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Assessing the breeding potential of newly developed inbred lines derived from India and Brazil germplasms in Southwest China

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Abstract

Introducing exotic maize germplasm can i) enrich the current genetic pool in Southwest China and ii) settle the severe situation of narrowing genetic diversity. To pave the path of the utilization of exotic germplasms from Brazil and India, 33 newly developed lines and six testers were genotyped with 32 simple sequence repeats (SSRs) for classifying heterotic groups. Ten out of those new lines were crossed to six testers according to the North Carolina II mating design, resulting in 60 single-cross hybrids. The 60 hybrids along with the commercial hybrid "Guinongyu889" were evaluated for 12 agronomic traits in two environments. The average polymorphism information content for the 32 SSRs was 0.73 and the average genetic similarity 0.52. The genetic analysis grouped the lines into six heterotic groups that are basically in line with information obtained from pedigree and specific combining ability effects-based groups. The lines 1862 and 363 had high general combining ability, and the hybrids 1862 × 223, Mo17 × 149, and 1862 × 363 exhibited the highest grain yield per plant; therefore, they could be used for the further commercial agronomic test. The high-yielding combinations suggested two promising heterotic patterns "Reid × PB (tem-tropical I)" and "Reid × Tropical", and exotic germplasms from India and Brazil could be utilized directly without bridging into elite germplasms in Southwest China. According to heterotic groups classified in the present study, bridging these exotic materials into elite base breeding populations is recommended for subsequent long-term breeding programs

Abbreviations

DTPS – days to pollen shed
DTS – days to silking
ED – ear diameter
EH – ear height
EL – ear length
ETB – ear tip-barrenness
GCA – general combining ability
GS – genetic similarity
GYPP – grain yield per plant
HKW – hundred kernels weight
KNPR – kernel number per row
NCII – North Carolina II mating design
PA – improved Reid
PB – tem-tropical I (abbreviated from the heterotic group temperate × tropical I)
PCR – polymerase chain reaction
PH – plant height
PIC – polymorphism information content
RHC – relative heterosis over the check
RKP – rate of kernel production
RN – row number
SCA – specific combining ability
SSR – simple sequence repeat
TCA – total combining ability
tem-tropical I – heterotic group temperate × tropical I
UPGMA – unweighted pair group method with arithmetic average

Introduction

Southwest China, mainly including Yunan, Guizhou, Sichuan, and Guangxi provinces, is the third maize-planting zone in China (Li 2009), with an average yield of around 9 tons/ha in the 2010s (Qin *et al.* 2016). The maize yield production in this mountainous region, with much more rainfall, still has a potential room to increase when compared with the Northeast and Yellow and Huai River maize zone. Currently, maize breeding in Southwest China uses few foundation parents and their descendants (Li and Wang 2010; Guo *et al*. 2020), which narrows the genetic diversity of maize germplasms and increases the genetic vulnerability to unpredictable biotic and abiotic stresses (McCouch *et al*. 2013; Allier *et al*. 2020). Besides, multiple complex microclimates within this region draw a high demand for abiotic and disease resistance of maize. A critical issue of maize breeding strategies is to broaden the germplasm base, particularly in the introduction of exotic genetic materials. Moreover, exotic germplasm can contribute new alleles to generate high-yielding hybrids (Menkir *et al*. 2006). Therefore, introducing exotic maize materials will harness favorable alleles from diverse resources that could be incorporated into elite germplasms.

The utilization of heterosis in maize is critical for boosting grain yield (Duvick 2005), and proper heterotic groups established from diverse germplasm resources according to the information of pedigree, phenotype, and molecular markers, played a key role in maize breeding (Zhang *et al*. 2002). Generally, traditional maize germplasm in China is classified into six heterotic groups, namely Tangsipingtou (a maize landrace group with the early-flowering trait), Lvdaredcob (a maize landrace group with red cob and thick ear derived from Lvshun district of Dalian city in Northeast China), Lancaster, the improved Reid (also abbreviated as PA), temtropical I (abbreviated from the heterotic group temperate × tropical I; also abbreviated as PB), and tropical subgroups (Li and Wang 2010). However, the Chinese heterotic patterns also varied among diverse ecological zones, especially in the shallow hill and mountainous regions of Southwest China (Peng *et al*. 2003; Pan *et al*. 2020). Hence, mastering pedigree information, general combining ability (GCA), and heterotic groups of new exotic germplasm will allow for designing efficient breeding programs.

In this study, 33 new inbred lines bred from India and Brazil, along with six testers, were genotyped with 32 simple sequence repeats (SSRs) for genetic diversity analysis. Ten out of 33 new lines were crossed to six testers stated above according to the North Carolina II mating design (NCII) (Comstock and Robinson 1948), resulting in 60 F1 hybrids. We evaluated 12 agronomic traits in these 60 hybrids and the commercial hybrid "Guinongyu889", as a check, under two environments. The objectives of the present study were (1) to use the SSRs markers to evaluate genetic diversity and heterotic groups of 33 new inbred lines, (2) to analyze GCA and specific combining ability (SCA) of those lines for 12 agronomic traits to see if it is consistent with heterotic groups of new lines classification based on SCA effects and molecular markers, and (3) to try to find the potential high-yielding crosses and their corresponding heterotic patterns, as well as pave more useful clues for maize breeding strategies with Indian and Brazilian resources in Southwest China.

Material and methods

Plant materials

In the current study, three inbred lines (F19, 171, and Ya8201) from Brazilian germplasm, and four hybrids (B06, K07, Bdsd 87-1, and Bdsd 265) from Indian maize resources, were used for the development of 33 new inbred lines (Supplementary Table S1). According to the NCII matting design (Comstock and Robinson 1948), ten new inbreds (133, 193, 223, 230, 236, 279, and 293 derived from Brazilian germplasm; 137, 149, and 363 bred from Indian materials) were crossed to six testers, Mo17 (Lancaster), 1862 (Reid), PN13-2 (PB), W527 (selected from a landrace from Guizhou), Dan340 (Lvdaredcob), and S37 (Suwan1 derived from a tropical population from Thailand) in the winter of 2015, in the Guizhou upland crops breeding base of Ledong, Hainan province (108.98°N,18.46°E). The resulting 60 testcross hybrids and the check Guinongyu889 (GP76 × GP16) were used for further evaluations.

Field trials and phenotypic trait measurements

The 60 F_1 hybrids along with Guinongyu889 were sown in the middle of April 2016, at two locations in China. The details of two locations are described as follows: the experimental farm of Guizhou University, Guiyang (106.68°N, 26.43°E; average annual rainfall: 1129.5 mm; annual average temperature: 15.3 °C; annual average sunshine hours: 1148.3 h; height above sea level: around 1100 m; tropical humid monsoon climate), and the experimental field of Shibing (108.13°N, 27.04°E; average annual rainfall: 1060 – 1200 mm; annual average temperature: 14 – 16 °C; annual average sunshine hours: 1197 h; height above sea level: around 800 m; tropical humid monsoon climate), Guizhou province. Each trial followed a randomized complete block design with three replications. Each plot included two 5 m long rows. Row spacing was 0.75 m, and each row contained 12 plants, reaching a plant density of 32,000 plants/ha. Field management including fertilizers, pesticides, fungicides, and weed control, followed local practices and actual needs.

Days to silking (DTS, day) and days to pollen shed (DTPS, day) were measured during maize flowering time as described by Buckler *et al*. (2009). Five plants were randomly selected in the middle of each row to measure plant height (PH, cm) and ear height (EH, cm) after 20 days from flowering time. All the ears of each plot were harvested after the maturity stage. Then, ear length (EL, cm), ear diameter (ED, mm), ear tip-barrenness (ETB, cm), row number (RN), kernel number per row (KNPR), hundred kernels weight (HKW, g), rate of kernel production (RKP), and grain yield per plant (GYPP) were assessed as described by Yi *et al.* (2020).

Genotyping and genetic analysis

Genomic DNA was isolated from the 33 new inbred lines and the 6 testers mentioned above. In order to do so, the 39 inbred lines were sown in a growth chamber and, after one week from planting, young leaves of five seedlings were collected for DNA extraction by using the cetyltrimethylammonium bromide method (Saghai-Maroof *et al.* 1984). Ninety SSR primers uniformly distributed across ten chromosomes were chosen from the maize genome database (http://www.maizegdb.org/) to examine clear and stable polymorphisms among the six testers. Then, 32 SSRs that showed clear and stable polymorphism between the 6 testers were selected for further genotyping of the 39 inbred lines. The polymerase chain reaction (PCR) mixture (10 µl) contained 1× Taq buffer, 3.0 mM $MgCl_2$, 0.24 mM dNTPs, 0.2875 µmol/µl of primers, 0.075 U of Taq polymerase, and 5.0 ng/µl of template DNA. The PCR reaction condition followed the program: one cycle of 94 °C for 5 min (an initial denaturation); then, followed by 35 cycles of 95 °C for 30 s (denaturation), 35 cycles of 58 °C for 30 s (annealing), 35 cycles of 72 °C for 30 s (extension). The final extension was performed at 72 °C for 10 min before cooling to 4 °C (modified from Williams *et al.* 1990). Finally, the 10 µl amplified products mixed with 2 µl of 10× DNA loading dye were electrophoresed on 10% denaturing polyacrylamide gels and visualized after silver staining (CIMMYT 2001). Assuming that each band position corresponds to a locus with two alleles, the states of the amplified DNA fragments can be scored for each SSR primer separately as one (1) for the presence or zero (0) for the absence of a particular allele, respectively (Devi and Singh 2011).

Genetic similarity (GS) was calculated following the equation below:

$$\mathrm{GS}=\mathrm{m}/(\mathrm{m}+\mathrm{n})$$

where m = the number of marker fragments shared between two individuals and n = the number of marker fragments differed between both individuals (Jaccard 1908). Polymorphism information content (PIC) was estimated using the method reported by Smith *et al.* (1997):

$$\mathrm{PIC}=1-\sum \mathrm{P}_i^2$$

where P_i is the frequency of the i^{th} allele. Finally, hierarchical cluster analysis was carried out using the Unweighted Pair Group Method with Arithmetic average (UPGMA) algorithm using the NTSYS-pc 2.10e program (Rohlf 2005).

Statistical analysis

Combined analysis of variance for each trait across both environments was estimated with a general linear model using DPS 7.05 software (Tang 2010):

$$Y_{abc}=\mu+B_c\,(L_a)+L_a+G_b+LG_{ab}+\varepsilon_{-abc}$$

where Y_{-abc} is the observed value, μ is the mean, B_c (L_a) is the block effect c within location a, L_a is the location effect a, G_b is the genotype effect b, LG_{ab} is the interaction effect between genotype b and location a, and ε_{-abc} is the residual effect.

GCA and SCA effects for 12 agronomic traits were calculated with the equations below (Ni *et al.* 2009) using DPS 7.05 software (Tang 2010):

$$g_i = \bar{x}_{i.} - \bar{x}_{..};$$

$$g_j = \bar{x}_{.j} - \bar{x}_{..};$$

$$s_{ij} = x_{ij} - \bar{x}_{..} - g_i - g_j;$$

$$T_{ij} = s_{ij} + g_i + g_j;$$

where g_i = GCA effect of new inbred line i; g_j = GCA effect of tester j ; s_{ij} = SCA effect between the new inbred line i and tester j ; $\bar{x}_{..}$ = overall mean; x_{ij} = phenotypic value of the testcross hybrid between the new inbred line i and tester j ; $\bar{x}_{i.}$ = average of the six hybrids between new inbred line i and the six testers; $\bar{x}_{.j}$ = average of the 10 hybrids between the 10 new inbred lines and tester j; T_{ij} = the sum of s_{ij} , g_i, and g_j. Relative heterosis over the check (RHC, %) = (F_1-*check*)/*check*, where F_1 is the testcross hybrid performance for GYPP, and check is the GYPP average of the commercial hybrid Guinongyu889. The correlations, r (GYPP, TCA for GYPP), r (GYPP, RHC for GYPP), r (GYPP, SCA for GYPP), r (GYPP, GCA of male parents), and r (GYPP, GCA of female parents) were performed with the R project (R Development Core Team 2010).

Results and discussion

Genetic diversity analysis of newly developed inbred lines

Among the 39 evaluated inbred lines, the average number of alleles per SSR marker for 32 selected SSRs was 4.59, with a minimum of three and a maximum of eight alleles per marker (Supplementary Table S2). The average alleles per locus in the present study (4.59) had a higher value than in previous studies showing around three alleles per locus (Badu-Apraku *et al.* 2013; Akinwale *et al.* 2014; Kamara *et al.* 2020), but a lower

Table 1 - Mean squares from the combined analyses of 60 testcross hybrids for 12 agronomic traits across two environments

Trait	Mean squares				
	Block(Location)	Location	Genotype	Genotype × Location	Residual error
PH	0.01	0.24**	0.18**	0.14**	0.01
EH	0.003	0.10**	0.09**	0.06**	0.002
DTS	2.38**	34.23**	82.07**	68.03**	0.51
DTPS	2.06*	52.14**	78.82**	71.58**	0.47
EL	1.67	15.21**	5.55**	4.52**	0.41
ED	0.04	0.05	0.35**	0.42**	0.12
RN	1.09	5.04**	7.46**	9.75**	0.51
GYPP	34.38	3676.17**	2861.58**	2098.70**	33.29
RKP	7.22	0.15	41.05**	26.85**	4.17
HKW	4.78	18.23	49.45**	49.64**	8.75
KNPR	4.85	3.25	46.58**	32.45**	2.94
ETB	0.01	0.12	0.47**	0.51**	0.06

Plant height (PH, cm) ear height (EH, cm), days to silking (DTS, day), days to pollen shed (DTPS, day), ear length (EL, cm), ear diameter (ED, mm), ear tip-barrenness (ETB, cm), row number (RN), kernel number per row (KNPR), hundred kernels weight (HKW, g), rate of kernel production (RKP), and grain yield per plant (GYPP). Block (Location) indicates block within location. * and **, significant at $p < 0.05$ and $p < 0.01$, respectively.

value than the 5.7 alleles/locus reported by Oyekunle *et al*. (2015). The mean PIC for these SSRs was 0.73, and PIC values ranged from 0.55 for umc1505 to 0.86 for umc1239. These 32 highly informative markers (Botstein *et al*. 1980) had a higher average than the values reported in other studies (Akinwale *et al*. 2014; Wu *et al*. 2019; Kamara *et al*. 2020), though we had a much lower marker density than the MaizeSNP50 BeadChip used by Wu *et al*. (2019). The GS analysis among these inbreds using the NTSYS-pc 2.10e program revealed a wide GS range from 0.16 between Indian material-derived 233 and Brazilian material-derived 377 to 0.84 between the F19 derivatives 250 and 263. Elite inbreds Ya8201 and F19 were selected from Brazilian materials. Altogether, these results indicate that these inbred lines, stated above, showed a large range of genetic diversity based on different geographical origins.

The UPGMA dendrogram of 33 maize inbred lines along with six testers was presented in Fig. 1. The 32 SSRs grouped 39 inbred lines into six groups. Group I contained four inbred lines from India, six from Brazil, and the tester PN13-2 derived from PB group, and PB group is widely used in Southwest China (Yi *et al*. 2019). Group II contained three inbred lines from India and the tester Dan340 (Lvdaredcob). Group III contained two inbred lines from India, two from Brazil, and the tester Mo17. Eight new lines from Brazil, one from India, and the tester 1862 from Reid group got assigned to group IV. Three new inbred lines from Brazil, three from India, and S37 (Suwan) were assigned to Group V. Group VI had W527 (Guizhou Landrace) and 131. The six groups are well in accordance with six conventional maize groups (Tangsipingtou, Lvdaredcob, Lancaster, PA, PB, and tropical groups) reported by previous studies in China (Li and Wang 2010). For instance, second cycle lines 393, 274, 285, 293, and 131 with female parents derived from Lancaster, Reid, and Landrace were assigned to groups III (Mo17), IV (1861), and VI (W527), which also highlighted the important contribution of donors (Qiao *et al*. 2009). Besides, several inbreds with the same pedigree within one group, such as new inbred lines 105 and 116 selected from the Indian hybrid Bdsd 265, confirmed the reliability of the SSR approach for the identification of genotypes. However, we also found some inconsistencies between clustering analysis and pedigree information. For example, 141 belonging to group IV was bred from S137 (Suwan) × F19 (Brazil). Several possible reasons could explain this discrepancy; for example, unlike previous studies with the high-density markers (Zhang et al. 2016; Leng et al. 2019; Wu et al. 2019), we used 32 SSRs that could be unevenly distributed across chromosomes, which may cause a limited coverage at marker loci for the genomes (Devi and Singh 2011).

General combining ability and specific combining ability effects in new lines

Combined analyses of the 60 hybrids for 12 agronomic traits across both environments indicated that genotype and the interaction between genotype and location were highly significant for all traits ($p < 0.01$; Table 1); therefore, there was genetic diversity among these inbred lines.

The GCA effects of 10 new inbred lines crossed by six testers for 12 agronomic traits are presented in Figures 2 and 3. High positive GCA effects would be favorable for GYPP, EL, ED, RN, KNPR, HKW, and RKP; conversely, for DTPS, DTS, PH, EH, and ETB high negative values would be desirable based on the current maize breeding trends (Duvick 2005; Kamara *et al*. 2020). The

Table 2 - Total combining ability (TCA) and relative heterosis over the check (RHC) of the 60 F_1 testcrosses for grain yield per plant (GYPP)

Rank	Hybrid combinations	GYPP(g)	TCA	RHC (%)	Rank	Hybrid combinations	GYPP(g)	TCA	RHC (%)
1	1862×223	291.83	18.93	14.41	31	S37×193	241.78	-1.47	-5.21
2	Mo17×149	287.58	17.20	12.75	32	PN13-2×363	241.35	-1.64	-5.38
3	1862×363	279.82	14.03	9.70	33	PN13-2×279	240.63	-1.94	-5.66
4	1862×193	275.22	12.16	7.90	34	1862×133	240.52	-1.98	-5.70
5	1862×236	273.77	11.57	7.33	35	S37×223	240.30	-2.07	-5.79
6	S37×363	272.42	11.02	6.80	36	S37×279	239.43	-2.42	-6.13
7	Mo17×279	271.12	10.49	6.29	37	PN13-2×293	238.87	-2.66	-6.35
8	W527×363	270.12	10.08	5.90	38	W527×293	236.92	-3.45	-7.11
9	Mo17×133	268.90	9.58	5.42	39	Dan340×279	236.68	-3.55	-7.21
10	1862×279	265.70	8.28	4.17	40	1862×293	236.43	-3.65	-7.31
11	Dan340×236	265.70	8.28	4.17	41	PN13-2×137	236.35	-3.68	-7.34
12	W527×149	265.23	8.09	3.98	42	Dan340×149	235.58	-3.99	-7.64
13	PN13-2×236	264.92	7.96	3.86	43	Dan340×293	234.57	-4.41	-8.04
14	1862×230	264.10	7.63	3.54	44	Mo17×293	233.27	-4.94	-8.55
15	PN13-2×193	260.27	6.07	2.04	45	S37×133	230.35	-6.13	-9.69
16	Dan340×133	260.20	6.04	2.01	46	Mo17×193	230.20	-6.19	-9.75
17	PN13-2×133	257.92	5.11	1.12	47	Mo17×236	229.73	-6.38	-9.93
18	1862×137	255.90	4.29	0.33	48	Mo17×363	229.05	-6.66	-10.20
19	W527×236	255.50	4.12	0.17	49	1862×149	228.75	-6.78	-10.32
20	Dan340×363	255.12	3.97	0.02	50	W527×137	228.32	-6.95	-10.49
21	W527×279	254.37	3.66	-0.27	51	S37×236	226.55	-7.67	-11.18
22	W527×223	254.32	3.64	-0.29	52	PN13-2×230	225.93	-7.93	-11.42
23	S37×230	253.50	3.31	-0.61	53	S37×293	224.97	-8.32	-11.80
24	S37×137	251.85	2.64	-1.26	54	Dan340×193	223.38	-8.97	-12.42
25	PN13-2×223	251.62	2.54	-1.35	55	PN13-2×149	220.10	-10.30	-13.71
26	S37×149	250.02	1.89	-1.98	56	W527×193	219.04	-12.88	-14.12
27	W527×133	249.35	1.62	-2.24	57	Mo17×230	213.57	-12.97	-16.27
28	Mo17×223	248.92	1.44	-2.41	58	Dan340×223	202.65	-17.41	-20.55
29	Dan340×137	246.92	0.63	-3.19	59	W527×230	200.45	-18.31	-21.41
30	Dan340×230	245.28	-0.04	-3.84	60	Mo17×137	192.07	-20.53	-24.70

new inbred lines that presented the highest positive GCA effects were 363 for GYPP, EL, HKW, and RKP, 279 for RN and ED, and 193 for KNPR, whereas the testers with the highest positive GCA effects were 1862 for GYPP and RN, W527 for KNPR and RKP, PN13-2 for EL and ED, and Dan340 for HKW. The highest favorable GCA effects belonged to the new inbred lines 236 for DTPS and DTS, 279 for PH and EH, and 223 for ETB; the testers Mo17 for PH and EH, 1862 for DTPS, W527 for DTS, and PN13-2 for ETB. Therefore, 363 and 279 within the new inbred lines exhibited the highest favorable GCA effects for up to four traits, whereas W527, 1862, and PN13-2 had the highest favorable GCA effects for three traits among the six testers, which potentially indicated a possible utilization in further breeding programs.

The SCA effects of the 60 F_1 hybrids for 12 agronomic traits across two locations are presented in Supplementary Table S3. Regarding GYPP, the SCA effects varied from different testers and origins. No specific trends were found between new inbred lines from India and Brazil-derived germplasms. The combinations with the high positive SCA effects for GYPP were 1862 × 193, 1862 × 233, Dan340 × 149, Dan340 × 230, Mo17 ×133, Mo17 × 149, Mo17 × 279, PN13-2 × 193, PN13-2 × 236, S37 × 137, S37 × 133, S37 × 230, S37 × 236, and W527 × 149. The correlated traits, such as PH and EH, showed a similar trend for SCA effects among different combinations, however other traits are not in keeping with such tendency. Favorable SCA effects for GYPP, RN, and RKP were found for the hybrid 1862 × 223 (heterotic pattern "Reid × PB"); SCA was also favorable for GYPP, RN, and DTPS for the hybrid Mo17 × 279 ("Lancaster × Reid"), and for DTPS, DTS, and RN for the cross Dan340 × 149 ("Lvdaredcob × PB").

Lines that presented positive SCA effects are in opposite heterotic groups whereas negative SCA effects indicate that the lines are in the same heterotic groups (Vasal *et al.* 1992). Concerning GYPP (Supplementary Table S3), the SCA effects of 1862 × 279 and 1862 ×

293 for GYPP are negative. The combinations between 293 and the testers, except Dan340 and 1862, exhibited positive SCA effects. Notably, Dan340 has been characterized as a potential genome donor for Zheng58, and Dan340 and Zheng58 were classified into Domestic Reid (Zhang *et al*. 2018). This is in agreement with the classified groups using clustering analysis. However, according to the clustering results, 236 also belongs to group IV (1862), but it had negative SCA effects for GYPP only when crossed with Mo17 or Dan340. Moreover, the SCA effect of the PB line PN13-2 with 149 or 230 was negative but positive with 137, 193, or 223. Group V (tropical) lines 133 and 363 differed for SCA effects when crossed to S37. These inconsistencies between classifications based on SCA and molecular markers could be explained by the low precisions of these classifications when considered separately. Fan et al. (2008) also found this similar phenomenon and concluded that GCA effect is a reliable criterion and better than SCA effect for classifying new germplasms into different heterotic groups because it provided more consistent results. Therefore, all criteria have to be considered, including pedigree information, clustering analysis, SCA and GCA effects, in the classification of heterotic groups for new lines.

Hybrid performance, total combining ability, and relative heterosis over the check for GYPP

In the current study, the evaluated hybrid combinations exhibited a considerable variation for GYPP (Table 2). Highly significant and positive correlations were observed between GYPP and TCA, RHC, or SCA for GYPP ($r \geq 0.85$, $p < 0.01$). Moreover, GYPP is simultaneously significantly and positively correlated to GCA effects of male ($r = 0.35$, $p < 0.01$) and female parents ($r = 0.40$, $p < 0.01$), though the correlations are quite weak, which is in agreement with the weak correlations among those traits reported in previous studies (Qi *et al*. 2013; Huang *et al*. 2013; Lu *et al*. 2020). Accordingly, the top ten high-yielding F_1 hybrids showed the highest TCA effects and had RHC values of > 4%. The crosses 1862 × 223 ("Reid × PB") with RHC = 14.41%, Mo17 × 149 ("Lancaster × PB") with RHC = 12.75%, and 1862×363 ("Reid × Tropical") with RHC = 9.7%, were the top three high-yielding hybrids. Additionally, high SCA effects were observed for GYPP in the crosses Mo17 × 149, Mo17 × 279, and Mo17 × 133, but their parents exhibited low GCA effects, emphasizing the importance of non-additive effects (Devi and Singh 2011; Kamara *et al*. 2020). However among most of the top ten, at least one parent with high GCA effects is required for producing high-yielding hybrids, and getting excellent combinations from high GCA parents would be much easier than obtaining the excellent cross between the parents with low × low GCA effects, especially under stress conditions (Kamara *et al*. 2020; Chiuta *et al*. 2020). Taking these results together, 1862 and 363 with high GCA effects were the best tester and new line, respectively.

Maize breeders pay specific attention to adopting the exotic germplasm to fuel breeding programs. The firstly successful application of the elite inbred line S37 deri-

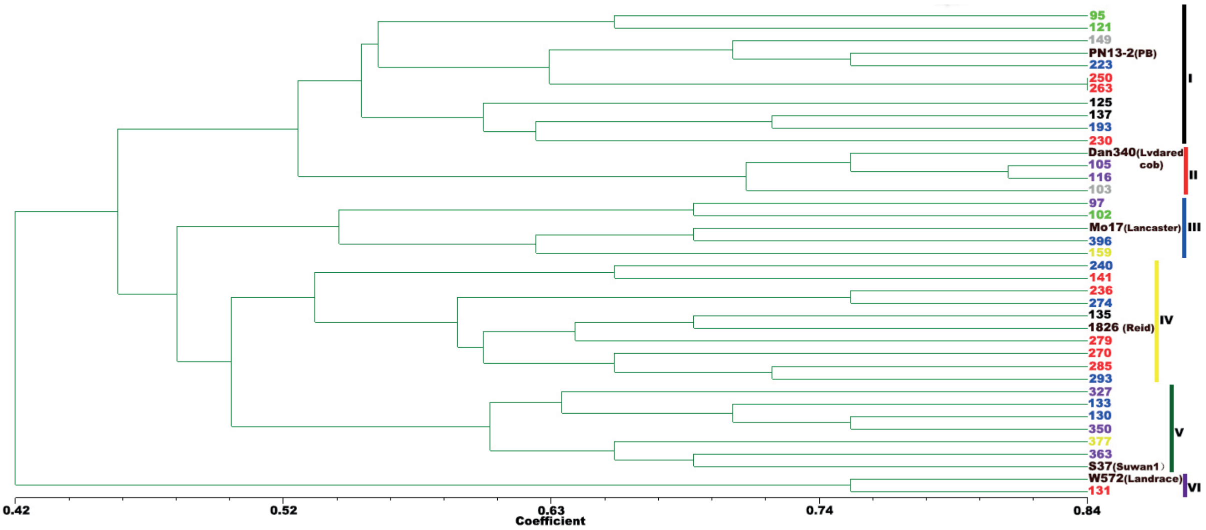

Fig. 1 - Unweighted Pair Group Method with Arithmetic average Dendrogram of 33 maize new inbred lines and six testers based on 32 SSR markers. New inbred lines derived from Brazilian materials F19, 171, and Ya8201 were indicated with red, yellow, and blue, respectively. New inbred lines derived from India materials B06, K07, Bdsd 265, and Bdsd 87-1 were indicated in black, green, purple, and grey, respectively. These new inbred lines were classified into five groups (I, II, III, IV, and V) based on the threshold of coefficient = 0.53

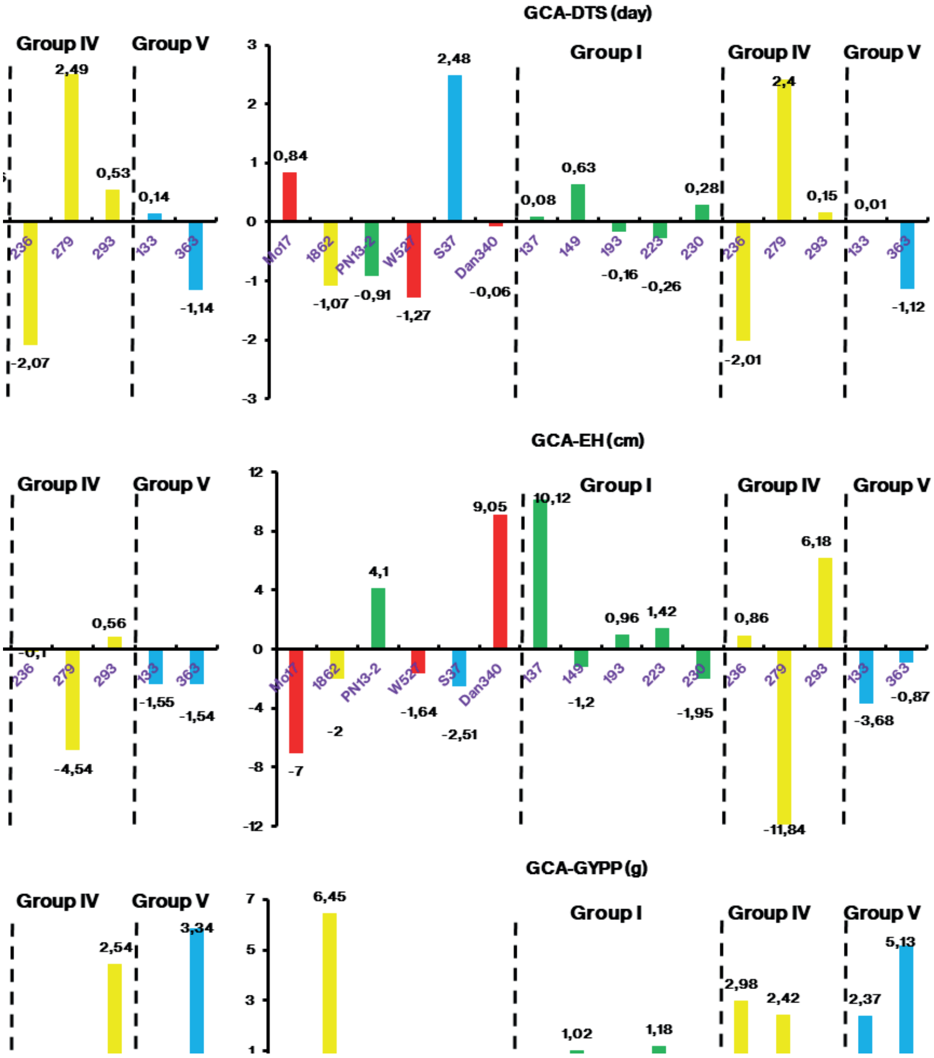

Fig. 2 -General combining ability (GCA) of 10 new inbred lines and six testers for days to pollen shed (DTPS), days to silking (DTS), plant height (PH), ear height (EH), ear length (EL), and grain yield per plant (GYPP) across two locations. These ten inbred lines were classified I, IV, and V based on Fig. 1. The GCA effects of inbred lines from groups I, IV, and V indicated with green, yellow, and blue, respectively. The GCA effects of Mo17, W527, and Dan340 indicated with red

ved from the Suwan-1 tropical population in Southwest China in the 1980s triggered a new insight that popular commercial hybrids could be produced using the heterotic patterns “temperate germplasm × tropical germplasm” in this region (Pan *et al*. 2020). Subsequently, more elite maize inbred lines were released from Suwan, Tuxpeño, ETO, and other tropical populations. Several classical inbreds, such as S37, T32, PA212, S1611, S11, QR273, and Nan99, were developed from the Suwan germplasm. M09 and PA31 were derived from Tuxpeño germplasm. Tropical maize exhibited a flourish and strong plant and root system, stay-green leaves, and tolerance to drought, lodging, and disease (Peng *et al*. 2004; Chen *et al*. 2013), and have been used to produce several popular commercial hybrids, containing Yayu2 (7922 × S37), Zhengda619 (F06 × F19), Dika007 (PA212 × PA31), and Guidan8 (P159 × T32) in Southwest China. The excellently new inbred line 336 and the tester S37 were classified into Group V in the present study, and the high-yielding combination 1862 × 363 conforms to the heterotic pattern “Reid × tropical germplasm”.

These results of high-yielding combinations and clustered groups in the present study further addressed the predominance of the heterotic pattern “Reid × PB” (Yi *et al*. 2019) and “Reid × tropical” in the low-planting zone of Southwest China, confirming the successful application of double and "triangle" heterotic pattern proposed by other breeders in this region (Fan *et al*. 2008, 2016; Chen *et al*. 2010; Pan *et al*. 2020). Fan *et*

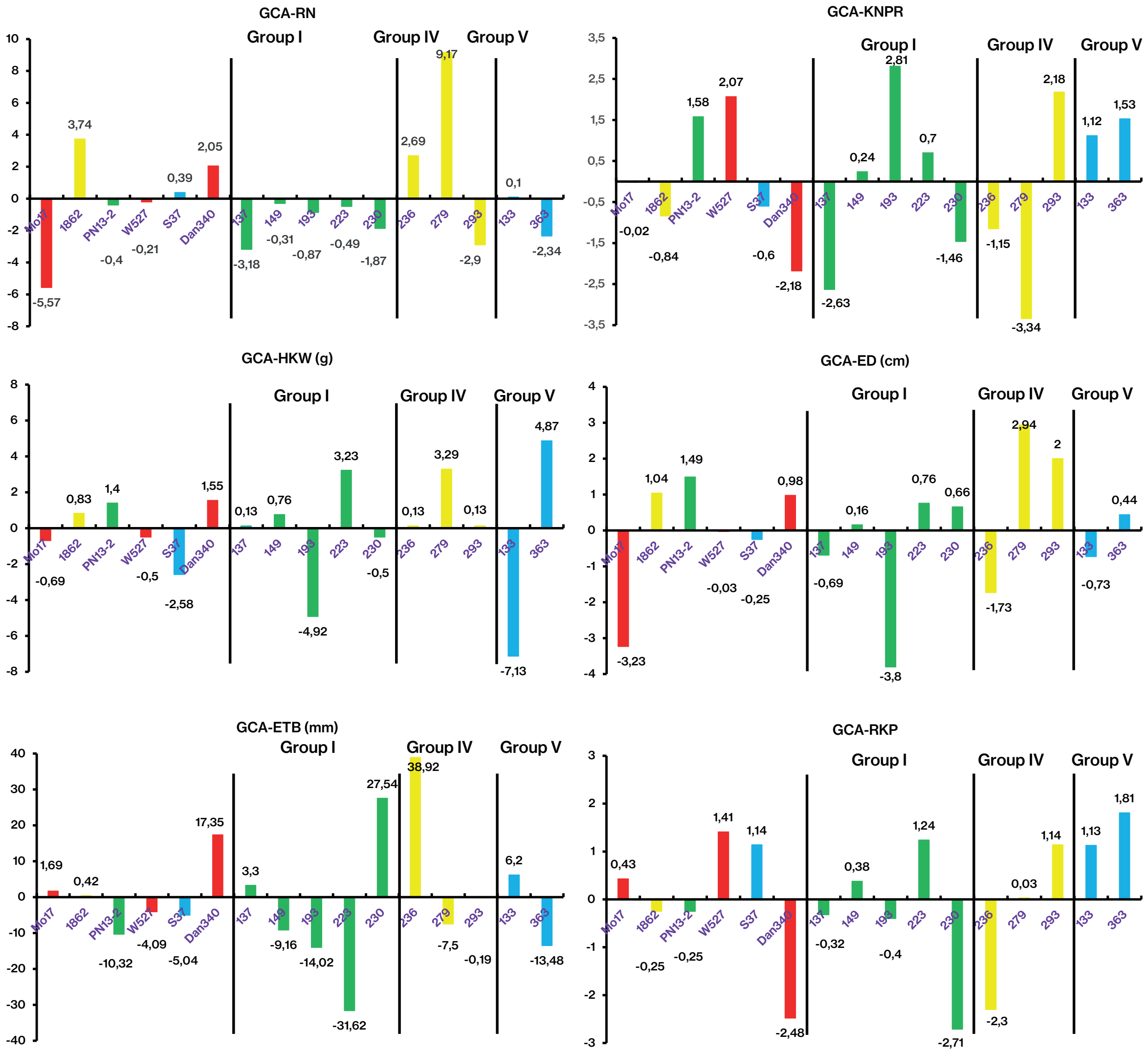

Fig. 3 -General combining ability (GCA) of 10 new inbred lines and six testers for row number (RN), kernel number per row (KNPR), hundred kernels weight (HKW), ear diameter (ED), ear tip-barrenness (ETB), and rate of kernel production (RKP) across two locations. These ten inbred lines were classified I, IV, and V based on Fig. 1. The GCA effects of inbred lines from groups I, IV, and V indicated with green, yellow, and blue, respectively. The GCA effects of Mo17, W527, and Dan340 indicated with red.

al. (2008, 2016) highlighted that three maize heterotic groups, namely Reid, non-Reid, and Suwan1, seemed to be an ideal number for improving maize breeding efficiency in Southwest China. Pan *et al*. (2020) reviewed the development process of maize breeding in the past several decades of this region and proposed a similar conclusion that a "triangle" heterotic pattern among Reid (Reid, PA, and Lvdaredcob), non-Reid (Lancaster and PB), and Tropical as well as landraces, could be more practicable. Chen *et al*. (2010) conducted a continuous breeding program, developed two balanced populations, Reid/Tuxpeño and Lancaster/Suwan, and recommended this promising heterotic pattern "Reid/Tuxpeño × Lancaster/Suwan", which is similar to the heterotic pattern "BSSS/Tuxpeño × non-BSSS/non-Tuxpeño" (Hallauer *et al*. 2010). These results also indicated that the exotic germplasm from India and Brazil could be utilized directly without bridging into elite germplasms in Guizhou province in the short term. Given that significantly higher mid- and long-term genetic gain could be obtained by using the bridging approach especially when introducing low-performing donors (Allier et al. 2020), bridging these exotic materials into elite lines from PB and Tropical germplasm is recommended for long-term pre-breeding programs. We also found three excellent combinations 1862 × 236 ("Reid × Reid"), S37 × 363 ("Tropical × Tropical"), and 1862 × 279 ("Reid × Reid") in the same heterotic groups. A possible reason is a large discrepancy of specific gene frequencies between testers and the exotic lines in the same groups. However, the complementary theory of differences in parental gene frequency for explaining heterosis (Chen *et al*. 2020) supports that outstanding maize hybrids are more likely generated from the crosses involving elite lines from diverse heterotic groups.

Conclusions

The cluster analysis based on SSRs grouped 33 newly developed lines derived from India and Brazil germplasms and six testers into six heterotic groups, and these groups are basically in line with information obtained from pedigree and specific combining ability effects-based groups. The lines 1862 and 363, with high general combining ability, resulted in the best lines; while 1862 × 223, Mo17 × 149, and 1862 × 363 exhibited the highest grain yield per plant. The high-yielding combinations suggested two promising heterotic patterns "Reid × PB" and "Reid × Tropical", and exotic germplasms from India and Brazil could be utilized directly without bridging into elite germplasms in short term in Southwest China. Bridging these exotic materials into elite PB or tropical germplasms is recommended for subsequent long-term pre-breeding programs.

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Table S1 - Pedigree of 33 new maize inbred lines derived from India and Brazil germplasm along with six testers

Inbred	Pedigree	Crossed with testers
95	Hybrid K07 (India)	
96 (Mo17)	187-2 × C103	
97	Hybrid Bdsd 265 (India)	
101 (1862)	Bred from Reid germplasm	
102	Hybrid K07 (India)	
103	Hybrid Bdsd 87-1 (India)	
105	Hybrid Bdsd 265 (India)	
116	Hybrid Bdsd 265 (India)	
121	Hybrid K07 (India)	
125	Hybrid B06 (India)	
130	Reid × Ya8201 (Brazil)	
131	Landrace × F19 (Brazil)	
133	HuangC × Ya8201 (Brazil)	√
135	Hybrid B06 (India)	
137	Hybrid B06 (India)	√
141	S137 × F19 (Brazil)	
149	Hybrid Bdsd 87-1 (India)	√
159	Variant plant from 171	
193	Variant plant from Ya8201	√
206 (PN13-2)	PN78599	
223	Variant plant from Ya8201	√
230	F19 × Mobai (Tuxpeño)	√
236	171 (Brazil) × F19 (Brazil)	√
240	Aoruijing × Ya8201 (Brazil)	
250	171 (Brazil) × F19 (Brazil)	
263	F19 × Mobai (Tuxpeño)	
270 (F19)	Brazil	
274	Reid × Ya8201 (Brazil)	
279	S1371 × F19 (Brazil)	√
285	Reid × F19 (Brazil)	
293	Reid × Ya8201 (Brazil)	√
327	Hybrid Bdsd 265 (India)	
340 (W527)	Guizhou Landrace	
342 (Dan340)	Lv9 × Wide Pod Corn	
350	Hybrid Bdsd 265 (India)	
363	Hybrid Bdsd 265 (India)	√
377	Variant plants from 171 (Brazil)	
393 (S37)	bred from Suwan1 population	
396	Lancaster × Ya8201	

Table S2 - Polymorphism information content (PIC), simple sequence repeat (SSR) marker, bin, and number of alleles

SSR marker	Bin$_a$	Number of alleles	PIC
bnlg667	1.10	*7*	*0.82*
umc1737	1.10	5	0.73
umc1681	1.11	7	0.72
umc1538a	1.11	5	0.81
bnlg125	2.02	5	0.74
umc0231	2.03	4	0.78
bnlg1931	3.06	5	0.77
umc2381	3.06	4	0.64
bnlg1189	4.07	6	0.84
umc1847	4.07	5	0.64
umc1167	4.08	3	0.72
umc1612	4.08	4	0.70
bnlg161	5.05	4	0.79
umc2164	5.05	6	0.85
umc1883	6.00	4	0.67
umc5809	6.00	4	0.58
umc2309	6.00	3	0.58
phi031	6.04	6	0.75
umc1505	6.04	3	0.55
umc1795	6.05	3	0.70
umc1127	6.07	6	0.78
phi299852	6.07	4	0.83
umc1412	7.04	6	0.79
umc2332	7.04	8	0.79
umc1149	8.05	3	0.68
phi022	9.03	3	0.71
phi065	9.03	4	0.74
umc1634	9.03	3	0.69
umc1494	9.05	5	0.79
umc1714	9.07	4	0.69
umc1863	10.03	4	0.70
umc1239	10.03	4	0.86

a *https://www.maizegdb.org/bin_viewer*

Table S3 - Specific combining ability of the 60 F$_1$ testcrosses for 12 agronomic traits across two locations

Trait	Inbred	137	149	193	223	230	236	279	293	133	363
GYPP	Mo17	*-14.7*	*18.08*	*-2.41*	*2.16*	*-6.35*	*-7.46*	*9.96*	*1.53*	*9.1*	*0.15*
	1862	1.77	-14.24	7.59	11.3	5.9	2.14	-0.59	-5.52	-10.8	3.49
	PN13-2	0.9	-10.67	8.59	2.01	-2.56	5.63	-3.71	2.56	3.38	1.04
	W527	-1.98	8.11	-9.96	3.5	-12.56	2.18	2.28	2.16	0.28	-0.18
	S37	6.51	-3.06	-5.14	-16.65	6.62	7.25	-4.02	2.11	5.61	-1.19
	Dan340	7.49	1.79	1.34	-2.33	8.95	-9.73	-3.92	-2.83	-7.58	-3.31
DTPS	Mo17	8.55	-2.99	2.71	-4.66	3.28	-4.23	-5.37	2.13	-5.16	10.01
	1862	-0.57	-1.21	-3.21	-2.24	-4.77	0.53	5.16	0.28	1.1	4.94
	PN13-2	-1.71	10.26	2.92	3.24	-3.34	-3.17	2.1	-0.21	-4.52	5.59
	W527	-1.3	1.91	-4.36	4.08	6.25	-4.68	-4.11	0.62	5.07	-3.47
	S37	-2.11	0.45	3.16	1.13	-3.11	6.26	-0.44	-3.61	3.84	-5.57
	Dan340	-2.86	-8.41	-1.22	-1.54	1.7	5.3	2.66	5.05	-0.33	-0.33
DTS	Mo17	8.19	-2.82	2.48	-3.57	2.65	-4.49	-5.21	-1.52	-4.05	8.36
	1862	-0.16	-0.5	-2.79	-2.07	-5.9	-0.33	4.9	0.8	0.73	5.34
	PN13-2	-1.76	8.36	2.99	3.3	-1.76	-2.34	1.66	0.02	-4.56	-5.9
	W527	-0.98	1.34	-3.41	3.87	6.2	-3.82	-4.13	-0.23	4.21	-3.07
	S37	-3.3	1.28	1.86	0.73	-2.68	5.96	0.94	-2.96	3.95	-5.79
	Dan340	-1.99	-7.66	-1.13	-2.26	1.5	5.02	1.84	3.89	-0.28	1.06
PH	Mo17	3.66	7.34	-4.34	6.69	0.26	-11.93	4.84	0.51	3.73	-10.75
	1862	7.89	-6.73	-10.75	-2.9	8.25	2.42	0.51	3.77	0.37	-2.82
	PN13-2	-5.76	-12.66	-0.01	-6.49	11.21	7.19	-3.35	1.67	0.21	7.98
	W527	-1.76	2.69	-0.36	3.92	-9.45	2.89	8.76	-7.44	0.51	0.24
	S37	-6.67	9.08	11.67	-1.82	-10.2	-3.64	-2.83	6.73	-1.74	-0.58
	Dan340	2.64	0.28	3.78	0.6	-0.06	3.07	-7.93	-5.25	-3.07	5.93
EH	Mo17	0.41	14.48	-12.63	7.33	-16.52	-17.71	17.51	11.32	1.74	-5.93
	1862	10.31	-11.42	-1.27	-0.43	9.58	4.83	8.94	2.27	-17.53	-5.27
	PN13-2	2.6	-14.92	-13.84	-20.78	22.29	15.75	-15.46	7.03	7.33	10.03
	W527	0.07	-5.62	10.2	4.88	-16.05	2.69	10.04	-9.76	-0.06	3.61
	S37	-6.02	19.07	4.27	12.24	-10.15	-12.63	-12.74	5.05	4.38	-3.45
	Dan340	-7.38	-1.57	13.28	-3.22	10.85	7.07	-8.27	-15.91	4.15	1.02
EL	Mo17	-10.09	8.88	-6.43	0.9	-7	-0.76	7.17	12.05	0.32	-5.04
	1862	7.74	-8.84	1.56	2.51	5.65	4.41	-6.65	-0.87	-2.53	-2.99
	PN13-2	2.23	-3.09	3.94	0.91	-3.2	-1.87	4.34	-4.87	-0.89	2.5
	W527	-2.66	-2.05	-6.34	0.66	0.59	3.7	4.64	-7.48	7.37	1.58
	S37	3.79	2.46	5.13	-1.9	-6.73	-1.05	-5.77	0.16	3.72	0.2
	Dan340	-1	2.64	2.14	-3.07	10.7	-4.43	-3.73	1	-7.99	3.74
ED	Mo17	-3.31	4.87	1.78	-1.72	-0.27	-6.02	-0.85	0.88	6.82	-2.2
	1862	2.19	1.65	-8.46	6.02	7.24	-6.71	3.78	-4.64	-7.07	5.99
	PN13-2	3.02	-4.4	5.16	-2.76	-3.78	1.81	-0.77	8.24	-4.25	-2.28
	W527	-2.51	3.46	-2.9	-0.27	-8.34	9.97	-0.43	-2	-1.57	4.6
	S37	-1.81	-2.18	4.34	-2.07	1.39	6.44	-1.59	-3.32	2.33	-3.55
	Dan340	2.41	-3.41	0.07	0.8	3.75	-5.49	-0.13	0.84	3.73	-2.57
RN	Mo17	-0.33	10.38	-5.78	-5.95	-2.68	-16.03	11.98	-3.54	7.88	4.06
	1862	-10.69	-1.03	5.13	10.45	6.81	10.61	-10.4	-0.52	-16.06	5.71
	PN13-2	-5.4	-3.15	3.68	1.21	0.71	-8.24	-2.28	8.11	6.89	-1.53
	W527	5.38	0.21	-6.75	-1.07	-3.76	4.11	5.99	-1.79	-5.84	3.5
	S37	9.38	-4.99	2.47	-1.32	3.89	1.42	-0.88	-5.53	3.73	-8.18
	Dan340	1.66	-1.42	1.24	-3.32	-4.97	8.13	-4.42	3.27	3.4	-3.56
KNPR	Mo17	-3.43	4.89	-4.72	1.18	-7.85	-1.8	-0.65	15.06	4.03	-6.72
	1862	7.69	-6.85	-6.31	2.58	-0.38	2.97	-3.13	-0.03	0.69	2.78
	PN13-2	0.57	-4.16	9.89	-6.37	6.38	0.55	-3.58	-12.72	7.21	2.23
	W527	-2.78	9.43	8.76	10.41	-4.08	3.71	-3.16	-5.44	-6.37	-10.48
	S37	0.47	-0.74	-4.22	-3.27	-1.04	0.65	4.62	-2.13	4.29	1.38
	Dan340	-2.52	-2.57	-3.39	-4.52	6.98	-6.08	5.9	5.25	-9.85	10.81

Table S3 - Specific combining ability of the 60 F_1 testcrosses for 12 agronomic traits across two locations

Trait	Inbred	137	149	193	223	230	236	279	293	133	363
ETB	Mo17	*68.4*	*-41.46*	*8.74*	*-9.03*	*0.31*	*6.37*	*-8.74*	*-68.61*	*34.85*	*9.19*
	1862	-86.27	53.72	129.33	7.93	-31.3	20.09	-0.25	-87.77	26.65	-32.13
	PN13-2	-31.2	68.45	5.3	-24.93	-36.26	-33.69	-31.36	104.57	22.94	-43.82
	W527	-1.55	30.33	-44.52	19.66	34.98	-69.31	-32.11	-30.95	-40.83	134.29
	S37	33.27	-40.21	-33.11	-16.01	-49.27	40.99	64	38.01	-21.7	-15.97
	Dan340	17.35	-70.83	-65.72	22.38	81.54	35.54	8.47	44.76	-21.92	-51.57
HKW	Mo17	2.9	4.16	2.27	5.49	1.64	1	-2.15	-12.26	6.37	-9.42
	1862	1.38	0.75	-3.04	3.97	3.91	-0.51	5.81	-2.41	1.07	-10.94
	PN13-2	-6.77	3.97	9.66	-9.86	-11.82	0.82	-0.45	10.29	0.5	3.66
	W527	4.61	-1.71	-14.98	-6.07	3.34	-4.87	-4.24	10.29	-1.4	15.03
	S37	-2.79	-9.1	9.85	3.59	1.64	6.69	-0.26	-2.79	-6.89	0.06
	Dan340	0.66	1.93	-3.76	2.87	1.3	-3.13	1.3	-3.13	0.35	1.61
RKP	Mo17	1.27	1.71	0.42	-0.86	0.61	-2.92	2.14	-1.79	-0.47	-0.1
	1862	0.23	-1.11	1.06	3.9	-4.86	2.78	2.45	-2.41	-1.64	-0.38
	PN13-2	0.24	0.84	3.22	-2.56	-1.99	1.01	-3.09	-2.73	2.77	2.29
	W527	0.54	2.23	0.38	-3.98	0.4	0.56	1.13	1.71	-0.77	-2.21
	S37	-2.02	-0.5	-3.9	-0.32	2.91	0.51	0.87	2.75	0.25	-0.55
	Dan340	-0.26	-3.16	-1.18	3.83	2.94	-1.93	-3.5	2.47	-0.14	0.94

Each cell indicates a cross between an inbred from the horizontal axis and an inbred from the vertical axis. Plant height (PH, cm) ear height (EH, cm), days to silking (DTS, day), days to pollen shed (DTPS, day), ear length (EL, cm), ear diameter (ED, mm), ear tip-barrenness (ETB, cm), row number (RN), kernel number per row (KNPR), hundred kernels weight (HKW, g), rate of kernel production (RKP), and grain yield per plant (GYPP).