

# Identification of combining ability loci for five yield-related traits in maize using a set of testcrosses with introgression lines

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**Abstract** Combining ability is essential for hybrid breeding in crops. However, the genetic basis of combining ability remains unclear and has been seldom investigated. Identifying molecular markers associated with this complex trait would help to understand its genetic basis and provide useful information for hybrid breeding in maize. In this study, we identified genetic loci of general combining ability (GCA) and specific combining ability (SCA) for five yield-related traits under three environments using a set of testcrosses with introgression lines (ILs). GCA or SCA of the five yield-related traits of the ILs was estimated by the performance of testcrosses with four testers from different heterotic groups. Genetic correlations between GCA of the traits and the corresponding traits per se were not significant or not strong, suggesting that the genetic basis between them is different. A total of 56 significant loci for GCA and 21 loci for SCA were commonly identified in at

least two environments, and only 5 loci were simultaneously controlling GCA and SCA, indicating that the genetic basis of GCA and SCA is different. For all of the traits investigated, positive and significant correlations between the number of GCA loci in the ILs and the performance of the corresponding GCA of the ILs were detected, implying that pyramiding GCA loci would have positive effect on the performance of GCA. Results in this study would be useful for maize hybrid breeding.

## Abbreviations

|     |                            |
|-----|----------------------------|
| GCA | General combining ability  |
| SCA | Specific combining ability |
| QTL | Quantitative trait loci    |
| ILs | Introgression lines        |
| WH  | Wuhan                      |
| BD  | Baoding                    |
| YP  | Yield per plant            |
| PH  | Plant height               |
| KN  | Kernel number per row      |
| RN  | Row number                 |
| KW  | 100-Kernel weight          |

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## Introduction

Maize (*Zea mays* L.) is one of the most important crops in the world, and the demand of maize production is increasing in recent years since it has been extensively used to feed animals or as a new source of bioenergy. Studies have suggested that the application of hybrids, especially single cross hybrids leads to dramatically increasing of the yield of crops. In maize, for instance, deployment of hybrid vigor or heterosis contributes 40 % of grain yield increase in China (Li 2009).

There are two distinguished steps in hybrid breeding, one is to develop inbred lines, and the other is to select proper parent inbred lines to combine elite hybrids (Lee and Tollenaar 2007). In breeding practice, it is well known that yield performance of hybrids could not be predicted by the performance of their parents per se (Hallauer 1990). On the contrary, it is largely controlled by the potential to create superior hybrids, or combining ability of the parental lines. Therefore, it is important to select inbreds with high combining ability in hybrid breeding. Combining ability was partitioned into general combining ability (GCA), which is primarily caused by additive effects, and specific combining ability (SCA), which mainly results from non-additive effects (Sprague and Tatum 1942). GCA of an inbred line is measured as the average performance of all hybrids with that inbred line as one parent, and SCA of specific combinations or crosses is measured by the deviation of the hybrid performance from the GCA of the parents (Xu 2010).

With the development of DNA markers, heterosis or hybrid performance can be predicted by genetic distance; however, it was not promising for predicting performance of inter-group hybrids (Melchinger 1999). Chromosomal loci or quantitative trait loci (QTL) associated with heterosis can be used to predict heterosis and possibly provide targets for marker-assisted selection in hybrid breeding (Cerna et al. 1997; Joshi et al. 2001; Jordan et al. 2003; Liu et al. 2004). For instance, a model designated TCSM (total contribution of the selected markers) was successfully developed to predict hybrid performance based on the QTL for hybrid performance in maize (Vuylsteke et al. 2000). Schrag et al. (2006) also detected some significant markers associated with single-cross hybrid performance for grain yield and grain dry matter content, and they found that the prediction efficiency increased based on the models enhancing the GCA approach with SCA estimates. Since combining ability has long been used in maize hybrid breeding, QTL mapping or identification significant loci for combining ability would be also useful for heterosis prediction and molecular breeding.

However, combining ability is a complex trait, and GCA and SCA of the maize grain yield interacted strongly with environment (Rojas and Sprague 1952; Walejko and Russell 1977). Moreover, it is expensive and time-consuming to conduct numerous repeated field trials to estimate GCA and SCA. Although QTL for heterosis has been identified using different types of molecular markers in several crops including maize (Schrag et al. 2006, 2010; Cerna et al. 1997; Joshi et al. 2001; Jordan et al. 2003; Liu et al. 2004), only few studies on QTL mapping for GCA have been reported in maize (Gu 2007; Lv et al. 2012) and rice (Qu et al. 2012) with populations of double haploid (DH), introgression lines (ILs) or backcross recombination inbred

lines (BCRIL) crossed with three testers. In addition, a whole-genome prediction model with accuracies ranged from 0.72 to 0.81 was built to predict the performance of hybrids by fitting effects for all single nucleotide polymorphisms (SNPs) in maize (Riedelsheimer et al. 2012). These studies suggest that it is possible to dissect the genetic basis of GCA with molecular markers. However, the association between QTL or marker loci and SCA of agronomic traits was seldom analyzed with segregating populations.

In comparison with other populations, ILs have relative simple genetic background and would have more advantages in dissection of the genetic basis of complex traits. In addition, estimation of GCA would be more accurate if more tester lines were used. In this study, genetic loci associated with combining ability (GCA and SCA) of five yield-related, agronomically important traits were analyzed with four testcrossing populations. The objectives of this study were to (a) estimate GCA of five yield-related traits in the ILs and analyze its correlation with the performance of their corresponding traits per se; (b) to evaluate SCA of ILs in different testcrosses (TCs); and (c) to detect molecular markers associated with combining ability of these traits, compare the genetic basis of GCA and SCA, and discuss the possibility of the application of these marker loci in maize hybrid breeding.

## Materials and methods

### Materials and planting

A set of ILs including 75 lines and four testers were used in this study. The ILs were constructed from a cross between Zong 3 and HB522 (Wang et al. 2007). Zong 3, a parent of an elite hybrid (Yuyu22) used in China, was used as recurrent parent, and HB522, a wax inbred, was the donor. A total of four cycles of backcrosses were conducted, and foreground and background selections were carried out by simple sequence repeat (SSR) markers at each generation. The 75 lines chosen out had introgressed fragments covering the whole genome with relatively uniform genomic background of the recurrent parent. The four testers are Huangzao 4 (HZ4), Mo17, B73 and Dan 340 (D340), which belong to different heterotic groups (Tang SPT, Lancaster, Reid and Zi330) (Teng et al. 2004). In the winter of 2009 and 2010, each IL was crossed to the four testers followed by the NCII mating design, and about 300 testcrosses were obtained in each year.

The TC hybrids were planted in three environments, Wuhan (WH, belongs to spring growing area in China) in 2010, Huanggang (HG, belongs to spring growing area) in 2011 and Baoding (BD, belongs to summer growing area in China) in 2011, following a field design of randomized

complete blocks with three replicates. Sowing dates in WH and HG were on 4 April 2010 and 1 April 2011, and that in BD were on 23 June 2011. Twelve or 24 seedlings were kept in each one- (in 2011) or two-row (in 2010) plot with a plant space of 25 cm within each row and 60 cm between adjacent rows (plant density was about 60,000 plants per ha), and the field management was same as the normal local maize field.

#### Trait measurements

At harvesting stage, plant height (PH, cm) was measured on the middle five to eight competitive plants in each plot, and ears on these plants were harvested for yield-related traits investigation. Four traits including yield per plant (YP, g), row number (RN), kernel number per row (KN), and 100-kernel weight (KW, g) were measured after the ears were dried by air. The former three traits were investigated on each ear, and two random 300-kernel samples from each plot were counted and weighed.

SCA and GCA effects were calculated with the method described by Ni et al. (2009):

$$s_{ij} = y_{ij} - \bar{y}_{..} - g_i - g_j;$$

$$g_i = \bar{y}_{i.} - \bar{y}_{..}$$

$s_{ij}$  = SCA effect;  $g_i$  = GCA effect of introgression line  $i$ ;  $g_j$  = GCA effect of tester  $j$ ;  $y_{ij}$  = phenotypic value of the TC hybrid between the introgression line  $i$  and tester  $j$ ;  $\bar{y}_{..}$  = overall mean; and  $\bar{y}_{i.}$  = average of the four hybrids between introgression line  $i$  and the four testers.

#### Genotyping

A total of 215 SSRs were employed to genotype the population. Besides the 121 SSR markers used for ILs construction (Wang et al. 2007), the other evenly distributed 94 polymorphic SSRs selected from 422 SSRs (<http://maizegdb.org>) were used to genotype the whole population.

#### Data analysis and marker-trait association detection

Two-factor ANOVA (analysis of variance) was conducted with the software DPS version 12.01 (<http://www.chinadps.net/>). Marker-trait associations were calculated with the method of single-factor ANOVA, and the mean values of each genotype and  $P$  values were given at each marker locus. For the significant loci clustered in a chromosomal region, only the locus with the least  $P$  value was selected.

#### Results

Performance of combining ability of the ILs and correlation analysis among the traits

Performance of combining ability of the five yield-related traits in the ILs is given in Table 1. The GCA and SCA of all the traits in the ILs were normally distributed (except for the GCA of kernel weight and plant height in the experiment of Huangguang in 2011) with averages around

**Table 1** Performance of GCA and SCA of the five traits in different environments

| Trait | Env <sup>a</sup> | GCA           |      |      | Range         |               |               |               |
|-------|------------------|---------------|------|------|---------------|---------------|---------------|---------------|
|       |                  | Range         | Skew | Kurt | SCA (B73)     | SCA (D340)    | SCA (HZ4)     | SCA (Mo17)    |
| YP    | 10WH             | −25.4 to 26.6 | 0.1  | −0.1 | −25.4 to 24.1 | −30.6 to 19.6 | −22.5 to 22.8 | −22.4 to 31.9 |
|       | 11HG             | −43.8 to 45.0 | 0.2  | −0.3 | −53.6 to 46.9 | −57.6 to 35.7 | −36.6 to 33.8 | −45.8 to 40.4 |
|       | 11BD             | −34.2 to 38.8 | 0.8  | 0.0  | −36.8 to 35.9 | −28.5 to 36.0 | −23.1 to 31.4 | −59.3 to 44.9 |
| KW    | 10WH             | −4.4 to 3.6   | −0.6 | −0.1 | −2.8 to 3.3   | −3.7 to 2.1   | −3.6 to 4.3   | −4.4 to 3.8   |
|       | 11HG             | −5.8 to 2.1   | 3.7  | −1.3 | −2.5 to 3.4   | −4.9 to 3.1   | −4.6 to 3.1   | −6.5 to 3.8   |
|       | 11BD             | −4.8 to 4.4   | 1.1  | −0.1 | −4.2 to 6.9   | −3.1 to 3.9   | −3.8 to 3.7   | −9.3 to 4.3   |
| RN    | 10WH             | −1.7 to 1.4   | −0.3 | 0.0  | −1.3 to 1.3   | −1.2 to 2.0   | −1.3 to 1.5   | −2.5 to 1.2   |
|       | 11HG             | −2.1 to 2.1   | −0.4 | 0.3  | −2.2 to 2.8   | −2.2 to 1.8   | −3.3 to 2.5   | −1.3 to 2.0   |
|       | 11BD             | −1.6 to 2.1   | 0.0  | 0.6  | −1.6 to 1.9   | −1.8 to 1.6   | −2.4 to 1.9   | −1.1 to 1.6   |
| KN    | 10WH             | −3.8 to 3.5   | −0.3 | −0.3 | −6.9 to 0.7   | −6.7 to 1.8   | −6.2 to 0.4   | −8.4 to 4.4   |
|       | 11HG             | −6.7 to 6.6   | 1.3  | −0.3 | −5.9 to 9.0   | −6.2 to 5.3   | −5.0 to 4.1   | −7.9 to 5.4   |
|       | 11BD             | −4.4 to 5.7   | −0.3 | 0.2  | −10.6 to 5.3  | −5.0 to 6.3   | −7.5 to 5.1   | −11.5 to 14.3 |
| PH    | 10WH             | −40.1 to 28.6 | 1.1  | −0.7 | −25.7 to 17.7 | −26.9 to 30.1 | −22.9 to 29.0 | −19.4 to 46.7 |
|       | 11HG             | −45.0 to 19.2 | 2.4  | −1.1 | −40.5 to 20.1 | −32.5 to 25.7 | −43.5 to 32.1 | −26.8 to 18.0 |
|       | 11BD             | −30.2 to 15.2 | 0.6  | −1.0 | −25.0 to 17.2 | −43.6 to 30.0 | −34.6 to 23.3 | −38.4 to 24.5 |

YP yield per plant, KW 100 kernel weight, RN row number, KN kernel number per row, PH plant height, Env environments

<sup>a</sup> 10WH, 11HG and 11BD are the tests at Wuhan, Baoding, and Huanggang in the year of 2010 and 2011, respectively

**Table 2** ANOVA for GCA and SCA of the five traits investigated

| Trait | Source | GCA  |      | SCA (B73) |      | SCA (D340) |      | SCA (HZ4) |      | SCA (Mo17) |      |
|-------|--------|------|------|-----------|------|------------|------|-----------|------|------------|------|
|       |        | F    | P    | F         | P    | F          | P    | F         | P    | F          | P    |
| YP    | G      | 1.47 | 0.03 | 1.40      | 0.05 | 1.08       | 0.34 | 1.14      | 0.27 | 0.87       | 0.74 |
|       | E      | 0.16 | 0.85 | 0.07      | 0.93 | 0.22       | 0.80 | 0.52      | 0.59 | 0.50       | 0.61 |
| KW    | G      | 2.39 | 0.00 | 1.49      | 0.03 | 1.47       | 0.03 | 1.60      | 0.01 | 1.38       | 0.06 |
|       | E      | 0.02 | 0.98 | 0.30      | 0.74 | 0.13       | 0.88 | 0.09      | 0.92 | 0.80       | 0.45 |
| RN    | G      | 5.64 | 0.00 | 1.88      | 0.00 | 1.94       | 0.00 | 1.65      | 0.01 | 1.11       | 0.30 |
|       | E      | 0.12 | 0.89 | 0.14      | 0.87 | 0.69       | 0.50 | 0.11      | 0.89 | 12.33      | 0.00 |
| KN    | G      | 1.95 | 0.00 | 0.68      | 0.96 | 1.29       | 0.10 | 1.53      | 0.02 | 1.34       | 0.08 |
|       | E      | 0.11 | 0.89 | 17.32     | 0.00 | 43.77      | 0.00 | 35.30     | 0.00 | 5.29       | 0.01 |
| PH    | G      | 3.07 | 0.00 | 1.96      | 0.00 | 1.39       | 0.05 | 1.39      | 0.06 | 1.79       | 0.00 |
|       | E      | 1.05 | 0.35 | 0.30      | 0.74 | 0.14       | 0.87 | 0.52      | 0.59 | 10.19      | 0.00 |

G genotype, E environment

zero (data not shown). Variations of the GCA of YP and PH in the ILs were larger than that for the GCA of other traits. In general, the SCA in the four TCs had similar distribution as that for GCA.

Two-factor ANOVA for the GCA and SCA of these traits was conducted, and significant difference was detected among ILs except for the SCA of KN in the TC (B73), YP and KN in the TC (D340), YP and PH in the TC (HZ4), as well as YP, RN, KW and KN in the TC (Mo17). This result suggests that the introgressed chromosomal fragments had significant effects on GCA and most of the SCA traits (Table 2). In addition, for the SCA of KN, significant difference was detected among environments in the four TCs, and significant difference was also detected among the environments for the SCA of PH and RN in the TC (Mo17), indicating that these traits were also significantly affected by environments especially for the trait of SCA of KN.

Table 3 presents the correlation coefficients between the combining ability of YP and the combining ability of other traits in all of the environments. The GCA of YP was positive and significantly correlated to the GCA of all other yield-related traits except for RN. The correlations between the GCA of YP and RN were also not significant in the environments of 10WH and 11BD or not strong ( $r = 0.40$ ) in 11HG. Moreover, the correlations between the GCA of YP and PH were relatively not strong as that between the GCA of YP and other traits in 10WH and 11BD ( $r \leq 0.35$ ).

In the four TCs, correlations between the SCA of YP and the SCA of other traits were generally similar as that for GCA. Correlations between the SCA of YP and the SCA of PH or RN were not significant or strong ( $r \leq 0.44$ ). However, the SCA of YP was strongly correlated to the SCA of KN and KW with several exceptions in certain TCs or in certain environments (Table 3). For instance, in the environment of WH in 2010, the correlations between the

**Table 3** Correlation analysis between GCA or SCA of YP and other traits investigated

|      | PH    | RN    | KN   | KW   |
|------|-------|-------|------|------|
| GCA  |       |       |      |      |
| 10WH | 0.35  | -0.08 | 0.50 | 0.74 |
| 11HG | 0.57  | 0.40  | 0.77 | 0.51 |
| 11BD | 0.26  | 0.16  | 0.66 | 0.40 |
| B73  |       |       |      |      |
| 10WH | -0.03 | 0.07  | 0.46 | 0.79 |
| 11HG | 0.15  | 0.30  | 0.71 | 0.65 |
| 11BD | 0.04  | 0.27  | 0.70 | 0.13 |
| D340 |       |       |      |      |
| 10WH | 0.13  | 0.12  | 0.64 | 0.37 |
| 11HG | 0.33  | 0.44  | 0.76 | 0.44 |
| 11BD | 0.20  | 0.18  | 0.71 | 0.45 |
| HZ4  |       |       |      |      |
| 10WH | -0.12 | 0.05  | 0.58 | 0.74 |
| 11HG | 0.41  | 0.13  | 0.64 | 0.63 |
| 11BD | 0.13  | 0.32  | 0.59 | 0.35 |
| Mo17 |       |       |      |      |
| 10WH | -0.27 | 0.37  | 0.02 | 0.50 |
| 11HG | 0.26  | 0.25  | 0.75 | 0.67 |
| 11BD | 0.08  | 0.30  | 0.81 | 0.16 |

Values larger than 0.23 and 0.30 are significant at the level of  $P < 0.05$  and  $P < 0.01$ , respectively

SCA of YP and the SCA of KN were not significant in the TC (Mo17).

Correlation analysis between the GCA of the five traits and the traits per se

In general, GCA was not significantly or strongly ( $r \leq 0.44$ ) correlated to their corresponding yield-related traits per se (Table 4). The GCA of RN was positively and

**Table 4** Correlation analysis between the five traits per se and their performance of GCA of the corresponding traits in different environments

| Environments | YP    | KW   | RN   | KN   | PH   |
|--------------|-------|------|------|------|------|
| 10WH         | −0.02 | 0.14 | 0.44 | 0.02 | 0.38 |
| 11HG         | −0.01 | 0.03 | 0.40 | 0.09 | 0.40 |
| 11BD         | 0.00  | 0.36 | 0.33 | 0.15 | 0.22 |

Values larger than 0.23 and 0.30 are significant at the level of  $P < 0.05$  and  $P < 0.01$ , respectively

significantly correlated to RN in all of the environments ( $0.33 \leq r \leq 0.44$ ), and significant correlations between GCA and the traits per se were also detected for KW and PH in one and two environments, respectively. However, the correlations were not strong ( $r \leq 0.40$ ).

#### Identification of GCA loci

For the five traits, a total of 75 loci were found to be associated with GCA at the significant level of  $P < 0.05$ . Fifteen to 22 GCA loci for KN, KW, YP and PH were detected, while only six GCA loci for RN were identified. Of them, 28 loci each were commonly identified under two and three environments, respectively. Tables 5 and 6 show the GCA loci detected in at least two environments.

**YP** Seventeen GCA loci distributed on all the chromosomes were detected for YP, seven and two of them were commonly detected in two and three environments, respectively. All of the introgressed alleles had negative effects, and they decreased the GCA of YP from 3.91 to 11.65. Alleles from the donor at two loci, bnlg1124 on chromosome 1 and bnlg1035 on chromosome 3, decreased GCA of YP more than 9.0 (Table 5).

**KW** Fifteen GCA loci for KW were detected, and seven and four of them were commonly detected in two and three environments. Of them, the introgressed allele at umc1770 had positive effect. However, the introgressed alleles at other loci decreased the GCA of KW from 0.52 to 1.64 (Table 5).

**RN** Of the six significant GCA loci for RN, one and five of them were detected in two and three environments, respectively. Of them, alleles from the donor at two and four loci had negative and positive effects, respectively (Table 5).

**KN** Fifteen GCA loci for KN were detected, and nine of them were commonly detected under two environments. Alleles from the donor at all the significant loci had negative effects, and they decreased the GCA of KN from 0.85 to 1.64 (Table 5).

**PH** Twenty-two GCA loci for PH were detected, and 17 of them were commonly detected in all the environments. Alleles of the donor at all but one significant loci had

negative effects. The introgressed alleles increased the GCA of PH from −15.14 to 7.12 (Table 6).

#### Identification of SCA loci

For the five traits, although a total of 108 SCA loci were identified in the four TCs at the significant level of  $P < 0.05$ , only 21 of them were commonly detected under multiple environments. Of them, five, nine, five and two were detected in the testcrossing populations of TC (B73), TC (D340), TC (HZ4) and TC (Mo17), respectively. Table 7 shows the 21 SCA loci detected in different TCs.

**TC (B73)** Among the five SCA loci detected in the TC(B73), three loci were associated with YP, and one SCA locus each for KW and RN were detected. The introgressed allele had negative effect at these loci (Table 7).

**TC (D340)** Three loci each were identified to be associated with SCA of YP, RN and PH, and alleles from the donor at all of these loci had negative effects.

**TC (HZ4)** Of the five SCA loci detected in the TC(HZ4), three loci were related to SCA of PH, and one locus each were associated with SCA of YP and KN. The introgressed alleles at the loci for SCA of YP and KN, and two of the three loci for SCA of PH had negative effects.

**TC (Mo17)** One locus each for SCA of YP and PH were identified in the TC(Mo17), and the introgressed alleles had positive effects.

#### Pleiotropism

The phenomenon of pleiotropism was found among most of the GCA traits especially between YP and PH, while relatively fewer cases were detected between SCA and GCA. In general, there were 13 and 2 marker loci simultaneously associated with 2 and 3 traits, respectively (Tables 5, 6, 7). For example, umc2208 on chromosome 6 was detected to be associated with the GCA of YP and PH, and the SCA of PH in the TC(D340) simultaneously. Of the 15 loci associated with at least two traits, 10 loci were among the GCA traits alone, while 5 were related to both GCA and SCA traits.

#### Correlations between the number of GCA loci and the performance of GCA in the ILs

For each trait, correlation between the number of GCA loci and the performance of GCA in the ILs was analyzed. As counting the number of GCA loci in each IL, the introgressed alleles with negative and positive effects were scored as “−1” and “1”, respectively, considering both negative and positive effects existed for some traits. As shown in Table 8, the number of GCA loci harboring in the ILs was significantly and positively correlated to their

**Table 5** Marker loci for GCA of yield and yield components commonly identified in at least two environments

| Traits | Chromosome | Loci     | 10WH             |       | 11HG   |       | 11BD  |      |      |
|--------|------------|----------|------------------|-------|--------|-------|-------|------|------|
|        |            |          | Add <sup>a</sup> | P     | Add    | P     | Add   | P    |      |
| YP     | 1          | bnlg1124 | -9.63            | 0.00  | -9.05  | 0.03  |       |      |      |
|        |            | umc2217  | -6.02            | 0.02  | -10.51 | 0.00  | -6.02 | 0.02 |      |
|        |            | bnlg1564 | -3.91            | 0.02  | -8.84  | 0.00  |       |      |      |
|        |            | phi011   | -5.95            | 0.00  | -7.08  | 0.01  |       |      |      |
|        | 2          | umc1516  | -4.06            | 0.01  | -9.72  | 0.00  |       |      |      |
|        | 3          | bnlg1035 | -9.51            | 0.00  | -11.65 | 0.01  |       |      |      |
|        | 4          | phi086   | -5.88            | 0.00  | -8.48  | 0.00  |       |      |      |
| KW     | 1          | umc2208  | -5.21            | 0.00  | -10.55 | 0.00  |       |      |      |
|        |            | umc1932  | -5.89            | 0.00  | -9.58  | 0.00  | -5.98 | 0.01 |      |
|        | 2          | bnlg1124 | -1.34            | 0.00  | -0.72  | 0.02  |       |      |      |
|        |            | umc1770  | 1.09             | 0.01  | 0.67   | 0.03  |       |      |      |
|        |            | bnlg1597 | -0.98            | 0.00  | -0.64  | 0.01  |       |      |      |
|        |            | umc1184  | -0.83            | 0.00  | -0.73  | 0.00  |       |      |      |
|        |            | phi083   | -1.09            | 0.00  | -0.76  | 0.01  | -0.76 | 0.02 |      |
| 4      | umc1065    |          |                  | -1.34 | 0.00   | -0.94 | 0.05  |      |      |
| RN     | 4          | umc1329  | -1.55            | 0.00  | -1.16  | 0.00  |       |      |      |
|        | 5          | umc1153  | -0.86            | 0.01  | -0.67  | 0.02  | -0.86 | 0.02 |      |
|        | 6          | umc1178  | -0.80            | 0.01  | -0.85  | 0.00  |       |      |      |
|        | 8          | umc1075  | -1.69            | 0.00  | -1.64  | 0.00  | -1.69 | 0.00 |      |
|        | 9          | umc1647  | -1.19            | 0.00  | -0.52  | 0.05  | -0.62 | 0.05 |      |
|        | KN         | 1        | umc1106          | -0.51 | 0.01   | -0.64 | 0.03  |      |      |
|        |            | 4        | umc1329          | 0.39  | 0.02   | 0.48  | 0.05  | 0.66 | 0.00 |
| 5      |            | umc1692  | 0.48             | 0.00  | 0.69   | 0.00  | 0.68  | 0.00 |      |
| 9      |            | bnlg127  | -0.31            | 0.04  | -0.44  | 0.01  | -0.31 | 0.04 |      |
| KN     | 10         | bnlg1129 | 0.53             | 0.00  | 0.63   | 0.01  | 0.56  | 0.01 |      |
|        |            | phi035   | 0.44             | 0.00  | 0.53   | 0.00  | 0.50  | 0.00 |      |
|        | 1          | umc1479  | -1.24            | 0.00  | -1.43  | 0.00  |       |      |      |
|        | 2          | Mmc0111  | -1.24            | 0.00  | -1.62  | 0.01  |       |      |      |
|        |            | Umc1042  | -1.46            | 0.00  | -1.33  | 0.00  |       |      |      |
|        | 4          | phi086   | -0.99            | 0.00  | -1.18  | 0.00  |       |      |      |
|        | 5          | Umc1852  | -0.97            | 0.02  | -1.69  | 0.00  |       |      |      |
|        | 6          | phi081   | -0.97            | 0.00  | -1.00  | 0.02  |       |      |      |
|        | 8          | Umc1858  | -1.08            | 0.00  | -0.98  | 0.02  |       |      |      |
|        | 9          | Umc2343  | -1.22            | 0.00  | -1.32  | 0.01  |       |      |      |
| 10     | Bnlg1526   | -0.88    | 0.00             | -0.85 | 0.01   |       |       |      |      |

<sup>a</sup> Add effects are averages of the means of ILs harboring alleles from the donor and the means of ILs possessing alleles of Zong 3 at the significant loci

performance of GCA for all of the traits investigated under the three environments ( $0.32 \leq r \leq 0.69$ ).

However, the correlations were relatively weak for the traits of YP and KN in the environments of 11BD ( $r \leq 0.37$ ). This might be because relatively fewer significant loci were detected for the two traits under this environment.

## Discussion

In this study, GCA and SCA of a set of ILs were estimated by evaluating testcrossing populations (the ILs were

crossed with four testers from different heterotic groups) under three environments. The GCA or SCA of the five yield-related traits varied largely especially for the traits of yield per plant and plant height. In general, all of these traits in the ILs fit to normal distribution, suggesting a complex genetic basis for GCA and SCA. It is interesting to note that, the performance of GCA was not significantly or strongly ( $-0.02 \leq r \leq 0.44$ ) correlated to the performance of the ILs per se for these traits. Moreover, in this study only two GCA and two SCA loci were simultaneously associated with four yield-related traits per se in the ILs (data unpublished), respectively. This also reflects

**Table 6** Marker loci for GCA of PH commonly identified in at least two environments

| Chromosome | Loci     | 10WH             |      | 11HG   |      | 11BD  |      |
|------------|----------|------------------|------|--------|------|-------|------|
|            |          | Add <sup>a</sup> | P    | Add    | P    | Add   | P    |
| 1          | bnlg1179 | -8.63            | 0.00 | -7.60  | 0.00 | -8.31 | 0.00 |
|            | umc2217  | -8.59            | 0.00 | -7.41  | 0.00 | -8.85 | 0.00 |
|            | bnlg1564 | -6.77            | 0.00 | -5.91  | 0.00 | -6.19 | 0.00 |
|            | phi011   | -7.89            | 0.00 | -4.36  | 0.02 | -6.01 | 0.00 |
| 2          | umc1024  | -5.86            | 0.00 | -4.99  | 0.01 |       |      |
|            | umc1156  | -15.14           | 0.00 | -11.22 | 0.00 | -6.77 | 0.01 |
|            | umc1736  | -7.12            | 0.00 | -5.93  | 0.00 | -7.12 | 0.00 |
| 3          | phi036   | -8.11            | 0.00 | -6.80  | 0.00 | -4.61 | 0.03 |
|            | bnlg1035 | -12.72           | 0.00 | -13.09 | 0.00 | -8.08 | 0.00 |
|            | umc1825  | -7.48            | 0.00 | -6.78  | 0.00 |       |      |
| 4          | bnlg2162 | -7.61            | 0.00 | -6.97  | 0.00 | -5.35 | 0.00 |
| 5          | umc2198  | -5.11            | 0.00 | -4.22  | 0.02 | -5.11 | 0.00 |
| 6          | umc2208  | -7.92            | 0.00 | -4.98  | 0.01 | -4.37 | 0.01 |
|            | mmc0241  | 3.49             | 0.02 | 7.12   | 0.01 | 6.55  | 0.01 |
| 7          | umc1932  | -8.28            | 0.00 | -7.87  | 0.00 | -5.33 | 0.01 |
|            | bnlg1805 | -5.27            | 0.01 | -6.02  | 0.00 | -5.23 | 0.00 |
|            | umc1154  | -6.36            | 0.00 | -5.41  | 0.03 | -6.36 | 0.00 |
| 8          | umc1858  | -7.33            | 0.00 | -5.25  | 0.01 | -6.05 | 0.00 |
|            | umc1724  | -5.79            | 0.00 |        |      | -4.76 | 0.01 |
|            | umc2343  | -10.85           | 0.00 | -7.95  | 0.00 | -8.47 | 0.00 |
|            | umc1104  | -13.57           | 0.00 | -8.85  | 0.01 |       |      |

<sup>a</sup> Add effects are averages of the means of ILs harboring alleles from the donor and the means of ILs possessing alleles of Zong 3 at the significant loci

**Table 7** SCA related loci commonly identified in at least two environments

| TC   | Traits | Chromosome | Loci      | 10WH             |       | 11HG   |       | 11BD  |      |
|------|--------|------------|-----------|------------------|-------|--------|-------|-------|------|
|      |        |            |           | Add <sup>a</sup> | P     | Add    | P     | Add   | P    |
| B73  | YP     | 2          | phi083    |                  |       | -9.82  | 0.03  | -8.54 | 0.01 |
|      |        | 3          | phi104127 |                  |       | -11.17 | 0.02  | -8.65 | 0.01 |
|      |        | 7          | umc1251   |                  |       | -13.25 | 0.01  | -9.01 | 0.02 |
|      | KW     | 9          | umc1231   |                  |       | -0.78  | 0.01  | -0.84 | 0.04 |
|      |        | 9          | umc1040   | -0.27            | 0.01  |        |       | -0.25 | 0.05 |
| D340 | YP     | 5          | mmc0282   | -4.12            | 0.00  | -6.59  | 0.03  |       |      |
|      |        |            | phi087    | -5.25            | 0.00  | -8.59  | 0.02  |       |      |
|      |        | 7          | umc1159   | -5.56            | 0.01  | -13.85 | 0.01  |       |      |
|      | RN     | 2          | umc1525   | -0.33            | 0.01  | -0.37  | 0.05  |       |      |
|      |        | 7          | umc1695   | -0.35            | 0.01  |        |       | -0.44 | 0.01 |
|      |        |            | umc1932   | -0.28            | 0.01  | -0.34  | 0.03  | -0.35 | 0.01 |
|      | PH     | 2          | umc1736   |                  |       | -4.54  | 0.01  | -5.50 | 0.00 |
|      |        | 6          | umc2208   |                  |       | -4.96  | 0.01  | -4.81 | 0.01 |
|      | 10     | umc1084    |           |                  | -4.56 | 0.01   | -4.90 | 0.00  |      |
| HZ4  | YP     | 7          | umc2635   |                  |       | -9.61  | 0.00  | -0.90 | 0.03 |
|      |        | 7          | umc2332   |                  |       | -1.09  | 0.00  | -0.84 | 0.05 |
|      | PH     | 3          | umc1286   | -3.94            | 0.03  | -6.98  | 0.01  | -4.50 | 0.01 |
|      |        | 7          | umc2160   |                  |       | 8.73   | 0.02  | 6.81  | 0.01 |
|      | 10     | Phi084     |           |                  | -6.32 | 0.01   | -3.45 | 0.03  |      |
| Mo17 | YP     | 3          | umc1825   |                  |       | 7.23   | 0.05  | 9.85  | 0.01 |
|      | PH     | 2          | mmc0001   | 9.07             | 0.00  | 3.84   | 0.03  |       |      |

<sup>a</sup> Add effects are averages of the means of ILs harboring alleles from the donor and the means of ILs possessing alleles of Zong 3 at the significant loci

**Table 8** Correlations between the number of significant loci for GCA in the ILs and the performance of their GCA

| Environments | YP   | KW   | RN   | KN   | PH   |
|--------------|------|------|------|------|------|
| 10WH         | 0.59 | 0.54 | 0.59 | 0.63 | 0.69 |
| 11HG         | 0.57 | 0.61 | 0.46 | 0.51 | 0.67 |
| 11BD         | 0.37 | 0.46 | 0.59 | 0.32 | 0.63 |

Values larger than 0.23 and 0.30 are significant at the level of  $P < 0.05$  and  $P < 0.01$ , respectively

the fact that the genetic basis of combining ability is different from that of the yield-related traits per se. Weak correlations between the performance of inbred lines and their GCA were also detected in a previous report (Lv et al. 2012), thus performance in testcrosses was still thought to be essential for the evaluation and selection of an inbred line. In addition, correlation analysis also revealed that the combining ability of YP was strongly correlated to the combining ability of KN and KW, in general; this indicates KN and KW instead of PH or RN should be paid more attention on the genetic improvement of the combining ability of maize yield.

Griffing (1956) first conducted diallel tests to understand the genetic basis of combining ability, and he proposed that GCA and SCA are mainly related to the additive and non-additive genetic effects, respectively. Moreover, breeding practice has proven that combining ability can be transmitted and accumulated across generations (Lv et al. 2012). Therefore, molecular markers could be used to dissect the genetic basis of GCA or SCA like the traits of yield and yield components per se. GCA related loci had been identified using various genetic populations including DHs, BCRIILs and ILs in maize or rice (Gu 2007; Lv et al. 2012; Qu et al. 2012). In this study, a total of 56 and 21 significant GCA and SCA loci for five yield-related traits were commonly detected at least in two environments. Four loci, umc1042, umc1770, umc1724 and umc1024, associated with the GCA of KN, PH and KW in this study were found to be located in four QTL regions for the GCA of yield and yield components in a previous report (Gu 2007). These results demonstrated that it is possible to dissect the genetic basis of GCA and SCA using molecular markers. Among the 56 GCA and 21 SCA loci identified in this study, only five of them controlling SCA and GCA simultaneously. In addition, among the significant SCA loci, only eight loci were detected in two testcrosses simultaneously. These results agree with the theory that GCA and SCA are caused by different genetic effects (Sprague and Tatum 1942). More significant loci were identified for GCA than SCA, implying that SCA might mainly result from the effects of dominance and epistasis which could not be detected in this study. In most of the cases, alleles from the donor line at

these significant loci had negative effects, which is consistent with the fact that the combining ability of the recurrent parent, Zong 3, was much better than the donor. In addition, of the 108 significant SCA loci, only 21 were commonly detected in multiple environments, which agrees to the data that the environment effects of some SCA traits were significant.

Recurrent selection is a strategy that uses repeating cycles of selection to improve the performance of a population. In practice, individuals from the population under selection are evaluated by the average performance of several testcrosses, and individuals showing hybrid vigor in the testcrosses are then inter-mated to form the next population. Yields of hybrids derived from the advanced populations were markedly superior to that of hybrids developed from the foundation populations (Genter and Eberhart 1974; Doerksen et al. 2003; Peng et al. 2004; Zou et al. 2010), and phenotype improvement might be due to the pyramiding of more favorable alleles in the advanced population (Schnell 1961; Johnson 1982). These results also imply that the GCA of an advanced population was increased with cycles of selection. In other words, the recurrent selection process is in company with the accumulation of favorable alleles for GCA (Lv et al. 2012). In rice, several favorite alleles related to combining ability were pyramided by marker-assisted selection, and combining ability of the selected lines was increased (Liu et al. 2004). Positive, significant correlations were detected between the number of GCA loci and the performance of GCA of the five yield-related traits in this study, also implying that pyramiding GCA loci would have positive effects on the performance of GCA. Therefore, the combining ability loci, especially for the GCA loci identified in multiple environments simultaneously in this study would be useful for maize hybrid breeding.

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