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Research Article

Genetic analysis and heterosis for quality traits in pearl millet [*Pennisetum glaucum* (L.) R. Br.]

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Abstract
The experiment was conducted with 60 hybrids developed by crossing two male sterile lines with 30 diverse testers studied for gene action and heterosis. ANOVA for combining abilities revealed that the parents are significantly different and diverse for all the traits under study. The ratio of *gca:sca* variance was found less than unity indicating the presence of non-additive gene action and the average degree of dominance was greater than unity indicated the predominance of over dominance. Among the parents, the tester 2325 was a good combiner and offers the development of genotypes with improved quality traits along with grain yield. The crosses ICMA 04999 × 2325 for iron content, ICMA 97111 × 2310 and ICMA 97111 × 2342 for zinc content, ICMA 97111 × 2395, ICMA 97111 × 2310 and ICMA 04999 × 2386 for protein content and ICMA 04999 × 2349 for rancidity exhibited significantly high standard heterosis, can be utilized for commercial cultivation after testing under multilocational trials.

Keywords: Pearl millet, GCA, SCA, gene action, quality traits, standard heterosis

INTRODUCTION

Pearl millet is an important staple food and fodder crop widely grown in arid and semi-arid regions of Africa and India. Globally it occupies sixth place in its importance after wheat, rice, maize, barley and sorghum, while in India it occupies fourth place after rice, wheat and maize. In the country, it is cultivated in acreage of 7.54 million hectares with 10.36 million tonnes of production and 1373.86 kg/ha of productivity (Ministry of Agriculture, 2019-2020). Because of its C_4 nature, it has high photosynthetic efficiency and has more potential for biomass production. Because of malnutrition number of people are suffering from nutrient deficiency, especially in developing countries. It needs improvement of nutritional quality along with grain yield through breeding programmes to sustain malnutrition. Pearl millet grains are of good nutritive value with 73% carbohydrates, 11% protein, 8.4% fat and rich source of minerals such as iron (6-7 mg/100 g) and zinc (3.4 mg/100 g) (Malik, 2015). Being it has high nutrient content its bioavailability through pearl millet flour is limited due to the presence of highly active lipases coupled with highly unsaturated fatty acids leads to rapid deterioration of flour during storage and becomes rancid. So there is a need to develop cultivars with high nutrient contents, low anti-nutritional factors along with more grain yield.

The protogynous and highly cross-pollinated nature of pearl millet along with the availability of several sources of male sterility helps in the successful development of hybrid cultivars at the field level. The development of hybrids through heterosis breeding requires information regarding the genetic architecture and behaviour of parents in hybrid combinations. The behaviour of parents in a hybrid combination can be evaluated by combining ability. Studies on combining ability help in the selection of suitable parents (GCA) for hybridization, specific cross combinations (SCA) and also provide information regarding the nature of gene action (Sprague and Tatum, 1942) which intern helps in the selection of suitable breeding methodology for further crop improvement. Among the biometrical techniques, Line × Tester analysis (Kempthorne, 1957) is one of the widely used techniques for testing combining ability (Solanki *et al*., 2017). This design helps in the evaluation of large number of germplasm at a time in terms of variance and effects (Sprague and Tatum, 1942). In view of the above points the present investigation was carried out to study the genetics and heterosis of quality traits for the selection of desired hybrids.

MATERIALS AND METHODS

The experimental material consists of 30 testers collected from four different states of India *i.e.*, Tamil Nadu, Maharashtra, Madhya Pradesh and Andhra Pradesh were crossed with two male sterile lines (ICMA 04999 and ICMA 97111) in L × T mating design at IIMR during *summer,* 2019 and generated 60 F_1 hybrids. The resultant 60 F_1's along with their 32 parents and six national checks (GHB 558, GHB 905, RHB 173, HHB 272, MPMH 21, HHB 67 improved) were evaluated in alpha lattice design (Patterson and Williams, 1976) with three replications during *Kharif*, 2019 at Indian Institute of Millet Research (IIMR). Each genotype was accommodated in two rows with two metres row length, 45 cm row spacing and 15 cm plant to plant spacing in each row. All the recommended agronomic and plant protection measures were followed to raise a good and healthy crop. The grain of each genotype was cleaned and powdered, resultant flour was used for the estimation of iron, zinc, protein and rancidity. Iron and zinc content was estimated from the extract of flour digested with diacid mixture (HNO_3 and $HClO_4$ in 9:4 ratio) by the use of Atomic Absorption Spectrophotometry (AAS), (Lindsay and Norvell, 1978) as described by Tandon (1999). Protein content was determined by estimating the nitrogen content (%) following the Micro-Kjeldhal method (Tandon, 1999) and multiplying it with the conversion factor (6.25) as given by Sadasivam and Manickam (1996). Rancidity was measured in terms of alcoholic acidity as per the IS 12711:1989 Method of Determination of Alcoholic Acidity. For this 5 g of bajra flour was mixed with 50 ml of neutral ethyl alcohol (90%) and allowed to stand for 24 hours with occasional swirling. Titrate the 10 ml of the alcoholic extract with standard sodium hydroxide solution to a pink endpoint using a phenolphthalein indicator. Grain yield was recorded on a plot basis.

Among the six national checks, the *per se* performance of RHB-173 was good and this was taken as a standard parent for estimation of standard heterosis. Statistical analysis was carried out using Genstat 12 edn and Indostat software packages.

RESULTS AND DISCUSSION

Analysis of variance for combining ability was presented in **Table 1**. The mean squares due to lines, testers and line × tester were significant for all the traits except for rancidity and grain yield in lines, iron content and grain yield in line × tester. The presence of significant differences indicates that the parents under study were diverse and significantly contribute toward combining ability and the resultant hybrids from those showed a substantial amount of genetic variability. The estimates of variances due to general combining ability and specific combining ability were non-significant and significant for all the traits, respectively. The ratio of GCA: SCA variance being less

Table 1. Analysis of variance for combining ability for yield and quality traits in Pearl millet

Source of variations	d.f.	Iron content	Zinc content	Protein content	Rancidity (Alcoholic acidity)	Grain yield
Replications	2	0.005	0.002	0.355**	0.00000	2.219
Treatments	91	2.100**	1.437**	11.128**	0.006**	2.764**
Lines	1	1.269**	0.277**	2.160**	0.00023	2.66667
Testers	29	1.550**	1.744**	13.354**	0.0051**	3.282**
Lines × Testers	1	0.0014	0.442**	1.806**	0.036**	2.236
Error	182	0.003	0.002	0.047	0.0002	0.81
Variance components						
σ^2 gca		0.0597	0.0402	0.0863	0.0001	0.0153
σ^2 sca		0.8445**	0.4173**	2.0946**	0.0023**	0.1602*
σ^2 gca / σ^2 sca		0.0706	0.0963	0.0412	0.0434	0.0955
σ^2 A		0.239	0.161	0.345	0.001	0.061
σ^2 D		3.378	1.669	8.378	0.009	0.641
$(\sigma^2 D: \sigma^2 A)^{0.5}$		3.76	3.22	4.93	3.00	3.24

** & * Significant at 1 and 5 per cent level, respectively than unity for all the traits indicating the importance of non-additive gene action in the inheritance of these traits and can be exploited by the production of hybrids through heterosis breeding. These results were in agreement with Pallavi *et al*. (2020) for rancidity and Solanki *et al*. (2017) for grain yield, iron content, zinc content and protein content. The average degree of dominance was greater than unity indicating the over dominance behaviour of interacting alleles for the five traits. These results were in accordance with Patel *et al*. (2018) for grain yield and protein content and Gavali *et al*. (2018) for iron content.

The results of estimates of *gca* effects for parents were presented in **Table 2**. Lines, ICMA 97111 for iron content (0.138) and zinc content (0.118), ICMA 04999 for protein content (0.010), rancidity (-0.005) and grain yield (0.080) recorded significant *gca* effects in desirable direction. Rancidity (Alcoholic acidity) with a lower value is desirable

Table 2. Estimates of general combining ability for yield and quality traits of parents

Parents	Iron content	Zinc content	Protein content	Rancidity (Alcoholic acidity)	Grain yield
Lines					
ICMA 04999	-0.138**	-0.118**	0.010**	-0.005**	0.080**
ICMA 97111	0.138**	0.118**	-0.010**	0.005**	-0.080**
C D at 5%	0.0062	0.0110	0.0440	0.0026	0.182
Testers					
2306	0.128**	-0.044**	-1.813**	-0.027**	0.724*
2309	0.843**	0.641**	1.837**	-0.002**	-0.159**
2310	0.378**	0.816**	2.387**	0.035**	-0.276**
2311	0.293**	0.281**	0.087**	0.047**	-0.909**
2368	-0.327**	-0.284**	-0.013**	-0.027**	0.324
2370	-0.707**	-0.329**	-0.713**	-0.002**	-1.176**
2318	0.873**	0.311**	0.287**	-0.027**	-1.193**
2381	1.038**	0.336**	-1.313**	-0.027**	-0.176**
2325	0.253**	0.121**	0.637**	-0.039**	0.874**
2327	0.313**	0.101**	-0.763**	0.010**	-0.059**
2328	0.148**	0.431**	0.137**	0.035**	0.207
2329	-0.487**	-0.344**	0.837**	-0.002**	0.441
2330	-0.097**	-0.339**	0.837**	0.010**	0.207
2331	-0.925**	-0.529**	-0.263**	0.022**	-0.409**
2332	-0.647**	-0.189**	0.687**	0.016**	-0.209**
2333	-0.582**	-0.354**	-0.713**	0.047**	0.391
2337	0.653**	0.206**	-0.513**	-0.027**	0.724*
2382	-0.095**	-0.024**	1.037**	0.071**	-0.476**
2342	0.618**	0.771**	1.037**	-0.021**	-0.176**
2386	0.658**	0.791**	1.937**	0.035**	-0.176**
2348	0.128**	0.176**	-1.813**	0.010**	0.686*
2352	-1.182**	-0.819**	-0.313**	-0.033**	-0.009**
2364	0.253**	-0.219**	0.187**	-0.051**	0.474
2365	-0.657**	-0.694**	-1.313**	-0.051**	0.041
2387	0.423**	0.146**	-0.513**	0.022**	-0.176**
2394	-1.022**	-0.719**	0.137**	-0.027**	0.674*
2395	0.293**	0.696**	2.187**	0.016**	-0.159**
2396	-1.077**	-0.774**	-1.363**	-0.002**	0.824*
2346	0.093**	-0.179**	-1.363**	0.035**	-0.643**
2349	0.408**	0.011**	-1.463**	-0.045**	-0.209**
CD at 5%	0.024	0.042	0.170	0.010	0.705

** & * Significant at 1 and 5 per cent level, respectively to increase the shelf life of flour. Among testers, 2381 (1.038), 2318 (0.873) and 2309 (0.843) for iron content; 2310 (0.816), 2386 (0.791) and 2342 (0.771) for zinc content, 2310 (2.387), 2395 (2.187) and 2386 (1.937) for protein content, 2364, 2365 and 2349 for rancidity and 2325 (0.874), 2396 (0.824), 2306 (0.724) and 2337 (0.724) for grain yield recorded the highest significant *gca* effect in desirable direction. It showed that these parental lines are having good general combining ability for improving respective traits, while crossing with other genotypes. The tester 2325 had a significant *gca* effect for all the traits in a desirable direction and offers the development of lines with improved grain yield along with good quality traits. Similar results were earlier reported by Nandaniya *et al*. (2016) and Solanki *et al*. (2017) for grain yield, iron content and zinc content and Patel *et al*. (2018) for protein content.

A perusal of *sca* effects (**Table 3**) revealed that out of 60 crosses, 29 crosses for iron content, 30 crosses for zinc content, 25 crosses for protein content and 23 crosses for rancidity recorded significant *sca* effects in a desirable direction. The crosses ICMA 04999 × 2309 for grain yield, ICMA 04999 × 2311 for iron content, ICMA 97111 × 2310 for zinc content, ICMA 97111 × 2395 for protein content and ICMA 97111 × 2331 for rancidity recorded the highest *sca* effects and were the best specific combinations for the respective traits. The crosses with high *sca* effects *viz.,* ICMA 04999 × 2368 and ICMA 04999 × 2311 for protein content and ICMA 04999 × 2309 for grain yield resulting from low × low *gca* combinations indicating the involvement of complementary gene action in the inheritance of these traits. The crosses with low × high combination were observed for ICMA 97111 × 2342, ICMA 97111 × 2306 and ICMA 04999 × 2318 for protein content indicating the involvement of one low combiner will result in high *sca* effects. Peng and Virmani (1990) reported the possibility of interaction between positive alleles from good combiners and negative alleles from poor combiners in high × low or low × high parental combinations and suggested for the exploitation of heterosis in F_1 generation as their high yield potential would be unfixable in succeeding generations. Similar results of significant *sca* effects were earlier reported by Karvar *et al.* (2017) for iron content, zinc content and grain yield and Patel *et al*. (2014) for protein content.

Table 3. Estimates of specific combining ability for yield and quality traits of hybrids

Crosses	Iron content	Zinc content	Protein content	Rancidity (Alcoholic acidity)	Grain yield
ICMA 04999 × 2306	0.498**	0.213**	-1.660**	0.005	-0.063
ICMA 04999 × 2309	-1.048**	-0.662**	-1.010**	-0.020**	1.120*
ICMA 04999 × 2310	-0.643**	-0.927**	-1.260**	0.041**	0.004
ICMA 04999 × 2311	1.393**	0.738**	1.340**	-0.044**	-0.730
ICMA 04999 × 2368	0.223**	0.173**	1.440**	0.005	-0.730
ICMA 04999 × 2370	0.403**	0.438**	-0.760**	0.029**	0.604
ICMA 04999 × 2318	0.933**	0.718**	1.540**	0.029**	0.187
ICMA 04999 × 2381	-0.573**	-0.177**	-0.060	-0.044**	0.004
ICMA 04999 × 2325	0.063**	0.288**	-0.010	0.042**	-0.080
ICMA 04999 × 2327	-1.108**	-0.352**	0.490**	0.017*	-0.646
ICMA 04999 × 2328	-0.263**	-0.582**	1.390**	-0.057**	0.287
ICMA 04999 × 2329	0.402**	0.273**	0.590**	0.005	-0.480
ICMA 04999 × 2330	1.103**	0.538**	-0.810**	-0.007	-0.380
ICMA 04999 × 2331	0.234**	0.268**	0.990**	0.078**	-0.096
ICMA 04999 × 2332	0.032	0.078*	-0.960**	-0.001	-0.296
ICMA 04999 × 2333	-0.703**	-0.437**	0.540**	0.029**	0.270
ICMA 04999 × 2337	0.643**	0.123**	-0.260*	0.029**	-0.630
ICMA 04999 × 2382	0.501**	0.283**	0.590**	-0.020**	-0.530
ICMA 04999 × 2342	-0.893**	-0.922**	-1.710**	0.060**	0.337
ICMA 04999 × 2386	0.147**	0.088**	1.090**	-0.057**	0.137
ICMA 04999 × 2348	-0.182**	-0.067*	0.540**	0.017*	0.508
ICMA 04999 × 2352	0.588**	0.498**	0.340**	-0.038**	0.270
ICMA 04999 × 2364	-0.568**	-0.352**	-0.560**	-0.020**	-0.280
ICMA 04999 × 2365	0.243**	0.543**	0.040	0.029**	-0.013

ICMA 04999 × 2387	-1.038**	-0.307**	0.340**	-0.020**	0.470
ICMA 04999 × 2394	0.348**	0.188**	0.490**	-0.020**	0.554
ICMA 04999 × 2395	-0.218**	-0.497**	-2.360**	-0.026**	0.487
ICMA 04999 × 2396	0.293**	0.303**	0.890**	0.005	0.170
ICMA 04999 × 2346	-0.308**	-0.352**	-1.010**	-0.008	0.004
ICMA 04999 × 2349	-0.502**	-0.112**	-0.210	-0.038**	-0.463
ICMA 97111 × 2306	-0.498**	-0.213**	1.660**	-0.005	0.063
ICMA 97111 × 2309	1.048**	0.662**	1.010**	0.020**	-1.120*
ICMA 97111 × 2310	0.643**	0.927**	1.260**	-0.041**	-0.004
ICMA 97111 × 2311	-1.393**	-0.738**	-1.340**	0.044**	0.730
ICMA 97111 × 2368	-0.223**	-0.173**	-1.440**	-0.005	0.730
ICMA 97111 × 2370	-0.403**	-0.438**	0.760**	-0.029**	-0.604
ICMA 97111 × 2318	-0.933**	-0.718**	-1.540**	-0.029**	-0.187
ICMA 97111 × 2381	0.573**	0.177**	-0.060	0.044**	-0.004
ICMA 97111 × 2325	-0.063**	-0.288**	0.010	-0.042**	0.080
ICMA 97111 × 2327	1.108**	0.352**	-0.490**	-0.017*	0.646
ICMA 97111 × 2328	0.263**	0.582**	-1.390**	0.057**	-0.287
ICMA 97111 × 2329	-0.402**	-0.273**	-0.590**	-0.005	0.480
ICMA 97111 × 2330	-1.103**	-0.538**	-0.810**	0.007	0.380
ICMA 97111 × 2331	-0.234**	-0.268**	-0.990**	-0.078**	0.096
ICMA 97111 × 2332	-0.032	-0.078*	0.960**	0.001	0.296
ICMA 97111 × 2333	0.703**	0.437**	-0.540**	-0.029**	-0.270
ICMA 97111 × 2337	-0.643**	-0.123**	0.260*	-0.029**	0.630
ICMA 97111 × 2382	-0.501**	-0.283**	-0.590**	0.020**	0.530
ICMA 97111 × 2342	0.893**	0.922**	1.710**	-0.060**	-0.337
ICMA 97111 × 2386	-0.147**	-0.088**	-1.090**	0.057**	-0.137
ICMA 97111 × 2348	0.182**	0.067*	-0.540**	-0.017*	-0.508
ICMA 97111 × 2352	-0.588**	-0.498**	-0.340**	0.038**	-0.270
ICMA 97111 × 2364	0.568**	0.352**	0.560**	0.020**	0.280
ICMA 97111 × 2365	-0.243**	-0.543**	-0.040	-0.029**	0.013
ICMA 97111 × 2387	1.038**	0.307**	-0.340**	0.020**	-0.470
ICMA 97111 × 2394	-0.348**	-0.188**	-0.490**	0.020**	-0.554
ICMA 97111 × 2395	0.218**	0.497**	2.360**	0.026**	-0.487
ICMA 97111 × 2396	-0.293**	-0.303**	-0.890**	-0.005	-0.170
ICMA 97111 × 2346	0.308**	0.352**	1.010**	0.008	-0.004
ICMA 97111 × 2349	0.502**	0.112**	0.210	0.038**	0.463
CD at 5%	0.034	0.060	0.241	0.014	0.997

** & * Significant at 1 and 5 per cent level, respectively

Out of 60 crosses, ICMA 04999 × 2318, ICMA 04999 × 2330 and ICMA 04999 × 2311 for iron content, ICMA 04999 × 2318, ICMA 97111 × 2395 and ICMA 97111 × 2309 for zinc content, ICMA 04999 × 2382 for protein content, ICMA 04999 × 2349 and ICMA 04999 × 2381 for rancidity and ICMA 04999 × 2309 for grain yield recorded significantly high heterosis over mid parent and better parent along with high and significant *sca* effects **(Table 4)**. Similar results were earlier reported by Athoni *et al*. (2016) and Kanfany *et al*. (2018) for grain yield, Jeeterwal *et al*. (2017) for iron content and zinc content and Patel *et al.* (2016) for protein content. Among the heterosis types, standard heterosis was economically important. The crosses that exhibited significantly high standard heterosis were: ICMA 04999 × 2325 for iron content, ICMA 97111 × 2310 and ICMA 97111 × 2342 for zinc content, ICMA 97111 × 2395, ICMA 97111 × 2310 and ICMA 04999 × 2386 for protein content and ICMA 04999 × 2349 for rancidity. These results are in accordance with Jeeterwal *et al*. (2017) for iron and zinc content, Pallavi *et al*. (2020) for rancidity and Jethva *et al*. (2012) for protein content.

Table 4. Range of heterosis and number of crosses showing significant heterosis in desirable direction (in per cent)

S. No.	Character	Mid parent heterosis		Heterobeltiosis		Standard heterosis	
		Range	Number of crosses	Range	Number of Crosses	Range	Number of Crosses
1.	Iron content	-34.16 to 45.47	33	-35.99 to 41.12	25	-43.11 to 18.88	5
2.	Zinc content	-45.00 to 53.12	22	-49.88 to 47.37	17	-54.52 to 22.36	5
3.	Protein content	-46.00 to 22.09	13	-48.65 to 12.63	6	-44.33 to 38.14	13
4.	Rancidity (Alcoholic acidity)	-65.25 to 153.26	12	-75.00 to 111.18	17	-55.59 to 111.18	38
5.	Grain yield	-71.11 to 304.37	18	-71.11 to 128.00	6	-71.11 to 34.44	0

Table 5. Top ranking genotypes based on *per se* performance, *gca* effects, *sca* effects and heterosis

Character	Best general combiners		Best specific combiners		Mid parent heterosis	Heterobeltiosis	Standard heterosis
	Based on *gca*	Based on *per se* performance	Based on *sca*	Based on *per se* performance			
Iron content	ICMA 97111, 2381, 2318, 2309	ICMA 97111, 2327, 2349, 2310	ICMA 04999 × 2311 ICMA 97111 × 2327 ICMA 04999 × 2330	ICMA 97111 × 2309 ICMA 97111 × 2381 ICMA 97111 × 2342	ICMA 04999 × 2318 ICMA 04999 × 2330 ICMA 04999 × 2311	ICMA 04999 × 2318 ICMA 04999 × 2311 ICMA 04999 × 2330	ICMA 04999 × 2325
Zinc content	ICMA 97111, 2310, 2386, 2342	ICMA 97111, 2327, 2381, 2349	ICMA 97111 × 2310 ICMA 97111 × 2342 ICMA 04999 × 2311	ICMA 97111 × 2310 ICMA 97111 × 2342 ICMA 97111 × 2309	ICMA 04999 × 2318 ICMA 97111 × 2395 ICMA 97111 × 2309	ICMA 04999 × 2318 ICMA 97111 × 2309 ICMA 97111 × 2395	ICMA 97111 × 2310 ICMA 97111 × 2342
Protein content	ICMA 04999, 2310, 2395, 2386	ICMA 97111, 2333, 2395, 2337, 2310	ICMA 97111 × 2395 ICMA 97111 × 2342 ICMA 97111 × 2306	ICMA 97111 × 2395 ICMA 97111 × 2310 ICMA 04999 × 2386	ICMA 04999 × 2382 ICMA 97111 × 2342 ICMA 04999 × 2318	ICMA 04999 × 2382 ICMA 04999 × 2328	ICMA 97111 × 2395 ICMA 97111 × 2310 ICMA 04999 × 2386
Rancidity (Alcoholic acidity)	ICMA 04999, 2364, 2365, 2349	ICMA 97111, 2318, 2329, 2382, 2386,	ICMA 97111 × 2331 ICMA 97111 × 2342 ICMA 04999 × 2328 ICMA 04999 × 2386	ICMA 04999 × 2349 ICMA 04999 × 2381 ICMA 04999 × 2352 ICMA 04999 × 2364 ICMA 97111 × 2325	ICMA 04999 × 2349 ICMA 04999 × 2381 ICMA 04999 × 2364	ICMA 04999 × 2349 ICMA 04999 × 2381 ICMA 04999 × 2364	ICMA 04999 × 2349
Grain yield	ICMA 04999 2325,2396, 2306, 2337, 2348, 2394	2348 2332 2331, 2370, ICMA 97111	ICMA 04999 × 2309	ICMA 04999 × 2394 ICMA 04999 × 2348 ICMA 97111 × 2337 ICMA 04999 × 2309	ICMA 04999 × 2309 ICMA 04999 × 2328 ICMA 04999 × 2333	ICMA 04999 × 2396 ICMA 04999 × 2309 ICMA 04999 × 2333	

In the present study, **Table 5** revealed that there is a lack of relation between *per se* performance, *sca* effects and heterosis which means that the cross recording high *sca* effect may not have high heterosis. Hence, consideration of these three criteria will be effective for the selection of best cross combinations.

From the present investigation, it can be concluded that all the characters are governed by non-additive gene action with over dominance and can be exploited through heterosis breeding. The identified general and specific combiners could be used in breeding programmes for the development of improved genotypes. In this study, even a single cross did not record significant heterosis for all the five traits indicating the presence of more genetic variation in the parental material and the possibility of genetic improvement through recurrent selection.

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