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RESEARCH

Reciprocal Diallel Crosses Impact Combining Ability, Variance Estimation, and Heterotic Group Classification

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ABSTRACT

Questions such as the following often arise: "Should reciprocal crosses be included in a diallel?" and "Would their inclusion in a diallel impact grain yield (GY), estimates of general (GCA) and specific combining ability (SCA) effects, and heterotic group classification in maize (*Zea mays* L.)?" We evaluated a 12-parent maize diallel cross (Griffing's Method 3 and Method 4) in three environments to determine (i) if reciprocal crosses impact GY of crosses and GCA and SCA effects, (ii) if reciprocal crosses influence the GCA and SCA and residual variance estimates in a diallel analysis, and (iii) if reciprocal crosses impact maize heterotic group classification. The results showed that inclusion of reciprocal crosses in a diallel greatly impacted GY and estimates of GCA and SCA effects. Under the assumption of a random-effects model, the inclusion of reciprocal crosses caused the residual and GCA variances to decrease and the SCA variances to increase as the number of parental lines increased in a diallel cross. Because inclusion of reciprocal crosses impacted GY and SCA estimates, reciprocal crosses would have great impact on maize heterotic group classification. The maize heterotic groups might be classified differently with and without the inclusion of reciprocal crosses. Based on our dataset from southwest China, three heterotic groups seemed to be an ideal number for improving maize-breeding efficiency.

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Abbreviations: F_GCA, general combining ability effects when lines were used as female parent; GCA, general combining ability; GCA_M3, general combining ability effects with reciprocal crosses in Method 3; GCA_M4, general combining ability effects without reciprocal crosses in Method 4; GY, grain yield; M_GCA, general combining ability effects when lines were used as male parent; MAT, maternal effect; NMAT, nonmaternal effect; REC, reciprocal effect; REML, restricted maximum likelihood; SCA, specific combining ability; SCA_REC, specific combining ability effects plus reciprocal effects; SSR, simple sequence repeat; TriHG, tri-heterotic group.

Griffing (1956) described statistical models for analyzing four different diallel mating designs (Method 1, Method 2, Method 3, and Method 4). Because of the availability of clearly defined general combining ability (GCA) and specific combining ability (SCA) effects and reciprocal effect (REC) and easy to understand statistical models as well as the availability of software to analyze diallel data (Burow and Coors, 1994; Magari and Kang, 1994; Zhang and Kang, 1997; Zhang et al., 2005), Möhring et al. (2011) have recently developed a restricted maximum likelihood (REML)-based diallel analysis method and SAS software (SAS version 9.2; SAS Institute Inc., 2009) to estimate variances of GCA, SCA, REC, and other related components from several diallel experiments. The model and SAS software

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have allowed researchers (e.g., Yao et al., 2013) to estimate related genetic variances among different diallel methods.

Griffing's methods have become popular among plant breeders to determine GCA and SCA effects in maize (Zhang et al., 1997; Kang et al., 1999, 2005; Phumichai et al., 2008; Yao et al., 2013) and other crops (Ragsdale and Smith, 2007; Zwart et al., 2008; Yang and Gai, 2009; Velu et al., 2011; Berger et al., 2012). The GCA and SCA effects have been further used for genetic diversity evaluation, inbred line selection, heterotic pattern classification, heterosis estimation, and hybrid development (Sughroue and Hallauer, 1997; Fan et al., 2002; Melani and Carena, 2005; Barata and Carena, 2006).

Reciprocal Crosses

Reciprocal crosses are included in Griffing's Method 1 and Method 3, which allow computation of REC as well as maternal effects (MAT) and nonmaternal effect (NMAT) (Zhang and Kang, 1997; Zhang et al., 2005). For various reasons, however, plant breeders may not always be able to include reciprocal crosses in experiments. Sometimes, reciprocal crosses may have failed altogether or may not have produced sufficient quantity of seed, and other times, their decision to include or not to include reciprocal crosses may depend on limitation of space and other resources. In crops such as maize, where grain yield (GY) is largely determined by endosperm, reciprocal crosses are important and it is crucial to know what impact the inclusion or noninclusion of reciprocal F_1 crosses in a diallel analysis will have on GY.

The studies by Mahgoub (2011) and Yao et al. (2013) have reported that RECs strongly influenced estimates of SCA effects. The SCA effects were different when a line was used as female from those when the same line was used as male (Mahgoub, 2011). The RECs have been shown to have a major impact on determination of hybrid yield (Yao et al., 2013).

Heterotic Group Classification

The SCA effects, pedigree, and GY information (Kauffman et al., 1982; Fan et al., 2001, 2009; Menkir et al., 2004) as well as molecular marker techniques (Fan et al., 2003, 2004; Barata and Carena, 2006; Yao et al., 2009; Delucchi et al., 2012) are frequently used in maize heterotic group classification. Fan et al. (2001) used a diallel design to study combining abilities among 10 maize lines, of which five lines were from the International Maize and Wheat Improvement Center (CIMMYT) and the other five were widely used lines in China. According to SCA and GY, they classified lines CML171, CML161, and CML166 into one heterotic group, lines Chang 631/o2 and Zhongxi 096/o2 into a second heterotic group, and line Qi 205 into a third heterotic group. Yao et al. (2009) used simple sequence repeat (SSR) markers as well as GY and pedigree information to classify 27 inbred lines into three heterotic groups, A, B, and C. Menkir et al. (2004) used two testers representing the flint and dent heterotic patterns to test 38 tropical maize inbred lines. The two testers successfully classified 23 of the 38 tested inbred lines into two heterotic groups based on the SCA and GY. Barata and Carena (2006) conducted a similar study to classify 13 elite North Dakota maize inbred lines into current U.S. Corn Belt heterotic groups. In addition, they evaluated the consistency in classification between the SSR and SCA plus GY methods in a diallel experiment. Results showed that heterotic groups of genetically similar germplasms could not be identified accurately and reliably with molecular markers. Therefore, extensive field evaluation was suggested to assign unrelated maize inbred lines to heterotic groups.

Mahgoub (2011) recently reported on how the MAT impacted GCA effects and how REC impacted SCA effects in Griffing's Method 1 and Method 3. This study theoretically demonstrated how REC influenced SCA, a key genetic statistic for maize heterotic group classification. None of the above studies reported on the impact of reciprocal crosses or RECs on heterotic group classification.

There are still many questions that need to be answered about reciprocal crosses and RECs. For example, (i) How will inclusion of reciprocal crosses in a diallel impact GCA and SCA effects and their variance estimates? (ii) How will inclusion of reciprocal crosses in a diallel impact maize heterotic group classification? (iii) How important is the influence of reciprocal crosses on GY of a hybrid? Therefore, the specific objectives of this study were to determine (i) how important is the inclusion of reciprocal crosses for improving GY of a hybrid, (ii) how reciprocal crosses influence GCA and SCA effects and their variance estimates, and (iii) whether maize heterotic group classification will be affected by inclusion or exclusion of reciprocal crosses in a diallel. Answering these questions should help plant breeders immensely in selecting the most appropriate maize breeding design, obtaining reliable genetic information, and improving breeding efficiency and productivity.

MATERIALS AND METHODS

Maize Materials

Twelve maize inbred lines used in this study are shown in Table 1. Four inbred lines were either introduced or improved tropical germplasm belonging to Suwan1 heterotic group and eight temperate germplasms were domestic elite maize lines, either from Reid or non-Reid heterotic groups. The 12 inbred lines produced 66 F_1 and 66 reciprocal F_1 crosses (132 total crosses) according to Griffing's Method 3 (Supplemental Table S1). The crosses were accomplished at Kunming, Kaiyuan, and Jinghong of Yunnan province, China, in 2008 and 2009. In 2010, the 132 crosses were planted at Kunming and Qujing in Yunnan province, China. The same experiment was repeated at Kunming in 2011. A randomized complete-block design with three replications was used. Each experimental unit was a single-row plot with a row-to-row spacing of 0.7 m and row length of 5 m. The distance between two adjacent plants was 0.25 m and the population density was approximately 57,140 plants ha^{-1}. At

Table 1. Inbred lines used for the diallel crosses and their origins and heterotic groups.

Code	Inbred line	Origin	Heterotic group
1	YML32	SW1(S)C8-#-1-10-2-1-1-1-1	Suwan 1
2	Ki50	SW1(S)C11-S8-#-5-3-2-1-2	Suwan 1
3	CML481	SW1(S)C11-14-1-3-3-B*4	Suwan 1
4	YML46	SW1(S)C10-#-1-1-2-1-2-1	Suwan 1
5	TR2	Hybrid of the United States	Non-Reid
6	Ye107	Derived from XL80	Reid
7	Zhong106	Derived from Yemen germplasm by transferring germplasms of oh43 and others	Non-Reid
8	Yuzi87-1	Pioneer Hybrid 87001	Reid
9	YML1218	Variation of K12 (Huangzao4 × Huaichun)	Non-Reid
10	81515	Huangzao4 × (Huafeng100 × Ai C103)	Reid
11	K22	K11 × Ye478	Reid
12	Xin9101-1/o2	Hybrid of the United States	Non-Reid

maturity, a 10-ear sample was harvested from 10 consecutive plants from the middle of each row. After harvest, kernels were air dried until constant moisture of 130 g kg^{-1} was achieved, and then data on ear length, ear diameter, number of kernel rows per ear, number of kernels per row, 100-kernel weight, and several other traits were collected. Grain yield per plot was determined.

Statistical Model and Analyses

The following statistical model was used for Griffing's Method 3 data analysis. For Griffing's Method 4, the model will be modified by removing the REC (r_{ij}) and its related components.

$$Y_{ijkl} = \mu + \alpha_l + b_{kl} + v_{ij} + (\alpha v)_{ijl} + e_{ijkl}$$

$$v_{ij} = g_i + g_j + s_{ij} + r_{ij}$$

$$r_{ij} = m_i + m_j + n_{ij}$$

in which Y_{ijkl} is the observed value from each experimental unit, μ is the population mean, α_l is the location effect, b_{kl} is the block or replication effect within each location, v_{ij} is the F_1 hybrid effect = the g_i plus g_j plus s_{ij} (in which g_i is the GCA for the *i*th parent with $\sum_i g_i = 0$, g_j is the GCA effect of the *j*th parent with $\sum_j g_j = 0$, s_{ij} is the SCA for the *ij*th F_1 hybrid with $S_{ij} = S_{ji}$ and $\sum_{i \neq j} s_{ij} = 0$ for each *j* in Method 3 and $\sum_{i<j} s_{ij} = 0$ for each *j* in Method 4), $(\alpha v)_{ijl}$ is the interaction effect between the *ij*th F_1 hybrid and location, e_{ijkl} is the random residual effect, r_{ij} is the REC of the *ij*th cross with $r_{ij} = -r_{ji}$, m_i is the MAT of *i*th parental line with $\sum_i m_i = 0$, m_j is the MAT of the *j*th parental line with $\sum_j m_j = 0$, and n_{ij} is the NMAT of the cross between the *i*th and *j*th parental lines with $\sum_{i<j} n_{ij} = 0$ for each *j* and $n_{ij} = n_{ji}$. The GCA and SCA effects and MAT and REC were estimated according to DIALLEL-SAS05 developed by Zhang et al. (2005), and GCA, SCA, and REC variances were estimated based on the methods and SAS software developed by Möhring et al. (2011).

The crosses with I < J (I and J are the lines ordered as shown in Supplemental Table S1) are F_1 crosses and the crosses with I > J are reciprocal crosses. The GYs of 66 F_1 crosses for Griffing's Method 4 and of 132 crosses (66 F_1 crosses plus 66 reciprocal crosses) for Griffing's Method 3 from the 12-parent diallel experiment in three environments were analyzed with appropriate general linear models shown above. Correlation analysis for the top 17 crosses for GY was performed among environments.

Table 2. Analysis of variance of overall experiment with 12 parent diallel at three environments (ENVs) and three replications (REPs).

Source	df	Sum of square	Mean square	*F* value	Pr† > *F*
ENV	2	466,828.5	233,414.20	518.67	<0.0001
REP(ENV)	6	98,980.7	16,496.79	36.66	<0.0001
Entry	131	449,409.7	3,430.61	7.62	<0.0001
Entry × ENV	262	314,559.3	1,200.61	2.67	<0.0001
Error	786	353,716.5	450.02		

† Pr, probability.

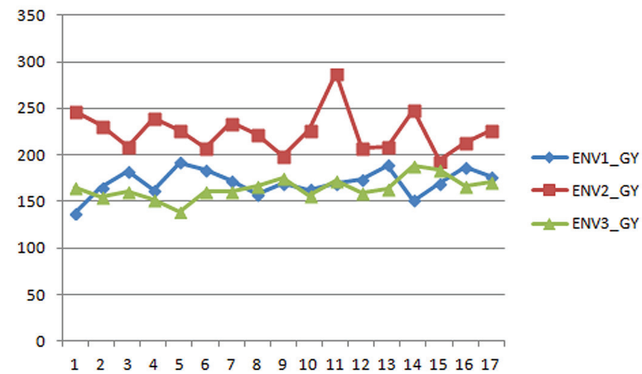

Figure 1. Grain yield mean of 17 crosses at three environments. ENV1_GY, ENV2_GY, and ENV3_GY are mean grain yields at environment 1, 2, and 3, respectively.

RESULTS AND DISCUSSIONS

Analysis of the Overall Experiments

Analysis of variance results showed that all sources of variation, including entry × environment, were statistically significant (Table 2). Entry variation was partitioned into GCA, SCA, REC, MAT, and NMAT. Rank correlation analysis conducted for GY of the top 17 crosses in three different environments showed no significant correlations among the mean GYs in the three environments, which suggested that the GYs of crosses were not consistent across these environments. The GY entry means for the top 17 crosses are shown in Fig. 1. This graph showed that both GY rank and magnitude were different in the three environments.

Impact of Reciprocal Crosses on Grain Yield of Crosses

The F_1 crosses and reciprocal crosses are shown in Supplemental Table S1. The GYs and SCA effects of the 66 F_1 crosses (Method 4) are listed in Supplemental Table S2 and the top 11 crosses with GY >180 g per plant are given in Table 3. The GYs and SCA effects of the 132 crosses for Method 3 are given in Supplemental Table S3 and the top

Table 3. The grain yield (GY) and specific combining ability (SCA) in 10 of 66 crosses from a 12-parent diallel with Griffing Method 4.

I	J	GY	SCA
8	9	195.67	21.63**
4	5	188.87	16.02*
2	8	185.94	10.10
2	7	184.75	21.04**
2	6	184.03	15.55*
3	12	183.91	26.19**
2	5	183.65	3.02
1	8	183.44	27.87**
4	9	182.30	13.42*
5	11	181.75	18.32**
5	8	181.26	3.24

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

17 crosses (high GY) (>180 g per plant) are listed in Table 4. By comparing the results from Table 3 and Table 4, six more crosses were found with high GY from Griffing's Method 3 than from Griffing's Method 4. A total of 24 reciprocal diallel crosses out of 132 crosses had statistically significant RECs (Supplemental Table S3). Because the only difference between Method 3 (REC included) and Method 4 (REC excluded) is reciprocal crosses, we concluded that by including reciprocal crosses (Method 3) in diallel analyses, additional high-GY hybrids could be identified.

Five (i.e., 6 × 2, 4 × 5, 3 × 12, 1 × 8, and 5 × 11) of the 17 crosses (Table 4) showed significant positive RECs that contributed to GY. Ranking of all 132 crosses by GY revealed that the top five high-GY crosses (Table 4) made substantial contributions to GY either through significant positive RECs or through significant positive SCA effects or both. Computation of percentages of SCA and RECs for GY showed that SCA effects accounted for −42.13% to +16.70% and RECs accounted for −13.07% to +10.36% (Supplemental Table S3). Correlation analysis showed that only combined effects of SCA and REC (i.e., SCA_REC [SCA effects plus RECs]) (Table 4) were significantly correlated with GY ($r = 0.653$, $P < 0.0045$), which strongly suggested that RECs played an important role in achieving high GY. Interestingly, the reciprocal cross (6 × 2) had the highest GY among all crosses (Table 4). Had reciprocal crosses not been included, we would not have been able to discover this important result. The GY of the 6 × 2 cross from Method 3 is about 7.2% higher than the GY of highest-yielding cross (8 × 9) from Method 4. Such solid high percentage increase in GY could be sufficient for a hybrid to be approved by a state or national variety evaluation committee.

The results from Supplemental Table S3 and Table 4 not only confirmed the results reported by Yao et al. (2013) but also suggested that much attention should be paid to choice of mating design (reciprocals vs. no reciprocals). To increase the chance of finding super GY hybrids, breeders need to include reciprocal crosses to benefit from

Table 4. Specific combining ability (SCA) effects and reciprocal effects (RECs) and grain yield (GY) for top 17 crosses out of the 132 crosses from a 12-parent diallel with Griffing method 3.

I	J	SCA	REC	SCA_REC†	GY
6	2	28.05**	12.84*	40.89	209.72
8	9	18.92**	6.63	25.55	195.67
12	4	20.65**	7.57	28.22	191.12
10	2	23.01**	8.72	31.73	188.99
4	5	−0.78	19.57**	18.79	188.87
8	2	12.47**	0.61	13.07	187.15
2	8	12.47**	−0.61	11.86	185.94
2	7	14.15**	5.08	19.24	184.75
2	6	28.05**	−12.84*	15.21	184.03
3	12	18.96**	10.94*	29.89	183.91
2	5	1.81	4.86	6.67	183.65
1	8	19.17**	10.23*	29.41	183.44
9	8	18.92**	−6.63	12.30	182.42
4	9	12.56**	2.62	15.18	182.30
5	11	10.89*	13.11**	24.00	181.75
5	8	7.34	0.83	8.17	181.26
6	4	12.57**	5.75	18.32	180.25

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

†SCA_REC, SCA effects plus RECs.

possible positive RECs that contribute to high GY. This study offers additional evidentiary support for the recommendation (Yao et al., 2013) that Griffing's Method 3 (F_1 crosses plus reciprocal F_1 crosses) was better than other diallel methods for obtaining super hybrids.

Some researchers use North Carolina Design II, which does not include reciprocal crosses. We suggest that a few reciprocal crosses be made on the side so that some high-performing combination or combinations is not missed.

From a commercial standpoint, in addition to high yield, maize breeders consider the amount of pollen produced by a male parent and synchronization of flowering between male and female parents. A good female parent is one that produces sufficient seed and is vigorous. So, the "good male" and "good female" characteristics also are important considerations.

Impact of Reciprocal Crosses on Estimation of General Combining Ability and Specific Combining Ability Effects

The GCA and SCA effects are important determinants of a hybrid's performance based on diallel cross designs (Griffing, 1956). The GCA effects from Method 3 and Method 4 from the 12-parent diallel and MAT effects from Method 3 are listed in Table 5.

Table 5 showed that GCA effects with reciprocal crosses in Method 3 (GCA_M3) might be higher (e.g., for lines YML32, Ki50, YML46, Ye107, Zhong106, and 81515) or lower (e.g., for lines CML481, TR2, Yuzi87-1, YML1218, K22, and Xin9101-1/o2) than those from Method 4 (general combining ability [GCA_M4]) because of random or

Table 5. General combining ability (GCA) and maternal effects (MAT) from Griffing Method 3 (GCA effects with reciprocal crosses in Method 3 [GCA_M3]) and GCA effects from Griffing Method 4 (GCA effects without reciprocal crosses in Method 4[GCA_M4]), GCA effects when lines were used as female parent (F_GCA) or as male parent (M_GCA) and MAT with the 12-parent diallel.

Line	GCA_M3	GCA_M4	MAT	F_GCA	M_GCA
YML32	−9.93**	−10.71**	0.89	−8.96	−10.91
Ki50	10.11**	9.55**	0.18	10.31	9.91
CML481	−5.67**	−3.85	2.88*	−2.51	−8.84
YML46	3.21*	1.77	1.14	4.47	1.95
TR2	9.12**	11.73**	1.46	10.72	7.51
Ye107	0.96	−0.43	1.12	2.20	−0.28
Zhong106	−2.35	−5.2*	−0.3	−2.68	−2.03
Yuzi87-1	6.21**	6.93**	−1.74	4.30	8.13
YML1218	6.15**	7.75**	−2.7	3.18	9.12
81515	−10.6**	−12.11**	2.63	−7.71	−13.49
K22	−9.12**	−7.65**	−3.71**	−13.21	−5.04
Xin9101-1/o2	1.93	2.21	−1.86	−0.11	3.97
Maximum	10.11	11.73	2.88	10.72	9.91
Minimum	−10.6	−12.11	−3.71	−13.21	−13.49

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

significant MAT. The GCA effects when lines were used as female parent (F_GCA) were equal to GCA_M3 plus MAT and the GCA effects when lines were used as male parent (M_GCA) were equal to GCA_M3 minus MAT for Method 3 (Mahgoub, 2011). When we checked GCA_M4, F_GCA, and M_GCA for CML481 and K22, we found that GCA_M4 values were closer to F_GCA for CML481 and closer to M_GCA for K22. This is because CML481 was used as female parent in 10 of the 11 crosses and K22 was used as male parent in 10 out of the 11 crosses in Method 4. The closeness between F_GCA and GCA_M4 for CML481 and between M_GCA and GCA_M4 for K22 tells us that the differences in GCA effects between Method 3 and Method 4 (Table 4) were largely caused by MAT or inclusion of reciprocal crosses in Method 3. That means that even when the same lines are used in Method 3 and Method 4, the GCA effects estimated from the two methods can be different because of MAT in Method 3.

To remove possible biased effects from the single 12-parent diallel experiment, 100 subsamples of diallel data with randomly selected 8 out of 12 lines were analyzed. The GCA effects from Method 3 and Method 4 were computed from the 100 resampled diallel datasets (data not shown). The results were similar to those that we obtained from the 12-parent diallel dataset. That is, when reciprocal crosses were included in a diallel, the GCA effects were different between Method 3 and Method 4 because MAT influenced GCA effects in Method 3. Furthermore, the simulated results also confirmed that the GCA effects from Method 4 tended to have more extreme values, as shown by the differences between maximum and minimum GCA effects (Table 5). The reason for the more extreme GCA values in Method 4 could be that GCA effects from Method 4 were confounded with MAT of the lines whereas the GCA effects from Method 3 had the MAT separated out in RECs (Mahgoub, 2011).

The SCA effect and RECs of 66 crosses from Method 3 and SCA effects from Method 4 for the 12-parent diallel and the differences in GCA effects between Method 3 and Method 4 are listed in Table 6. Correlation analysis showed that the differences in SCA effects between Method 3 and Method 4 were statistically significantly correlated with RECs calculated from Method 3 ($r = 0.954$, $P < 0.0001$). Therefore, the difference in SCA effects between Method 3 and Method 4 was greatly impacted by reciprocal crosses in a diallel. This result implied that although there might not be significant differences in direction (i.e., positive or negative) of SCA effects between Method 3 and Method 4, as found by Yao et al. (2013), the magnitudes of SCA effects with and without reciprocal crosses were different because of RECs. In some cases, when RECs were larger than SCA effects, it might even change direction of the SCA effects (e.g., crosses 4 × 5, 3 × 9, 1 × 9, and 7 × 8) between Method 3 and Method 4 (Table 6).

Impact of Reciprocal Crosses on Variances of General Combining Ability, Specific Combining Ability, and Residual

Assuming that lines for diallel crosses were randomly selected from a maize population, we applied a REML method developed by Möhring et al. (2011) to compute differences in variances of GCA, SCA, and residual from the diallel experiments with parent numbers ranging from 4 to 12 for Method 3 and Method 4. Results (Fig. 2) revealed that inclusion of reciprocal crosses in a diallel might have little or no impact on variance of GCA for any number of parental lines in a diallel cross. The differences in variances of SCA between Method 3 and Method 4 showed a mild increase as the number of parental lines increased. This increase in the SCA variance estimates from Method 3 to Method 4 can be explained by the fact that in Method 4 the MAT were confounded with SCA effects, which increases the variance for SCA as compared with Method 3. While the differences in variances of residual or error variances fluctuated widely for the diallel experiments with different numbers of parents, the fluctuation became less erratic as the number of parental lines increased in a diallel cross.

One hundred subsamples of the diallel dataset with 4, 5, 6, 7, 8, 9, 10, and 11 parental lines were randomly extracted and analyzed. The mean differences in variances of GCA, SCA, and residual between Method 3 and Method 4 were calculated from the 100 subsamples and the differences in GCA, SCA, and residual variances with the different number of parent lines are shown in Fig. 3.

By comparing Fig. 2 and 3, we found that (i) when a single dataset was used (Fig. 2), the differences in residual variances between Method 3 and Method 4 tended to fluctuate more erratically; (ii) the mean differences in the

Table 6. Reciprocal effects (RECs) and specific combining ability for Griffing Method 3 (SCA_M3), specific combining ability for Griffing Method 4(SCA_M4), and the differences of specific combining abilities between Griffing Method 3 and Griffing Method 4(M3 – M4).

I	J	SCA_M3	SCA_M4	REC	M3-M4
1	2	−31.11**	−26.38**	5.00	4.73
1	3	−14.24**	−16.02*	0.87	−1.78
1	4	−33.04**	−33.86**	−1.44	−0.82
1	5	0.30	−6.18	−3.05	−6.48
1	6	14.78**	12.23	−3.12	−2.55
1	7	−3.29	0.54	1.79	3.83
1	8	19.17**	27.87**	10.23*	8.70
1	9	1.26	−8.47	−7.31	−9.73
1	10	9.28*	9.97	−0.01	0.69
1	11	23.53**	23.28**	2.04	−0.25
1	12	13.35**	17.02**	4.77	3.66
2	3	−11.00*	−12.67*	1.19	−1.67
2	4	−37.08**	−38.12**	−1.44	−1.04
2	5	1.81	3.02	4.86	1.21
2	6	28.05**	15.55*	−12.84*	−12.50
2	7	14.15**	21.04**	5.08	6.89
2	8	12.47**	10.10	−0.61	−2.37
2	9	−9.13*	−3.20	8.58	5.93
2	10	23.01**	14.76*	−8.72	−8.25
2	11	9.92*	15.59*	8.18	5.67
2	12	−1.09	0.31	2.73	1.40
3	4	−15.52**	−19.31**	−1.81	−3.79
3	5	−11.20*	−24.82**	−7.59	−13.62
3	6	4.69	13.07*	10.42*	8.39
3	7	6.87	10.81	4.51	3.94
3	8	−18.85**	−15.77*	7.22	3.08
3	9	−0.55	11.31	16.89**	11.86
3	10	28.12**	24.96**	−1.25	−3.16
3	11	12.73**	2.24	−5.60	−10.49
3	12	18.96**	26.19**	10.94*	7.23
4	5	−0.78	16.02*	19.57**	16.80
4	6	12.57**	8.05	−5.75	−4.52
4	7	10.04*	5.02	−7.71	−5.02
4	8	−8.70	−2.41	7.17	6.29
4	9	12.56**	13.42*	2.62	0.86
4	10	20.52**	26.98**	5.11	6.46
4	11	18.79**	11.58	−5.58	−7.20
4	12	20.65**	12.64*	−7.57	−8.01
5	6	4.00	8.13	6.95	4.13
5	7	0.67	−8.12	−7.43	−8.79
5	8	7.34	3.24	0.83	−4.10
5	9	−1.19	−14.74*	−7.74	−13.55
5	10	−0.93	6.68	10.30*	7.60
5	11	10.89*	18.32**	13.11**	7.43
5	12	−10.91*	−1.55	13.85**	9.35
6	7	7.65	10.20	−0.09	2.55
6	8	−6.93	−2.77	5.09	4.16
6	9	8.62	4.81	−2.00	−3.81
6	10	−40.55**	−50.56**	−11.31*	−10.01
6	11	−19.79**	−10.10	11.37*	9.69
6	12	−13.08**	−8.62	4.96	4.47
7	8	0.06	−7.39	−7.98	−7.45
7	9	−12.90**	0.76	14.01**	13.65
7	10	−6.36	−7.63	−4.03	−1.27
7	11	−14.10**	−17.80**	−3.48	−3.69
7	12	−2.77	−7.43	−5.62	−4.65
8	9	18.92**	21.63**	6.63	2.71
8	10	−12.10**	−12.50*	0.41	−0.40
8	11	−18.68**	−22.50**	−0.03	−3.82
8	12	7.31	0.50	−4.21	−6.81
9	10	0.28	−5.16	−3.74	−5.44
9	11	−6.14	−2.92	7.89	3.22
9	12	−11.73**	−17.43**	−2.21	−5.70
10	11	−8.87*	−1.79	8.64	7.08
10	12	−12.41**	−5.72	7.06	6.69
11	12	−8.27	−15.90*	−4.28	−7.63
Maximum		28.12	27.87	19.57	16.80
Minimum		−40.55	−50.56	−12.84	−13.62

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

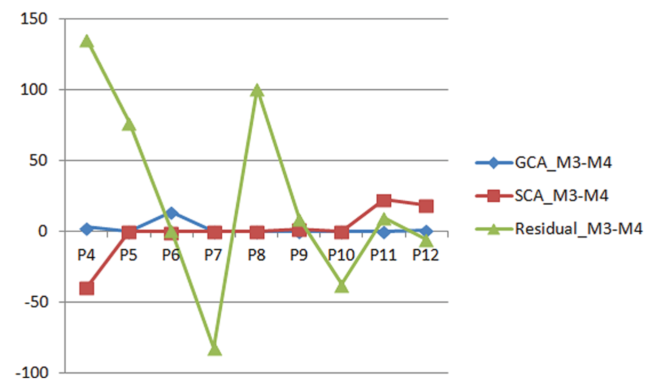

Figure 2. The differences of variances of general combining ability, specific combining ability, and residual from diallel experiments with parent numbers being from 4 to 12 (P4 to P12). GCA_M3-M4, the differences of general combining ability variances between Method 3 and Method 4 for parent numbers varying from 4 to 12; Residual_M3-M4, the differences of residual variance between Method 3 and Method 4 for parent numbers varying from 4 to 12; SCA_M3-M4, the differences of specific combining ability variances between Method 3 and Method 4 for parent numbers varying from 4 to 12.

variances of GCA and SCA between Method 3 and Method 4 with 100 subsamples tended to fluctuate in diallels having less than eight parental lines and tended to stabilize when the number of parental lines was eight or more. This may be because most of the genetic diversity among the lines in the target population is likely accounted for when number of parents reaches eight in a diallel. When eight lines are randomly selected, there will be a high probability of lines being included from all three heterotic groups; (iii) the variance of SCA was higher and of GCA lower for Method 3 than those for Method 4 when number of parents was more than eight. One possible reason for this is that although the GCA and SCA variances for Method 3 were less than those for Method 4, the relative changes in the SCA variances were usually larger than those in GCA variances as shown by maximum and minimum GCA and SCA effects (Tables 5 and 6); and (iv) the differences in residual variances between Method 3 and Method 4 decreased much

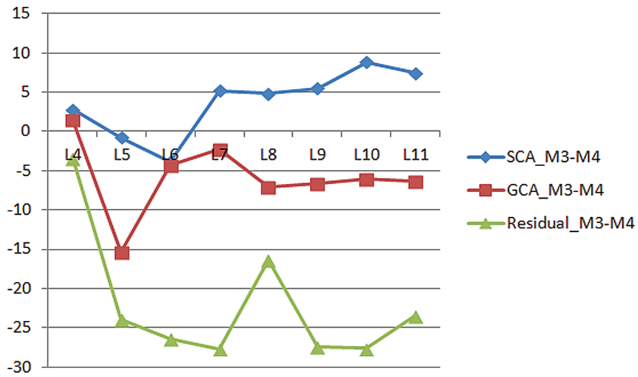

Figure 3. The average differences of variances of general combining ability, specific combining ability, and residuals between Griffing method 3 and Method 4 from 100 resamples. GCA_M3-M4, the differences of GCA variances between Method 3 and Method 4 for parent numbers varying from 4 to 12 with 100 samples; Residual_M3-M4, the differences of residual variance between Method 3 and Method 4 for parent numbers varying from 4 to 12 with 100 samples; SCA_M3-M4, the differences of SCA variances between Method 3 and Method 4 for parent numbers varying from 4 to 12 with 100 samples.

for four to five parents and became stabilized when number of parents reached nine or more. More degrees of freedom with higher number of parental lines in a diallel experiment could explain the differences in the residual variances.

Impact of Reciprocal Crosses on Maize Heterotic Group Classification

The SCA effects and GYs of crosses have been widely used for heterotic group classification (Kauffman et al., 1982; Li et al., 2001; Bhatnagar et al., 2004; Fan et al., 2004, 2009). This study showed that reciprocal crosses and computed RECs from reciprocal crosses greatly impacted GYs and SCA estimates. For example, when we compute SCA effects by Method 4 (Supplemental Table S2) and Method 3 (Supplemental Table S3) and then use combined information on SCA and GY to classify the 12 lines into heterotic groups, we might find some differences when using SCA effects from Method 3 and Method 4. The basic steps or rules for using SCA and GY information are (i) find crosses with lower GY; if they have significant negative SCA, the two lines involved in the cross should be assigned to the same heterotic group; and then (ii) check the crosses with high GY and make sure no two lines with top high GY crosses are assigned to the same heterotic group. When we apply these two steps to data in Supplemental Table S2 with SCA and GY information for Method 4, the following heterotic groups can be classified: lines 1, 2, 3, and 4 get assigned to group 1, lines 9 and 12 to group 2, lines 6 and 10 to group 3, and lines 7 and 11 to group 4. However, we had difficulty assigning lines 5 and 8. Line 5 could be assigned to group 1 because cross 3 × 5 had a significant negative SCA effect (–24.82) or to group 2 because cross 5 × 9 also had a significant negative SCA effect (–14.74). A similar situation was found for line 8 because it could be assigned to group 1 as the SCA effect for cross 3 × 8 was negative (–15.77) or to group 4 because SCA effect for cross 8 × 11 was negative (–22.50). When we apply the same steps to the data in Supplemental Table S3 containing SCA and GY information for Method 3, the heterotic groups could be classified as follows: lines 1, 2, 3, and 4 could be assigned to group 1, lines 6, 10, and 11 to group 2, and lines 7 and 9 to group 3; this method leaves three lines (i.e., lines 5, 8, and 12) unassigned. Based on SCA effects, both lines 5 and 8 could be assigned to group 1 because SCA effects for crosses 3 × 8 (–18.85) and 3 × 5 (–11.20) were significantly negative. However, several high GY crosses suggested that lines 5 and 8 should not be in group 1 (i.e., 4 × 5, 2 × 5, 8 × 2, 2 × 8, and 1 × 8). Line 12 could be assigned to group 2 because SCA effects from crosses 12 × 10 (–12.41) and 12 × 6 (–13.08) were significantly negative or lines 5 and 12 could form another group because SCA effect for cross 5 × 12 was significantly negative. Apparently, with the SCA and GY information, both Method 3 and Method 4 could not clearly assign all 12 lines to different heterotic groups. The different heterotic groups for the same 12 lines were found when they were classified using SCA and GY values from diallel with and without reciprocal crosses. This result indicated that the reciprocal crosses clearly influenced heterotic group classification. When SCA effects are obtained from diallel experiments with and without reciprocal crosses, it is not uncommon to see heterotic groups classified differently even when the same lines are used in diallel crosses.

The REC estimation methods have been described in Griffing (1956) and elsewhere (Kang, 1994; Zhang and Kang, 1997), but the impacts of reciprocal crosses and RECs on GCA and SCA effect estimations, their variances, and residual variances have not been reported. The results from this study suggested that RECs derived from reciprocal crosses greatly impacted GCA, SCA, and their variance estimations. To further investigate the underlying causes, RECs were partitioned into MAT and NMAT. Then, correlations between REC and MAT and NMAT were analyzed, which showed that NMAT but not MAT were highly correlated with RECs ($r = 0.945$, $P < 0.001$). Because NMAT are attributable to specific interactions between genes in the nucleus and cytoplasm (Zhang and Kang, 1997; Mahgoub, 2011), the impacts of RECs on SCA effects and their variances should be mainly attributable to interaction between nuclear genes and cytoplasmic factors. Till today, in most of the molecular marker analyses, no marker has been reported for tracing the cytoplasmic "genome." For exploring hybrid potential in maize hybrid breeding programs, more studies are needed on cytoplasmic genes related to GY and other important agronomic traits.

The RECs derived from reciprocal crosses impact GCA (via MAT) and SCA (via NMAT) effects and their corresponding variances as well as GY. Therefore, in maize breeding programs, breeders may intentionally need to test some reciprocal crosses because it may greatly increase chances of finding super high GY hybrids. This study showed that both Method 3 and Method 4 could not perfectly group the 12 lines into different heterotic groups and the heterotic groups classified by widely used SCA and GY method will give different results because SCA effects are different from a diallel experiment with (Method 3) and without (Method 4) reciprocal crosses.

The purpose of maize heterotic group classification is to improve maize breeding efficiency by reducing crosses among intragroup lines by avoiding missing potential super hybrids. Based on pure mathematics, we know that with same numbers of parental lines, the more the heterotic groups, the more intergroup crosses will need to be made and thus breeding efficiency will be reduced. For example, if 36 inbred lines are separated into two, three, four, and six heterotic groups with equal number of lines in each group, the intergroup crosses will be 648, 864, 972, and 1080, respectively, with a total of 1260 possible crosses. From the standpoint of improving breeding efficiency, the fewer the heterotic group crosses, the higher the breeding efficiency. Therefore, breeders should classify all lines into as few heterotic groups as possible.

Based on GY and SCA effects calculated from Method 3 and Method 4, the 12 parental lines used in our study were classified into at least three groups, with some lines unassigned. Plant breeders with many years of field experience would know that there are always some lines that cannot be assigned to a defined heterotic group. That is very reasonable because heterotic groups do not naturally exist; they are a man-made classification based on a long history of practice for improving breeding efficiency. What researchers can do is to try their best to group maize lines into as few heterotic groups as possible, aiming at improving breeding efficiency. From this standpoint, with any diallel or North Carolina mating design II experiments, researchers may have to force some lines into certain heterotic groups by keeping as many high GY crosses as possible. For example, with the current 12-parent diallel, according to the data in Tables 4 and 5 and Supplemental Tables S2 and S3, we first tried to classify the 12 lines into two groups: with lines 1, 2, 3, 4, 5 in group 1 and lines 6, 7, 8, 9, 10, 11, and 12 in group 2 or with lines 1, 2, 3, 4, 8 in group 1 and rest of them in group 2. With both groupings, however, about 20% of the super crosses would be missed (Supplemental Table S3) if only interheterotic crosses were made. Losing 20% of possible super hybrids would not be acceptable in actual breeding programs, especially when one of them is a top performer in GY (e.g., cross of 8 × 9) (Table 4). Therefore, classifying 12 maize lines into two heterotic groups would not be acceptable. What if we place the 12 lines into three heterotic groups? Again, the following two types of three groups could be obtained: Type A, with lines 1, 2, 3, and 4 in group 1 (Suwan1), lines 6, 8, 10, 11, 12 in group 2 (Reid), and lines 5, 7, 9 in group 3 (non-Reid), and type B, with lines 1, 2, 3, and 4 in Suwan1, lines 6, 8, 10, 11 in Reid, and lines 5, 7, 9, and 12 in non-Reid. If we check

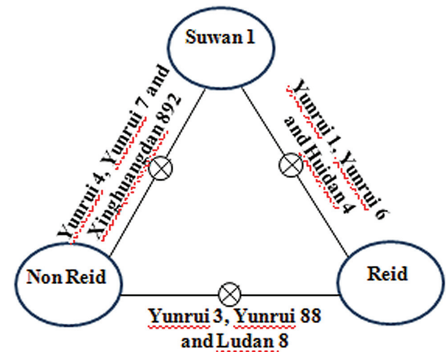

Figure 4. Three heterotic groups of maize in southwest China with some released maize hybrids in the region.

the GY for all crosses in Supplemental Table S2, with any of the two types of three groups, no super high GY crosses were missed (Table S2). It seems that classifying these 12 lines into three heterotic groups was best for both improving breeding efficiency (less intergroup crosses) and effectiveness (not miss any super hybrids).

Fan et al. (2007, 2008) postulated that three heterotic groups (tri-heterotic group [TriHG]) in maize might be the most suitable number for improving both maize breeding effectiveness (missing fewer super hybrid) and efficiency or productivity (i.e., produce more super hybrids with certain number of crosses). With the application of this TriHG theory to their maize breeding programs, many super maize hybrids have been developed and released in southwest China; a few released and widely used maize hybrids in the region were shown in Fig. 4. Because the data from this study showed that two heterotic groups were not sufficient, as too many super hybrids were missed, we are of the opinion that most of the maize lines may be grouped according to TRiGH in southwest China. Germplasms from the Suwan1 group are from the tropical and subtropical region and different mega-environments may cause them to be much different from Reid and non-Reid groups from temperate regions (Reif et al., 2004). Although more experimental data are needed to verify TriGH in maize in other maize-production regions, we are of the opinion that with more tropical and subtropical maize germplasms used in temperate maize-breeding programs, TriHG may be useful in the future.

Supplemental Information Available

Supplemental material is available at http://www.crops.org/publications/cs.

Supplemental Table S1. F_1 crosses and reciprocal F_1 crosses with the top 17 high green yield crosses.

Supplemental Table S2. Includes the grain yield (GY) and specific combining ability (SCA) of all 66 crosses from a 12-parent diallel with Griffing Method 4.

Supplemental Table S3. The specific combining ability (SCA) effects and reciprocal effects (RECs) of all 132 crosses from a 12-parent diallel with Griffing method 3.

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