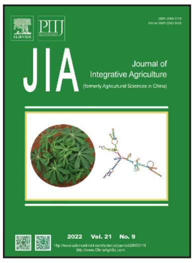
Journal of Integrative Agriculture 2022, 21(9): 2477–2491

Available online at www.sciencedirect.com

ScienceDirect



RESEARCH ARTICLE

Heterosis and heterotic patterns of maize germplasm revealed by a multiple-hybrid population under well-watered and drought-stressed conditions

SANG Zhi-qin[1, 2, 3], ZHANG Zhan-qin[2], YANG Yu-xin[3], LI Zhi-wei[3], LIU Xiao-gang[3], XU Yun-bi[3, 4], LI Wei-hua[1]

[1] *Key Laboratory of Oasis Eco-Agriculture, Xinjiang Production and Construction Corps/Agricultural College, Shihezi University, Shihezi 832003, P.R.China*
[2] *Xinjiang Academy of Agricultural and Reclamation Science, Shihezi 832000, P.R.China*
[3] *Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing 100081, P.R.China*
[4] *International Maize and Wheat Improvement Center (CIMMYT), El Batan Texcoco 56130, Mexico*

Abstract

Understanding the heterosis in multiple environments between different heterotic groups is of fundamental importance in successful maize breeding. A total of 737 hybrids derived from 41 maize inbreds were evaluated over two years, with the aim of assessing the genetic diversity and their performance between heterotic groups under drought-stressed (DS) and well-watered (WW) treatments. A total of 38 737 SNPs were employed to assess the genetic diversity. The genetic distance (GD) between the parents ranged from 0.05 to 0.74, and the 41 inbreds were classified into five heterotic groups. According to the hybrid performance (high yield and early maturity between heterotic groups), the heterosis and heterotic patterns of Iowa Stiff Stalk Synthetic (BSSS)×Non-Stiff Stalk (NSS), NSS×Sipingtou (SPT) and BSSS×SPT were identified to be useful options in China's maize breeding. The relative importance of general and specific combining abilities (GCA and SCA) suggests the importance of the additive genetic effects for grain yield traits under the WW treatment, but the non-additive effects under the DS treatment. At least one of the parental lines with drought tolerance and a high GCA effect would be required to achieve the ideal hybrid performance under drought conditions. GD showed a positive correlation with yield and yield heterosis in within-group hybrids over a certain range of GD. The present investigation suggests that the heterosis is due to the combined accumulation of superior genes/alleles in parents and the optimal genetic distance between parents, and that yield heterosis under DS treatment was mainly determined by the non-additive effects.

Keywords: maize, drought stress, heterosis, heterotic group, heterotic pattern, genetic distance

1. Introduction

Maize (*Zea mays* L.) is one of the oldest domesticated crop species in the world, and it is also an important feedstock and industrial raw material (Makumbi *et al.* 2018; Yang *et al.* 2021). As the demand for maize continues to increase, expanding its production is an

---

Received 26 December, 2020 Accepted 1 April, 2021
SANG Zhi-qin, E-mail: sangzhiqin@126.com; Correspondence LI Wei-hua, Mobile: +86-18997736665, E-mail: lwh_agr@shzu.edu.cn; XU Yun-bi, E-mail: y.xu@cgiar.org

doi: 10.1016/j.jia.2022.07.006

urgent challenge. Since 2007, maize planting area and total grain output have ranked first in China (FAO 2019). Chinese maize germplasms have been introduced from different regions and countries, and breeding projects in China have always integrated local varieties and exotic germplasm to broaden the germplasm resources. However, there is no complete record of the entire selection process (Zhang *et al.* 2016, 2017). Based on these considerations, an in-depth understanding of maize germplasm in China, including the genetic relationships and heterotic patterns, is very important (Choukan *et al.* 2006; Zhang *et al.* 2018), particularly through high-throughput SNP genotyping.

Drought has always been one of the major stresses that affect plant growth and reduce crop production (Toker *et al.* 2007; Hu and Xiong 2014), and it is most recalcitrant to breeding. Even in the areas with sufficient rainfall for maize production, intermittent drought is almost certain to occur during one or more plant growth stages, especially during the most sensitive flowering and grain filling phase (Menkir and Akintunde 2001; Oyekunle *et al.* 2015). While drought limits the crop growth and ultimate performance at any stage, it can decrease grain yields by up to 17% and reduce the average yield by more than 90% when severe stress coincides with the flowering and grain-filling stages (NeSmith and Ritchie 1992). Located in the center of Eurasia, Xinjiang is a typical arid and semi-arid farming region in China, with an average annual precipitation of about 150 mm. Therefore, there is an increasing demand for breeding maize with enhanced tolerance to drought and improved yield in water-deficient environments.

Generally, maize germplasm in China can be assigned to eight heterotic groups: Lancaster, Reid, Iowa Experiment Station Reid Yellow Dent (Iodent), Lvda Red Cob (LRC), Sipintou (SPT), group A germplasm derived from modern US hybrids (PA), group B germplasm derived from modern US hybrids (PB), and Tropical (Xie *et al.* 2007; Lu *et al.* 2009; Zhang *et al.* 2016, 2018; Xu *et al.* 2017). The Reid group primarily originated from the US southern dent type, while the Lancaster group originated from the US northern flint type. This difference in geographical origin laid the foundation for the heterosis observed between these two groups (Troyer and Palmer 2006). Aiming at early maturity and yield traits, Iodent was successfully selected from the open-pollinated varieties of Reid Yellow Dent lines by ear-to-row selection (Smith *et al.* 2004). With the development of the commercial breeding process, the heterotic groups in US have undergone great changes. The Reid group was gradually replaced by the Iowa Stiff Stalk Synthetic (BSSS) germplasm, thus establishing a female parent bank with BSSS as the main germplasm. On the contrary, the other germplasm was merged into Non-Stiff Stalk (NSS) as a paternal group (Mikel *et al.* 2008, 2011). The SPT group was bred from Tangshan Sipingtou Chinese landrace, which initiated a large-scale cultivation of compact maize in China. The PB group was derived from modern US hybrids, including 78599, 78641, 78698, and 87001, containing tropical and subtropical genetic germplasm, and has the advantages of good greenness, mature living culm, strong culm, resistance to leaf spot and stem rot, etc.

The exploitation of heterotic patterns provides a vital source of information for hybrid breeding (Laude *et al.* 2015). Although the genetic gain of maize inbred lines is the primary focus, breeders often want to produce superior performance of hybrids when one line is crossed with another from a different group (Ertiro *et al.* 2017). However, this does not necessarily imply that all hybrids produced between parents of different heterotic groups would always obtain high yield or heterosis, but only parental crosses between certain specific groups actually do (Wang *et al.* 2015). As a result, maize heterosis models are difficult to predict and are not consistent with the test materials and evaluation environments, and thus their application is limited. Therefore, it is essential for breeders to understand the genetic relationship between groups, because it guides the direction of germplasm resource improvement and the choices for hybridization. When data on the hybrid performance of different heterotic groups become available (Zhao *et al.* 2015), we can identify the most promising heterotic pattern based on existing breeding information.

Previous studies have mainly focused on the classification of heterotic groups of Chinese germplasm, with no attention given to the performance of the hybrids from different heterotic groups with contrasting responses to drought stress and non-stress conditions. Thus, the major objectives of this study were to: (i) analyze the combining ability and heterosis using 41 representative maize inbred lines and their hybrids across well-watered and drought-stressed conditions; (ii) determine the genetic relationships and heterotic patterns in a broad and diverse set of maize inbreds; and (iii) assess the relationships between hybrid performance, heterosis, and SNP-based genetic distance (GD) under distinct water treatments.

2. Materials and methods

2.1. Plant materials

A total of 41 maize inbred lines were selected from a diverse panel of 593 maize inbreds (Appendix A), representing temperate and tropical maize germplasm from the current breeding programs in different locations in China. These inbred lines show normal maturity in Shihezi, Xinjiang, China and their germplasm information was described in detail elsewhere (Wang *et al.* 2017; Yu *et al.* 2020). Among the 28 temperate lines, B73, Mo17, Dan340, Ye478, HZ4, and Qi319 have been used as common testers for six Chinese heterotic groups. The remaining nine Chinese temperate inbreds are key donors for developing both inbreds and hybrids across maize regions in China. The 23 tropical lines have been playing a very important role as the parents across worldwide breeding programs in China and International Maize and Wheat Improvement Center (CIMMYT), three of which (Jiao51, Chuan29 female, and 18-599) are from China. The parents were selected based on three criteria: (1) representativeness of the original population structure of the subgroups, (2) possessing a maximum allelic variation and (3) their wide cultivation in temperate regions with normal fertility and maturity. The parents comprised 13 conventional inbred lines (C), nine drought-tolerant lines (D) and 19 drought susceptible lines (S) (Appendix B) (Derera *et al.* 2008; Oyekunle *et al.* 2015). The conventional lines were selected for high general combining ability (GCA) for yield potential with GCA>6.5 in multi-environment trials (Shunyi, Beijing during 2013–2015 and Shehizi during 2017–2018). Drought tolerant and susceptible lines were selected, respectively, with drought tolerance index (DTI)≤20 and DTI>20 under managed drought-stressed condition at Shehezi (2017–2020). DTI was calculated as a percentage of grain weight per plant (GWPP) under drought stress compared to that under full irrigation, using the following equation: DTI (%)=[(Yield under well-watered treatment–Yield under drought)/Yield under well-watered treatment]×100 (Derera *et al.* 2008).

The 41 parents were crossed in an incomplete diallel to develop a multiple-hybrid population with 737 F_1 hybrids in Sanya, Hainan Province, China in Winter 2016 (Kempthorne and Curnow 1961). Partial tropical–tropical crosses were not included, because they cannot mature normally in Xinjiang. Five hybrids were excluded because their flowering was too late. The multiple-hybrid population can be divided into two subsets, 378 temperate hybrids derived in Griffing IV using 28 temperate parental lines, and 359 North Carolina Design II (NC II) hybrids generated between 28 temperate and 13 tropical parental lines (Appendix C). The breeding lines were genotyped with the Maize 55K SNP chip in a previous study (Wang *et al.* 2017; Xu *et al.* 2017). Compared with the widely-used Illumina MaizeSNP 50 BeadChip, the 55K array has lower missing and heterozygous rates and more SNPs with lower minor allele frequency (MAF) in tropical maize, facilitating the in-depth dissection of rare but possibly valuable variations in tropical germplasm resources. Initially, 50 812 SNPs evenly distributed on the maize chromosomes were genotyped for each line. Next, SNPs with missing data >20%, heterozygosity >20%, and minor allele frequency <0.05 were excluded, leaving a total of 38 737 SNPs that were used in the final data analysis.

2.2. Field trials

The 737 hybrids were planted in a 10×80 alpha lattice design, while the 41 parents were planted in a randomized complete block design, each with two replications, in 2017 and 2018 under two water treatments in Shihezi, Xinjiang (44°27´N, 85°94´E), with average annual precipitation 160 mm. Xianyu 335 was used as a control to evaluate the consistency of the environmental treatments. The rainfall data for the two years are included in Appendix D. The trials of each treatment were implemented in two-row, 3-m long plots each with 26 plants. The irrigation regimes were created using drip irrigation starting at the seeding period. To initiate drought stress, average days to anthesis (DTA) were predicted based on previous data of hybrids under well-watered conditions (Wang *et al.* 2017). In the well-watered (WW) regime, sufficient water (12 cm) was supplied in each 10-day interval after June 10 (45 days after planting). In the drought-stressed (DS) regime, the hybrids were classified into three sets (≤60, 60–70, and ≥70 d) based on the DTA with a 5-m isolation zone, and irrigation was given until the 15 days before the expected anthesis date in each set. The DS condition was maintained until 20 days after 90% of the hybrids flowered, and then irrigation was provided in 10-day intervals with one-third of the water amount as the WW regime.

2.3. Phenotyping

Hybrids and their parents were characterized for eight traits under both treatments. DTA and days to silking (DTS) were recorded when at least 50% of the plants reached anthesis and silking, respectively. The anthesis–silking interval (ASI) was measured for each plot as the difference between DTS and DTA (i.e., ASI=DTS–DTA). After female flowering, plant height (PH) was recorded as the distance between the ground and the tassel tip, and ear height (EH) was recorded as the distance between the ground surface and the stem knot of representative ears. PH and EH values were recorded and averaged from five plants in the middle of the plot. Grain weight per plant (GWPP) was evaluated from an average of 10 plants.

Row number (RN) and kernel number per row (KPR) were measured and averaged for each plot from 10 ears.

2.4. Population structure analysis

The analyses of population structure and relationships among the 41 target inbred lines were conducted using admixture (Alexander *et al.* 2009) and MEGA version 7.0.26 (Kumar *et al.* 2016). The genetic divergence between each pair of 41 parents was measured as Nei's (1972) GD using MEGA version 7.0.26. A dendrogram was constructed using the Neighbor-Joining (NJ) tree method based on the shared allele genetic distance matrix of all individuals using MEGA.

2.5. Statistical analysis

Genetic variation among parental lines was statistically tested by analysis of variance for each trait under both treatments using the linear model:

$$y_{ikl}=u+g_i+l_k+(gl)_{ik}+r_{lk}+e_{ikl}$$

where y_{ikl} is the observed value of ith genotype (parental line i) in the lth replication in the kth year, u is the grand mean, g_i is the effect of the ith genotype, l_k is the effect of the kth year, $(gl)_{ik}$ is an interaction effect between ith genotype with kth year, r_{lk} is the effect of the lth replication within the kth year, and e_{ikl} is the random residual error (Makumbi *et al.* 2018). The genotypic effect was considered a fixed effect, while the others were considered random effects. The model was fit to the data using the R "lmer" and "lmerTest" packages (R Core Team 2013; Bates *et al.* 2015; Kuznetsova *et al.* 2017).

The total variance of hybrids was divided into the variances due to GCA effects of parental lines and specific combining ability (SCA) effects of crosses and their interactions. The variance components for hybrids were estimated using the mixed effect model:

$$y_{ijklm}=u+l_k+g_i+g_j+s_{ij}+(g_il)_{ik}+(g_jl)_{jk}+(s_{ij}l)_{ijk}+r_{lk}+b_{mlk}+e_{ijklm}$$

where y_{ijklm} is the phenotypic performance of the ijth cross in the mth incomplete block of the lth replication in the kth year, l_k is the effect of the kth year, g_i and g_j are the GCA effect of the ith and jth parental lines, respectively, s_{ij} is the SCA effect of crosses between lines i and j, $(g_il)_{ik}$ and $(g_jl)_{jk}$ are GCA×year effects of the lines, $(s_{ij}l)_{ijk}$ is the SCA×year interaction effects, r_{lk} is the replication effect, b_{mlk} is the effect of the incomplete blocks in the lth replication nested in the kth year, and e_{ijklm} is the residual error. The variance components of GCA and SCA were estimated using the PROC MIXED procedure of SAS (2008) following partial diallel cross analysis (Kempthorne and Curnow 1961). The relative importance of GCA and SCA (GCA–SCA ratio) was calculated as the ratio (Makumbi *et al.* 2011):

$$\text{GCA–SCA ratio}=\frac{2\delta^2_{\text{GCA}}}{(2\delta^2_{\text{GCA}}+\delta^2_{\text{SCA}})}$$

where δ^2_{GCA} and δ^2_{SCA} are the variances for GCA and SCA, respectively.

The GCA and SCA effects were estimated following Griffing's (1956) method 2 model I using the R program (R Core Team 2013):

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

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

where $\bar{y}_{i.}$ is the average of the hybrids among the ith line crossed with a series of parents, $\bar{y}_{..}$ is the overall mean, g_i and g_j are the GCA effects for the ith and jth lines, respectively, s_{ij} is the SCA effect for the ijth hybrid, and y_{ij} is the trait value of the ijth hybrid.

The mid-parent heterosis (MPH) for each hybrid was calculated as MPH=100×(F_1–MP)/MP, where F_1 is the hybrid mean performance, MP is mid-parent value and MP=(P_1+P_2)/2, and P_1 and P_2 represent the mean performances of Parent 1 and Parent 2, respectively. Better parent heterosis (BPH) was calculated as BPH=100×(F_1–BP)/BP, where BP represents the better-performing parental line. The Pearson correlation of GWPP with parental GD, MP values, and hybrid performance were calculated using the R "Hmisc" package (Harrell and Dupont 2016).

3. Results

3.1. Analysis of parental genetic diversity

An admixture model-based clustering was performed using the genotypes of 38 737 SNPs from the Maize 55K chip with improved genome coverage by the Admixture Software to infer the population structure with a fixed number of groups k that varied from 1 to 10. For k=5, the CV error was relatively low (Appendix E). When only the lines from Chinese maize breeding projects were considered, k=5 resulted in the optimal partition, which is highly consistent with the known heterotic groups established in maize breeding programs in China.

The GD values for the 41 inbred lines ranged from 0.05 (NK764 *vs.* PHG83) to 0.74 (FAPW *vs.* HZ4) with an average GD of 0.56. The results of the cluster analysis using an NJ phylogenetic tree based on GD revealed a distinct separation of the lines into five groups. Besides the tropical subgroup, other temperate inbred lines could be clustered into NSS, BSSS, PB, and SPT, represented by the inbred lines Mo17, B73, Qi319, and HZ4, respectively (Fig. 1). The NSS group could be subdivided into the Lancaster and Iodent groups, while

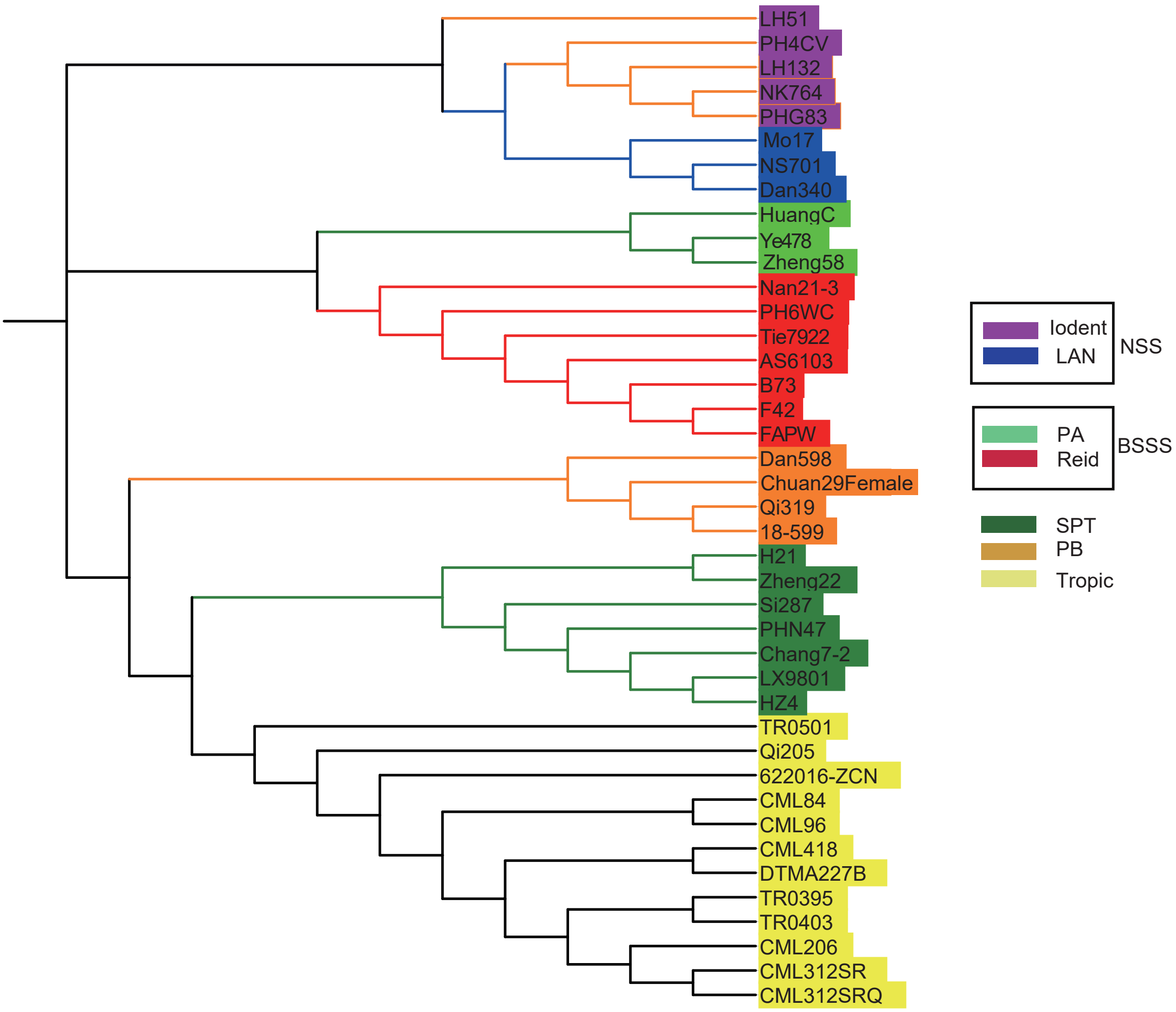


Fig. 1 Neighbor-Joining dendrogram of 41 maize inbreds with 38 737 SNPs. NSS, Non-Stiff Stalk; Iodent, Iowa Experiment Station Reid yellow Dent; LAN, Lancaster group; BSSS, Iowa Stiff Stalk Synthetic; PA, group A germplasm; Reid, Reid group; SPT, Sipingtou; PB, group B germplasm; Tropic, Tropical.

the BSSS group could be subdivided into the Reid and PA groups. Among the five subgroups, PB had the least allelic variation, with an average GD of 0.36, and Tropical had the highest GD value (Table 1). Among the inter-groups, the least allelic variation was found between Tropical and PB with an average GD of 0.53, while GD values were relatively higher between BSSS and SPT, SPT and NSS, and BSSS and NSS. The average GDs between inter-group parents were significantly higher than those between intra-group parents.

3.2. Performance of maize inbred lines under DS and WW treatments

Results of the analysis of variance for the inbreds under WW and DS treatments showed significant genotype effects for grain yield and all other measured traits (Appendix F). GWPP values of the inbred lines ranged from 22.3 g/plant for CML206 to 123.2 g/plant for Tie7922 under the WW treatment, and from 18.3 g/plant for TR0403 to 91.2 g/plant for PH6WC under the DS treatment (Appendix B). While 18–599 had the highest drought tolerance, H21 had the lowest drought tolerance. PH6WC, Tie7922, Zheng58, and PHN47 had the four highest GWPP values under drought, and their ASI values were 5.8, 2.8, 3.5, and 2.0 days.

3.3. Performance of hybrids under DS and WW treatments

Significant phenotypic differences were observed among the hybrids under both the WW and DS conditions

Table 1 Genetic distance (GD) among groups and the performance of grain weight per plant (GWPP) and the other traits under well-watered (WW) and drought-stressed (DS) conditions[1)]

Heterosis model[2)]	SUM	GD	GWPP (g/plant)		DTI	DTS (d)		ASI (d)		PH (cm)		KPR	
			WW	DS	(%)	WW	DS	WW	DS	WW	DS	WW	DS
PB×BSSS	28	0.56	173.2 a	112.9 a	34.8	70.8 ab	76.1 b	4.3 b	7.6 b	322.0 cd	269.7 a	34.4 ab	29.3 a
NSS×PB	5	0.56	170.7 ab	112.4 a	34.2	68.6 bc	73.1 c	4.9 b	7.3 b	318.4 de	265.1 ab	34.8 a	29.4 a
SPT×BSSS	45	0.62	170.0 ab	107.9 ab	36.5	62.3 f	67.4 f	1.9 f	5.0 d	302.5 ef	253.1 bc	34.5 ab	28.9 a
PB×SPT	21	0.56	169.6 ab	108.2 ab	36.2	70.9 ab	76.6 b	4.6 b	7.7 b	335.0 ab	277.5 a	34.2 ab	29.0 a
Tropic×Tropic	11	0.51	167.2 ab	107.7 ab	35.6	67.8 c	72.7 c	3.3 c	6.2 bc	328.9 abc	272.9 a	31.3 ab	27.2 a
BSSS×Tropic	79	0.61	164.9 abc	104.4 ab	36.7	67.4 cd	72.1 c	3.1 c	5.7 cd	333.6 ab	274.0 a	34.7 ab	29.8 a
SPT×NSS	56	0.59	164.6 abc	103.2 ab	37.3	61.5 f	66.3 f	2.3 ef	5.6 cd	301.1 ef	254.0 bc	34.3 ab	30.1 a
SPT×Tropic	95	0.56	163.4 abc	107.1 ab	34.5	62.5 f	66.8 f	2.6 de	5.6 cd	299.7 ef	251.4 bc	34.8 a	29.5 a
BSSS×NSS	32	0.59	158.3 bcd	102.7 ab	35.1	66.4 d	70.7 d	3.4 c	5.9 cd	325.8 bcd	271.1 a	33.8 ab	29.5 a
Tropic×NSS	26	0.60	156.5 bcd	97.8 ab	37.5	74.3 a	80.5 a	5.7 a	9.5 a	338.5 a	280.5 a	32.3 ab	29.2 a
Tropic×PB	70	0.53	156.5 bcd	97.8 ab	37.5	74.3 a	80.5 a	5.7 a	9.5 a	338.5 a	280.5 a	34.8 a	30.9 a
NSS×NSS	118	0.47	151.8 cd	100.1 ab	34.1	62.1 f	66.7 f	3.0 cd	6.1 bcd	297.2 fg	244.7 c	34.3 ab	29.6 a
BSSS×BSSS	83	0.41	147.3 d	103.5 ab	29.7	64.3 e	69.1 e	2.4 de	5.6 cd	286.0 g	238.0 c	31.6 ab	32.5 a
SPT×SPT	40	0.37	145.5 d	88.0 b	39.5	62.1 f	66.9 f	1.8 f	5.1 cd	296.2 fg	241.3 c	32.0 ab	28.9 a
PB×PB	28	0.36	137.6 d	110.6 ab	19.6	75.4 a	80.8 a	5.4 ab	8.9 ab	320.5 cde	263.2 abc	32.0 ab	28.9 a
Summarized by inter- or intra-group													
Inter-group	627	0.59	165.4 a	106.2 a	35.8	66.2 a	71.1 a	3.2 a	6.2 a	319.4 a	266.0 a	34.3 a	29.5 a
Intra-group	110	0.43	150.0 b	100.8 b	32.8	64.4 b	69.3 b	2.8 b	6.0 a	297.2 b	245.6 b	32.9 b	29.0 a
Summarized by parental groups involved in hybrids													
PB			167.1 a	108.8 a	34.9	71.1 a	76.5 a	4.8 a	7.9 a	327.0 a	272.1 a	34.1 a	29.8 a
SPT			165.3 a	104.3 a	36.9	64.6 d	69.6 d	2.9 d	5.9 c	310.6 c	259.1 b	34.2 a	29.8 a
BSSS			165.0 a	107.7 a	34.7	65.4 c	70.2 c	2.6 e	5.7 c	315.0 b	261.7 b	34.2 a	29.0 a
Tropic			163.4 a	104.9 a	35.8	67.9 b	72.7 b	3.1 c	5.9 bc	310.2 c	259.3 b	34.3 a	29.6 a
NSS			161.7 a	104.7 a	35.3	64.2 d	68.7 e	3.5 b	6.3 b	330.1 a	273.5 a	33.9 a	29.3 a

[1)] SUM, count the number of hybrids; DTI, drought tolerance index; DTS, days to silking; ASI, anthesis–silking interval; PH, plant height; KPR, kernel number per row.

[2)] PB, group B germplasm; BSSS, Iowa Stiff Stalk Synthetic ; NSS, Non-Stiff Stalk; SPT, Sipingtou; Tropic, Tropical.

Different letters in the same column indicate significant differences at the 5% level.

(Appendix G). GCA and SCA effects were significant for all traits under both WW and DS treatments. Further results showed that the GCA×year effects were significant for all traits, and the SCA×year effects were significant for all traits except KPR under WW treatment. The ratio of variance components revealed that GCA effects were much higher than SCA effects for all traits except the KPR and GWPP under DS treatment, ranging from 0.58 for KPR to 0.90 for EH under WW treatment and ranging from 0.46 for GWPP to 0.95 for RN under DS treatment. Thus, the relative contribution of the GCA effect is much higher.

Hybrid performance was strongly affected by genotype under both DS and WW treatments (Fig. 2). Under the DS treatment, GWPP ranged from 52.92 to 165.13 g/plant (Table 2), whereas under the WW treatment, it ranged from 72.39 to 236.23 g/plant. The mean GWPP (106.14 g/plant) under DS was 34.76% less than that under the WW treatment (Table 2). Among the 15 top-yielding single-cross combinations under the DS treatment, two were from within-group crosses, while the others were from between-group crosses (Table 2). In contrast, out of the 10 worst single-cross hybrids under the DS treatment, five were from within-group crosses. Meanwhile, out of the 15 top-yielding hybrids under the DS treatment, one hybrid was from C×D cross, six were from C×C crosses, three were from S×C crosses, and three were from S×D crosses. In contrast, out of the 10 worst single-cross hybrids under the DS treatment, four were from S×S crosses. The best single-cross hybrid (Dan340×PH6WC) was from a C×C and NSS×BSSS cross, generating yields higher than the control Xianyu 335 by 19% under DS but by –0.02% under the WW treatment.

With the highest yield hybrid model, PB×BSSS produced an average yield of 173.2 g/plant with 143.6%, 89.6% and 2.6 g/plant of MPH, BPH, and SCA under the WW treatment, and it produced 112.9 g/plant with 102.2%, 68.6% and –1.6 g/plant of MPH, BPH, and SCA under the DS treatment (Table 1; Fig. 3; Appendix H). The lowest yield hybrids were from the crossing patterns of PB×PB under WW treatments and SPT×SPT under DS treatments. The earliest hybrids were from the crossing patterns of SPT×NSS, SPT×Tropic, NSS×NSS, BSSS×NSS and SPT×BSSS under both treatments, and the lowest PH hybrids were from SPT×BSSS, SPT×NSS, SPT×Tropic, BSSS×NSS, BSSS×BSSS and NSS×NSS.

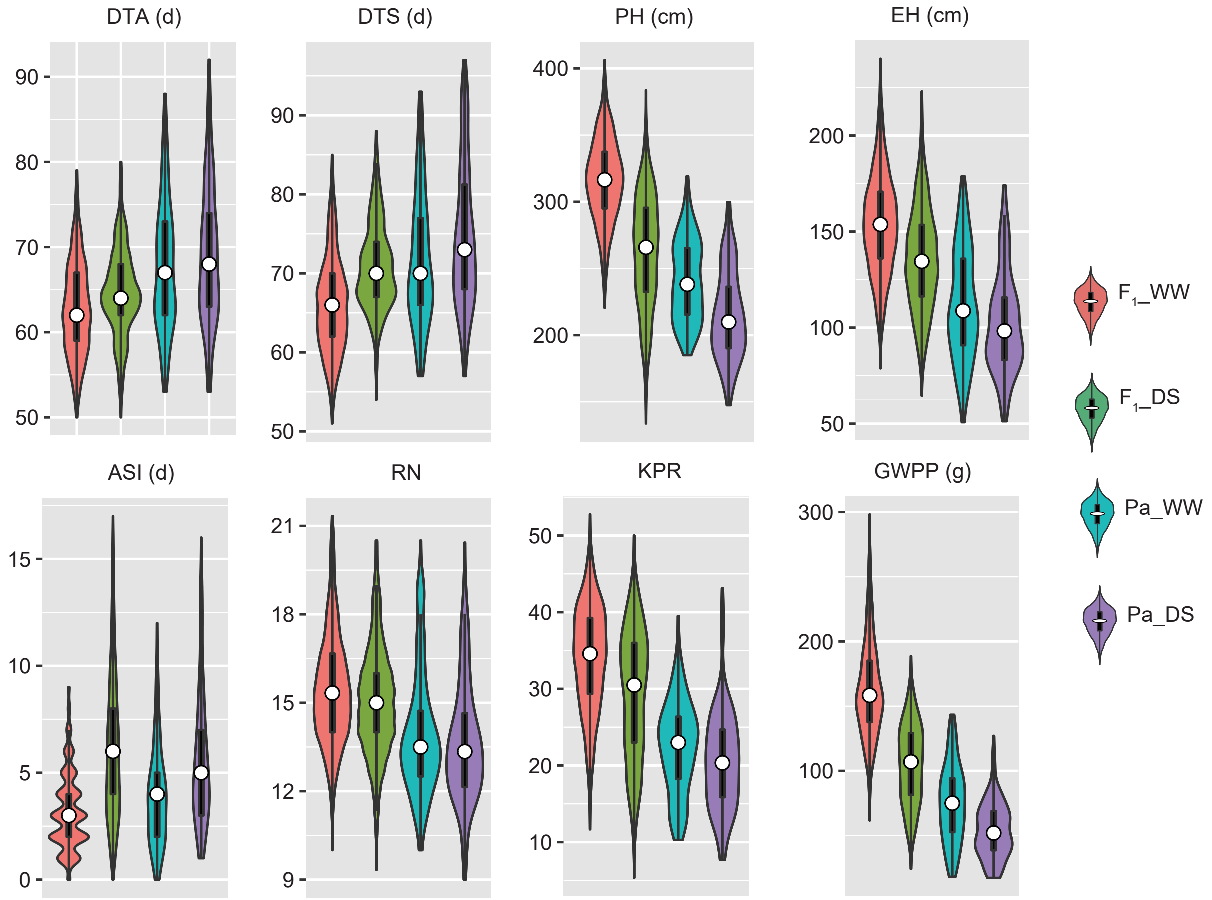


Fig. 2 Phenotype distributions of eight agronomic traits in F_1 hybrids (F_1) and their parents (Pa) under well-watered (WW) and drought-stressed (DS) conditions. DTA, days to anthesis; DTS, days to silking; ASI, anthesis–silking interval; PH, plant height; EH, ear height; RN, row number; KPR, kernel number per row; GWPP, grain weight per plant.

3.4. Heterosis

The level of MPH varied widely among the tested traits. MPH was positive for GWPP, RN, KPR, and PH, but negative for DTA and DTS under both the WW and DS treatments (Table 3). The average MPH values for KPR and GWPP were much higher than other traits under both treatment conditions. The MPH values for GWPP, KPR, RN, and PH were higher under the WW treatment than those under the DS treatment. The hybrids from BSSS×Tropic, Tropic×NSS, Tropic×PB, and SPT×Tropic had relatively higher MPH values for GWPP under the WW and DS conditions, while other hybrids involving tropical parents performed poorly, indicating a poor yielding potential of tropical parents (Fig. 3).

The GCA effects of the 41 inbred lines for each trait under the WW and DS treatments were shown in Appendix I. The top five positive GCA effects for GWPP under the WW treatment were from inbred lines H21, Qi205, PH6WC, DAN340, and Tie7922. Under the DS treatment, the highest and most significant GCA effects for GWPP were from inbred lines PH6WC, Zheng58, Tie7922, Dan598, Qi205, and F42. The SCA effect for GWPP ranged from –70.93 g/plant (F42×FAPW) to 74.12 g/plant (TR0423×HZ4) under the WW treatment, and it ranged from –2.85 g/plant (F42×B73) to 59.01 g/plant (LH132×Qi319) under the DS treatment. The hybrids from PB×BSSS and NSS×PB had high SCA effects for GWPP (>2.00 g/plant) under the WW treatments, while NSS×PB had high SCA effects for GWPP (>2.00 g/plant) under the DS treatment (Appendix H). The SCA effects were negative for the within-heterotic group hybrids and greater for between-heterotic group hybrids than within-heterotic group hybrids (Appendix H).

3.5. Correlations among phenotypic traits, heterosis, and genetic distance of F_1 hybrids

GWPP in F_1 hybrids was significantly positively correlated with PH, EH, RN, KPR, BPH, SCA, and MPH under

Table 2 Grain yield and other agronomic traits in 25 maize single-cross hybrids (the worst 10 and the best 15 based on grain yield) evaluated under well-watered (WW) and drought-stressed (DS) treatments[1)]

Hybrid	Category[2)]	Pattern[3)]	GWPP (g/plant)		DTS (d)		ASI (d)		PH (cm)		KPR	
			WW	DS	WW	DS	WW	DS	WW	DS	WW	DS
NK764×HuangC	S×S	NSS×BSSS	149	53	57.0	64.0	2.0	7.5	279	198	30.8	12.7
PHN47×HZ4	S×D	SPT×SPT	101	55	61.0	63.5	2.3	4.5	267	236	26.5	23.8
NK764×LH132	S×S	NSS×NSS	74	56	59.8	62.7	3.8	6.7	234	190	20.1	15.9
Ye478×Si287	S×S	BSSS×SPT	175	56	61.5	67.0	3.5	10.0	287	231	29.0	9.7
HZ4×Chang7-2	D×D	SPT×SPT	107	57	64.8	69.0	2.3	6.8	279	231	29.7	23.8
H21×HZ4	C×D	SPT×SPT	141	57	61.5	70.0	1.5	5.5	314	202	20.2	27.3
Nan21-3×Chuan29Female	S×D	BSSS×PB	102	58	76.7	79.5	7.0	7.5	312	265	30.3	18.4
Si287×Dan598	S×C	SPT×PB	136	62	58.0	66.5	3.0	10.5	291	245	28.5	14.8
Nan21-3×CML84	S×S	BSSS×Tropic	97	62	75.0	77.0	7.0	11.3	319	253	28.4	24.0
AS6103×B73	D×S	BSSS×BSSS	126	63	62.3	65.0	2.0	3.5	261	219	25.8	22.5
B73×Chuan29Female	S×D	BSSS×PB	193	151	70.0	76.8	3.8	8.8	343	241	42.0	34.6
LH51×Dan598	C×C	NSS×PB	236	151	66.8	74.0	4.3	8.5	313	240	40.1	36.1
LX9801×PH4CV	D×C	SPT×NSS	197	152	64.8	69.5	1.0	4.5	330	267	37.0	41.1
Dan598×18-599	C×C	PB×PB	153	154	73.0	81.3	5.3	9.0	326	269	34.6	28.8
Zheng58×18-599	S×C	BSSS×PB	184	155	72.3	79.0	5.0	9.3	328	300	38.3	41.5
AS6103×Qi319	D×S	BSSS×PB	196	156	69.5	72.3	4.5	6.8	310	260	35.9	34.6
Qi205×Dan598	C×C	Tropic×PB	211	157	71.8	78.5	4.5	10.0	326	267	35.2	34.5
PH6WC×Tie7922	C×C	BSSS×BSSS	175	159	65.5	72.3	2.0	7.0	332	272	29.9	32.8
Dan340×Qi319	C×S	NSS×PB	224	161	70.3	75.5	5.0	9.5	324	264	35.7	32.3
F42×PHN47	S×S	BSSS×SPT	181	162	59.3	63.3	1.8	5.0	314	263	38.0	28.8
Mo17×Qi319	C×S	NSS×PB	213	162	66.3	71.5	4.0	7.5	315	247	41.7	34.3
H21×PH4CV	C×C	SPT×NSS	207	162	63.0	70.8	1.5	5.0	343	277	34.7	38.9
LH132×Qi319	S×S	NSS×PB	181	163	63.3	68.3	4.5	8.3	303	239	36.3	28.5
LX9801×B73	D×S	SPT×BSSS	175	164	63.3	68.8	2.5	2.8	323	286	42.1	35.6
Dan340×PH6WC	C×C	NSS×BSSS	200	165	66.0	68.8	3.0	5.8	313	278	29.3	38.1
Xianyu 335			204	138	63.3	71.3	0.8	6.0	337	262	38.9	33.6
Mean			163	106	66.0	70.9	3.2	6.2	316	262	34.0	29.6

[1)] GWPP, grain weight per plant; DTS, days to silking; ASI, the anthesis–silking interval; PH, plant height; KPR, kernel number per row.

[2)] The parents in each hybrid were classified as drought-tolerant (D), high general combining ability (GCA) (C) and drought-sensitive (S).

[3)] NSS, Non-Stiff Stalk; BSSS, Iowa Stiff Stalk Synthetic; SPT, Sipingtou; PB, group B germplasm; Tropic, Tropical.

both DS and WW treatments ($P<0.01$; $r>0.2$) (Fig. 4); but negatively correlated with ASI under both treatments ($P<0.01$). Similarly, MPH and BPH had significantly positive correlations with PH, DTS, DTA and KPR under both treatments ($P<0.01$; $r>0.2$). SCA and GD were significantly positively correlated with GWPP, PH and ear-related traits, but negatively correlated with DTA, DTS, and ASI under both water treatments ($P<0.01$; $r>0.2$). The GD estimates were highly correlated between the pairwise parental lines and the hybrid performance for SCA, MPH, BPH, PH, and GWPP.

Regression analysis revealed that the GD between the parental lines within heterotic groups was significantly correlated with hybrid grain yield under WW and DS treatments (Fig. 5-A; Appendix J), and hybrid yield increased with the increase of genetic distance, but no relationship between GD and hybrid grain yield was found between heterotic groups (Table 4). We found that under the WW and DS treatments, the degree of GD was significantly positively correlated with MPH within heterotic groups, but not between heterotic groups (Appendix K), suggesting that a medium level of GD may contribute the maximum increasing effect on the heterosis of grain yield. To test this hypothesis, we classified the hybrids into groups based on their levels of GD: ≤0.40, 0.41–0.45, 0.46–0.50, 0.51–0.55, 0.56–0.60, 0.61–0.65, and ≥0.66. Hybrids with 0.55–0.60 GD had the highest MPH of grain yield under both water treatments (Fig. 5-C), and the MPH under the WW condition was consistently higher than that under the DS condition. In addition, because SCA is an indicator of non-additive effects, the relationship between SCA and GD under both water treatments was investigated. We found that SCA for grain yield was correlated positively with GD under both water treatments. With the further increase of GD, however, the rate of increase of SCA decreased gradually (Fig. 5-B and C).

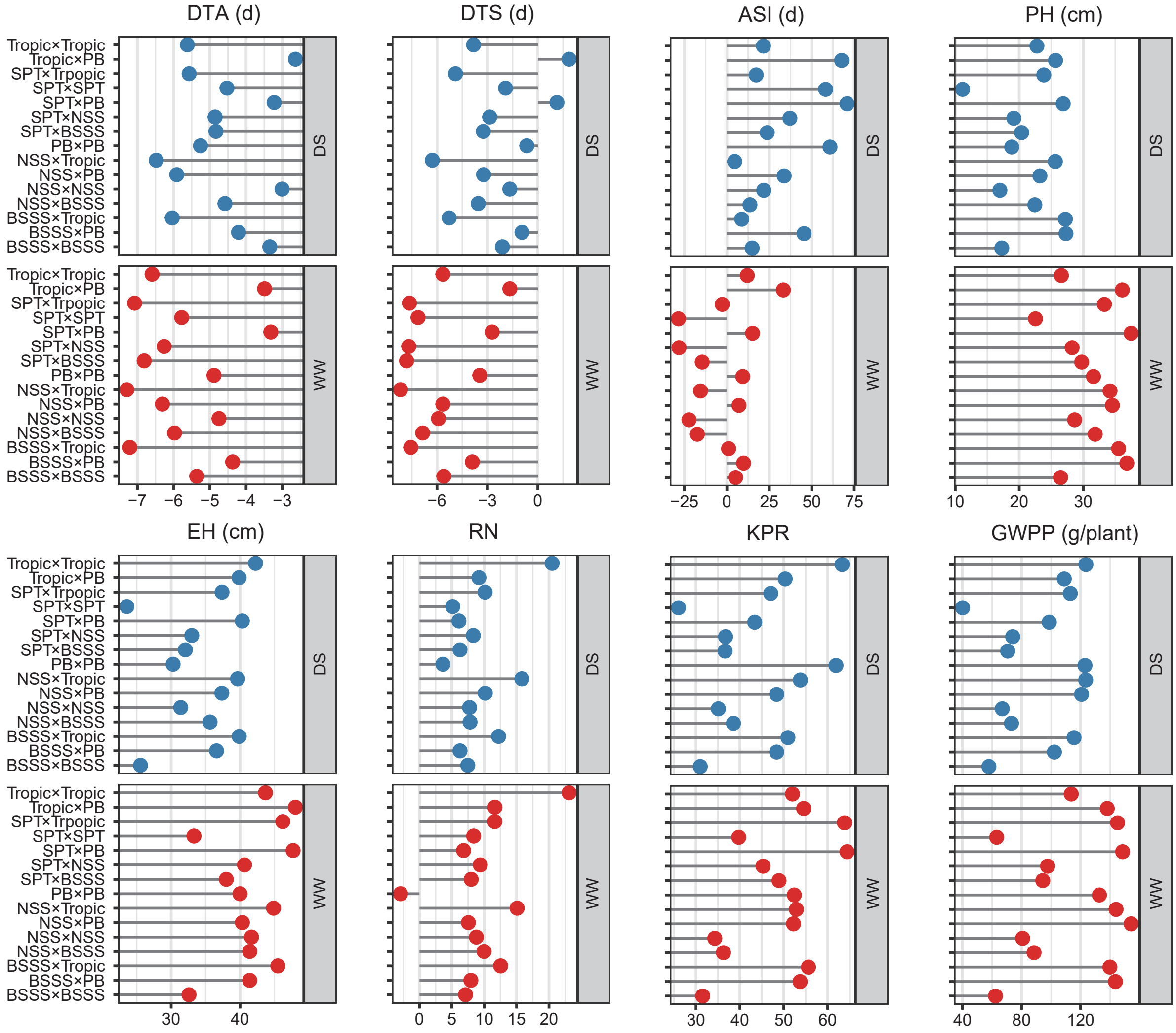


Fig. 3 Mid-parent heterosis between heterotic groups under well-watered (WW) and drought-stressed (DS) treatments. DTA, days to anthesis; DTS, days to silking; ASI, anthesis–silking interval; PH, plant height; EH, ear height; RN, row number; KPR, kernel number per row; GWPP, grain weight per plant. Tropic, Tropical; PB, group B germplasm; SPT, Sipingtou; NSS, Non-Stiff Stalk; BSSS, Iowa Stiff Stalk Synthetic.

The SCA for grain yield under the DS condition was higher than under the WW condition. It is interesting that when the GD between parents was relatively small, the hybrid had a higher SCA.

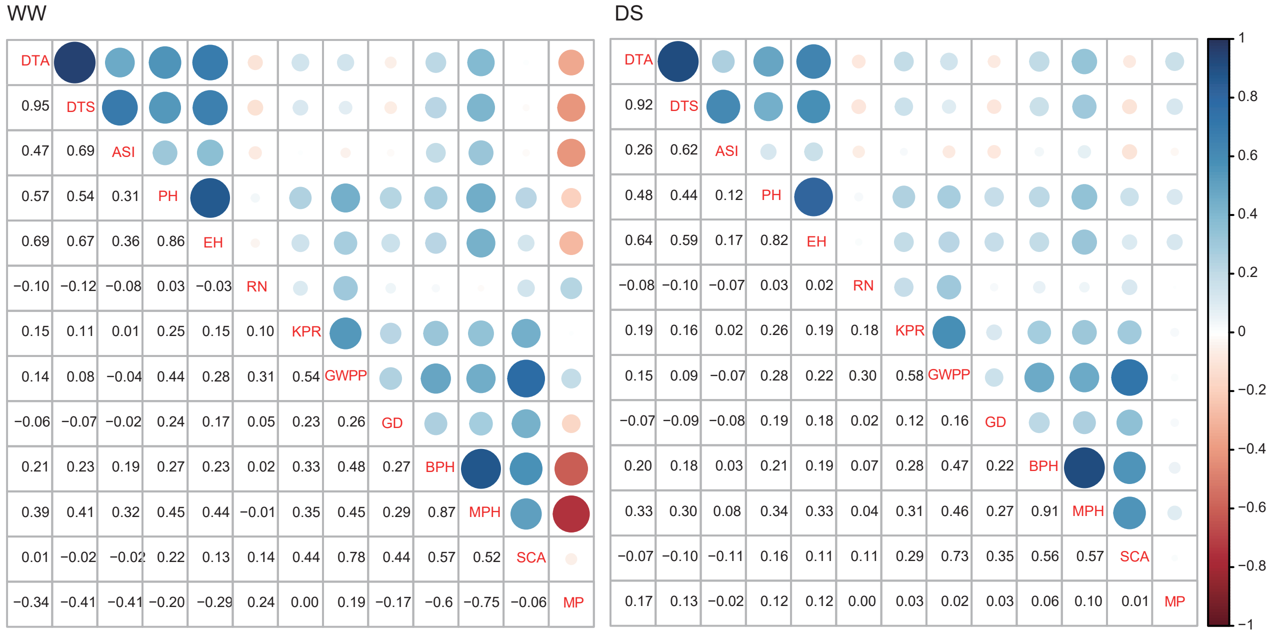
4. Discussion

The significant variations among inbred lines and hybrids in GWPP and other traits indicate that substantial genetic variation exists among the maize parents and their hybrids. The significant genotype×environment interactions for grain yield and other traits reveal that the expression of these traits would not be consistent across the test environments. Similar findings have been reported in other studies (Oyekunle *et al.* 2015; Makumbi *et al.* 2018), suggesting the need to evaluate inbreds and hybrids in various environments to identify drought-tolerant genotypes with consistent favorable responses to unpredictable growing environments. Decreased PH, EH, GWPP, and ear traits and increased ASI under drought were also reported in several earlier studies (Bolaños and Edmeades 1993; Adebayo *et al.* 2017). Here, we monitored the stress level imposed on experimental hybrids under drought stress for two consecutive seasons in order to obtain sufficient stress intensity to evaluate the differential responses of hybrids. The average GWPP recorded for hybrids under DS decreased by 35.76% compared to the WW treatment. A yield decrease in the range of 20–30% has been considered as severe drought stress (Bolaños and Edmeades 1996; Campos *et al.*

Table 3 Heterosis for agronomic traits under well-watered (WW) and drought-stressed (DS) treatments

Trait[1)]	WW			DS		
	Min.	Max.	Mean±SD (*n*=737)	Min.	Max.	Mean±SD (*n*=737)
MPH (%)						
DTA (d)	–13.82	2.97	–6.22±2.55	–13.03	7.34	–5.02±3.32
DTS (d)	–14.99	4.87	–6.70±3.30	–14.96	10.91	–3.50±4.33
ASI (d)	–90.91	135.00	–6.09±38.98	–75.64	280.00	23.59±46.84
PH (cm)	–3.68	59.59	32.40±7.31	–10.20	50.92	23.05±9.96
EH (cm)	2.71	100.37	42.36±13.69	–2.52	100.25	35.89±14.07
RN	–14.93	45.39	10.56±8.73	–16.77	47.54	9.77±9.14
KPR	–46.27	187.98	49.92±28.74	–46.67	161.32	44.28±27.91
GWPP (g/plant)	–17.59	388.27	119.39±57.63	–20.22	305.80	95.66±48.57
SCA (g/plant)						
DTA (d)	–3.26	6.22	0.31±0.05	–5.18	8.93	0.28±0.06
DTS (d)	–3.75	9.61	0.35±0.06	–5.60	12.37	0.27±0.09
ASI (d)	–2.33	4.90	0.06±0.03	–3.95	4.59	0.00±0.05
PH (cm)	–67.55	31.88	–3.90±0.47	–53.88	41.57	–2.90±0.60
EH (cm)	–40.32	26.56	–2.04±0.36	–33.21	28.00	–1.83±0.35
RN	–2.93	2.32	–0.08±0.03	–2.79	2.52	–0.10±0.03
KPR	–12.78	10.68	–0.71±0.13	–11.79	9.97	–0.61±0.12
GWPP (g/plant)	–80.97	62.95	–5.04±0.73	–63.30	41.97	–3.41±0.53

[1)] MPH, mid-parent heterosis for grain yield; SCA, specific combining ability for grain yield. DTA, days to anthesis; DTS, days to silking; ASI, the anthesis–silking interval; PH, plant height; EH, ear height; RN, row number; KPR, kernel number per row; GWPP, grain weight per plant.



WW

DTA												
0.95	DTS											
0.47	0.69	ASI										
0.57	0.54	0.31	PH									
0.69	0.67	0.36	0.86	EH								
−0.10	−0.12	−0.08	0.03	−0.03	RN							
0.15	0.11	0.01	0.25	0.15	0.10	KPR						
0.14	0.08	−0.04	0.44	0.28	0.31	0.54	GWPP					
−0.06	−0.07	−0.02	0.24	0.17	0.05	0.23	0.26	GD				
0.21	0.23	0.19	0.27	0.23	0.02	0.33	0.48	0.27	BPH			
0.39	0.41	0.32	0.45	0.44	−0.01	0.35	0.45	0.29	0.87	MPH		
0.01	−0.02	−0.02	0.22	0.13	0.14	0.44	0.78	0.44	0.57	0.52	SCA	
−0.34	−0.41	−0.41	−0.20	−0.29	0.24	0.00	0.19	−0.17	−0.6	−0.75	−0.06	MP

DS

DTA												
0.92	DTS											
0.26	0.62	ASI										
0.48	0.44	0.12	PH									
0.64	0.59	0.17	0.82	EH								
−0.08	−0.10	−0.07	0.03	0.02	RN							
0.19	0.16	0.02	0.26	0.19	0.18	KPR						
0.15	0.09	−0.07	0.28	0.22	0.30	0.58	GWPP					
−0.07	−0.09	−0.08	0.19	0.18	0.02	0.12	0.16	GD				
0.20	0.18	0.03	0.21	0.19	0.07	0.28	0.47	0.22	BPH			
0.33	0.30	0.08	0.34	0.33	0.04	0.31	0.46	0.27	0.91	MPH		
−0.07	−0.10	−0.11	0.16	0.11	0.11	0.29	0.73	0.35	0.56	0.57	SCA	
0.17	0.13	−0.02	0.12	0.12	0.00	0.03	0.02	0.03	0.06	0.10	0.01	MP

Fig. 4 Correlation among genetic distance (GD), mid-parent heterosis for grain yield (MPH, %), better parent heterosis for grain yield (BPH, %), grain yield, and other agronomic traits under drought-stressed (DS) and well-watered (WW) conditions. DTA (d), days to anthesis; DTS (d), days to silking; ASI (d), anthesis–silking interval; PH (cm), plant height; EH (cm), ear height; RN, row number; KPR, kernel number per row; GWPP (g/plant), grain weight per plant; SCA (g/plant), specific combining ability for grain yield; MP (g/plant), mid-parent for grain yield. Blank, not significant at *P*<0.01.

2006). The significant variation in GWPP under drought observed in this study is an indicator for distinguishing tolerance from drought in maize hybrids.

In this study, a larger contribution of the GCA sum of squares was found for most traits compared to that from the SCA sum of squares (Appendix G). This result suggests that additive gene action was predominant over non-additive gene action for most of the observed

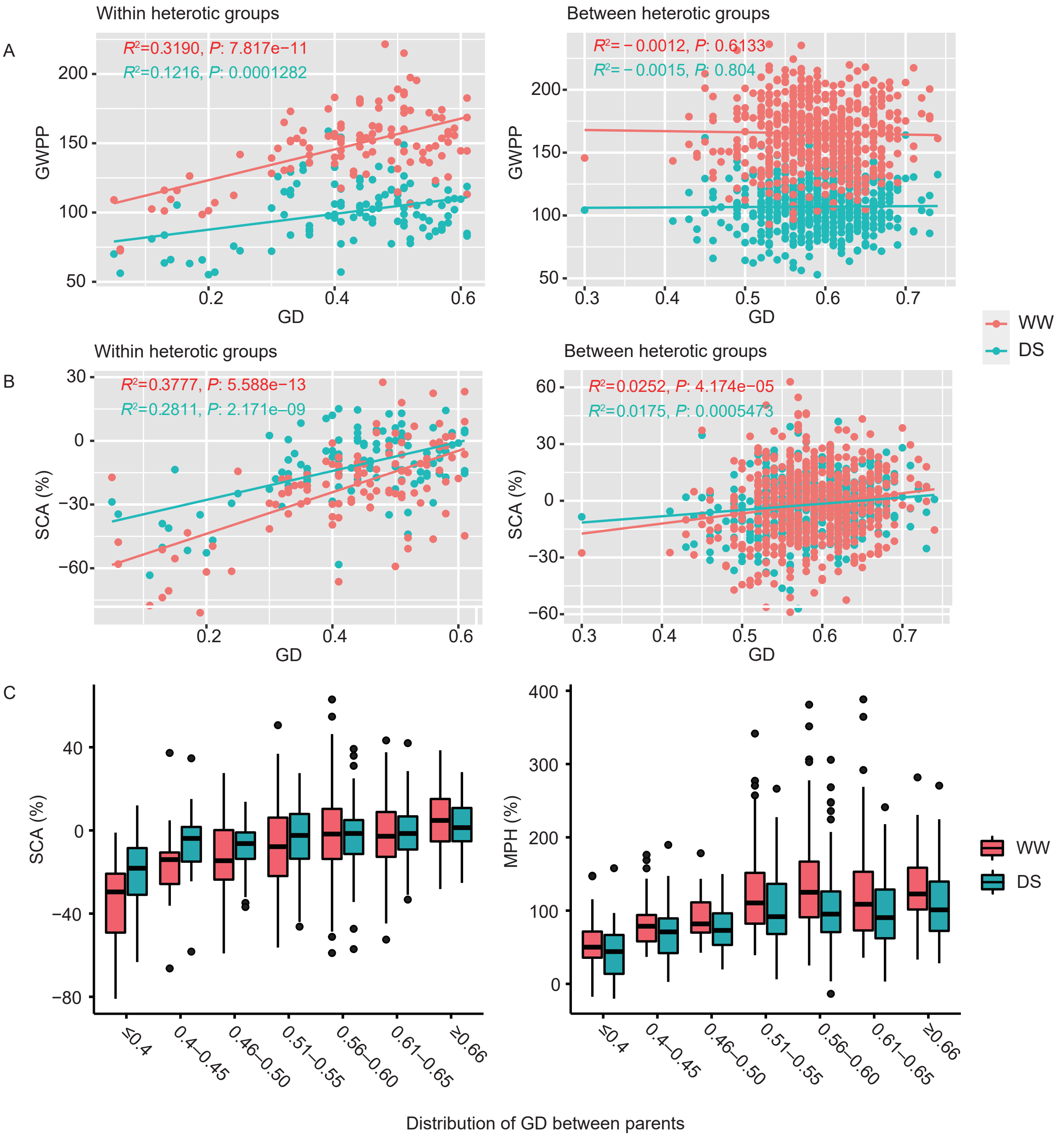


Fig. 5 The relationships between heterosis and the genetic distance (GD) between parents under well-watered (WW) and drought-stressed (DS) conditions. A, relationship between grain weight per plant (GWPP) and GD. B, relationship between the specific combining ability for grain yield (SCA) and GD. C, comparison of grain yield heterosis with the distribution of GD. MPH, mid-parent heterosis for grain yield.

traits under both conditions, which is contradictory with several studies (Bolaños *et al.* 1993; Njeri *et al.* 2017), but consistent with others (Amiruzzaman *et al.* 2010; Ahmad *et al.* 2016; Dermail *et al.* 2020). The difference between this study and the others could be largely attributed to the differences in the germplasm used, as our study included a large number of tropical maize inbreds with a higher level of genetic diversity. The GCA–SCA ratio for KPR and GWPP under the WW treatment was higher than that under the DS treatment, suggesting that additive effects play a major role for yield traits under the WW treatment. On the contrary, non-additive effects appeared to play an important role in grain yield under the DS treatment (Appendix G; Fig. 5-C), and non-additive effects were relatively higher, despite the close genetic distance between parents.

Previous reports suggested that tropical germplasm had higher diversity and larger GD than temperate germplasm (Lu *et al.* 2009; Zhang *et al.* 2016). Because the tropical germplasm was mainly introduced from CIMMYT with distinct genetic diversity, a systematic comparison of GD and heterosis was performed and higher GD was confirmed in the tropical germplasm, indicating that modern temperate maize inbred lines have experienced a long period of intense breeding selection. Only favorable alleles for target agronomic traits were selected and maintained during the breeding process, resulting in a decrease in genetic diversity. In this study, all the hybrids containing tropical parents had higher MPH and BPH for GWPP than the other hybridization models under the DS treatment (Table 1; Fig. 3). High percentages of heterosis recorded for grain yield, particularly in the high-yielding hybrids between tropical and temperate germplasms under DS treatment, suggests that tropical and temperate maize inbreds possess the complementary genes/alleles. The diversity hosted by the temperate and tropical lines surveyed in this study can be exploited for developing maize hybrids with enhanced heterosis under DS treatment, and promising lines could be derived by crossing the temperate group with tropical lines to enhance their levels of drought tolerance.

The parents selected for this study were from elite inbreds currently used in the temperate regions. They are widely used as hybrid parents or excellent donors in breeding programs across maize-growing regions. They can generally represent the production level and genetic diversity of the existing elite maize germplasm in China. The dendrogram from the NJ tree method based on SNP markers identified five distinct groups, which is consistent with previous studies (Teng *et al.* 2004; Xie *et al.* 2007; Lu *et al.* 2009; Zhang *et al.* 2016). A slight difference in our study was that the LRC group was merged into Lancaster, which included the Chinese landraces from Chinese maize breeding programs, including Dan340. Chuan29Female as a Tropic line was grouped into PB. The drought resistance of the hybrids under the drought stress condition could explain the drought tolerance observed in this group of germplasms. In commercial maize breeding in China, the BSSS group is considered as a female group, while other groups are regarded as paternal. In this study, the BSSS group showed better GCA effects for GWPP, KPR, and other traits under stress when compared with the paternal group (Appendix I). Therefore, yield stability under multiple environmental stresses is an important criterion during the breeding of female inbred lines.

Extensive information in divergent pools can be generated from the diallel mating design (Reif *et al.* 2005), providing useful information for understanding the genetic relationships among genotypes, and thus a more strategic breeding plan could be developed to improve specific traits. Based on our field data (Table 1), the PB group manifested high GWPP, late maturity, and high PH. The SPT, BSSS, and NSS groups showed high GWPP, early maturity, and low PH, which was consistent with the research in northern China (Zhang *et al.* 2004; Meng *et al.* 2010). The most explored heterotic pattern is the crosses between BSSS and NSS (Mikel 2008, 2011). In the early and middle 1990s, the major heterotic pattern was domestic inbreds×SPT in the North Spring Maize Region, while in the Huang–Huai–Hai Summer Maize Region the major pattern was domestic inbreds×Pioneer hybrids in China. The major heterotic patterns then became Reid×Tem-tropic I, Reid×Zi330, Reid×SPT, Zi330×Tem-tropic I and Lancaster×SPT in the early 21st century (Wang *et al.* 1997; Teng *et al.* 2004). With the increase of mechanized planting, the combination of early maturity with high yield gained more popularity. In this study, we studied the heterotic patterns of BSSS×NSS, NSS×SPT, and BSSS×SPT, which are the most popular in China's maize breeding with some important hybrids. During 2005–2019, the two most commercialized hybrids were Zhengdan 958 and Xianyu 335. The parents of Xianyu 335, PH6WC and PH4CV, belong to the BSSS×NSS pattern, while the parents of Zhengdan 958, Zheng58 and Chang7-2, are in the SPT×BSSS pattern.

A superior grain yield was observed in the hybrids involving drought-tolerant inbred lines or conventional inbred lines compared to those involving tolerant×conventional lines, conventional×conventional lines, tolerant×sensitive lines or conventional×sensitive lines, indicating additive gene effects and the significance of dosage effects of drought-tolerant genes in the parental lines. This result is consistent with the previous studies (Kirkham *et al.* 1984; Derera *et al.* 2008; Oyekunle *et al.* 2015), where hybrids

Table 4 Correlations of genetic distance (GD) with heterosis and grain yield under drought-stressed (DS) and well-watered (WW) conditions1)

GD	KPR2)	GWPP (g/plant)	BPH (%)	MPH (%)	SCA (g/plant)
Between-group (WW)	0.07**	0.11**	0.20**	0.24**	0.31**
Between-group (DS)	0.10**	–0.02**	0.02**	0.03**	0.16**
Within-group (WW)	0.51**	0.57**	0.49**	0.48**	0.62**
Within-group (DS)	0.28**	0.36**	0.29**	0.38**	0.51**

1) KPR, kernel number per row; GWPP, grain weight per plant; BPH, better parent heterosis for grain yield; MPH, mid-parent heterosis for grain yield; SCA, specific combining ability for grain yield.

**, significant at *P*<0.01, respectively.

with at least one drought-tolerant parental line have higher yields than hybrids with two susceptible parental lines. It can be concluded that at least one parent should be either drought-tolerant or a good GCA line to produce single-cross hybrids with drought tolerance, and that parents should be selected from different heterotic groups so that the hybrids can be formed with complementary inbred lines. In our study, drought-tolerant hybrids showed more KPR, higher PH and shorter ASI than susceptible hybrids (Table 2; Fig. 4). Therefore, these three traits can be used as selection criteria for drought-tolerant maize breeding. PH6WC and Tie7922 are two outstanding inbreds because when crossed with other lines their hybrids performed well under both WW and DS treatments. These inbred lines can be used as superior donors in the development of elite inbred lines in Chinese and worldwide breeding programs.

The genetic distance between parental lines has been used to improve the identification of heterotic hybrids in several crops (Betrán *et al.* 2003). Previous studies have reported that the correlation between parental GD and hybrid performance was significant for grain yield and other traits under DS and WW conditions (Badu-Apraku *et al.* 2013; Suwarno *et al.* 2014; Laude and Carena 2015), which is contrary to several other reports (Balestre *et al.* 2008; Oyekunle *et al.* 2015; Su *et al.* 2017). Within a certain range, greater genetic distance is associated with greater heterosis. On the other hand, a GD that is too large is harmful owing to genetic incompatibility (Wei *et al.* 2018). In this study, more markers and a larger population were used to estimate the GDs among the 41 parental lines. Positive correlations of parental GD with GWPP, MPH and BPH were observed under both treatments ($P<0.01$ and $0.2<r<0.4$) (Fig. 4). Based on the parents' heterotic groups, the hybrids could be classified into within-group and between-group categories. The GD between parents was relatively small for within-group hybrids, and MPH for grain yield increased with GD. With the increase of GD, however, the level of MPH for grain yield between heterotic groups remained largely unchanged (Fig. 5-C). Therefore, the optimal genetic distance should be studied further for breeding super hybrids.

5. Conclusion

This study shows that the inbreds were clustered into five groups, and the heterosis patterns of BSSS×NSS, NSS×SPT and BSSS×SPT with early maturity and high yield were determined to be useful in Chinese maize breeding. A larger contribution of the GCA sum of squares to genetic variation was found for most traits under both WW and DS conditions, suggesting that additive gene action was predominant, but GWPP and KPR under DS treatment are mainly controlled by non-additive effects, suggesting that SCA would be a good selection criterion for future drought resistance breeding. Tropical and temperate inbreds possess complementary genes/alleles, and thus promising inbred lines could be derived by crossing these two groups to enhance drought tolerance. This study reveals that at least one of the parental lines with drought tolerance and high GCA effect would be required to achieve the ideal hybrid performance under drought stress. GD had a significant positive correlation with yield and yield heterosis in within-group hybrids across a certain range of GD. There are a large number of genes governing the heterosis, and high-yielding hybrids should have more superior genes (Huang *et al.* 2015). The present investigation suggests that the heterosis is due to the accumulation of superior genes/alleles between parents and the optimal genetic distance between parental lines. In maize drought resistance breeding, superior genes/alleles should be aggregated into parents based on their heterotic groups, and super hybrids could be produced by crossing different heterotic groups.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (31760424) and the Scientific and Technological Project of Xinjiang Production and Construction Corps of China (2019AB021). Research activities of CIMMYT staff were supported by the Bill and Melinda Gates Foundation and the CGIAR Research Program on Maize.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Appendices associated with this paper are available on http://www.ChinaAgriSci.com/V2/En/appendix.htm

References

Adebayo M A, Menkir A, Blay E, Gracen V, Danquah E. 2017. Combining ability and heterosis of elite drought-tolerant maize inbred lines evaluated in diverse environments of lowland tropics. *Euphytica*, **213**, 43.

Ahmad M, Saleem M, Ahsan M, Ahmad A. 2016. Genetic analysis of water-deficit response traits in maize. *Genetics and Molecular Research*, **15**, 15017459.

Alexander D H, Novembre J, Lange K. 2009. Fast model-based estimation of ancestry in unrelated individuals. *Genome Research*, **19**, 1655–1664.

Amiruzzaman M, Islam M A, Hassan L, Rohman M. 2010. Combining ability and heterosis for yield and component characters in maize. *Academic Journal of Plant Sciences*, **3**, 79–84.

Badu-Apraku B, Oyekunle M, Akinwale R O, Aderounmu M. 2013. Combining ability and genetic diversity of extra-early white maize inbreds under stress and nonstress environments. *Crop Science*, **53**, 9–26.

Balestre M, Machado J C, Lima J L, Souza J C, Nóbrega F. 2008. Genetic distance estimates among single cross hybrids and correlation with specific combining ability and yield in corn double cross hybrids. *Genetics and Molecular Research*, **7**, 65–73.

Bates D, Mächler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, **1406**, 133–199.

Betrán F J, Ribaut J M, Beck D, Leon D, De G. 2003. Genetic diversity, specific combining ability, and heterosis in tropical maize under stress and nonstress environments. *Crop Science*, **43**, 797–806.

Bolaños J, Edmeades G O. 1993. Eight cycles of selection for drought tolerance in lowland tropical maize. II. Responses in reproductive behavior. *Field Crops Research*, **31**, 253–268.

Bolaños J, Edmeades G O. 1996. The importance of the anthesis-silking interval in breeding for drought tolerance in tropical maize. *Field Crops Research*, **48**, 65–80.

Campos H, Cooper M, Edmeades G O, Loffler C, Schussler J R, Ibanez M. 2006. Changes in drought tolerance in maize associated with fifty years of breeding for yield in the US corn belt. *Maydica*, **51**, 369–381.

Choukan R, Hossainzadeh A, Ghannadha M R, Warburton M L, Talei A R, Mohammadi S A. 2006. Use of SSR data to determine relationships and potential heterotic groupings within medium to late maturing Iranian maize inbred lines. *Field Crops Research*, **95**, 212–222.

Derera J, Tongoona P, Vivek B S, Laing M D. 2008. Gene action controlling grain yield and secondary traits in southern African maize hybrids under drought and non-drought environments. *Euphytica*, **162**, 411–422.

Dermail A, Suriharn B, Chankaew S, Sanitchon J, Lertrat K. 2020. Hybrid prediction based on SSR-genetic distance, heterosis and combining ability on agronomic traits and yields in sweet and waxy corn. *Scientia Horticulturae*, **259**, 108817.

Ertiro B T, Semagn K, Das B, Olsen M, Labuschagne M, Worku M, Weganry D, Azmach G, Ogugo V, Abebe B, Chibsa T, Menkir A. 2017. Genetic variation and population structure of maize inbred lines adapted to the mid-altitude sub-humid maize agro-ecology of ethiopia using single nucleotide polymorphic (SNP) markers. *BMC Genomics*, **18**, 777.

FAO (Food and Agriculture Organization). 2019. Online statistical database: food balance. FAOSTAT. [2019-07-03] http://www.fao.org/faost at/en/#data.

Griffing B. 1956. Concept of general and specific combining ability in relation to diallel crossing systems. *Australian Journal of Biological Sciences*, **9**, 463–493.

Harrell F E, Dupont C. 2016. Hmisc: Harrell miscellaneous. R package version 4.0-0. https://cran.r-project.org/web/packages/Hmisc/index.html.

Hu H, Xiong L. 2014. Genetic engineering and breeding of drought-resistant crops. *Annual Review of Plant Biology*, **65**, 715–741.

Huang X, Yang S, Gong J, Zhao Y, Feng Q, Gong H, Li W, Zhan Q, Cheng B, Xia J, Chen N, Hao Z, Liu K, Zhu C, Huang T, Zhao Q, Zhang L, Fan D, Zhou C, Lu Y, *et al.* 2015. Genomic analysis of hybrid rice varieties reveals numerous superior alleles that contribute to heterosis. *Nature Communications*, **6**, 6258.

Kempthorne O, Curnow R N. 1961. The partial diallel cross. *Biometrics*, **17**, 229–250.

Kirkham M B, Suksayretrup K, Wassom C E, Kanemasu E T. 1984. Canopy temperature of drought-resistant and drought-sensitive genotypes of maize. *Maydica*, **29**, 287–303.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, **33**, 1870–1874.

Kuznetsova A, Brockhoff P B, Christensen R H B. 2017. lmerTest package: tests in linear mixed effects models. *Journal of Statistical Software*, **82**, 1–26.

Laude T P, Carena M J. 2015. Genetic diversity and heterotic grouping of tropical and temperate maize populations adapted to the northern US Corn Belt. *Euphytica*, **204**, 661–677.

Lu Y, Yan J, Guimaraes C T, Taba S, Hao Z, Gao S, Chen C S, Li J, Zhang S, Vivek B S, Magorokosho C, Mugo S, Makumbi D, Parentoni S N, Shah T, Rong T, Crouch J H, Xu Y. 2009. Molecular characterization of global maize breeding germplasm based on genome-wide single nucleotide polymorphisms. *Theoretical and Applied Genetics*, **120**, 93–115.

Makumbi D, Assanga S, Magorokosho C, Asea G, Worku M, Bänziger M. 2018. Genetic analysis of tropical midaltitude-adapted maize populations under stress and nonstress conditions. *Crop Science*, **58**, 1492–1507.

Makumbi D, Betrán J F, Bänziger M, Ribaut J M. 2011. Combining ability, heterosis and genetic diversity in tropical maize (*Zea mays* L.) under stress and non-stress conditions. *Euphytica*, **180**, 143–162.

Meng Y, Yan J, Teng W, Li J. 2010. Trends in genetic diversity among widely used inbreds from 1991 to 2001 in China and application of three major germplasm groups in maize breeding. *Scientia Agricultura Sinica*, **43**, 670–679. (in Chinese)

Menkir A, Akintunde A O. 2001. Evaluation of the performance of maize hybrids-improved open-pollinated and farmers' local varieties under well-watered and drought stress conditions. *Maydica*, **46**, 227–238.

Mikel M A. 2008. Genetic diversity and improvement of contemporary proprietary North American dent corn. *Crop*

Science, **48**, 1686–1695.

Mikel M A. 2011. Genetic composition of contemporary US commercial dent corn germplasm. *Crop Science*, **51**, 592–599.

Nei M. 1972. Genetic distance between populations. *The American Naturalist*, **106**, 283–292.

NeSmith D, Ritchie J. 1992. Effects of soil water-deficit during tassel emergence on development and yield components of maize (*Zea mays* L.). *Field Crops Research*, **28**, 251–256.

Njeri S G, Makumbi D, Warburton M L, Diallo A, Jumbo M B, Chemining'wa G. 2017. Genetic analysis of tropical quality protein maize (*Zea mays* L.) germplasm. *Euphytica*, **213**, 261.

Oyekunle M, Badu-Apraku B, Hearne S, Franco J. 2015. Genetic diversity of tropical early-maturing maize inbreds and their performance in hybrid combinations under drought and optimum growing conditions. *Field Crops Research*, **170**, 55–65.

R Core Team. 2013. *R: A language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Reif J C, Hallauer A R, Melchinger A E. 2005. Heterosis and heterotic patterns in maize. *Maydica*, **50**, 215–223.

Smith J S C, Duvick D N, Smith O S, Cooper M, Feng L. 2004. Changes in pedigree backgrounds of pioneer brand maize hybrids widely grown from 1930 to 1999. *Crop Science*, **44**, 1935–1946.

SAS (Statistical Analysis System). 2008. Institute SAS/STAT version 9.2 user's guide. SAS Institute, Cary, NC, USA.

Su J, Zhang F, Yang X, Feng Y, Yang X, Wu Y, Guan Z, Fang W, Chen F. 2017. Combining ability, heterosis, genetic distance and their intercorrelations for waterlogging tolerance traits in chrysanthemum. *Euphytica*, **213**, 42.

Suwarno W B, Pixley K V, Palacios-Rojas N, Kaeppler S M, Babu R. 2014. Formation of heterotic groups and understanding genetic effects in a provitamin A biofortified maize breeding program. *Crop Science*, **54**, 14–24.

Teng W, Cao J, Chen Y, Chen X, Liu X, Jing X, Zhang F, Li J. 2004. Analysis of maize heterotic groups and patterns during past decade in China. *Scientia Agricultura Sinica*, **37**, 1804–1811. (in Chinese)

Toker C, Canci H, Yildirim T. 2007. Evaluation of perennial wild *Cicer* species for drought resistance. *Genetic Resources and Crop Evolution*, **54**, 1781–1786.

Troyer A F, Palmer L S. 2006. Background and importance of Troyer Reid corn. *Crop Science*, **46**, 2460–2467.

Wang H, Xu C, Liu X, Guo Z, Xu X, Wang S, Xie C, Li W, Zou C, Xu Y. 2017. Development of a multiple-hybrid population for genome-wide association studies: theoretical consideration and genetic mapping of flowering traits in maize. *Scientific Reports*, **6**, 40239.

Wang K, Qiu F, Larazo W, Paz M A D, Xie F. 2015. Heterotic groups of tropical *indica* rice germplasm. *Theoretical and Applied Genetics*, **128**, 421–430.

Wang Y, Wang Z, Wang Y, Wang Y, Zhang X, Lu L. 1997. Studies on the heterosis utilizing models of main maize germplasms in China. *Scientia Agricultura Sinica*, **30**, 16–24. (in Chinese)

Wei X, Zhang J. 2018. The optimal mating distance resulting from heterosis and genetic incompatibility. *Science Advances*, **4**, eaau5518.

Xie C, Zhang S, Li M, Li X, Hao Z, Bai L, Zhang D, Liang Y. 2007. Inferring genome ancestry and estimating molecular relatedness among 187 Chinese maize inbred lines. *Journal of Genetics and Genomics*, **34**, 738–748.

Xu C, Ren Y, Jian Y, Guo Z, Zhang Y, Xie C, Fu J, Wang H, Wang G, Xu Y, Li P, Zou C. 2017. Development of a maize 55 K SNP array with improved genome coverage for molecular breeding. *Molecular Breeding*, **37**, 20.

Yang Y, Sang Z, Du Q, Guo Z, Li Z, Kong X, Xu Y, Zou C. 2021. Flowering time regulation model revisited by pooled sequencing of mass selection populations. *Plant Science*, **304**, 110797.

Yu K, Wang H, Liu X, Xu C, Li Z, Xu X, Liu J, Wang Z, Xu Y. 2020. Large-scale analysis of combining ability and heterosis for development of hybrid maize breeding strategies using diverse germplasm resources. *Frontiers in Plant Science*, **11**, 660.

Zhang R, Xia J, Xue J, Shi G. 2004. Improvement and utilization of maize 78599 germplasm. *Chinese Agricultural Science Bulletin*, **20**, 128–130. (in Chinese)

Zhang R, Xu G, Li J, Yan J, Li H, Yang X. 2018. Patterns of genomic variation in Chinese maize inbred lines and implications for genetic improvement. *Theoretical and Applied Genetics*, **131**, 1207–1221.

Zhang X, Yong H, Zhou Z, Zhang C, Lu M, Sun Q, Zhang L, Li M, Zhang D, Weng J, Hao Z, Zhang S, Wang Z, Li X. 2017. Heterosis and combining ability of seven maize germplasm populations. *Euphytica*, **213**, 45.

Zhang X, Zhang H, Li L, Lan H, Ren Z, Liu D, Wu L, Liu H, Jaqueth J, Li B, Pan G, Gao S. 2016. Characterizing the population structure and genetic diversity of maize breeding germplasm in Southwest China using genome-wide SNP markers. *BMC Genomics*, **17**, 697.

Zhao Y, Li Z, Liu G, Jiang Y, Maurer H P T, Würschum T, Mock H, Matros A, Ebmeyer E, Schachschneider R, Kazman E, Schacht J, Gowda M, Login C F H, Reif J C. 2015. Genome-based establishment of a high-yielding heterotic pattern for hybrid wheat breeding. *Proceedings of the National Academy of Sciences of the United States of America*, **112**, 15624–15629.

Executive Editor-in-Chief ZHANG Xue-yong
Managing Editor WANG Ning