemed to play a key role in DGY stability. Our results confirm the key role of water availability and flowering earliness on grain yield stability in maize.

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Introduction

Yield in maize is known to be a complex trait, the expression of which highly depends on environmental conditions (biotic and mostly abiotic factors). Some of these limiting factors can be controlled to a large extent by the use of irrigation, fertilizers, pesticides, etc. However, one can observe a general trend towards a reduction of inputs, for economical reasons in some areas and environmental reasons in others. Selecting genotypes with stable performance is therefore increasingly considered as a key objective for maize breeding.

Understanding the genetic basis of genotype × environment (G×E) interaction for grain yield is a major issue to achieve this goal. Many studies have been carried out to quantify and try to understand G×E interaction (see for instance Crossa et al. 1990; Epinat-Le Signor et al. 2001). Statistical models of the interaction have been proposed, such as the regression on environmental mean (Finlay and Wilkinson 1963), additive main effects and multiplicative interaction [(AMMI), Gauch 1992] models or biclassification of genotypes and environments (Corsten and Denis 1990). Compared to a simple analysis of variance (ANOVA), these models allow one to better describe the interaction term with fewer parameters, which facilitates biological interpretation. When external information on genotypes or environments is available, factorial regression (Denis 1988) enables one to go further by determining the factors (genotypic or environmental) that are influencing the interaction. The shortcoming of all these strategies is that the interaction is evaluated across the entire genome, whereas one can expect that only some quantitative trait loci (QTL) are involved in the interaction.

The development of molecular markers in the early eighties allowed one to localise QTL involved in the variation of many traits of economical interest and to estimate their effects. Analysing the stability of these effects across environments is particularly important in the context of marker-assisted selection. Several strate-

gies have been proposed to study QTL × environment interaction. Until recently the most usual strategy consisted of comparing the QTL detected with the same population in several environmental conditions (Stuber et al. 1992; Hayes et al. 1993; Koester et al. 1993; Schön et al. 1994). Nevertheless, it is now well known that, due to sampling problems, different QTL can be detected in different environments, even if there is no interaction (Beavis 1994; Melchinger et al. 1998). More refined strategies have been developed, such as including QTL × environment terms in the QTL detection model (Jansen et al. 1995; Sari-Gorla et al. 1997) or considering multiple environments as multiple traits (Jiang and Zeng 1995). When a large number of environments are considered, these models require the estimation of many parameters, which may decrease detection power and complicates biological interpretation. To avoid such drawbacks, Romagosa et al. (1996) proposed to detect QTL of the principal components (PC) of an AMMI model and applied this strategy to barley. More recently, Korol et al. (2001) proposed a multivariate approach based on the use of multivariate complexes of quantitative traits performed interval per interval which permits to detect QTL without increasing the number of parameters estimated whatever the number of traits (or environments) considered. Other strategies derived from the regression on the mean performance in each environment (Korol et al. 1998) or from the factorial regression (Crossa et al. 1999; Van Eeuwijk et al. 2002) were also proposed. Nevertheless, factorial regression requires additional variables to define environmental or genotypic covariates, which are not always available.

The magnitude of QTL × environment interaction for yield in maize is still poorly known. Many studies underlined an important global G×E interaction for yield. Surprisingly however, QTL detected for yield often showed consistent positions and effects in different environments (Stuber et al. 1992; Ragot et al. 1995; Kraja and Dudley 2000), even if some environment-specific QTL were detected (Agrama et al. 1999; Ribaut et al. 1997; Bertin and Gallais 2001). To study grain yield stability, it is important to analyse simultaneously related traits such as yield components and earliness, which are known to be important factors for environmental adaptation in maize. Indeed, at flowering time, maize is particularly susceptible to abiotic stresses that can have a major negative effect on yield. Flowering precocity is often strongly related with grain yield, especially in the case of drought stress, and coincidences were observed between the QTL detected for these traits (Ribaut et al. 1996, 1997; Veldboom and Lee 1996a, b).

The aim of this study was to detect QTL for yield and related traits (yield components and precocity) in a population evaluated in a wide range of environments (several locations in 2 years) in order to evaluate QTL × environment interactions and thus identify factors influencing yield stability. Compared to other studies, we did not focus on the interaction induced by a specific stress but evaluated the interaction in a large range of environmental conditions. Among the different approaches that have been proposed in the literature to study QTL × environment interaction, we chose to first evaluate the importance of the G×E interaction on yield by an ANOVA and then to partition this interaction, using a classification process of the trials (Corsten and Denis 1990). This allowed us to identify groups of environments, to detect QTL within each group and finally to test the QTL × group interaction. This test was compared to a QTL × trial interaction. We also used the approach based on an AMMI model as was done by Romagosa et al. (1996) on barley that had the advantage compared to other methods proposed in the literature to be easy to implement for our specific population (F_3). The chromosome areas identified by those approaches were later compared. Particular attention was paid to the coincidences between the areas involved in the G×E interaction of yield and the QTL detected for related traits, especially earliness.

Materials and methods

Plant materials and field experiments

A population of 300 F_3 plants was obtained by single-seed descent from the F_1 hybrid between two maize inbred lines: F2, an early European flint inbred line, and F252, an early dent line with US origin. Each F_3 was self-fertilized to obtain an $F_{3:4}$ line. Each line was crossed with an inbred line tester, MBS847, a dent line with US origin but unrelated to F252. These testcross progenies were evaluated in 1995 in 14 trials planted at 11 different locations in northern France (Table 1). Among these trials, three were deliberately conducted so as to generate a stress condition: low nitrate input (trial 10), early sowing to induce low-temperature stress during germination and early growth (trial 11) and no irrigation to induce drought stress (trial 3). In each of these three locations, another trial was conducted in standard conditions to provide a reference to evaluate the effect of stress (trials 4, 9 and 12). These last trials and all the others were conducted following the agricultural practice adapted to the location (with irrigation for trials 2, 4, 5, 6, 7 and 8, without for the others). In 1996, six trials (3b, 4b, 9b, 10b, 11b and 12b) were conducted in the same locations and experimental conditions as trials 3, 4, 9, 10, 11 and 12, respectively. Seed stocks were not sufficient to test testcross progenies of all the $F_{3:4}$ in all the trials. So in a given trial, only a subset of testcross progenies of 280 $F_{3:4}$ lines was evaluated. This subset consisted in testcross progenies of 243 $F_{3:4}$ lines that were grown in all trials and testcross progenies of 37 $F_{3:4}$ lines chosen at random among the remaining testcross of 57 $F_{3:4}$ lines, so that each of these 57 testcross progenies was evaluated in at least three trials. Following recommendations of Knapp and Bridges (1990) and Moreau et al. (2000), each testcross progeny was generally not replicated within a given trial. Nevertheless, in order to evaluate trial accuracy, a

Table 1 Average testcross performance of the $F_{3:4}$ population in the different trials for the traits: grain yield (*DGY*), grain moisture (*GM*), silking date (*SD*), kernel weight (*KW*) and the number of kernels per plant (*NKP*)

Year	Trial	Location (longitude; latitude)	Irrigation	Specific condition	Traits				
					DGY (t/ha)	GM (%)	SD (days)	KW (mg)	NKP
1995	1	−01°40′; 48°06′	No	–	7.31	35.1	24.6	229.8	308.7
	2	00°04′; 47°15′	Yes	–	5.94	24.1	27.1	235.1	272.3
	3	00°07′; 46°26′	No	Drought	1.74	27.9	27.7	179.4	112.9
	4	Idem	Yes	–	8.69	31.0	23.3	244.6	406.7
	5	01°19′; 47°35′	Yes	–	8.72	37.8	–	214.4	480.0
	6	01°20′; 48°04′	Yes	–	9.35	34.6	21.0	–	–
	7	01°39′; 48°24′	Yes	–	10.01	37.5	31.8	250.6	376.9
	8	01°25′; 48°30′	Yes	–	9.46	37.6	34.3	236.8	392.9
	9	02°08′; 48°42′	No	–	8.68	35.6	27.6	236.8	362.1
	10	Idem	No	Low nitrate input	6.84	37.9	31.1	224.3	303.1
	11	02°56′; 49°26′	No	Early sowing	7.13	33.3	24.6	246.5	375.7
	12	Idem	No	–	6.56	30.6	32.2	246.6	336.5
	13	03°18; 48°42′	No	–	6.48	37.7	–	–	–
	14	07°45′; 48°43′	No	–	9.16	34.3	22.8	260.3	407.2
1996	3b	00°07′; 46°26′	No	Drought	3.69	33.2	29.3	231.7	180.2
	4b	Idem	Yes	–	7.60	34.5	25.0	263.9	325.6
	9b	02°08′; 48°42′	No	–	7.93	38.7	29.2	243.7	326.4
	10b	Idem	No	Low nitrate input	7.56	37.6	27.8	225.5	335.2
	11b	02°08′; 49°26′	No	Early sowing	6.04	39.8	32.7	232.0	288.1
	12b	Idem	No	–	6.04	40.1	36.1	203.1	309.1

random subset of testcross progenies (26 or 28, depending on the trial) was replicated twice within a given trial. The single-cross hybrids F2 × MBS847 and F252 × MBS847 (parental lines evaluated in test) were used as checks and replicated 17 or 19 times within each trial. Elementary plots consisted of two seeded rows, spaced 0.8 m apart and, depending on the location, 5–6 m long. More details on the experimental design can be found in Moreau et al. (1999). Each plot was harvested in bulk to evaluate dry grain yield [(DGY) in tons per hectare, at 0% of grain moisture] and grain moisture at harvest [(GM) percentage of the fresh grain weight]. GM at harvest is important from an economical standpoint, since it determines post-harvest drying cost. In all the trials but trials 5 and 13, the silking date (SD) was determined as the date (in days after the first of July) when 50% of the plants of the plot exhibited silks. The kernel weight [(KW) milligrams] was evaluated in all trials but trials 6 and 13 from a sample of 200 kernels. When available, this trait was used to estimate the number of kernels per plant (NKP) as: $NKP = (GW) \times 10^6/(KW \times NP)$, where GW is the dry grain weight harvested (in kilograms) per plot and NP is the number of plants per plot. As this material generally produced a single ear per plant, NKP is approximately equivalent to the number of kernels per ear.

Population genotyping and construction of the linkage-map

Each $F_{3:4}$ line was characterized for RFLP markers. Based on a map previously developed by Causse et al. (1996), 70 RFLP probes were chosen to provide polymorphic molecular markers evenly spread on the chromosomes. $F_{3:4}$ lines were sown, and genomic DNA was extracted from leaves of approximately 20 plants per line. The selected probes were used to detect polymorphism following established procedures (see Causse et al. 1996 for more details). A genetic map was constructed from the marker data set, using MAPMAKER, version 3.0b (Lander et al. 1987), and an LOD threshold of 3.0 in two-point analyses. Recombination frequencies were expressed in map distances using the Haldane mapping function. The genetic map obtained with the RFLP data included 93 loci and had a total length of 1,549 cM (Fig. 1). Several markers showed significant segregation distortions. As segregation distortions may lead to false linkages between markers (Lorieux et al. 1995), the significance of the linkage between markers was checked by χ^2 tests, accounting for the observed frequencies of each genotypic class. Marker orders on each chromosome were checked using the *ripple* option.

Analysis of G×E interactions

For each trait, an ANOVA was performed using the GLM procedure of SAS (SAS Institute 1989), with a model including a trial effect, T, defined as the combination of year, location and condition (stressed or unstressed). This effect was considered as fixed. The genetic effect, g, and the genotype × trial interaction effect, $g \times T$, were considered as random. For the random effects of the model, REML estimates of the variance components were obtained using the VARCOMP procedure of SAS (SAS Institute 1989). Prior to the analysis, the error variance was estimated for each trial, and its homogeneity among trials was checked with a Bartlett's test ($P = 0.05$). Two severely stressed trials (2 and 3b) showed

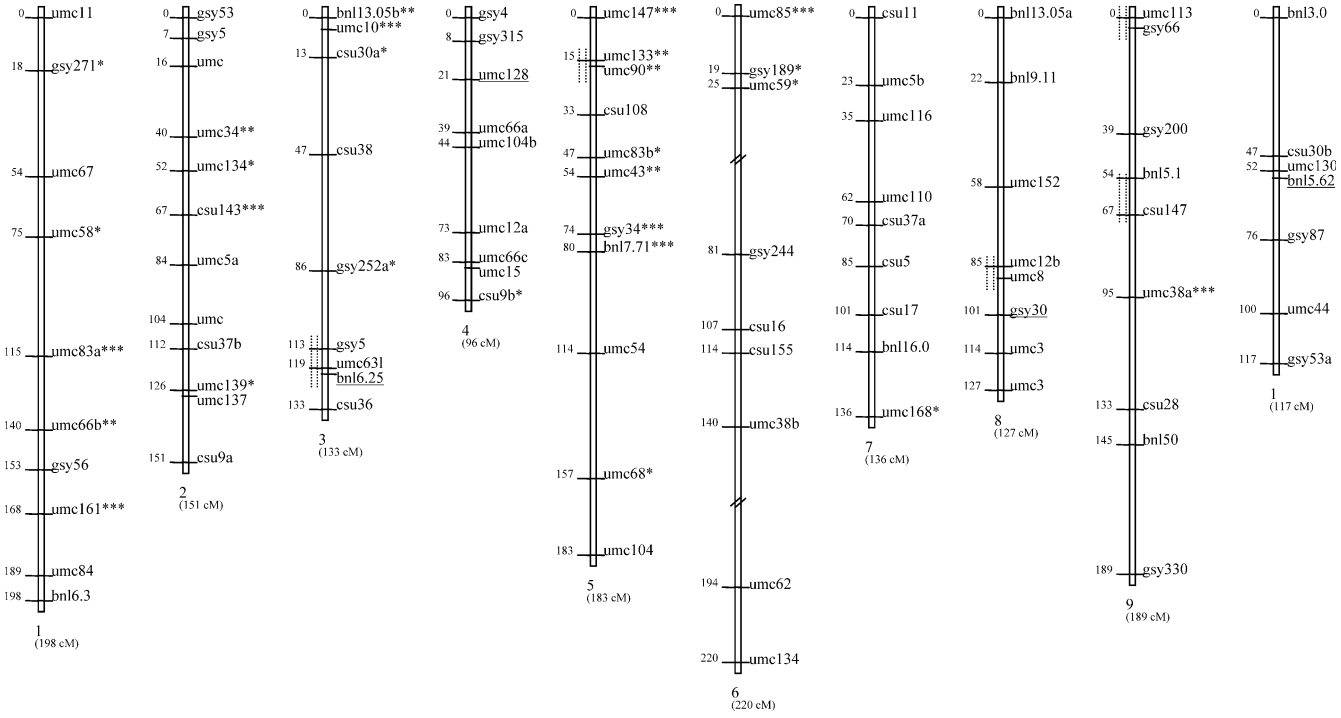

Fig. 1 Linkage map of the F_3 population based on 93 RFLP marker loci. Markers located on chromosomes different from those expected from literature are *underlined*. *Dotted lines* indicate chromosome segments where the marker order is not significant. The total length of each chromosome and the position of the markers were computed using the Haldane's mapping function. The symbols *, ** and *** indicate markers showing segregation distortion significant at the 5, 1 and 0.1% levels, respectively

very high error variances for DGY and were discarded from the global ANOVA. Trial 2 suffered from a severe attack of tassel smut (*Sphacelotheca reiliana*) and bad conditions during sowing. Trial 3b was stressed by the lack of irrigation. Among the 18 remaining trials, trials 3 and 13 also showed significantly higher error variances than the other ones, but to a lesser extent. They were kept in the analysis. Because error variances were not homogeneous across all the trials, probabilities associated with significance tests may not be exact.

The genotype × trial interaction effect was partitioned using a classification on the 'trial' factor. The average testcross performance of the progeny, i, in the trial, t, $Y_{it.}$ was modelled as:

$$Y_{it.} = \mu + g_i + T_t + R_{it}$$

where g_i is the genotype effect, T_t, the trial effect and R_{it}, the residual of the additive model, which includes the genotype × trial interaction term $(g{\times}T)_{it}$ and the error term e_{it}. The classification consisted in an ascending hierarchical clustering of trials based on R_{it}, minimising at each step the average within group mean square. This was achieved by using the Ward criterion of the CLUSTER procedure of SAS (SAS Institute 1989). This process corresponds to the one proposed by Corsten and Denis (1990), except that we did not include a classification on the 'genotype' factor. Following these authors, the clustering process was stopped at the step before which the cumulative G×E interaction within groups became significant. To avoid the problem of missing values, the analysis was restricted to the subset of genotypes that had a DGY performance in all the trials (212 among the 243 that were sown in all the trials). At the end of the process, the significance of the group and the genotype × group interaction effects were tested by means of ANOVA in the whole population. The proportion of G×E interaction variance accounted for by the classification was estimated. An AMMI model (Gauch 1992) was applied to the same data set (212 individuals) as the one used for the classification. The residual of the additive model, R_{it} was decomposed into r components as:

$$R_{it} = \sum_r \gamma^r \alpha_i^r \beta_t^r + (g \times T)_{it}^* + e_{it}$$

where α_i^r, β_t^r and γ^r are respectively the genotypic, trial and scale factor associated with the rth component and $(g{\times}T)_{it}^*$ the residual interaction. This analysis was performed through a principal component analysis using the *principal* option of the FACTOR procedure of SAS (see Romagosa et al. 1996, for more details).

For each trait, the broad-sense heritability and its confidence interval at 95% were estimated according to Knapp et al. (1985) on the whole data set and within each group of trials obtained with the clustering process. A multiple traits analysis was performed using the *MANOVA* option of the GLM procedure (SAS Institute 1989) to estimate the genetic correlation between the

traits. The genetic correlations between different groups of trials were obtained by dividing the phenotypic correlations by the square root of the product of the heritabilities in each group.

QTL detection

QTL detection was performed on the progeny mean testcross performance, adjusted for trial effect (within a group of trials or within the whole data set) and on the PC of the AMMI model. The method of composite interval mapping [(CIM) Zeng 1994; Jansen and Stam 1994] implemented in PlabQTL software (Utz and Melchinger 1996) was used. An LOD threshold of 2.4 was considered for declaring a putative QTL significant. Based on permutation tests (Churchill and Doerge 1994), this threshold corresponds to a global type I risk of 10% at the level of the whole genome. The covariates introduced in the CIM were chosen by selecting markers with a stepwise multiple regression, considering an F-to-enter and an F-to-drop value equal to 7. This value was chosen empirically to select as covariates markers close to the most important QTL detected with a simple interval mapping. It corresponds to an individual type I risk of 0.8%. The confidence intervals of the QTL positions were determined by a one-LOD unit fall. The question of the confidence interval of the QTL positions in CIM is not completely resolved (Melchinger et al. 1998), and the intervals determined by a one-LOD unit fall must be considered as underestimates of the true confidence interval at a 5% probability level. For agronomic traits, QTL with overlapping confidence intervals in different subsets of trials were considered as identical. For QTL detected in different subsets of trials, we considered the average of the estimated positions over subsets of trials as its most likely position. In order to compare the effects of a given QTL in the different environments, all QTL positions found significant in at least one subset of trials were simultaneously included in a multiple regression model. A backward selection was then performed in each subset, and only QTL significant at the 1% level were kept in the model. Using this regression model, we derived in all the subsets of trials: the LOD score value of each QTL, the additive effect associated with the F2 allele (which corresponds to half the difference between the testcross value of the homozygous genotypes). We computed the global percentage of genotypic variance explained by all the QTL (R_G^2) by dividing the ratio of the sum of squares explained by the QTL over the total sum of squares [i.e. the percentage of phenotypic variance explained by the QTL (R_P^2)] by the heritability of the trait (h^2). For PC QTL, we estimated the additive effect, which needs to be interpreted in the light of the linear combination of trials that defines the axis. A positive additive effect means that the individuals carrying the F2 allele at this locus are more adapted to the trials with a positive value for this PC. For PC, we only computed the phenotypic percentage of variance explained by QTL. Digenic epistasis between all pairs of markers was tested by a two-way ANOVA. As no clear evidence of epistasis was found (about 5% of the tests were significant at the 5% type I risk level), the results are not presented.

For all the agronomic traits, QTL × group interactions were tested at each detected QTL position with the model:

$$Y_{itk} = \mu + a_q\theta_{qi} + g_i^* + E_l + T(E)_{tl} + (a \times E)_{ql}\theta_{qi} + (g^* \times E)_{li} + e'_{tik}$$

where Y_{itk} is the k^{th} testcross performance of the genotype i in the trial t. As testcross progenies were usually not replicated within a given trial, k is equal to one in most of the cases. μ is the overall mean. E_l and $T(E)_{tl}$ are the group and the trial within group effects, respectively. a_q is the additive effect associated with the F2 allele at QTL q. θ_{qi} indicates the expected genotype of i at the QTL position q, knowing the genotypes at flanking markers, derived from the formulae developed by Hospital et al. (1996). The values 1 and −1 correspond to homozygous genotypes F2 and F252, respectively. g_i^* is the residual genetic effect of i not explained by the QTL. $a{\times}E_{ql}$ is the interaction between the QTL and the group and $g^*{\times}E_{li}$ is the residual interaction effect not explained by the QTL. Both g_i^* and $g^*{\times}E_{li}$ were considered as random. e'_{tik} is the residual of the model. The F-test of QTL × group interaction was performed using the mean square of the $g^*{\times}E_{li}$ effect for the denominator. All the QTL × group interactions significant at the 5% level risk were then simultaneously included in a global model to compute the total percentage of the genotype × group interaction explained. We also tested for each QTL detected the significance of the QTL × trial effect. Because of the large number of individuals (300) and trials (18), we were not able to include the genotype × trial interaction effect in the model in order to test the QTL × trial effect, since the estimation of such model required too much memory for our computer. We simplified the model by working on the average testcross line performance within trial ($Y_{it.}$).

$$Y_{it.} = \mu + a_q\theta_{qi} + g_i^* + T_t + (a \times T)_{qt}\theta_{qi} + \varepsilon_{ti}$$

where ϵ_{ti} included both the $(g^*{\times}T)_{ti}$ interaction effect and the error term.

For both models, we computed for each QTL the ratio of the sum of squares of the QTL × group (or QTL × trial) effect over the sum of squares of the total genotype × group (or genotype × trial) effect. Actually, the genotype × group (or genotype × trial) sum of squares depended not only on the genotype × group (or genotype × trial) variance but also on the error variance (σ_e^2) and the genotype × trial (within group) variance ($\sigma_{g \times T(E)}^2$) for the genotype × group interaction sum of squares. In order to estimate the proportion of genotype × group (or genotype × trial) variance explained by the QTL, these ratios were divided by the proportion of the phenotypic interaction due to the genotype × group

(or genotype × trial) interaction (i.e. $\sigma_{g\times E}^2/(\sigma_{g\times E}^2+\sigma_{g\times T(E)}^2/nk+\sigma_e^2/n)$ for the QTL × group interaction and $\sigma_{g\times T}^2/(\sigma_{g\times T}^2+\sigma_e^2/k)$ for the QTL × trial interaction, where k is the average number of replication per trial and n is the average number of trials within groups). This is analogous to the computation of the percentage of genotypic variance explained by QTL, R_G^2, based on the ratio between R_P^2 and h^2 (see above).

Results and discussion

Average performances and genotype × environment interaction

The average testcross performances on the whole experimental design illustrated the complementarities of the two parental lines (Table 2). F2 × MBS847 produced more than F252 × MBS847 (higher DGY) but presented higher GM at harvest. The difference between the DGY of the two parental hybrids was mostly due to the difference in KW, since both hybrids showed close values for NKP. The difference for SD was low (only 3 days), F2 × MBS847 being earlier than F252 × MBS847. For all the traits, the average testcross performance of the $F_{3:4}$ population was intermediate between the performances of the parental hybrids, and transgressive segregations were observed. The testcross performance of the $F_{3:4}$ population strongly varied from one trial to another (Table 1). Yield was very low in the trials submitted to drought stress (3 and 3b) when compared to other trials. The effect of limited nitrogen supply and early sowing was lower. On average, the phenotypic correlation between trials for DGY was rather low (0.23) and ranged from 0 (between trials 3 and 7) to 0.68 (between trials 4b and 9b). The grain yield components (KW and NKP) were also affected by the environment and were both low in the severely stressed trials (2, 3, 10 and 3b). The differences between trials 3 and 4 and between trials 3b and 4b (sown and harvested at the same dates in the same location) show that drought stress delayed SD and reduced GM.

Trial, genotype and genotype × trial effects were significant at the 0.1% level for all traits. The genotype × trial effect was particularly important for DGY. Its variance was almost as high as the genetic variance (Table 3). Despite this large genotype × trial interaction, the h^2 on the whole experimental design (18 trials) was high for all the traits, and varied from 0.84 to 0.91 (Table 2). This is clearly a consequence of the high number of trials that were considered.

The clustering process of the trials based on the residuals of the additive model was carried out with the 212 individuals evaluated in each of the 18 trials. The criterion used to stop the clustering process led us to consider three groups of trials (Fig. 2). The first level of classification clearly separated trials performed in 1996 (except trial 12b), which will be further referred to as the group 1, from those performed in 1995 (plus trial 12b). The second level of classification split the 1995 trials into two groups: group 2 composed of trials 4, 5, 6, 7, 8, 14 and group 3, composed of trials 1, 3, 9, 10, 11, 12, 13 and 12b. Apart from trial 14, all the trials included in group 2 were irrigated during 1995, whereas all the trials included in the group 3 were not (particularly trial 3 that was voluntarily not irrigated in a location where irrigation is usually required). This classification into three groups is consistent with very contrasted climatic conditions in France between the 2 years. It was rather cold during summer of 1996, whereas 1995 was a dry, warm year. Consistently, the second level of classification separates the 1995 trials according to the amount of water supply, which was an important limiting factor

Table 2 Adjusted means of the testcross performances of the two parental lines (F2 and F252) and the $F_{3:4}$ population in the whole experimental design (except trials 2 and 3b) for the different traits

Trait	Parental lines		F_3 population				
	F2	F252	Mean	Inf.	Max.	h^{2a}	CI[b]
DGY (t/ha)	7.77	7.04	7.44	5.43	8.62	0.85	0.82–0.87
GM (%)	35.9	33.8	35.6	33.3	38.0	0.91	0.89–0.92
SD (days)	26.3	29.2	28.3	24.8	32.0	0.91	0.90–0.93
KW (mg)	244.7	218.2	233.8	199.2	271.4	0.91	0.90–0.93
NKP	329.4	343.0	338.2	250.6	402.4	0.84	0.82–0.87

[a] h^2 Broad-sense heritability
[b] CI 95% Confidence interval

Table 3 Analysis of variance (ANOVA) for DGY in the whole experimental design (except trials 2 and 3b)

Factors	*df*	Mean squares III	Fisher test	Variance (SE)[a]
Trials	17	1,030.750	2,114.8[b]	–
Genotype	299	3.006	6.17[b]	0.150 (0.015)
Genotype × trials	4,694	0.493	1.42[b]	0.134 (0.021)
Error	460	0.347		0.347 (0.021)

[a] Estimated variances and their standard errors (SE) obtained by the REML method are added for random effects
[b] Effect significant at the 0.1% level

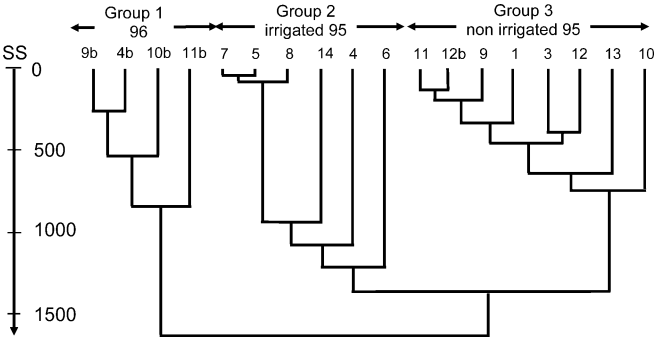

Fig. 2 Classification of trials based on genotype × trial interaction. Groups of trials are identified by a hierarchical clustering process performed on the residual of the additive model for grain yield. The *vertical axis* corresponds to the cumulated within-group sum of squares

this year. The position of trial 14 among the irrigated trials can be explained by its location in an eastern region of France that received more precipitation than the others. The reason for the position of trial 12b among the non-irrigated 1995 trials is less clear. The effect of this partition into three groups was highly significant when considering the whole dataset (Table 4). The genotype × groups variance accounted for 59% of the total DGY genotype × trials interaction variance [equal to the sum of the genotype × group and genotype × trials (within groups) interaction variances]. It can be noted that in this analysis the genotype × trials (within groups) interaction effect was significant at the 1% level risk, whereas this effect was not significant in the analysis used to define the groups of trials. This can be related to the superior number of progenies used in this analysis (300 instead of 212). Even if the classification of trials was based on DGY performances, the genotype × group interaction was also found significant for the other traits (results not shown). Hence, for all the traits, the QTL detection was performed separately for each group of trials in order to identify QTL showing differential adaptation to the different types of environment. Mean performances of the population within each group of trials showed that DGY was lower in the group 1 and 3 than in the group 2 (Table 5). This low DGY was associated with low NKP (in group 1 and 3) and low KW (in group 3 only). The trait heritabilities within each group were rather high (between 0.65 and 0.86). This should ensure us to have a good power of QTL detection within each group of trials.

Four significant (at a 5% level) PCs were found with the AMMI model of the genotype × trial interaction. The trial positions according to the first and second axes corroborated the result of the clustering process. The first PC clearly separated trials of the group 1 from those of the groups 2 and 3 (Fig. 3). The second PC separated the most favourable trials of group 2 from the least favourable trials of groups 1 and 3. The next two axes were mostly associated with only one trial (trial 8 for the third PC and trial 14 for the fourth PC) and were not considered further on. The first two PCs (PC1 and PC2) explained 28% of the sum of squares of the residual of the additive model (Table 6). It has to be noted that this sum of squares includes the experimental error, so that the percentage of genotype × interaction variance explained by the two PCs should be over 28%.

Thus, both interaction models (hierarchical classification and AMMI) showed that the major part of the interaction was associated with three contrasting groups of trials. These correspond to different years and to different amounts of water supply for the dry year 1995. This clearly highlights that, in a somewhat limited area such as northern France, the genotype × year interaction can be more important than the genotype × location interaction. Similar climatic events are generally observed in the whole area a given year. This is consistent with results observed on other crops in the United Kingdom (Talbot 1984). The effect of irrigation in 1995 confirms the well-known importance of this factor on maize yield (Hall et al. 1981; Westgate and Boyer 1986; Epinat Le Signor et al. 2001). Despite the genotype × group interaction, correlations between groups of trials were medium to high for all traits (Table 7), even for DGY. The positive correlation between irrigated and non-irrigated groups is in agreement with results found by Frova et al. (1999) but differs from the results observed by Ribaut et al. (1997) in severe drought stress conditions. In our case, drought stress was certainly more moderate. The majority of non-irrigated trials were performed in areas where rainfalls are usually sufficient to prevent from severe drought stress.

Table 4 ANOVA of grain yield including the effect of the groups of trials determined by the hierarchical clustering analysis

Factors	*df*	Mean squares III	Fisher test	Variance (SE)
Group of trials	2	3,976.879	3,860.1[a]	–
Trials (group)	15	613.522	1,469.7[a]	–
Genotype	299	3.316	3.22[a]	0.138 (0.017)
Genotype × group	572	1.030	2.47[a]	0.098 (0.010)
Genotype × trials(group)	4,122	0.417	1.20[b]	0.068 (0.020)
Error	460	0.347		0.347 (0.020)

[a]Effect significant at the 0.1% level
[b]Effect significant at the 1% level

Correlations between traits

Considering the whole experimental design level, DGY was positively correlated with its two components, KW and NKP (Table 8). The higher correlation with NKP suggests that the variability of DGY in the population was mostly due to differences in NKP. The strong neg-

Table 5 Average adjusted means and h^2 in the different groups of trials (1, 2 and 3) for the measured traits

Trait (unit)	Group 1		Group 2		Group 3	
	Means	h^2 (CI)	Means	h^2 (CI)	Means	h^2 (CI)
DGY (t/ha)	7.29	0.85 (0.82–0.88)	9.24	0.72 (0.67–0.77)	6.36	0.66 (0.60–0.72)
GM (%)	37.7	0.68 (0.62–0.74)	35.5	0.80 (0.76–0.83)	34.8	0.85 (0.82–0.87)
SD (in days)	28.7	0.73 (0.67–0.77)	26.6	0.75 (0.71–0.80)	29.1	0.86 (0.84–0.88)
KW (mg)	241.2	0.80 (0.76–0.84)	241.4	0.81 (0.77–0.84)	223.9	0.81 (0.78–0.84)
NKP	319	0.78 (0.73–0.82)	413	0.75 (0.71–0.80)	302	0.65 (0.59–0.71)

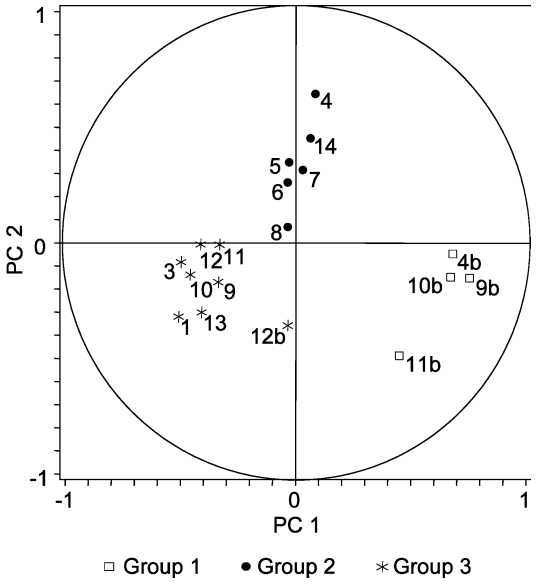

Fig. 3 Position of the 18 trials according to their coordinates along the first two principal components (PC 1 and PC 2) of the multiplicative model of interaction model of genotype × trial interaction. Trials belonging to the same group of trials, determined by the clustering analysis (see Fig. 2), are plotted with the same symbol

Table 6 Decomposition of the grain yield genotype × trial interaction by the first two principal components (PCs), PC1 and PC2, obtained with the additive main effects and multiplicative interaction (AMMI) model

Factors	*df*	Sum of squares	Mean squares	Fisher test
Trial	17	12,944.862	761.462	1,670.9[a]
Genotype	211	614.040	2.910	6.39[a]
Genotype × trial + error	3,587	1634.719	0.456	
Interaction decomposition				
PC1	227	292.871	1.290	3.4[a]
PC2	225	163.580	0.727	1.9[a]
Residual	3,135	1,178.268	0.376	

[a]Effect significant at the 0.1% level

Table 7 Genetic correlations between groups of trials for the different traits

	DGY	GM	SD	KW	NKP
Group 1/group 2	0.78	0.80	0.85	0.85	0.72
Group 1/group 3	0.57	0.94	0.87	0.87	0.59
Group 2/group 3	0.66	0.89	0.99	0.93	0.86

ative correlation between the two DGY components was certainly due to a competition between kernels during grain filling. The magnitude of these correlations slightly varied according to the group of trials that was considered. In the group 1, DGY was more correlated to NKP and less correlated to KW than in the other groups, and the correlation between these two components was lower (but still negative). Hence, NKP was clearly the limiting factor in this group and there was less competition during grain filling. In group 3 (characterized by water deprivation), both NKP and KW were low and they were strongly negatively correlated.

Table 8 Genetic correlations between the different traits in the whole experimental design (18 trials) and in each group of trials (1, 2 or 3)

	Group of trials	Traits			
		DGY	GM	SD	KW
GM	18	0.44			
	1	0.37			
	2	0.40			
	3	0.14			
SD	18	0.41	0.55		
	1	0.38	0.62		
	2	0.64	0.39		
	3	−0.02	0.65		
KW	18	0.28	0.27	−0.09	
	1	0.23	0.20	−0.08	
	2	0.30	0.34	−0.07	
	3	0.28	0.18	−0.15	
NKP	18	0.60	0.14	0.37	−0.56
	1	0.76	0.18	0.34	−0.26
	2	0.66	0.05	0.55	−0.58
	3	0.53	−0.01	0.11	−0.63

DGY was positively correlated with traits related to earliness (SD and GM), when considering the whole experimental design level. These correlations strongly varied according to the group considered, particularly for that between DGY and SD. A high positive correlation (0.64) between these two traits was observed in group 2 (well-irrigated trials). Conversely, this correlation was slightly negative in the group 3 of non-irrigated trials, which is consistent with results observed in drought-stressed conditions by Ribaut et al. (1997).

QTL detection for agronomic traits

The QTL detection was carried out for the different agronomic traits on the average performance within the whole experimental design and within each group of trials (Table 9).

A total number of 61 QTL were detected for yield and related traits in this study (14 for DGY, 13 for KW, 15 for NKP, 10 for GM and 9 for SD). These QTL generally displayed small individual effects, except one QTL of GM located on chromosome 4 which explained more than 20% of the phenotypic variance (result not shown). For all traits, both parental lines carried favourable alleles, which is consistent with the transgressions observed in the $F_{3:4}$ population. The detected QTL explained between 38% (for SD in group 2) and 79% (for KW in the whole set of trials) of the genetic variance, with an average close to 50%. R^2 are known to be overestimated, especially when the power of QTL detection is low due to a low population size and/or a low trait heritability (Beavis 1994; Melchinger et al. 1998). We can assume that this over-estimation was only

Table 9 QTL of DGY, KW, NKP, GM, and SD detected in the whole data set (18 trials) or in each groups of trials. Values in italic correspond to QTL with a LOD between 1.5 and 2.4 that were kept in the model because they were significant (LOD > 2.4) in at least one subset of trials

Trait	Chr[a]	Pos[b]	CI	18 trials		Group 1		Group 2		Group 3		QTL × group	QTL × trial
				LOD	Add[c]	LOD	Add	LOD	Add	LOD	Add	r^2	r^2
DGY (t/ha)	1	0	0–8	3.7	0.088	–	–	–	–	4.5	0.096	NS	NS
	1	166	164–174	8.4	−0.142	10.0	−0.308	*2.2*	*− 0.101*	3.0	−0.087	5.3***	3.7***
	2	46	42–52	*1.8*	*0.083*	3.2	0.183	–	–	–	–	NS	2.1*
	2	78	72–90	2.5	0.101	–	–	6.7	0.199	8.6	0.15	NS	2.9**
	2	124	118–126	4.4	0.109	6.6	0.245	*2.0*	*0..03*	–	–	4.1***	4.6***
	3	14	8–22	5.4	−0.106	–	–	2.9	−0.110	5.7	−0.111	NS	NS
	4	2	0–6	2.4	0.081	–	–	3.9	0.134	–	–	NS	2.8**
	4	56	42–66	2.9	0.120	5.3	0.297	–	–	9.8	0.179	2.8**	3.2***
	4	94	86–94	5.8	0.122	*1.9*	*0.137*	7.5	0.168	–	–	2.4*	4.0***
	6	56	38–68	5.8	0.179	*2.1*	*0.212*	4.8	0.232	–	–	2.5*	2.5***
	7	66	64–78	4.2	−0.095	4.3	−0.190	–	–	2.8	−0.080	1.7*	2.4*
	8	14	0–22	*2.0*	*0.074*	3.0	0.193	*1.7*	*0.103*	–	–	NS	NS
	8	62	58–72	–	–	3.5	−0.242	*1.9*	*− 0.125*	*1.5*	*0.063*	16.9***	9.5***
	8	92	88–98	8.9	−0.142	*2.1*	*− 0.165*	3.0	−0.141	–	–	14.0***	7.4[f]
R_G^2				62		52		54		55		41	38
KW (mg)	1	100	94–114	7.7	5.13	–	–	4.7	4.73	10.3	7.49	15.7***	8.4***
	1	194	190–196	6.5	3.47	*1.5*	*2.26*	5.3	3.63	5.3	3.87	NS	NS
	2	46	42–62	*1.7*	*2.03*	2.6	3.56	–	–	–	–	7.8***	7.4***
	2	96	88–102	2.6	2.74	*1.7*	*3.17*	6.2	4.08	3.3	3.17	4.4*	NS
	2	146	140–150	*1.5*	*1.89*	2.8	3.55	–	–	–	–	NS	5.2*
	3	20	12–26	12.0	−5.18	5.7	−4.93	14.5	−6.50	7.5	−5.02	3.8*	5.2*
	4	6	4–8	15.9	5.23	12.8	6.51	12.3	5.31	7.1	4.31	4.2*	5.8**
	5	40	20–46	3.9	2.93	–	–	6.0	4.21	3.4	3.39	9.7***	5.4**
	5	138	124–152	3.5	−3.34	3.0	−4.27	–	–	–	–	5.7**	5.6**
	7	72	66–80	6.3	−3.28	3.12	−3.18	3.6	−2.87	4.7	−3.50	NS	5.2*
	8	86	74–88	–	–	2.8	−2.96	–	–	–	–	9.3***	10.9***
	9	64	62–72	8.4	3.77	5.2	4.01	7.2	4.01	3.9	3.18	NS	NS
	10	109	92–116	2.6	2.34	*1.5*	*2.36*	3.2	3.00	*1.8*	*2.41*	NS	NS
R_G^2				79		58		60		53		53	57
NKP	1	92	84–104	4.8	−8.70	*2.1*	*− 8.4*	3.3	−10.9	6.3	−10.2	NS	NS
	1	170	166–178	*1.9*	*− 4.79*	8.4	−12.4	–	–	4.0	−6.0	3.1*	2.3*
	1	196	190–196	*2.3*	*− 5.04*	–	–	4.7	−7.8	–	–	NS	NS
	2	78	68–98	–	–	–	–	–	–	2.4	4.77	NS	NS
	2	126	114–128	2.7	5.46	2.8	6.6	–	–	–	–	NS	3.1**
	2	150	144–150	*2.0*	*− 4.70*	–	–	–	–	3.2	−5.06	4.4**	4.7***
	4	8	8–18	–	–	2.9	−7.5	–	–	*1.7*	*− 3.94*	NS	NS
	4	52	44–72	3.3	5.68	7.3	13.9	–	–	4.8	7.9	NS	NS
	5	46	40–52	2.9	−5.01	–	–	3.2	−7.4	3.2	−5.5	4.7**	4.1***
	6	18	14–24	4.4	5.57	2.6	6.3	3.3	7.0	*1.6*	*3.5*	NS	NS
	6	186	166–206	–	–	2.4	−7.4	–	–	–	–	NS	NS
	8	62	58–72	–	–	4.6	−9.1	–	–	–	–	17.3***	11.2***
	8	92	86–96	3.3	−4.99	–	–	6.0	−9.4	–	–	12.9***	6.8***
	9	70	58–80	2.6	−4.32	5.3	−9.3	–	–	–	–	2.8*	3.6**
	10	100	84–112	3.4	−4.82	*2.2*	*− 5.7*	6.2	−9.4	–	–	4.0**	3.1*
R_G^2				45		46		41		49		37	32
GM (%)	2	126	122–128	9.8	0.30	*2.1*	*0.17*	9.7	0.33	9.9	0.39	8.8***	3.8**
	3	86	72–98	3.5	0.19	–	–	5.3	0.26	*1.9*	*0.18*	NS	3.6**
	4	4	2–6	31.7	0.56	17.1	0.52	34.0	0.63	19.5	0.57	NS	8.4***
	5	78	74–92	–	–	–	–	2.5	0.18	–	–	4.4*	4.2**
	6	164	144–184	–	–	–	–	2.9	0.28	–	–	NS	NS
	7	108	102–116	4.9	−0.19	4.6	−0.27	*1.5*	*− 0.17*	2.8	−0.21	NS	3.7**
	8	62	42–74	6.5	−0.26	*1.7*	*− 0.17*	–	–	8.3	−0.38	13.8***	4.2***
	8	110	102–126	–	–	–	–	3.5	−0.20	–	–	NS	2.9*
	9	42	32–46	11.0	0.34	4.3	0.27	13.4	0.41	6.4	0.33	3.8*	5.5***
	10	100	96–114	*1.8*	*− 0.13*	–	–	–	–	3.2	−0.22	8.2***	5.0***
R_G^2				56		47		63		53		38	42
SD (days)	1	188	180–194	5.7	−0.37	*1.7*	*− 0.24*	3.8	−0.27	5.0	−0.47	6.4**	7.1***
	2	46	42–66	2.7	0.27	–	–	*2.3*	*0.23*	4.4	0.47	10.7***	5.3**
	5	50	42–54	*2.1*	*− 0.23*	3.0	−0.32	–	–	–	–	NS	NS
	8	58	46–70	6.5	−0.43	2.9	−0.34	4.1	−0.31	5.9	−0.57	12.7***	14.0***
	8	92	86–96	8.0	−0.57	6.7	−0.62	3.6	−0.30	5.8	−0.67	7.5***	8.8***
	8	122	116–126	2.6	0.30	3.0	0.38	–	–	*1.7*	*0.33*	NS	NS
	9	34	18–48	3.6	0.32	2.4	0.31	3.8	0.30	2.6	0.37	NS	NS

Table 9 (Contd.)

Trait	Chr[a]	Pos[b]	CI	18 trials		Group 1		Group 2		Group 3		QTL × group	QTL × trial
				LOD	Add[c]	LOD	Add	LOD	Add	LOD	Add	r^2	r^2
	9	180	164–188	4.3	−0.37	*1.7*	*− 0.27*	3.5	−0.30	4.2	−0.50	NS	4.8*
	10	100	90–100	5.8	−0.35	4.1	−0.34	2.8	−0.30	5.6	−0.48	5.6**	6.1**
R_G^2				45		38		39		44		42	43

[a]Maize chromosome
[b]Position on the map
[c]Additive effect associated with the F2 allele
*Significant at the 5% level
**Significant at the 1% level
***Significant at the 0.1% level
R_G^2 Percentage of genetic variance explained by the QTL

moderate in this experiment, since trait heritabilities were high and the population size was rather large. The power of QTL detection in this experiment was indeed about 75% for a QTL, explaining 5% of the variation [computation based on equations given by Charcosset and Gallais (1996) and an individual type I error risk of 0.1%]. Nevertheless, the estimated R^2 clearly showed that despite the large size of the experimental design that was carried out (300 genotypes, 18 trials), a large part of the genetic variance remained unexplained. The map density in this experiment was rather good (one marker every 20 cM on average), but the map was sparse on some chromosome segments (especially on chromosome 6). This could have affected the power of QTL detection in these areas. Nevertheless, despite the low marker density, we were able to detect four QTL (for DGY, NKP and GM) on chromosome 6.

More QTL were detected for yield (DGY) and its components (KW and NKP) than for earliness related traits (SD and GM). This is consistent with the fact that DGY is the final product of plant development and should therefore be affected by a high number of QTL. However, the small number of QTL detected for SD and the low R^2 is surprising, considering that h^2 was very high for this trait. Parental lines were both early, so we did not expect to observe a QTL of major effect in segregation in the population. The variation within the population is more likely to be due to numerous QTL of small individual effects that could hardly be detected. Many detected QTL for different traits appeared to be located on the same chromosome segments. DGY is determined by the product of NKP and KW, so it is logical that the majority of the DGY QTL were detected on areas also involved in the variation of KW and NKP. Slightly more coincidences were found between DGY and NKP than between DGY and KW, which is consistent with the genetic correlations and confirms the importance of NKP in the determinism of DGY. The negative correlation between KW and NKP certainly explains why QTL involved in both traits but with antagonist allelic effects had no significant effects on DGY (as was the case on chromosomes 5 and 9). SD and GM are also expected to be correlated. SD determines the length of the desiccation period, since all the genotypes were harvested at the same date. However, only three chromosome regions had significant effects for both traits (two on chromosome 8, one on chromosome 10). The region located on chromosome 4 which had a major effect on GM had no significant effect on SD. So even if differences in SD may explain part of GM variation, the majority of GM QTL seems specific to kernel maturation. Some chromosome areas affected both (1) yield and its components and (2) traits related to earliness (SD and GM). This is in particular the case for the area located around *umc84* (position 189) on chromosome 1 (involved in DGY, KW, NKP and SD), the area located at position 126 on chromosome 2 (involved in DGY, GM and NKP) and most of all, the area located on chromosome 8 near position 92, which is involved in all the traits analyzed. As the effects of QTL located in the same chromosome areas were generally in good agreement with the agronomic correlations between traits, it is reasonable to assume that some of these coincidences are due to QTL with pleiotropic effects rather than to closely linked QTL. Nevertheless, the precision of QTL position estimates does not allow us to decide with certainty between these two hypotheses.

Stability of QTL in the different groups of trials

The number of QTL detected within a group of trials or on the mean of the 18 trials is lower than the total number of QTL detected. Generally speaking, QTL detected in a given group of trials were also detected when considering the whole experimental design. However some QTL were only detected within a group (3, 3, 6, 4 and 2 QTL for GY, KW, NKP, GM and SD, respectively). Thus, the QTL detection performed group by group allowed us to detect additional QTL that were not detected on the mean performances over the whole experimental design. Very few QTL were detected in all the groups of trials: none for DGY and NKP, two for GM and four for KW and SD. This is consistent with the highest G×E variance observed for DGY and NKP. When detected in several groups of trials, the contribution of parental lines was always consistent. Only one inversion of effect was detected for a DGY QTL located on chromosome 8 (position 62), but this QTL was only significant with a LOD score >2.4 in the group 1 of

trials. In the group 3, its effect is opposite to the one found in group 1, but it is only sub-significant (LOD score = 1.5). Only a limited number of QTL displayed a significant effect in all the groups of environments. This result differs from the relative stability of QTL effects found in some studies for DGY or its components (Stuber et al. 1992; Schön et al. 1994). Sampling and experimental errors could result in the detection of different QTL in different environments without any G×E interaction (Jansen et al. 1995). For this reason, the simple comparison between QTL detected in different groups of environments is not sufficient to study the stability of QTL effects. To better understand the genetic basis of the interaction, the QTL × group and QTL × trial interactions were tested for all the detected QTL.

A large majority of QTL that were detected in this experiment displayed a significant QTL × group (54% on average over the different traits) or QTL × trial (70% on average over the different traits) interaction at the 5% level risk. Some of these interactions are highly significant: 26% and 34% of the detected QTL showed a significant QTL × group and QTL × trial interaction at the 0.1% level risk. Considering the number of tests performed (61), an individual risk of 5% corresponds to a global risk of 95%. Thus, some of these significant interactions are false positive. Nevertheless, at a 0.1% level risk, the global risk level that at least one interaction is spurious is only about 6%. So a large proportion of QTL presents a significant QTL × group or QTL × trial interaction. This result differs from the lack of significant interaction found in some studies for similar traits (Melchinger et al. 1998). We detected more QTL × trial interaction than QTL × group interaction. It was not obvious to predict such a result. The QTL × group interaction test is more parsimonious than the QTL × trial interaction test (more parameters needed to be estimated), but the genotype × group interaction only represents 59% of the genotype × trial interaction. Even if in our data set, the QTL × group interaction test was less powerful than the QTL × trial interaction test, QTL that showed the highest QTL × trial interaction (significant at the 0.1% level risk) also showed significant QTL × group interaction, at least at a 5% level risk. Moreover, the QTL × group interaction is easier to interpret, since it is associated with three types of contrasting environments (1996 vs 1995 irrigated and 1995 non-irrigated trials). The percentage of QTL showing significant interaction was almost the same for the different traits, whereas the genotype × trial interaction was higher for DGY and its components than for GM and SD. This may be explained by the fact that these last two traits are more accurately measured than DGY in a given trial, which increased the power to detect interactions and compensated for the smallest interaction variance. The level of significance of the QTL × group and QTL × trial interaction was not always consistent with the stability of the QTL detection in the different groups. For instance, the SD QTL that showed the highest QTL × trial and QTL × group interaction was a QTL of large effect detected in all the groups but with various effects (chromosome 8, position: 58 cM). On the other hand, one of the three DGY QTL for which the test of interaction was not significant was only detected in one group of trials (chromosome 8 position 14 cM). The lack of detection in the other groups is thus certainly due to a lack of power rather than to a real QTL × group interaction.

The percentage of genotype × trial and genotype × group interaction variances explained by the QTL × trial and QTL × group interaction varied between 32% and 57%. Some chromosome segments appeared to play an important role and explained more than 10% of the genotype × group or genotype × trial interaction variances. It is striking to note that for all the traits, major interaction effects were found on chromosome 8. For DGY, three segments (located on chromosomes 1, 2 and 8) presented both a QTL × group and QTL × trial interaction significant at the 0.1% level risk. These areas coincided with NKP QTL with significant interaction effects. Hence, environmental conditions affecting NKP had a much stronger impact on DGY than those affecting KW. The establishment of final NKP occurs in a 2-week period following flowering (Claassen and Shaw 1970). NKP is especially susceptible to environmental conditions during and just after flowering, particularly to solar radiation (Otegui and Bonhomme 1998) and water availability (Hall et al. 1981; Westgate and Boyer 1986). This also explains why chromosome segments involved in DGY genotype × trial interaction in this study often correspond to areas where SD QTL were detected. The most important interaction for DGY was found on chromosome 8, where three SD QTL explaining the largest part of SD variation were detected. In this area, the F2 allele of QTL located at positions 58 and 92 decreased SD and had (1) a negative effect on DGY in the groups 1 and 2 of well-watered trials and (2) no effect for position 92 or even a slightly positive (but not significant) effect for position 58 in the group 3 of non-irrigated trials. The same type of results was observed for the area located on chromosome 1. This region was also identified by Bertin and Gallais (2001) as being involved in QTL × nitrogen interaction. Such areas are maybe responsible for the lack of positive correlation observed between SD and DGY in the group 3 of non-irrigated trials. The relationship between flowering time and drought susceptibility can be explained in several ways. First, earliness is known to be involved in drought escape. Early genotypes that flower at the beginning of a water deprivation are less penalized than others, which compensates for their shorter vegetative period. Second, drought stress often generates a delay in silk emergence relative to anthesis [this delay is measured by the anthesis-silking interval (ASI)], which can be correlated with important DGY losses (Westgate and Boyer 1986; Ribaut et al. 1997; Sari-Gorla et al. 1999). The genotypes that are able to maintain a sufficient silk growth rate under stress (and thus not delay SD) may have a biomass parti-

tioning to the developing ear shoot which results in fewer kernel abortions (Bolanos et al. 1993; Edmeades et al. 1993). In this experiment, male flowering date was only recorded in a few trials, which did not allow us to estimate precisely ASI and distinguish between these two phenomena (drought escape or drought tolerance). Nevertheless, very interestingly, QTL involved in precocity have often been detected on chromosome 8 near the marker *umc89* in a lot of different genetic backgrounds (Abler et al. 1991; Stuber et al. 1992; Koester et al. 1993; Beavis et al. 1994; Veldboom and Lee 1996b; Ribaut et al. 1996; Rebaï et al. 1997; Sari-Gorla et al. 1999; Vladutu et al. 1999). Some studies have shown that this area was also involved in ASI (Ribaut et al. 1996; Veldboom and Lee 1996b; Sari-Gorla et al. 1999) and drought tolerance (Ribaut et al. 1997; Frova et al. 1999; Betran et al. 2003), which is consistent with our results. Despite the differences between experiments, the significant effects reported for this chromosome region on stress tolerance could indicate that it corresponds to an important genetic component of yield stability. As for chromosome 8, ASI QTL related to drought tolerance have been detected on chromosome 1 in the literature but not exactly in the same chromosome segments. Because of the large confidence intervals, it is not easy to determine if the QTL detected in this study coincide with QTL positions found in the literature. A statistical approach has been recently proposed to analyze simultaneously QTL results from different studies in order to determine the most likely positions of QTL on chromosomes (Goffinet and Gerber 2000), but its implementation requires the projection of QTL on a composite map and tools needed to perform such analysis are not yet available.

QTL for PCs

Five QTL were detected for PC1 and explained 24.6% of the variation of this axis (Table 10). The largest QTL was located on chromosome 8 (position 58). Only one QTL was detected for PC2 on chromosome 10 (position 98) and explained 6% of the variation. PC1 discriminated between the trials of the group 1 (which had a positive value on this axis) and the trials of the group 3 (which had a negative value on this axis), whereas PC2 discriminated between the best trials of the group 2 (positive value) and the worst trials of the other groups (negative value). Thus, the negative QTL effect for the PC1 QTL detected on chromosome 8 position 58 means that progenies with the F2 genotypes at this QTL were less adapted to the group 1 than to the group 3 of trials. This is consistent with the occurrence of a DGY QTL at this position with a negative effect of F2 allele in group 1 and a positive effect in group 3. All the QTL but two detected on PCs corresponded to DGY QTL that showed the most significant QTL × group interaction (at position 166 on chromosome 1, 124 on chromosome 2, 56 on chromosome 4 and the position 62 on chromosome 8), which confirms the importance of these segments on DGY stability, especially the role of chromosome 8. The two exceptions (located on chromosomes 9 for PC1 and 10 for PC2) consisted in segments that had no significant effects on DGY, but had significant effects on DGY components NKP and KW and indeed showed a significant QTL × group interaction for NKP. Thus such QTL which have a small effect on average on DGY and were not detected as QTL for this trait may play an important role in DGY stability.

Table 10 QTL of PC1 and PC2 obtained with the AMMI model

Trait	Chr	Pos	CI	Flanking markers[a]	LOD	Add
PC1	1	170	162–180	*umc161–umc84*	3.17	−0.03
	2	136	128–144	*umc137–csu9a*	4.54	0.038
	4	52	40–70	*umc104b–umc12a*	2.74	0.029
	8	58	54–76	*umc152–umc12b*	6.04	−0.038
	9	88	76–110	*csu147–umc38a*	2.76	−0.028
R^2_P[b]					25	
PC2	10	98	80–102	*gsy87–umc44*	3.21	−0.031
R^2_P[b]					6	

[a]Markers flanking the estimated position of the QTL
[b]R_P^2 Percentage of phenotypic variance explained by the QTL

Conclusion

The results of the QTL detection were consistent with the important G×E interaction found at the agronomic level. The different approaches developed in this study were complementary. The clustering processes of trial as well as the AMMI model both lead to the identification of three contrasting groups of trials that explain the major part of the DGY genotype × trial interaction. The high h^2 values within groups of trials gave us sufficient power to detect QTL specific to some of these groups that were not detected on the global mean. Among those QTL, one located on chromosome 8 explained a large part of the genotype × trial interaction for DGY and related traits. The QTL detected on PC derived from the AMMI model allowed us to confirm the key role of some chromosome segments in DGY stability and identify two other regions that had no significant effect on DGY (but an effect in DGY components) but that were important in DGY stability. Even though no inversion of effect was found (with the exception of a non-significant one on chromosome 8), the lack of stability of DGY QTL effect can have strong consequences on the success of marker-assisted selection (MAS). An MAS experiment is being carried out on this population based on QTL effects presented in this study (1995 and 1996 trials). Only little research has been done on the best way to include QTL × environment interaction in MAS (Chapman et al. 2003). A better understanding of the factors that determine G×E interaction is certainly needed to

achieve this goal. Since our results highlight the importance of earliness and water availability in this experiment, it would have been interesting to record data on temperature, precipitation or radiation within the trials in order to better characterize these environments and evaluate which factors were limiting. The use of such data could be of great help to study more precisely the effect of environmental factors on the stability of QTL (as was done by Crossa et al. 1999) in order to include such information in the genetic value prediction. Further work is therefore needed on this major issue in order to optimize experimental designs and strategies for the MAS of stable genotypes.

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