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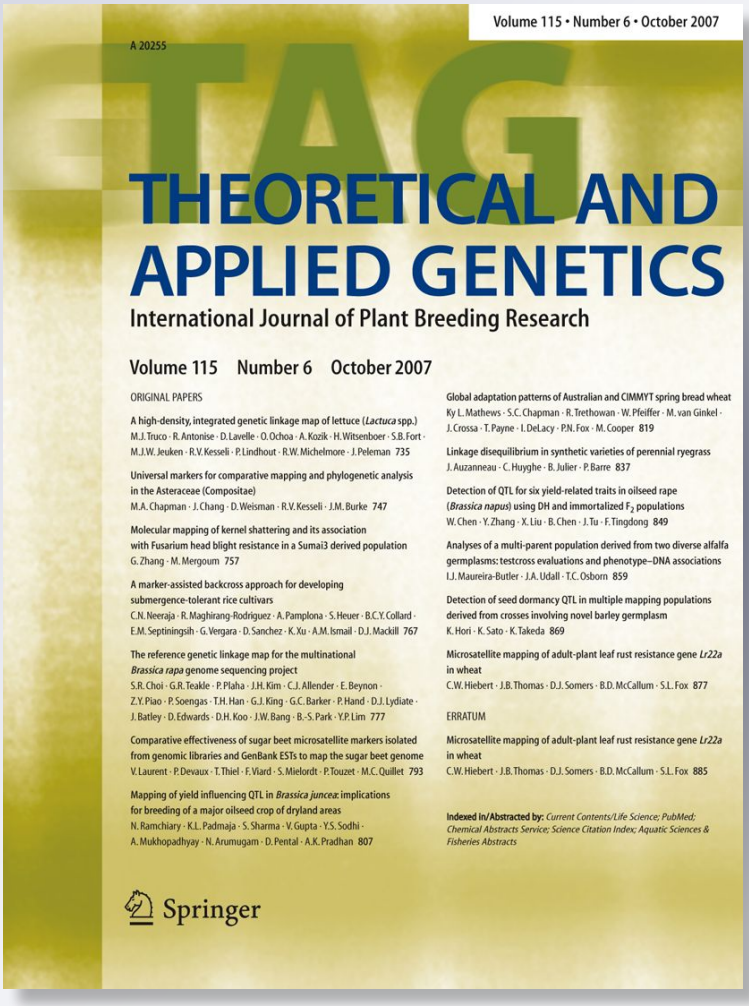
**Theoretical and Applied Genetics**
International Journal of Plant Breeding Research

ISSN 0040-5752
Volume 124
Number 1

Theor Appl Genet (2012) 124:35-47
DOI 10.1007/s00122-011-1685-6




Springer

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DOI 10.1007/s00122-011-1685-6

ORIGINAL PAPER

# Characterization of heterotic quantitative trait loci in maize by evaluation of near-isogenic lines and their crosses at two competition levels

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Received: 15 November 2010 / Accepted: 12 August 2011 / Published online: 28 September 2011


**Abstract** In a previous study on a maize (*Zea mays* L.) population of recombinant inbreds derived from B73 × H99, we identified several quantitative trait loci (QTL) for agronomic traits with high dominance-additive ratio. Then, for four of these QTL, we developed families of near-isogenic lines (NILs) homozygous either for the QTL allele from B73 (BB) or from H99 (HH); for two of these QTL, the NILs' families were produced in two different genetic backgrounds. The present study was conducted to: (1) characterize these QTL for agronomic traits and (2) verify whether their effects were influenced by the genetic background, inbreeding level and plant density (PD). The six NILs' families were tested across 3 years and in three experiments at different inbreeding levels as NILs per se and their reciprocal crosses (Experiment 1), NILs crossed to related inbreds B73 and H99 (Experiment 2) and NILs crossed to four unrelated inbreds (Experiment 3). Experiment 2 was conducted at two PDs (4.5 and 9.0 plants $m^{-2}$). Results of Experiments 1 and 2 confirmed previous findings as to QTL effects, with dominance–additive ratio superior to 1 for several traits; as a tendency, dominance effects were more pronounced in Experiment 1. The QTL effects were also confirmed in Experiment 3. The interactions involving QTL effects, families and PD were generally negligible, suggesting a certain stability of the QTL. Results emphasize the importance of dominance effects for these QTL, suggesting that they might deserve further studies, using the NILs' families and their crosses as base materials.

Communicated by M. Bohn.

**Electronic supplementary material** The online version of this article (doi:10.1007/s00122-011-1685-6) contains supplementary material, which is available to authorized users.

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## Introduction

Heterosis is a term coined by Shull in 1914 to indicate the superiority of hybrids over their parents. Several studies conducted ever since have led to the formulation of three main hypotheses concerning the gene actions accounting for heterosis, namely, dominance, overdominance and epistasis (for review, see Reif et al. 2005). The classical quantitative genetics did not clarify the relative importance of these gene actions, mainly because the statistical procedures could reveal only the net effects across the various loci involved. All such issues on the gene actions determining heterosis led to an inconclusive controversy (Crow 2008); but now, with the advent of the genomic era, we are endowed with powerful tools to study heterosis, as we can identify the chromosome regions (quantitative trait loci, QTL) determining heterosis, map their position, estimate their effects and elucidate the molecular bases of heterosis.

For maize (*Zea mays* L.), the pioneer studies of Stuber et al. (1992) and of Lu et al. (2003) are noteworthy, as they detected several QTL, most of which were characterized by overdominant gene action. However, in a re-analysis of the data of Stuber et al. (1992), Cockerham and Zeng (1996) pointed out a prevalence of dominance with a contribution of epistasis among linked loci. Moreover, in a subsequent fine-mapping study on one of the overdominant QTL, Graham et al. (1997) found that the QTL included two loci

linked in repulsion phase, each showing dominant gene action. To provide further information concerning the identification of QTL involved in the control of heterosis in maize, Frascaroli et al. (2007) conducted a study on a population of 142 recombinant inbred lines (RILs, as $F_{12:13}$) derived from the heterotic single cross B73 × H99. B73 is an inbred line of the Stiff Stalk Synthetic (SSS) heterotic group, while H99 is an inbred line belonging to the opposite heterotic group Lancaster (LAN) (Melchinger et al. 1991). RILs were investigated per se and as crosses with the two parental inbreds and their $F_1$, produced according to the triple testcross scheme. For grain yield, 21 QTL were detected and 16 of them showed a marked effect on the expression of heterosis, with the ratio between dominance and additive effects being superior to 1 (heterotic QTL). Moreover, most of these QTL overlapped with heterotic QTL for other agronomic traits, thus suggesting that, besides linkage effects, the underlying genes might exert pleiotropic effects on the overall plant vigor by controlling a sequence of causally related events.

These findings prompted us to focus our investigations on the detected heterotic QTL for improving the comprehension of the genetic basis of heterosis for complex traits and their components. As a first step toward the undertaking of these investigations, we developed pairs (families) of near-isogenic lines (NILs) for the heterotic QTL of greater interest (Pea et al. 2009). Families of NILs were produced differing specifically for the parental alleles at the target QTL, while sharing in homozygosity the rest of the genome. Therefore, such NILs' families are suitable materials to accurately investigate the direct and associated QTL effects for a number of reasons: (1) biases due to the genetic background would be negligible (Paterson et al. 1990); (2) due to the peculiar breeding scheme adopted in our case, more than one NILs' family could be produced for two of the target heterotic QTL, thus allowing the investigation of the epistatic interactions of these QTL with the genetic background; (3) NILs can be crossed to each other as well as to testers, both related and unrelated, thus allowing the analyses of QTL effects across inbreeding levels and a range of heterotic combinations; (4) finally, NILs and/or their crosses can be tested at varying stress levels, an objective that could be of particular interest, as heterosis can play an important role in maize responses to a wide range of stress conditions, such as drought (Betrán et al. 2003), excessive soil moisture (Zaidi et al. 2007), and high plant density (Duvick 2005; Liu and Tollenaar 2009).

Here, we present the results of an extensive study in which families of NILs for heterotic QTL and their crosses to related and unrelated inbred lines were analyzed to: (1) characterize the QTL for complex traits and their components, by assessing the relative importance of the QTL additive and dominance effects, and (2) verify whether the QTL effects are affected by the genetic background, the inbreeding level and the competition among plants as determined by low versus high plant density. The high plant density factor was chosen because it can bring about stress conditions, which involve adaptive mechanisms at least partly common to other stress factors (Bruce et al. 2002; Echarte and Tollenaar 2006).

## Materials and methods

### Plant materials: development of the NILs' families

A description of the NILs' families for the heterotic QTL herein analyzed is summarized because a detailed description has been already given (Pea et al. 2009). Among the QTL identified in the study of Frascaroli et al. (2007), six QTL, located in bins 3.05, 4.10, 7.03, 8.03, 8.05 and 10.03, were selected to be introgressed into NILs (Pea et al. 2009). Four of these QTL were chosen for this study because they showed overdominance for plant height and kernel weight (7.03) or for grain yield and number of kernels per plant (3.05, 4.10 and 10.03). The base materials for the NILs production was represented by $F_{4:5}$ lines obtained during the development of the corresponding RILs investigated by Frascaroli et al. (2007) and that were still heterozygous for the two marker loci flanking the target QTL. Because of the adopted introgression procedure (Pea et al. 2009), each pair (family) of NILs is homozygous at the two marker loci, for the alleles of either B73 or H99, but is a mosaic of homozygous recombinant blocks coming from B73 or H99 in the background, as is the corresponding RIL. For QTL 3.05 and 4.10, it was possible to produce two NILs' families (pairs), so as to allow the evaluation of the QTL effects in two different genetic backgrounds. For QTL 7.03 and 10.03, it was possible to produce only one NILs' family each, due to the lack of more than one suitable $F_{4:5}$ line per QTL (i.e., still heterozygous for the two marker loci flanking the target QTL). The NILs' families were named first on the basis of the target QTL (e.g., 3.05), then of the code number of the corresponding introgression RIL (e.g., R8 for the RIL 8) and finally on the basis of their genotype at each of the two marker loci used for QTL introgression, i.e., BB or HH for the NIL homozygous for the B73 and H99 QTL alleles, respectively. The length of the introgressed chromosome segments ranged from 13 cM (QTL 4.10) to 33 cM (QTL 7.03) (Pea et al. 2009).

The six investigated NILs' families were: 3.05_R8, 3.05_R40, 4.10_R40, 4.10_R55, 7.03_R35 and 10.03_R63. They were evaluated per se (except families 3.05_R8 and 10.03_R63 because of seed shortage) and as crosses, thus giving rise to materials with different inbreeding coefficient ($F$). These materials were tested in three experiments,

distinguished as 1, 2 and 3 on the basis of the materials' $F$ values.

### Experiment 1: evaluation of NILs per se and of their reciprocal crosses ($F \approx 1$)

For the four NILs' families, 3.05_R40, 4.10_R40, 4.10_R55 and 7.03_R35, the two NILs per se and their two reciprocal crosses were tested (see Table S1 for a summary of the genotypes tested for each family). All these materials are assumed to be highly homozygous, except for the target QTL in the two reciprocal crosses, and hence have an $F$ value very close to 1. The experimental design was a randomized complete block with two replications. Plots were single rows spaced 0.85 m, including 19 plants (after thinning) at a density of 6.0 plants $m^{-2}$.

For each NILs' family, the genotypes' source of variation (3 *df*) was partitioned into the following components (Table S1): between mean values of NILs (BB vs. HH, 1 *df*, estimating the variation due to twice the QTL additive effect), between reciprocal crosses (RCs, 1 *df*, estimating the variation due to reciprocal effects) and mean value of NILs BB and HH versus mean value of RCs (1 *df*, estimating the variation due to the QTL dominance effect). Moreover, for the two NILs' families concerning the QTL in bin 4.10, the variation between families (FAM) and its interactions with additive, reciprocal and dominance effects were considered. As to the QTL effects, the additive effect ($a$) was calculated, for each family, as (HH − BB)/2; the dominance effect ($d$) was calculated as the difference between the mean value of the two RCs and the mean value of the two NILs.

### Experiment 2: evaluation at two plant densities of NILs crossed to the two related testers ($F = 0.5$)

The NILs of the six families were crossed to both parental inbred lines B73 and H99, thus obtaining crosses whose average $F$ value was 0.5. Crosses were tested at two plant densities (PD), i.e., 4.5 and 9.0 plants $m^{-2}$. The experimental design was a split-split-plot with three replications; the two PD were the main plots, the two testers the sub-plots (such a distinction was necessary because of the expected large difference in plant height between B73 and H99), and the two NILs were the sub-sub-plots. Two border rows were used to separate the two main plots as well as the two sub-plots. Sub-sub-plots were single rows spaced 0.85 m between rows, including (after thinning) 15 and 27 plants for the low and high PD, respectively.

For each NILs' family, the genotypes' source (3 *df*) was partitioned into the following components (Table S1): between mean values of the two related inbred line testers (TS) across the two NILs' (1 *df*), between mean values of the NIL BB and the NIL HH across the two testers (BB vs. HH, 1 *df*, estimating the variation due to the average effect of the QTL allele substitution) and interaction TS × (BB vs. HH) (1 *df*, estimating the variation due the QTL dominance effect). For the two pairs of NILs' families concerning bins 3.05 and 4.10, the variation between FAM and all the corresponding interactions involving FAM were also considered.

The average effect of the QTL allele substitution was calculated as the difference between the crosses'mean value of HH and the crosses' mean value of BB [i.e., (HH × B73 + HH × H99)/2 − (BB × B73 + BB × H99)/2]. According to Falconer and McKay (1996), this average effect is equal to: $a + d(q - p)$, where $p$ and $q$ are the average allelic frequencies over the two related testers. Because both average allelic frequencies are equal to 0.5 ($p$ being equal to 1 for one tester and equal to 0 for the other), the average effect of the QTL allele substitution can be equaled to $a$. The $d$ effect was calculated as the difference between the mean value (BB × H99 + HH × B73)/2 and the mean value (BB × B73 + HH × H99)/2.

### Experiment 3: evaluation of NILs crossed to four unrelated testers ($F \approx 0$)

The six families of NILs were also crossed to four unrelated inbred line testers, i.e., A632 and Lo1016, of the SSS heterotic group, and Mo17 and Va26, of the LAN heterotic group. These inbreds were chosen because they were well adapted to our environments and because they differed from one another and from the two parental inbreds, B73 and H99, in both molecular and agronomic characteristics (Livini et al. 1992; Pejic et al. 1998; Frascaroli et al. unpublished data). Therefore, the $F$ value of such crosses is expected to be very close to 0. The experimental design was a randomized complete block with two replications. Plots were single rows spaced 0.85 m, including 19 plants at 6.0 plants $m^{-2}$. For each NILs' family, the genotypes' source (7 *df*) was partitioned into the following components (Table S1): among TS (3 *df* estimating the variation due to the four testers), BB versus HH (1 *df*, due to the average effect of the QTL allele substitution), interaction TS × (BB vs. HH) (3 *df*, due to the QTL dominance effects). Moreover, the 3 *df* for TS and for its interactions were partitioned into three sources with 1 *df* each: between heterotic groups (i.e., SSS vs. LAN), within SSS (i.e., A632 vs. Lo1016) and within LAN (i.e., Mo17 vs. Va26). For QTL in bins 3.05 and 4.10, the variation between FAM and all the corresponding interactions were also considered.

The average effect of the QTL allele substitution was again calculated as the difference between the crosses' mean value of HH and the crosses' mean value of BB. In this case, the QTL allelic frequencies over the four testers

are not known and more than two alleles could be involved. Hence, it follows that the average effect of the QTL allele substitution is not comparable to the *a* value estimated in the previous two experiments, unless the QTL allelic frequencies across testers are equal to 0.5 (as was the case for Experiment 2), and/or *d* is equal to zero. As to the interaction TS × (BB vs. HH), the component (SSS vs. LAN) × (BB vs. HH) is of particular interest, as it reflects the change of dominance effects from crosses within heterotic group to crosses between heterotic groups. The crosses within heterotic group are the ones involving NILs homozygous for a given QTL allele (e.g., BB) and the two inbred testers of the same heterotic group as that of the QTL allele donor parent (A632 and Lo1016 of the SSS group as the donor B73), while the crosses between heterotic groups are the ones involving the same NILs and the two inbred testers of the opposite heterotic group (e.g., BB combined with Mo17 and Va26). The effect associated with this interaction component was calculated as the difference between the mean value of the four crosses between heterotic groups (i.e., the two crosses BB × LAN and the two crosses HH × SSS) and the mean value of the four crosses within heterotic groups (the two crosses BB × SSS and the two crosses HH × LAN).

### Field techniques common to all trials

The three experiments were always conducted at Cadriano (Bologna, Italy; 44°33′ N lat., 11°24′ E long.) for 3 years (2008–2010); in each year (environment), the trials of Experiments 1, 2 and 3 were adjacent in the same field. Trials were treated using the same standard techniques for maize cultivation in the region. Sowing was made at mid-end of April. Fertilizer rates were 45 kg $ha^{-1}$ for P (all applied before sowing) and 200 kg $ha^{-1}$ for N (half before sowing and half after thinning). Weed control was made mechanically and by hand when needed. To attain favorable growing conditions, four to five irrigations were made from the mid-end of stem elongation (1–2 weeks before silking) to the mid-end of the milk stage (2–3 weeks after silking), providing on the whole 60–80 mm of water in each trial. Trials were hand-harvested in the first half of September, by discarding the first and the last plant of each row in Experiments 1, 2 (low PD) and 3, or by discarding the first two and the last two plants in Experiment 2 (high PD).

### Data collection and statistical analysis

In all trials, data were taken at the single plot level for the following traits: (1) juvenile vigor (JV, cm, at approximately the 10th leaf stage), estimated as distance from ground to the tip of the uppermost leaf; (2) days to pollen shedding (PS, days), as interval between sowing date and PS date (assessed when 50% of plants had extruded anthers); (3) anthesis-silking interval (ASI, days), as difference between silking date (when 50% of plants had extruded silks) and PS date; (4) plant height (PH, cm), measured at the base of the tassel; (5) largest stalk diameter (SD, mm), measured on the second elongated internode; (6) kernel moisture at shelling (KM, %); (7) ears per plant (EP, no.); (8) grain yield per plant (GYP, g); (9) kernel weight (KW, mg); (10); number of kernels per plant (KP, no.), calculated as ratio between GYP and KW; (11) number of kernels per ear (KE, no.), calculated as ratio between GYP and the product between EP and KW. JV, PH and SD were investigated on five competitive plants per plot, while all other traits were investigated at the whole plot level; KW was the mean of a sample of 200 kernels per plot. Both GYP and KW were adjusted to 15.5% KM. JV was investigated in only one environment in Experiment 1, whereas SD was not investigated in Experiment 3. Because ears were kept for a few days in a drier (at 35°C) before shelling, the KM values have no biological meaning and, hence, they are not presented and discussed.

The analysis of variance (ANOVA) of each experiment was conducted separately for each QTL and family of NILs. For those two QTL represented by two families (i.e., QTL 3.05, Experiments 2 and 3, and QTL 4.10, all the three experiments), the ANOVA was then combined across families. The effects due to years (or environments, EN, 2 *df*) and all the interactions involving environments and the other sources of variation were also investigated. A mixed model of ANOVA was followed, considering plant densities (only for Experiment 2) and genotypes as fixed, and environments as random factors.

## Results

### Comparison among environments (years) within experiment

The ANOVA (Table S2) pointed out that the differences among environments within each experiment were significant for most traits, thus indicating that, despite the investigation being always conducted in the same location, genotypes were grown across widely different environmental conditions. As a general trend, the most favorable conditions were attained in the first year and the least favorable in the third (means not shown). Despite the significant differences among environments, the genotype × environment interaction was significant only in some instances (considering all families and traits, 13% for Experiment 1, 37% for Experiment 2 and 7% for Experiment 3), likely because of the irrigation supplied during the

summer season (thus reducing the effects of the rainfall vagaries) and because of the peculiarity of the investigated genotypes, which were derived from inbreds well adapted to our environments. Therefore, the results provided by the genotypes tested in each experiment are presented and discussed as means across the three environments.

### Comparison among genotypes within experiments and between plant densities

In Experiment 1, the differences among the four genotypes of each NILs' family were significant for most traits (Table S2). The significance of such differences was primarily due to the sources estimating both the additive and dominance effects, while the difference between reciprocal crosses was significant in 2 cases out of 40 (i.e., 5%), thus indicating that maternal and/or cytoplasmic effects were negligible. The mean values of NILs and of their crosses are given in the supplemental materials (Table S3).

In Experiment 2, the difference between plant densities (PDs) within each family was significant in almost all instances (87% considering all families and traits, Table S2). To summarize, the mean values of the two PDs across all genotypes are given in Table S4. From 4.5 to 9.0 plants $m^{-2}$, there was an increase in PS, ASI, PH and KE, and a decrease in SD, GYP and its components EP, KW and KP. The increase for KE at high PD, in contrast to the other yield components, was due to the absence of small secondary ears, which were rather numerous at the low PD. These findings thus indicate that, as compared to the low PD, high PD led to a stress level appreciable for all the traits of the adult plant (in fact, the PD effect was not significant only for JV measured during the vegetative developmental stage). Interestingly, GYP, i.e., a trait whose expression is affected throughout the plant's entire life cycle, showed the most pronounced decline due to the increase in PD. This decline, however, was lower than 50% (i.e., 39%) and, hence, the higher mean value for yield as expressed per unit area (not shown) was detected in the high PD (8.78 and 7.19 Mg $ha^{-1}$ for high and low PD, respectively).

The differences among crosses within each family in Experiment 2 were always significant and largely due to the different effects of the two related testers. To summarize, the mean values of B73 and H99 across all NILs and across the two PDs are presented in Table S4. The comparison between the two inbred testers was highly significant for all traits; in particular, the mean value of B73 was always higher than that of H99, with the exception of EP. The PD × crosses interaction within each family was significant in several instances, but almost always due to the component PD × TS; this interaction was always of size, as the difference between the mean values of the two testers from low to high PD was enhanced for PS, ASI and PH, while it was reduced for all other traits. The mean values over the two PDs of the four crosses within each of the six families are given in the supplemental materials (Table S5).

In Experiment 3, the differences among crosses of each NILs' family with the four unrelated testers were significant for most traits (Table S2). A large part of the variation among crosses was due to the effects of the four inbred testers, with Lo1016 always later and taller and often more productive than the other three inbreds (Table S6).

### Comparison among experiments

The mean values across environments and families of each experiment are presented in Table 1; to make the experiments comparable, the mean values of only the four families of NILs common to the three experiments are shown. However, the three experiments should be compared cautiously, mainly because these were conducted as different trials (although the one adjacent to the other) and because of the peculiar characteristics of the testers in Experiment 2 and 3. Nonetheless, it is interesting to note that the mean values of Experiment 2 were generally intermediate between those of Experiment 1 (which were the lowest for several traits) and Experiment 3, consistently with the heterozygosity level of the materials tested in each experiment. On the contrary, the coefficients of variation (CVs) were generally the highest in Experiment 1 (Table 1). These latter findings are indicative of the greatest reaction to the uncontrolled sources of variation of the less vigorous inbred materials grown in Experiment 1.

### Analysis of the QTL effects

The effects of the six QTL in the three experiments are presented in Table 2. As to the QTL 3.05_R8, in Experiment 2, the additive effect ($a$) was significant only for SD, while the dominance effect ($d$) was significant for JV, GYP and its components KW and KP. For all these traits, the $|d/a|$ ratio was superior to 1 (thus indicating overdominance) and, in particular, it was 2.4 for GYP. The interactions PD × $a$ and PD × $d$ effects (Table S2) were not significant for any trait. In Experiment 3, the average effect of the QTL allele substitution was significant for EP; the interaction (SSS vs. LAN) × (BB vs. HH) was significant for KP and KE and the effect was positive, indicating that the NILs BB and HH performed relatively better with the two inbred testers of the opposite heterotic group (i.e., with LAN and SSS, respectively).

The effects of the QTL in bin 3.05 was also studied in the family R40 (i.e., 3.05_R40) and in all the three experiments. The $a$ effect for GYP was significant in both Experiments 1

**Table 1** Mean values and coefficient of variations (CV) for the traits investigated in Experiment 1 (NILs per se and their reciprocal crosses), Experiment 2 (NILs crossed to the two related testers) and Experiment 3 (NILs crossed to four unrelated testers) across three environments (years) and across the four families of NILs common to the three experiments

| Trait | Mean | | | CV (%) | | |
|---|---|---|---|---|---|---|
| | Experiment | | | Experiment | | |
| | 1 | 2 | 3 | 1 | 2 | 3 |
| JV (cm) | 111 | 129 | 110 | 5.2 | 5.4 | 4.9 |
| PS (days) | 59.5 | 61.3 | 60.2 | 2.1 | 1.8 | 2.2 |
| ASI (days) | 2.4 | 0.5 | 0.4 | – | – | – |
| PH (cm) | 138 | 196 | 242 | 4.6 | 2.6 | 4.1 |
| SD (mm) | 23.2 | 24.2 | – | 5.7 | 4.6 | – |
| GYP (g) | 64 | 135 | 157 | 17.9 | 8.5 | 10.2 |
| KW (mg) | 189 | 245 | 298 | 5.3 | 4.2 | 4.9 |
| KP (no.) | 344 | 495 | 531 | 17.0 | 8.6 | 10.8 |
| EP (no.) | 1.25 | 1.22 | 1.04 | 14.7 | 7.9 | 8.6 |
| KE (no.) | 276 | 412 | 509 | 14.8 | 9.2 | 8.2 |

*JV* juvenile vigor (as distance from ground to the tip of the uppermost leaf), *PS* pollen shedding (as interval from sowing), *ASI* anthesis-silking interval, *PH* plant height, *SD* largest stalk diameter (measured on the second elongated internode), *GYP* grain yield per plant, *KW* kernel weight, *KP* number of kernels per plant, *EP* number of ears per plant, *KE* number of kernels per ear

and 2, and was mainly due to the component KW; moreover, the $a$ effect was always positive, indicating that the increasing allele was of H99 origin. Positive $a$ effects were also found for ASI and SD in Experiment 2. The $d$ effect was significant in both experiments for several traits and, in particular, for GYP and its components KP and KE. The $|d/a|$ ratio for GYP was in the overdominance range: 1.4 for both Experiments 1 and 2. The interactions PD $\times$ $a$ and PD $\times$ $d$ were not significant for any trait (Table S2). In Experiment 3, the average effect of the QTL allele substitution was significant and positive for ASI as well as for GYP and its components KW and KE. The interaction (SSS vs. LAN) $\times$ (BB vs. HH) was significant for ASI, GYP, KE and KP and the effect was always positive.

A combined analysis of the 3.05 QTL effects across the two families was conducted for Experiments 2 and 3. The results (not shown) confirmed the significance of both the $a$ and $d$ effects in Experiment 2 for GYP; the overdominant gene action for GYP was also confirmed ($|d/a|$ ratio of 1.6). The interactions of both $a$ and $d$ effects with family was not significant in almost all instances, suggesting that the gene action of the QTL 3.05 was not much affected by the genetic background in crosses with related testers. For Experiment 2, the combined ANOVA also revealed the significance of the interaction PD $\times$ $a$ effect for GYP; the interaction was of size, with $a$ being larger at low than at high PD. In contrast, the $d$ effect did not significantly vary from low to high PD and, hence, the $|d/a|$ ratio proved to be much higher at 9.0 rather than at 4.5 plants $m^{-2}$ (3.0 and 1.2, respectively). To have a better insight of these trends, $a$ and $d$ effects for GYP at the two PDs are presented in Fig. 1a. In Experiment 3, the significance of the interaction (SSS vs. LAN) $\times$ (BB vs. HH) was confirmed for GYP, KE and KP. An exemplifying graphic representation of this type of interaction for GYP is shown in Fig. 2.

For QTL 4.10_R40, the $a$ effect in Experiment 1 was significant only for PH and KW. The $d$ effect was significant for several traits, including GYP; the $|d/a|$ ratio for GYP was largely superior to 1. In Experiment 2, the $a$ effect was significant in most instances, including GYP, and generally negative, indicating that the increasing allele was provided by B73. The $d$ effect was significant for ASI ($d$ negative), PH, GYP, KP, EP and KE ($d$ positive). The $|d/a|$ ratio for GYP was again superior to 1, though much lower than the ratio obtained for GYP in Experiment 1. The PD $\times$ $a$ interaction was significant for ASI, while the PD $\times$ $d$ interaction was significant for GYP, KW and KP (Table S2). In Experiment 3, the average effect of the allele substitution was significant for some traits but not for GYP, whereas the interaction (SSS vs. LAN) $\times$ (BB vs. HH) was not significant for any trait.

For QTL 4.10_R55, the $a$ effect, when significant, was always negative (except for SD) in both Experiments 1 and 2, thus confirming that the increasing allele for QTL 4.10 was of B73 origin. The $d$ effect, when significant, was always positive (except for PS). For GYP, the $a$ effect was significant only in Experiment 2, whereas the $d$ effect was significant in both experiments, with the $|d/a|$ ratio largely superior to 1 in Experiment 1 and slightly lower than 1 in Experiment 2. No significant interactions involving PD and $a$ and $d$ effects were found (Table S2). In Experiment 3, nonsignificant effects were found for almost all traits.

**Table 2** Effects and dominance ratios of the six QTL investigated in Experiments 1, 2 and 3

| QTL and trait | Experiment 1 | | | Experiment 2[a] | | | Experiment 3 | |
|---|---|---|---|---|---|---|---|---|
| | $a$[b] | $d$[b] | $\|d/a\|$ | $a$[c] | $d$[c] | $\|d/a\|$ | [d] | [e] |
| 3.05_R8 | | | | | | | | |
| JV (cm) | – | – | – | 3.8 | 6.9** | 1.8 | −3.3 | 1.9 |
| PS (days) | – | – | – | 0.4 | −0.1 | 0.2 | 0.1 | 0.3 |
| ASI (days) | – | – | – | 0.1 | 0.0 | 0.2 | 0.1 | 0.0 |
| PH (cm) | – | – | – | 1.6 | 2.3 | 1.5 | 4.4 | -2.7 |
| SD (mm) | – | – | – | 1.6** | 0.2 | 0.1 | – | – |
| GYP (g) | – | – | – | 3.8 | 9.2** | 2.4 | −1.1 | 3.6 |
| KW (mg) | – | – | – | −4.7 | 6.7** | 1.4 | −10.0 | −12.6 |
| KP (no.) | – | – | – | 20.3 | 21.6* | 1.1 | 13.7 | 39.8* |
| EP (no.) | – | – | – | 0.03 | 0.01 | 0.3 | 0.06* | 0.00 |
| KE (no.) | – | – | – | 4.5 | 12.6 | 2.8 | −15.2 | 33.7* |
| 3.05_R40 | | | | | | | | |
| JV (cm) | −0.9 | 2.1 | 2.3 | −2.4 | −1.1 | 0.5 | −3.7 | 2.0 |
| PS (days) | 0.3 | −0.7 | 2.3 | 0.2 | 0.1 | 0.4 | −0.1 | 0.0 |
| ASI (days) | 0.1 | −0.2 | 1.9 | 0.7** | −0.3 | 0.4 | 0.6** | 0.9** |
| PH (cm) | −2.5 | 6.0** | 2.4 | 0.8 | 2.7 | 3.4 | −3.2 | 0.7 |
| SD (mm) | 0.9 | 1.0* | 1.1 | 2.0** | −0.2 | 0.1 | – | – |
| GYP (g) | 9.4* | 13.2** | 1.4 | 6.0* | 8.3** | 1.4 | 9.4** | 10.8** |
| KW (mg) | 13.8* | 5.5 | 0.4 | 6.9** | 4.7 | 0.7 | 9.7** | 3.1 |
| KP (no.) | 27.1 | 59.0** | 2.2 | 8.8 | 23.7** | 2.7 | 16.6 | 33.7** |
| EP (no.) | 0.06 | 0.05 | 0.8 | 0.02 | −0.02 | 0.9 | −0.02 | 0.02 |
| KE (no.) | 6.8 | 28.6** | 4.2 | 0.8 | 20.3** | 24.1 | 28.4** | 25.0* |
| 4.10_R40 | | | | | | | | |
| JV (cm) | −4.0 | 5.4 | 1.4 | −4.3** | 2.4 | 0.6 | 3.7 | 1.6 |
| PS (days) | −1.9 | −0.3 | 0.1 | −1.2** | −1.0** | 0.9 | −1.2** | 0.0 |
| ASI (days) | −0.8 | 0.1 | 0.2 | −0.3 | 0.1 | 0.2 | 0.2 | 0.1 |
| PH (cm) | −9.8** | 8.1** | 0.8 | −4.1** | 3.6** | 0.9 | −2.4 | −0.8 |
| SD (mm) | −0.4 | 1.5** | 4.4 | 1.7** | 0.1 | 0.1 | – | – |
| GYP (g) | 2.9 | 20.1** | 7.0 | −8.2** | 16.5** | 2.0 | −5.1 | 4.1 |
| KW (mg) | 9.1** | −7.0 | 0.8 | 19.7** | −1.3 | 0.1 | 30.1** | −3.1 |
| KP (no.) | 4.1 | 118.3** | 28.7 | −73.1** | 64.3** | 0.9 | −68.8** | 14.5 |
| EP (no.) | −0.04 | 0.02 | 0.5 | −0.03 | 0.09** | 3.0 | 0.01 | 0.00 |
| KE (no.) | 16.6 | 103.5** | 6.2 | −51.8** | 23.2* | 0.4 | −73.6** | 13.8 |
| 4.10_R55 | | | | | | | | |
| JV (cm) | −3.8 | 7.1 | 1.9 | 0.6 | 4.2* | 7.2 | −2.2 | 6.4** |
| PS (days) | −0.4 | −0.3 | 0.7 | 0.4 | −0.9** | 2.4 | 0.1 | 0.1 |
| ASI (days) | 0.1 | −0.1 | 1.0 | −0.1 | 0.2 | 1.2 | 0.3 | 0.3 |
| PH (cm) | −6.1* | 3.3 | 0.5 | −7.7** | 1.0 | 0.1 | 0.6 | 2.5 |
| SD (mm) | 0.3 | 1.1 | 3.4 | 1.9** | −0.2 | 0.1 | – | – |
| GYP (g) | −8.8 | 18.8** | 2.1 | −7.5** | 6.8** | 0.9 | 2.2 | −5.0 |
| KW (mg) | 1.4 | −0.8 | 0.6 | −4.4* | 4.0 | 0.9 | −8.8* | 4.9 |
| KP (no.) | −64.1 | 128.3** | 2.0 | −24.3** | 22.2* | 0.9 | 20.1 | −27.3 |
| EP (no.) | −0.10 | 0.21 | 2.2 | 0.02 | 0.04* | 2.4 | 0.02 | −0.04 |
| KE (no.) | −33.5 | 80.4** | 2.4 | −27.3** | 3.5 | 0.1 | 10.4 | −0.9 |
| 7.03_R35 | | | | | | | | |
| JV (cm) | −8.1 | 2.3 | 0.3 | −1.9 | −0.4 | 0.2 | −1.8 | −0.7 |
| PS (days) | −0.6 | 0.4 | 0.6 | −0.2 | −0.2 | 1.3 | −0.2 | 0.3 |

**Table 2** continued

| QTL and trait | Experiment 1 | | | Experiment 2[a] | | | Experiment 3 | |
|---|---|---|---|---|---|---|---|---|
| | $a$[b] | $d$[b] | $\|d/a\|$ | $a$[c] | $d$[c] | $\|d/a\|$ | [d] | [e] |
| ASI (days) | 0.1 | −0.9 | 7.0 | −0.3 | 0.3 | 1.0 | 0.0 | 0.1 |
| PH (cm) | −7.7* | 15.7** | 2.0 | −8.6** | 2.9* | 0.3 | −14.8** | 7.5* |
| SD (mm) | 0.4 | −0.69 | 1.8 | 2.6** | 0.3 | 0.1 | – | – |
| GYP (g) | −3.1 | 7.5 | 2.4 | −10.1** | 4.0 | 0.4 | −14.4** | 7.3* |
| KW (mg) | −9.4** | 8.3* | 0.9 | −12.1** | 0.1 | 0.0 | −0.2 | 0.1 |
| KP (no.) | −6.6 | 23.2 | 3.5 | −9.9 | 12.5 | 1.3 | −42.3** | 19.3 |
| EP (no.) | 0.05 | 0.13 | 2.4 | −0.01 | −0.01 | 0.9 | 0.00 | 0.03 |
| KE (no.) | −15.9 | 1.2 | 0.1 | −4.1 | 17.3 | 4.3 | −43.8** | 9.6 |
| 10.03_R63 | | | | | | | | |
| JV (cm) | – | – | – | −2.4 | 11.7** | 4.9 | −1.8 | −0.9 |
| PS (days) | – | – | – | −0.2 | −1.1** | 5.9 | 0.0 | 0.1 |
| ASI (days) | – | – | – | −0.6* | −0.8** | 1.2 | 0.4 | 0.1 |
| PH (cm) | – | – | – | 0.5 | 1.3 | 2.4 | 1.8 | 3.5 |
| SD (mm) | – | – | – | 1.6** | 0.2 | 0.1 | – | – |
| GYP (g) | – | – | – | 2.8 | 22.4** | 8.0 | 1.2 | 4.0 |
| KW (mg) | – | – | – | −9.8** | 5.9* | 0.6 | −5.6 | −3.1 |
| KP (no.) | – | – | – | 25.3 | 67.4** | 2.7 | 10.0 | 17.3 |
| EP (no.) | – | – | – | 0.06 | 0.10** | 1.8 | −0.03 | 0.04 |
| KE (no.) | – | – | – | 5.8 | 38.6** | 6.7 | 23.3* | 4.3 |

*, ** : effect significant at $P \leq 0.05$ and $P \leq 0.01$, respectively

[a] Mean values across two plant densities

[b] $a$: additive effect calculated as (HH – BB)/2; $d$: dominance effect calculated as the difference between the mean value of the two RCs and the mean value of the two NILs

[c] $a$: additive effect calculated as the difference between the crosses'mean value of HH and the crosses' mean value of BB [i.e., (HH × B73 + HH × H99)/2 − (BB × B73 + BB × H99)/2]; $d$: dominance effect calculated as the difference between the mean value (BB × H99 + HH × B73)/2 and the mean value (BB × B73 + HH × H99)/2

[d] Average effect of the QTL allele substitution calculated as the difference between the crosses' mean value of HH and the crosses' mean value of BB

[e] Effect of the (SSS vs. LAN) × NILs interaction, calculated as the difference between the mean value of the four crosses between heterotic groups (i.e., BB × LAN and HH × SSS) and the mean value of the four crosses within heterotic groups (BB × SSS and HH × LAN)

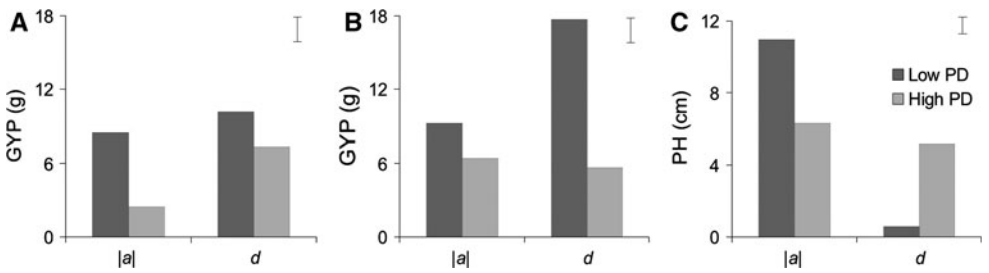


**Fig. 1** Additive ($a$) and dominance ($d$) effects at low and high PD (4.5 and 9.0 plants $m^{-2}$, respectively) in Experiment 2 for **a** QTL 3.05 and **b** QTL 4.10 (both across two NILs' families) in GYP and for **c** QTL 7.03 in PH (one NILs' family). Additive effects for both QTL 4.10 and 7.03 are negative. *Vertical bars* indicate standard errors

The combined analysis of the two NILs' families of QTL 4.10 (not shown) confirmed that the effects estimated in Experiments 1 and 2, when significant, were generally negative for $a$, while these were always positive for $d$ (except for PS). For GYP, the $d$ effect was significant in both experiments, with the $|d/a|$ ratio superior to 1, especially in Experiment 1. The combined ANOVA also revealed the significance of the interaction PD × $d$ effect

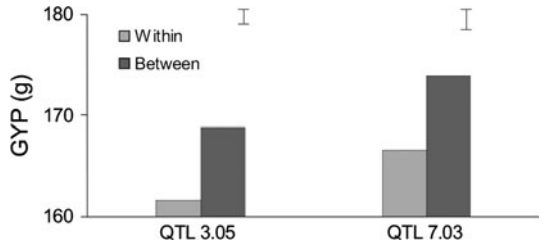


**Fig. 2** Mean values of the four crosses within heterotic groups (as BB × SSS and HH × LAN) and the four crosses between heterotic groups (as BB × LAN and HH × SSS) in Experiment 3, for GYP in QTL 3.05 (across two NILs' families) and QTL 7.03 (one NILs' family). *Vertical bars* indicate standard errors

for GYP, with $d$ being larger at low PD; consequently, the $|d/a|$ ratio was reduced from low to high PD (1.9 in the former case and 0.9 in the latter). For a better insight, $a$ (as absolute values) and $d$ effects at the two PDs are presented in Fig. 1b. No significant effects were found in Experiment 3. With regard to the interactions with families, the one involving the $a$ effects was significant in several instances (five out of ten) for both Experiments 1 and 2; the interaction involving the $d$ effect was not significant in Experiment 1 and significant for two traits in Experiment 2. For GYP, in particular, the significance of the interaction with families was attained for $a$ and $d$ effects (in Experiments 1 and 2, respectively), indicating that the genetic background was important in this respect.

For QTL 7.03_R35, in both Experiments 1 and 2, the significant $a$ effects were always negative, whereas the significant $d$ effects were always positive. In particular, for PH (i.e., the main trait for which this QTL was selected) the $a$ and $d$ effects were significant in both experiments, with the $|d/a|$ ratio largely superior to 1 in Experiment 1 but lower than 1 in Experiment 2. For GYP, the $a$ effect was significant only in Experiment 2, whereas the $d$ effect was not significant. Both interactions PD × $a$ and PD × $d$ were significant for PH, with $a$ (as absolute value) being larger at low PD and $d$ larger at high PD. As a result, the $|d/a|$ ratio was very close to 0 at low PD and was 0.8 at high PD; for a better insight, the $a$ (as absolute values) and $d$ effects are presented in Fig. 1c. In Experiment 3, the average effect of allele substitution was significant for several traits, including PH and GYP, and was always negative. The interaction (SSS vs. LAN) × (BB vs. HH) was significant for PH and GYP with positive effect in both instances (the interaction concerning GYP is presented in Fig. 2).

For QTL 10.03_R63, the $a$ effect in Experiment 2 was significant only for few traits (GYP was not included); the $d$ effect was significant and negative for PS and ASI and positive for JV, GYP and all its components. As a result, the $|d/a|$ ratio for GYP was much greater than 1, indicating a marked overdominance. The PD × $a$ interaction was significant for SD, while the PD × $d$ interaction was significant for KE (Table S2). In Experiment 3, the effects were not significant in almost all instances.

## Discussion

### Characterization of the heterotic QTL and relative importance of their additive and dominance effects

The effects of the selected QTL were consistent with the effects that they had exhibited in the previous studies of Frascaroli et al. (2007) and of Pea et al. (2009). In fact, QTL 3.05, 4.10 and 10.03 showed sizable $d$ effects for GYP and for some of its components, whereas QTL 7.03 showed a sizable $d$ effect for PH. This confirmation is an important issue, given the risk of obtaining false positive and/or inflated estimates of QTL effects, especially (1) when a mapping population not large in size is used (e.g., $N < 200$), (2) when QTL mapping and estimates of their effects are made by using the same data, and (3) when dealing with complex traits (for review, see Beavis et al. 1994; Kearsey and Farkuhar 1998; Melchinger et al. 1998). Moreover, with regard to PH for QTL 7.03, the present study allowed the detection of a significant and negative $a$ effect, which was not detected in the previous QTL analysis of Frascaroli et al. (2007), thus emphasizing the importance of the NILs approach for studying the QTL main effects, given the absence of biases due to the genetic background.

In addition, the $a$ effects for GYP were positively associated with the $a$ effects of its component KP as well as of PH (especially, in the two families of QTL 4.10 and in the family of QTL 7.03); such positive associations among $a$ effects were also found in previous QTL analyses (Frascaroli et al. 2007; Stuber et al. 1992) and were ascribed to close linkage and/or to pleiotropy. The $d$ effects were consistent when considering GYP, its component KP and, to some extent, the other component KE. In all other cases, the consistency of the $d$ effects was weaker or even negligible, mainly because of the modest importance of the $d$ effects for the other two GYP components, KW and EP, as well as for the other investigated traits. It should be noted that GYP and its main component KP are the outcome of a multiplicative function of their simpler components, which can show from negligible to complete dominance. These findings thus indicate that the high heterotic level of complex traits (especially fitness-related traits) can be notable not only when dealing with the whole contribution over many loci in crosses between different inbreds (e.g., Tollenaar et al. 2004; Yan et al. 2006), but also when dealing with the crosses of NILs' families differing in just one QTL (Melchinger et al. 2007; Semel et al. 2006). In fact, Falconer and Mckay (1996) pointed out that

heterosis for a complex trait can arise even in case of a single gene acting additively on the two components and affecting them pleiotropically in the opposite directions. The present study also shows that *d* effects, when significant, were always negative for PS and ASI and always positive for all other traits. Also these findings are in accordance, because the negative *d* effects for PS and ASI are indicative of a more rapid growth and of a better synchronization between male and female flowering. Therefore, our data confirmed that the dominant alleles are the ones more favorable and that the unidirectionality of the *d* effects is an essential prerequisite to attain a high heterotic level in hybrids.

Influence of the genetic background on the QTL effects

Since for QTL 3.05 and 4.10 the effects were investigated in two families, the interaction FAM × QTL effects could be analyzed. For QTL 3.05, these interactions proved to be negligible in almost all instances, thus suggesting that the QTL is quite stable across genetic backgrounds, i.e., not much prone to epistatic interactions; however, we cannot draw a general conclusion from this finding because only two families were investigated. For QTL 4.10, the interactions FAM × QTL effects (both *a* and *d*) were significant for some traits, especially GYP. Therefore, epistatic interactions seem to have some importance and, hence, marker-assisted selections (MAS) for this QTL might lead to inconsistent results depending on the recipient's genetic background. The role of the genetic background on MAS was investigated in several studies on different species (Bouchez et al. 2002; Chaïb et al. 2006; Reyna and Sneller 2001) and lack of consistency of QTL effects was not rare, especially when the QTL were transferred into unrelated genetic backgrounds and complex traits were considered. On the other hand, inconsistent results in different NILs' families could also arise from the contribution of small chromosome segments (relics) independent of the target QTL and fixed at random across the genome (Paterson et al. 1990). If this were true also for the NILs herein investigated, the effects of these relics could bias both the effect of the QTL under investigation and its interaction with the genetic background. Pea et al. (2009) characterized the NILs' families herein investigated for 19 SSR markers (i.e., one marker for each chromosome arm different from the one carrying the introgressed QTL) and results pointed out that the two NILs of each family were always identical with the only exception of family 4.10_R55, as the two NILs differed for the marker alleles identifying the long arm of chromosome 2. Therefore, such a difference could have at least partly contributed to the significant interaction FAM × QTL effects detected for some traits as to QTL 4.10.

Influence of the inbreeding level on the QTL effects

A clear consistency in *a* effects was observed from Experiment 1 to Experiment 2, thus indicating that these effects were not much influenced by inbreeding coefficients. A somewhat different situation was noted for *d* effects, as they were still consistent from one experiment to the other, though they were more pronounced for the inbred materials tested in Experiment 1, especially in case of GYP and KP for QTL 4.10 (both families) and of PH for QTL 7.03. To a certain extent, also the $|d/a|$ ratio showed a trend similar to that of *d* effect, attaining higher values more often in Experiment 1 than Experiment 2. These findings cannot be ascribed to scaling effects (as the mean values of Experiment 1 were much lower than those of Experiment 2); instead, they suggest the existence of epistatic interactions which might affect the *d* estimate of the single QTL at varying homozygosity levels in the background. Such epistatic interactions could be, at least partly, accounted for by assuming that the superiority of the heterozygote at the single QTL is less pronounced in crosses than in highly inbred materials, because in the former condition there can be a greater biochemical versatility that allows the attainment of the same QTL function by following different pathways. This hypothesis is consistent with the observation that heterosis can be affected by dosage-dependent regulatory genes operating in hierarchical networks and interacting with genes expressed downstream (Birchler et al. 2010).

For all four investigated QTL, *a* effects detected in Experiment 1 and/or 2 also showed a certain consistency with the average effects of allele substitution detected for the same traits in Experiment 3; in fact, when significant, these effects were often of similar size and always of the same sign. This finding is noteworthy because the effects of *a* and the average effects of allele substitution are comparable only when $p = q = 0.5$ across the four testers (especially in case the *d* effects are not negligible as for these heterotic QTL), thus indicating that the four unrelated testers do not carry all the same dominant alleles at the QTL in question. In fact, in case of complete dominance, homozygosity for the same dominant alleles in all inbred testers (implying $p = 1$ and $q = 0$) would have led to the cancelation of the effects of the QTL allele substitution. The importance of the role played by the testers in affecting the QTL effects, even leading to a change of their signs from one tester to the other in case of QTL showing overdominance, was emphasized by Frascaroli et al. (2009). Moreover, the significance of the interaction TS × (BB vs. HH), especially for QTL 3.05 and 7.03, was mainly due to the component (SSS vs. LAN) × (BB vs. HH), with the other two components [(A632 vs. Lo1016) × (BB vs. HH) and (Mo17 vs. Va26) × (BB vs. HH)] being negligible in

almost all instances. The effect of the former interaction, when significant, was always positive, thus indicating the relative superiority of the crosses that, at the QTL in question, carry alleles derived from opposite heterotic groups. In fact, the NIL BB, homozygous for the QTL allele of SSS origin, performed relatively better when combined with testers of the LAN group, whereas the NIL HH performed relatively better with the SSS inbred testers. These results, besides further stressing the importance of dominance effects for the investigated QTL, suggest that for each QTL the two unrelated inbred testers of a given heterotic group (e.g., A632 and Lo1016 for SSS) are homozygous for the same (or similar) allele/s as that provided by the parental inbred of the same group (i.e., B73). The same should be likely true for the other two inbred testers (Mo17 and Va26), which can be assumed to be homozygous for the same (or similar) complementary allele/s as that provided by the other parental inbred (H99). This hypothesis is consistent with the one expressed by Schön et al. (2010), who studied the congruency of heterotic QTL detection and estimate of their effects in three different mapping populations; such populations included the one investigated by Frascaroli et al. (2007) and arose from the same heterotic pattern SSS × LAN. Schön et al. (2010) suggested that for important loci affecting heterosis, complementary alleles are fixed in the two opposite heterotic groups and that they likely remain unchanged in the subsequent intra-group selections, until new genetic variation is developed by introducing in these groups genetic material of different origin.

### Influence of the competition level (low vs. high PD) on QTL effects

Despite the large effects of the two PDs in Experiment 2 on almost all traits, the interactions between PD and QTL effects were often negligible. This finding could represent a further confirmation of the QTL stability, even though one could argue that the competition level among plants at 9.0 plants $m^{-2}$ in our quite favorable environments was not as high as needed to attain a rather discriminative growing condition. Previous studies (Duvick 2005; Liu and Tollenaar 2009) emphasized the role of plant density in affecting heterosis, as an increase of the former was followed by an increase of the latter. On the other hand, the discrepancy between those and our findings could be accounted for, at least partly, by the fact that we studied the effects of the single QTL, whereas the cited papers studied the effects of a multitude of QTL acting together and, hence, also took into account the possible contribution of the complex interactions among them. However, some important exceptions were noted in our study, mainly concerning GYP (for the *a* effect of QTL 3.05 and the *d* effect of QTL 4.10, both across families) and PH (for both *a* and *d* effects of QTL 7.03_R35). Therefore, at least for these traits and QTL, the competition level among plants played a certain role in influencing their effects and this aspect should not be neglected in possible future studies on such QTL. The role exerted by plant density on the single QTL effects was also investigated by Gonzalo et al. (2006); they tested segmental introgression lines (derived from the cross B73 × Tx303) and their hybrids with Mo17 and found that the QTL effects for inbreds and their crosses varied depending on PD. On the other hand, in a study conducted on a population of RILs derived from the cross B73 × Mo17, LeDeaux et al. (2006) found that heterotic QTL were rather stable at varying stress levels, including low versus high plant density, with very few QTL being affected. A possible explanation for these contrasting findings could be that in the study of LeDeaux et al. (2006), both parents were well adapted to temperate climatic regions, whereas in the study of Gonzalo et al. (2006) one parent was of subtropical origin.

### Prospects of future studies on these QTL

The importance of the effects of the investigated QTL provides the stimulus to conduct further studies on the materials herein presented. In particular, studies of fine mapping could be made, so as to gain useful information on the cause of the association among traits (linkage vs. pleiotropy) and the cause of the QTL heterotic effect (true overdominance vs. pseudo-overdominance). McMullen et al. (2009) pointed out that centromeric regions are characterized by low recombination rate and thus can be associated with heterotic phenomena determined by linkage of favorable alleles in the repulsion phase (pseudo-overdominance). In this connection, it is noteworthy that bins 3.05 and 10.03 are centromeric and that bin 7.03 is adjacent to the centromeric bin 7.02. Moreover, the average length of the introgressed chromosome segments was of ca. 22 cM (Pea et al. 2009) and, hence, the possibility that two or even more linked genes controlling the same trait are included in these segments should not be neglected. This could be likely the case for GYP in QTL 10.03, which showed the highest dominance effect of all investigated QTL associated with a negligible additive effect, a finding suggestive of genes linked in repulsion. Also QTL 3.05 and 4.10 are of great interest for fine mapping, because of the importance of their dominance effects and because two different NILs' families are available for each QTL. In particular, in QTL 4.10, the significance of both the FAM × *a* and the FAM × *d* interactions for GYP suggests that a careful choice of the NILs' family to be used as the base material for fine mapping should be made. In this respect, family 4.10_R55 seems to be more suitable

than 4.10_R40, because the former proved to be less prone to interactions with PD. QTL 7.03 seems to be the most appealing of the investigated QTL for fine mapping, because the phenotyping can be made on plant height, i.e., a trait easily measurable, with high heritability and less affected by inbreeding depression as compared to grain yield. Moreover, plant height is an interesting model trait for relating the expression of genes and the manifestation of heterosis, as pointed out by Uzarowska et al. (2007).

The above NILs' families can also represent a valuable base material to undertake studies aimed at elucidating the molecular bases of heterosis. Structural genome diversity between inbred lines, as well as gene and allelic expression diversity between parental lines and their corresponding $F_1$ hybrids, have been described in relation to heterosis (reviewed in Hochholdinger and Hoecker 2007 and Springer and Stupar 2007). In recent studies, high levels of structural genome diversity, which may contribute to heterosis, have been detected on the whole maize genome (Beló et al. 2010; Springer et al. 2009). Moreover, the application of next-generation high-throughput sequencing has further widened the possibilities of genome-wide comparisons (see Lai et al. 2010 for an example). Nevertheless, extensive experiments dedicated to evaluate structural genome diversity effect on plant phenotypes should be performed to elucidate its role in determining heterosis. As recently pointed out, also results of studies on gene expression diversity still do not allow a consensus view, since varying levels of additive and non-additive gene actions were shown in heterotic hybrids (Birchler et al. 2010). Such studies have been so far prevailingly conducted by comparing parental lines of different origins and their hybrids, thus taking into account a multitude of possible causative genes and chromosomal regions spread all over heterogeneous genomes. In this respect, the NILs' families herein described are unique in that they carry heterotic QTL within near-isogenic contexts. Therefore, investigations of structural and genic/allelic expression diversity on such materials might clarify the complex picture by focusing on restricted chromosome regions bearing already validated and well-characterized heterotic QTL for specific phenotypic traits. This latter aspect might also help in bridging the genotype-to-phenotype gap by allowing hypothesis-driven phenotypic validation of heterotic effects.

## Conclusion

The present study allowed the validation and characterization of four heterotic QTL, which showed sizable dominance effects especially for GYP, its main component KP and other important traits such as PH. These findings were corroborated by the consistency of the QTL effects exhibited across genetic backgrounds, levels of inbreeding and of competition among plants, as determined by low and high PD. Significant interactions of QTL effects with genetic background and PD were found in some instances, especially for GYP, but they were always of size and led to moderate changes of $a$ and $d$ effects. The $d$ effects and the $|d/a|$ ratios for the investigated QTL tended to be higher in the inbred materials, suggesting the importance of the role played by the homozygosity level of the overall genetic background in modulating such effects. The importance of dominance effects at least for two QTL was also confirmed in crosses with unrelated inbred testers belonging to opposite heterotic groups, suggesting that complementary QTL alleles were fixed in these groups. All these findings provide the stimulus to carry out further studies on such QTL aimed at their fine mapping, so as to gain information on the role played by true- and pseudo-overdominance in affecting heterosis. Moreover, these NILs' families and their crosses can represent a valuable material for use in conducting studies aimed at elucidating the molecular bases of heterosis.

**Acknowledgments** This study was conducted with the financial support of the Italian Ministry of University and Research, PRIN (*Progetti di Ricerca di Interesse Nazionale*), Project: "Sequence variability and heterosis in crop plants" (2007) and Project: "Towards the elucidation of the molecular bases of heterosis in crop species: characterization and fine mapping of QTL for heterosis in maize" (2010), and with the financial support of the University of Bologna, Grant RFO (2008-9).

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