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COMPARISONS OF FOUR TESTERS IN EVALUATING 27 CIMMYT AND CHINESE MAIZE POPULATIONS

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ABSTRACT - The choice of testers is important for evaluating combining ability, and defining heterotic groups and patterns of maize germplasm effectively and accurately. This research was conducted on 108 testcrosses among 27 populations and four testers in order to (i) determine the effectiveness of testers to estimate general combining ability; (ii) select the best testers to determine heterotic groups and patterns. Seven trials were carried out in China. Each trial was arranged in α-lattice design (11 × 11) with three replications. Data for grain yield and agronomic traits were recorded. The variations among locations and genotypes were highly significant for all seven traits evaluated. The testers and populations showed significant variation for all seven traits evaluated. A high correlations between testers was observed for all traits. Testers HZ4 and Ye478 had large genetic distance and lowest correlation for grain yield when crossed with 27 populations, a finding that indicated these two lines as the best testers for classifying new germplasm into different heterotic groups and defining heterotic patterns in China.

KEY WORDS: Maize; Tester; Heterotic group; Heterotic pattern.

INTRODUCTION

Increasing efforts have been focusing on evaluating maize (*Zea mays* L.) genetic resources for combining ability and heterotic grouping. A large number of maize inbred lines and populations are available from many breeding programs, and breeders cannot evaluate the combing ability of too many maize germplasm in diallel crosses because the number of potential hybrids is prohibitive. HALLAUER *et al.* (1988) stated that the relative performance of inbred lines and populations in testcrosses with divergent testers of known origin can be used as a practical tool in determining heterotic patterns. Breeders can obtain the information on heterotic groups of new germplasm by testing crosses obtained with the effective testers.

Many studies have provided definitions of the best or the most convenient testers to evaluate combining ability and breeding values of new germplasm. RAWLINGS and THOMPSON (1962) defined a good tester as one that correctly classifies the relative performance of lines and discriminates efficiently among lines under test. HALLAUER (1975) suggested that criteria for choosing a tester should include simplicity in use, the ability to correctly rank lines, and maximization of genetic gain. ABEL and POLLAK (1991) compared eight testers and concluded that at least two testers should be used to evaluate relevant agronomic traits of maize genetic resources. HOLLAND and GOODMAN (1995) drew the conclusion that an initial testing of tropical accessions by a single temperate tester would be appropriate. The testers used by breeders will be dictated by the stage of development of breeding programs (HALLAUER *et al.*, 1988). For evaluating heterosis and combining ability between exotic and domestic germplasm, the testers should represent the basis of domestic germplasm. In China, most of the Chinese hybrids are derived from four heterotic groups: Non-Reid, Reid, Sipingtou, and Lvda Red Cod (Non-Reid and Reid heterotic groups are U.S germplasm, Sipingtou and Lvda Red Cob heterotic groups were developed from Chinese varieties) (LIU *et al.*, 1997; PENG *et al.*, 1998; LI *et al.*, 2002; ZHANG S.H. *et al.*, 2002). In the current study, we compared four testers (representing four heterotic groups in China), combined with 14 CIMMYT and 13 Chinese maize populations. The objectives of the research were to (i) determine the effectiveness of testers to estimate general combining ability of exotic

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germplasm, (ii) identify the best tester to evaluate heterotic groups and patterns between exotic populations and domestic germplasm.

MATERIALS AND METHODS

Materials

The materials for this study consisted of 108 testcrosses developed by crossing four testers with 27 populations. The tester Dan340 is an inbred line derived from Lv9×podcorn, and Huanghao4 (HZ4) is an inbred line in the Sipingtou group. These two lines represent Lvda Red Cob group and Sipingtou group, which are the dominant germplasm used in development of commercial hybrids in China. The tester Ye478, an inbred line derived from U8112×Shen5003, is from the Reid Yellow Dent heterotic group in origin and the tester Mo17 is an inbred line of the Lancaster heterotic group. These two lines represent Reid and Non-Reid germplasm in China. Four commercial hybrids, CAU 108, Tangkang5, Ludan50, and Yedan13, were included as checks in the experiment. In addition, nine populations were included for field design, yielding a total of 121 entries to be organized following a α-lattice design (11×11).

The 27 populations included 14 CIMMYT populations and 13 domestic populations. The 14 CIMMYT populations were introduced in China and have been improved from the south to the north along the Corn Belt by Prof. Zhang since 1996. Some of them were considered adapted to northern China, for example populations 45 and 46. The 13 Chinese domestic populations were collected from all over the country. These populations, which were developed by different institutes and universities, should represent the diversity of maize germplasm from China. The populations are listed in Table 1.

TABLE 1 - *The germplasm name, origin and description of the 27 maize populations crossed to four testers.*

Germplasm name	Origin	Description
Domestic Populations		
Jilin Syn A	Jilin AAS*	Synthetic composed of lines derived from Yinglizi.
Liaolv Syn	Liaoning AAS*	Synthetic composed of lines derived from Lvda Red Cob.
CSyn 5	CAAS**	Synthetic composed of lines derived from Sipingtou germplasm.
CSyn 3	CAAS**	Synthetic composed of lines derived from Lvda Red Cob.
CSyn 4	CAAS**	Synthetic composed of anti-disease lines derived from USA germplasm.
Yu Syn 5	Henan AU***	Synthetic composed of intermediate maturing and dent lines derived from USA germplasm.
Golden Queen	Shanxi AAS*	A yellow dent variety introduced from USA in 1931.
Shaan Syn 1	Shaanxi AAS*	Synthetic with long-ear, big kernel, including the Lancaster Sure Crop germplasm.
Shaan Syn 3	Shaanxi AAS*	Synthetic with short plant and big ear.
Dongnong Pop	Northeast AU***	Synthetic with Lvda Red Cob germplasm.
WBM-C4	Huazhong AU***	Population including some BSSS, CIMMYT Tropical germplasm and flint germplasm from southern China.
CPop 13	CAAS**	Improved from Pool 33QPM.
CPop 14	CAAS**	Improved from Pool 34QPM.
CIMMYT Populations		
Population 21	CIMMYT	Tropical, late maturing, white dent. Tuxpeno-based material.
Population 28	CIMMYT	Tropical, late maturing, yellow dent. Includes some material from Mexico and Colombia.
Population 32	CIMMYT	Tropical-subtropical, intermediate to late maturing, ETO-based material.
Population 43	CIMMYT	Tropical, late maturing, white dent. La Posta-based material.
Population 45	CIMMYT	Subtropical, early to intermediate maturing, yellow dent. Includes some material from Mexico and Colombia.
Population 46	CIMMYT	Subtropical, early maturing, yellow flint. Includes some material from Europe and America.
Population 49	CIMMYT	Tropical-subtropical, intermediate maturing, white dent.
Population 69	CIMMYT	Subtropical, intermediate to late maturing, yellow flint, QPM.
Population 70	CIMMYT	Subtropical, intermediate to late maturing, yellow semident, QPM.
Population 501	CIMMYT	Subtropical, intermediate maturing, white semident.
Population 502	CIMMYT	Subtropical, intermediate maturing, white semident.
Stay Green	CIMMYT	Tropical, late maturing, white semident.
Pool 19	CIMMYT	Tropical, intermediate maturing, white flint.
Suwan 1	CIMMYT	Tropical, intermediate maturing, yellow flint.

*: AAS: Academy of Agricultural Sciences.
**: CAAS: Chinese Academy of Agricultural Sciences.
***: AU: Agriculture University.

Procedure and field experiments

The testcrosses between 27 maize populations and 4 testers were produced in the southern provinces, Hainan in 2000 and Yunnan in 2001. The testers as female were pollinated with the populations. A minimum of 40 tassels determined arbitrarily from each population were used as pollen donors.

The 121 materials (108 testcrosses, 4 checks, and 9 populations) were evaluated in four environments: Sanya in Hainan Province, Zhengzhou in Henan Province, Ya'an in Sichuan Province, and Beijing City in 2001 and 2002. Sanya is the most southern city in China. Ya'an is in the southwest. Zhengzhou is the center. Beijing, the capital of China, is the northeast of China. The testcrosses were arranged in α-lattice design (11×11) with 3 replications and planted in double-row plots, 5 m long with 0.65 m between rows, and 0.30 m between plants within a row. Plant density was adjusted by thinning to one plant per hill (51,308 plants ha^{-1}). Fertilizer and field management practices recommended for optimum maize production were used at each location. The experiment at Sanya in Hainan Province was destroyed by a storm in 2002. Plots were hand harvested when feasible at all locations.

Data collection

The traits evaluated were grain yield, number of kernels per row, number of ear rows, 100-kernel weight, days to silking, plant height, and percentage lodging. In each plot, grain yield (kg ha^{-1}) was calculated as weight adjusted to 14% moisture after shelling. Number of kernels per row and number of ear rows were counted as the average number of kernels in one row and average number of rows in one ear based on 10 random ears for each plot. Days to silking (d) was recorded as the number of days from planting to the day when 50% of the plants exhibited silks. Plant height (cm) was measured on 10 random plants as the distance from the ground to the height of the first tassel branch in each plot. Percentage lodging was counted as the rate of leaning stalks with an angle more than 45°. Data of 100-kernel weight, days to silking, and percentage lodging were collected from six locations.

The genetic relationsbips analysis

The genetic relationships of four testers were analyzed using SSR markers (simple sequence repeats). DNA samples from 15 plants of each inbred line were extracted from young leaves using a modified CTAB procedure (SAGHAI-MAROOF *et al.*, 1984). Sixty-four SSR primer pairs were selected based on their distribution in the genome, profile quality, and polymorphism level (Table 2; LI *et al.*, 2006). PCR amplifications, conditions, and electrophoretic separation of PCR reaction products were conducted according to YUAN *et al.* (2001) and TUBEROSA *et al.* (2002). PCR products were separated in denaturing polyacrylamide (SequaGel XR, Polymed) gels in 1X TBE buffer; 2-4 µl of each PCR product were pooled and diluted from 1:3 to 1:10 (according to the relative PCR product concentration) with 0.1% (TE) solution.

Statistical analysis

Analyses of variance (ANOVA) over locations and years were made for all seven agronomic traits using plot mean data. Population and tester effects were considered as fixed and replication and location-year environmental effects were treated as random. Sum of squares due to testcrosses were divided into populations, testers, and population x tester interaction. Estimates of GCA were calculated from the means of crosses according to GUO (1993). The model for the GCA was:

$GCA_i = T_i/fr\text{-}T_c/mfr$

(f = No. of populations, m = No. of testers, r = No. of replications).

The T_i/fr and T_c/mfr correspond to the mean of the ith tester and the grand mean, respectively. The coefficients of linear correlation were calculated between GCA in crosses with four testers and in crosses with two testers for all traits across seven environments. The correlation among entry yields across the four testers was calculated.

The genetic similarity (GS_{ij}) between lines i and j was calculated according to the following equation (SNEATH and SOKAL, 1973): $GS(i,j) = m/n$, where m is the number of loci with allelic variants of the same molecular weight for lines i and j, and n is the total number of loci, excluding loci with missing data. In some cases (less than 1%), SSR loci showed the presence of a residual non-uniformity within lines (i.e. the presence of two different alleles per locus): in such cases, the two alleles are considered to contribute equally to the genetic make-up of the lines.

All computations were carried out with appropriate procedures of the software package SAS 2002 and NTSYS-pc vers.2.0 (ROHLF, 1997).

TABLE 2 - *Partial mean squares from the combined ANOVA of 108 testcrosses across seven environments for grain yield (GY).*

Source of variation	df	GY
Replications®/E	14	3628.19**
Enviroment (E)	6	269344.61**
Genotypes(G)	120	4620.41**
ExG	720	1461.40**
Error	1673	501.12**
Populations(P)	26	10414.74**
Testers(T)	3	4362.84**
PxT	78	1522.38**
Error	1924	412.88

**, * Significance at the 0.01 and 0.05 probability levels, respectively.

RESULTS

Analyses of variance

Highly significant effects of replications were observed for grain yield in the combined ANOVA (Table 2), perhaps due to large plant lodging (0.02-0.09% on average, depending on the tester; Table 3). Fortunately, the variations among genotypes were highly significant for grain yield and the other traits evaluated (data not shown). The source of variation due to 108 testcrosses was divided into populations, testers, and their interaction. The testers and populations showed significant variation at the 0.01 or 0.05 probability levels for all seven

TABLE 3 - *Means of testers for grain yield (GY), 100-kernel weight (KW), number of row kernels (NK), number of ear rows (NR), days to silking (DS), plant height (PH), and percentage lodging (PL) on 108 testcrosses in seven environments.*

Tester	GY(kg ha^{-1})	KW(g)	NK	NR	DS(d)	PH(cm)	PL
Dan340	6550.47	29.70	32.43	17.30	71.74	246.80	0.06
Ye478	6376.42	31.73	35.10	14.64	69.55	232.54	0.02
Mo17	6145.35	30.59	36.82	13.98	69.76	247.58	0.05
HZ4	6379.50	29.65	32.32	15.53	67.54	242.88	0.09

TABLE 4 - *The coefficients of correlation between GCA effects in crosses with different testers and in crosses with four testers among seven agronomic traits: grain yield (GY), 100-kernel weight (KW), number of row kernels (NK), number of ear rows (NR), days to silking (DS), plant height (PH), and percentage lodging (PL).*

Testers	GY	KW	NK	NR	DS	PH	PL
Dan340 & Ye478	0.95	0.99	0.90	0.94	0.99	0.99	0.92
Dan340 & Mo17	0.96	0.98	0.92	0.92	0.99	0.98	0.97
Ye478 & HZ4	0.94	0.97	0.90	0.91	0.99	0.98	0.95
Mo17 & HZ4	0.93	0.98	0.88	0.93	0.99	0.99	0.97

traits evaluated. The interaction (testers x populations) showed significant differences at P 0.01 for all seven traits except for plant lodging.

Mean values of testers

A good tester should possess satisfactory agronomic traits. Mean values for seven agronomic traits of testers evaluated across all other factors are presented in Table 3. The tester Dan340 showed the latest silking dates, the highest grain yield, and number of ear rows among all four testers. The tester Ye478 possessed the highest 100-kernel weight, the lowest plant height, and plant lodging. The tester Mo17 displayed the highest plant height and number of row kernels, the lowest grain yield, and number of ear rows. The tester HZ4 performed the earliest silking dates, the lowest number of row kernels, and the highest plant lodging.

The correlations among GCA effects of populations in crosses with different testers

To compare GCA effects in crosses with different testers, we estimated GCA effects in crosses with pairs of testers that correspond to different heterotic patterns: Dan340 and Ye478, Dan340 and Mo17, Ye478 and HZ4, Mo17 and HZ4. These four heterotic patterns have been utilized predominantly in Chinese commercial hybrids. The correlations between GCA effects in crosses with different testers and in crosses with four testers among seven agronomic traits are shown in Table 4. High correlations were found for all traits with coefficients ranging from 0.88 (number of row kernels) to 0.99 (many traits). There is no difference among the coefficients of correlation (0.99) for silking dates between GCA effects in crosses under four heterotic patterns and in crosses with four testers. The highest coefficients of correlation between GCA effects in crosses with Dan340 and Ye478 and in crosses with four testers was shown by number of ear rows (0.94), 100-kernel weight (0.99), while the lowest correlation was shown by plant lodging (0.92). The GCA effects in crosses with Dan340 and Mo17 showed the highest correlation with GCA effects in crosses with four testers for grain yield (0.96), for number ot kernel rows (0.92), and for plant lodging (0.97). The lowest correlation was displayed between GCA effects in crosses with Ye478 and HZ4 and in crosses with four testers for number of ear rows (0.91). The lowest correlation was displayed between GCA effects in crosses with Mo17 and HZ4, and in crosses with four testers for grain yield (0.93), number of row kernels (0.88), and the highest correlation for plant lodging (0.97). The coefficients of correlation were similar in traits of plant height, 100-kernel weight, and ranged from 0.97 to 0.99.

The genetic relationships among testers

The genetic similarity estimates among testers varied from 0.329 (Mo17 vs. HZ4) to 0.472 (Dan340

TABLE 5 - *The genetic similarity estimates between four testers.*

	Mo17	HZ4	Dan340	Ye478
HZ4	0.329	1		
Dan340	0.473	0.333	1	
Ye478	0.412	0.333	0.333	1

TABLE 6 - *The coefficients of correlation for grain yield among the four testers.*

Testers	Dan340	Ye478	Mo17	HZ4
Dan340		0.79	0.72	0.46
Ye478			0.83	0.43
Mo17				0.44
HZ4				

vs. Mo17), with a mean value of 0.369 (Table 5). The highest similarity observed between lines Mo17 and Dan340 showed these two lines had the closest genetic relationship among four testers. The lines Ye478 also had a closer genetic relationship with Mo17, but the lines HZ4 had low genetic similarity with the others testers.

A comparison of grain yield among 27 populations in crosses with four testers

There were high coefficients of correlation for grain yield in crosses with 27 populations among the testers Dan340, Ye478, and Mo17, and lower coefficient of correlation between HZ4 and the other testers (0.46, 0.43, and 0.44, respectively; Table 6). The coefficients of correlation for grain yield across Dan340, Ye478, and Mo17 were r = 0.79, 0.72, and 0.83, respectively. HOLLAND and GOODMAN (1995) had a similar result that the coefficient of correlation between TR(A632Ht x B73Ht, representing the Reid Yellow Dent heterotic group) and TL(Mo17Ht x Oh43E, representing the Lancaster Sure Crop hetrotic group) was r = 0.78. The lowest correlation was shown between HZ4 and Ye478 (0.43). The testers Dan340, Ye478, and Mo17 were somewhat consistent in ranking the populations (Table 7). The Populations 21, 43, 28, 501, 502, Suwan 1, and CSyn 5 were ranked in the top 45% by the testers Dan340, Ye478, and Mo17. The tester HZ4 showed inconsistencies in ranking accessions. For example, the populations 502, Suwan 1, and CSyn 5 were ranked in the bottom 50% by the tester HZ4, the Liaolv Syn was ranked in the 8th by the tester HZ4 and in bottom 55% by the others testers, the Jilin Syn A was ranked 7th by the tester HZ4, 18th by the testers Ye478 and Mo17, and 22nd by the tester Dan340.

DISCUSSION

The choice of tester usually involves many alternatives, such as broad genetic-base vs. narrow genetic-base, high alleles frequency vs. low allele frequency, general combining ablility vs. specific combining ability (SPRAGUE and TATUM, 1942). But what kind testers can be selected to estimate combining ability and heterotic groups and patterns?

Agronomic traits of testers

ABEL and POLLAK (1991) concluded two agronomic standards to choose effective testers for germplasm evaluation. First, an effective tester should be a good pollen donor, so that crossing to candidate entries is easy. In our study, we found an effective tester also should be a good kernel producer. In China, the tester Mo17 produced little kernels. All other testers produced sufficient kernels for crossing. Second, a tester should possess good agronomic characteristics, such as little root and stalking lodging. We share the same opinion. The tester Ye478 had the least plant lodging in cross with populations. The current study suggested the days to maturation is also an important trait for an effective tester because germplasm evaluation can be performed in broader environmental conditions with the shorter maturation. The more environments the more accurate are the results and the information of germplasm evaluation can be used in more locations. In the current study, the tester HZ4 had the earliest silking date.

An effective tester to estimate GCA effects of germplasm

For breeding efficiency, breeders want to use a small quantity of testers in maize breeding effort. Our study compared GCA effects of populations in crosses with different testers, four testers and two testers. The results showed every two testers, which represent different heterotic patterns, could evaluate GCA effects of seven traits of 27 populations as accurately as four testers. ABEL and POLLAK (1991) also had a similar result that at least two testers can be used to evaluate relevant agronomic traits of maize genetic resources. So we suggested an accurate and efficient testing of GCA effects of populations by

TABLE 7 - *Grain yield and ranks for 27 maize populations when evaluated by four testers across seven environments.*

Populations	Dan340 GY(kg ha^{-1})	rank	Ye478 GY(kg ha^{-1})	rank	Mo17 GY(kg ha^{-1})	rank	HZ4 GY(kg ha^{-1})	rank
Population 21	7659.78	4	7350.30	3	7275.01	2	6958.12	3
Population 32	7605.21	5	6511.88	10	6107.20	16	6397.61	18
Stay Green	6220.67	19	7180.38	4	6724.14	7	6678.07	8
Population 43	7664.01	3	7580.52	2	7396.30	1	6860.90	5
Population 49	7207.06	6	7143.69	6	6563.71	9	7202.56	2
Pool 19	6598.64	10	6472.68	13	5517.62	23	6603.70	10
Population 28	6799.92	8	7139.32	7	6776.77	5	6950.62	4
Population 45	6413.18	14	6151.38	17	6251.63	12	6498.75	16
Population 46	6284.88	16	5441.93	25	5652.71	22	5763.72	25
Population 501	7923.96	2	7699.77	1	7006.88	3	7336.21	1
Population 502	6477.16	12	6485.32	12	6692.18	8	6460.49	17
Population 69	6239.39	18	6325.04	16	6186.52	14	6375.33	19
Population 70	5691.53	26	5278.02	26	5327.38	24	5964.02	24
Suwan 1	7938.93	1	6973.85	8	6779.39	4	6516.32	14
Jilin Syn A	5972.44	22	6106.93	18	5944.02	18	6762.26	6
Liaolv Syn	6121.20	20	6046.61	19	6212.26	13	6718.73	7
CSyn 5	6678.41	9	7164.68	5	6743.65	6	4095.61	27
CSyn 3	5799.08	24	6504.30	11	5944.59	17	6498.93	15
CSyn 4	6088.30	21	5876.81	21	6135.23	15	6637.66	9
Yu Syn 5	6546.42	11	5490.17	24	5708.71	20	6519.42	12
Golden Queen	4963.69	27	4556.44	27	4380.99	27	4906.58	26
Shaan Syn1	6261.19	17	6425.24	15	4958.44	26	6079.15	22
Shaan Syn 3	5826.74	23	5670.04	22	5033.49	25	6042.31	23
Dongnong Pop	5721.55	25	5658.17	23	6338.32	11	6180.36	21
WBM-C4	6887.13	7	6617.27	9	6519.95	10	6517.97	13
CPop 13	6398.15	15	6465.34	14	5917.89	19	6212.03	20
CPop 14	6422.44	13	5952.44	20	5678.66	21	6577.87	11
LSD0.05	607.7		540.93		585.61		551.02	

two testers, corresponding to a heterotic pattern, would be appropriate.

The genetic relationship between testers HZ4 and Ye478

MELCHINGER (1999) proposed that, when a large number of inbred lines are available and proven testers exist, the relative performance of the lines in testcrosses with proven testers can be used as a main criterion for grouping of the lines. The best testers should have large genetic distance and quite different yield with others germplasm so that materials which be tested could be classified into different heterotic groups clearly. Based on the results of SSRs and grain yield analyses, testers HZ4 and Ye478 seems to be a good compromise to consider as the convenient testers. Testers HZ4 and Ye478 had large genetic distance (Table 5) and a lowest correlation for grain yield in cross with 27 populations (Table 6), which can be important to classify the new germplasm into different heterotic groups. The cross, HZ4 and Ye478, is a main heterotic pattern which has been used widely in Huang-huai-hai summer maize region of China (ZHANG *et al.*, 2002; LI *et al.*, 2004).

The tester HZ4

The line HZ4 was selected by Maize Program of Chinese Academy of Agricultural Sciences (CAAS) and Beijing Academy of Agricultural Sciences from a local germplasm which planted in Tangshan City, Hebei Province in 1970s. Many recycled lines derived from HZ4, for instance, Chang 7-2 and LX9801, showed very good combining ability and used predominantly in China. The result of grain yield in cross with 27 populations showed the tester HZ4

had lower correlation with the others three testers, while the testers Dan340, Ye478 and Mo17 had similar coefficients of correlation (table 6). Lower coefficient of correlation for yield between the tester HZ4 and the other testers implied that some genetic alleles controlling yield in HZ4 were quite different with the other three lines. Tester HZ4 should be paid more attention in breeding and research program in China, and that experiments should be conducted to find the genes underlying yield of HZ4.

CONCLUSION

Our results suggested that every two testers, which represent different heterotic patterns, could evaluate GCA of 27 populations as accurately as four testers. The tester Ye478 had the least plant lodging and HZ4 had the earliest silking date in crosses with 27 populations and there was the lowest correlation for grain yield in cross with 27 populaitons between Ye478 and HZ4 (r = 0.43). So the testers Ye478 and HZ4 are the best ones to evaluate GCA and heterotic groups and patterns of maize germplasm in China.

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