



# Association Analysis in Rice Germplasm Lines for High Temperature Tolerance

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## ABSTRACT

**Background:** Relative to other abiotic factors like salinity and drought, breeding rice cultivars that are resistant of high temperatures has received a little attention. The purpose of this study is to find parents and genotypes that are suitable for utilizing in high temperature stress breeding program.

**Methods:** The genetic material for this study comprised of a set of germplasm collections (293 accessions) from different sources such as International Rice Research Institute germplasm. These populations were raised at the Department of Rice, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore. The Heat tolerance Indices (TOL) viz., Heat tolerance index (HTI) and Heat susceptibility index (HSI) and association analysis were estimated among germplasm lines for yield attributes under normal and high temperature stress condition.

**Result:** Out of 293 genotypes, sixty nine genotypes significantly exceeded for both HTI and HSI. Association studies revealed that, single plant yield had highly significant and positive association with panicle exertion, number of total tillers per plant, number of productive tillers per plant, panicle length, number of filled grains per panicle and total dry matter production. Spikelet sterility and high temperature susceptibility index had highly significant and negative association with single plant yield. Number of productive tillers had significant and positive correlation with total dry matter production, panicle length, number of filled grains per panicle, days to maturity, hundred grain weight, grain breadth and high temperature susceptible index. Hence these traits may be considered as selection indices for yield and high temperature tolerance improvement programme. Hence it may be possible to combine single plant yield and heat susceptibility index by specific breeding programme for high temperature tolerance in rice.

**Key words:** Association analysis, Germplasm lines, High temperature tolerance, Rice.

**Abbreviations:** DFF- Days to fifty per cent flowering; DM- Days to maturity; GB- Grain breadth; G- Grain length; GLBR- Grain length breadth ratio; GFR- Grain filling rate; HGW- Hundred grain weight; HTI- Heat tolerance index; HIS- Heat susceptibility index; NTT- Number of total tillers; NPT- Number of productive tillers; NFG- Number of filled grains per panicle; PE- Days to panicle exertion; PH- Plant height; PL- Panicle length; SPY- Single plant yield; SS-Spikelet sterility percentage.

## INTRODUCTION

Rice is the prime crop in research and developmental activities in Asia from green revolution to gene revolution. The crop is grown in a wide range of environments starting from higher elevations as upland rice to shallow lowlands and deep water situations. Due to global climatic changes, the crop has to withstand higher temperatures in the near future (Mahendran *et al.*, 2015).

When exposed to high temperatures, rice germplasm shows significant variation. The ability of a plant to tolerate heat stress under field conditions depends on a number of physiological factors and mechanisms, including amendments to critical processes like photosynthesis, chlorophyll content, canopy temperature depression and concurrent increases in transcripts encoding protective protein. Higher photosynthetic rates, enhanced membrane thermostability and heat avoidance are frequently traits of a heat-tolerant variety (Nagarajan *et al.*, 2010; Scafaro *et al.*, 2010). Identification of heat tolerant germplasm lines were used in several breeding programmes to exploit the variation in both genotypic and morphological characters associated

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with heat tolerance. The majority of popular rice varieties are extremely sensitive to high temperatures during

flowering, according to a research of those varieties conducted in high temperature conditions (Vanitha *et al.*, 2023; Baidya *et al.*, 2020). To increase high temperature tolerance in present cultivars, a number of strategies should be actively employed, including as the identification and use of new genes and alleles, increased breeding effectiveness and marker-assisted selection (Vanitha and Mahendran 2022). Although there have been limited observations on specific high temperature stress in tropical and subtropical areas, there has not yet been a comprehensive study on monitoring and evaluating heat stress-induced yield losses globally (Matsushima *et al.*, 1982; Osada *et al.*, 1973).

## MATERIALS AND METHODS

The field experiment was conducted at Department of Rice, Centre for Plant Breeding and Genetics (CPBG), Tamil Nadu Agricultural University, Coimbatore during *kharif* 2016 control (S1) and summer 2017; summer 2018 high temperature conditions (S2 and S3) (Table 1). This area is situated at latitude of 11°N and longitude of 77°E with clayey soil of pH 7.8. The experiment was laid out in randomized complete block design with two replication, a spacing of 20 × 20 cm.

Observations were recorded on five plants selected at random from a row in each accession. Data were recorded for 16 quantitative characters *viz*, days to panicle exertion (PE- days), plant height (PH- cm), days to fifty per cent flowering (DFF- days), number of total tillers (NTT- No.), number of productive tillers (NPT- No.), panicle length (PL- cm), number of filled grains per panicle (NFG- No.), days to maturity (DM- days), hundred grain weight (HGW- g), spikelet sterility percentage (SS%), total dry matter production (TDMP- g), grain length (GL- mm), grain breadth (GB- mm), grain length breadth ratio (GLBR- mm), grain filling rate (GFR- g d<sup>-1</sup>), single plant yield (SPY- g) and 8 physiological characters *viz*, chlorophyll meter (SPAD) readings at fifty per cent flowering stage and grain filling stage, leaf gas exchange parameters, leaf temperature (T: °C), panicle temperature (T: °C), ambient temperature (T: °C), stay green for rice, canopy temperature depression (CTD) for leaf and CTD for panicle.

### Heat tolerance indices (TOL)

#### Heat tolerance index (HTI)

The Heat tolerance index (HTI) was calculated for all the genotypes under stress conditions using the formula suggested by Rosielle and Hamblin (1981).

$$HTI = x_p - x_s$$

Where:

$x_s$  = Trait value of the genotype under stress.

$x_p$  = Trait value of the genotype under non-stress.

#### Heat susceptibility index (HSI)

The Heat susceptibility index (HSI) was calculated for all the genotypes under stress conditions using the formula suggested by Fischer and Maurer (1978).

$$HSI = \frac{1 - (x_s/x_p)}{1 - (X_s/X_p)}$$

Where:

$x_s$  = Trait value of the genotype under stress.

$x_p$  = Trait value of the genotype under non-stress.

$X_s$  = mean values of the trait of all the genotypes under stress.

$X_p$  = mean values of the trait of all the genotypes under non-stress.

#### Association analysis

The genotypic correlations between yield and its component traits and among themselves as well as between characters were worked out as per the methods suggested by Johnson *et al.* (1955).

#### Genotypic correlation coefficient

$$r_g(xy) = \frac{\text{Cov}_g(xy)}{\sqrt{(\sigma_g^2x)(\sigma_g^2y)}}$$

Where:

$r_g(xy)$  = Genotypic correlation coefficients.

$\text{Cov}_g(xy)$  = Genotypic covariance between the traits 'x' and 'y'.

$\sigma_g^2x$  = Genotypic variance of the trait 'x'.

$\sigma_g^2y$  = Genotypic variance of the trait 'y'.

$x$  = Dependent variable x and

$y$  = Independent variable y.

The significance of genotypic correlation coefficient was tested by referring to the standard table given by Snedecor (1961).

## RESULTS AND DISCUSSION

In rice, extreme maximum temperature is particularly important during flowering which usually lasts for 2-3 weeks. Exposure to high temperature for a few hours can greatly reduce pollen viability and therefore cause yield loss (Mirza, 2013; Raghunath and Beena, 2021). In comparison, breeding rice cultivars resistant to salinity and drought has gotten more attention than breeding types resistant to high temperatures. Only region-specific

**Table 1:** Details of season, mean temperature and temperature range at grain filling stage.

Season	Mean temperature at grain filling stage (°C)	Temperature range (°C)
Control (S1) 2016	30.58	27.3-32.1
Summer (S2) 2017	34.97	31.6-35.9
Summer (S3) 2018	34.70	31.5-36.4

breeding initiatives have attempted to address rice's high temperature tolerance since a thorough study conducted in the early 1980s, with limited success. (Mackill, 1981; Mackill *et al.*, 1982; Mackill and Ni, 2001). The periods of high temperature negatively affected the sexual reproduction in rice (Zakaria *et al.*, 2022). Hence, there is an urgent need to address high temperature induced yield losses in rice to face a changing climate.

Heat tolerance is influenced by several genes rather than a single gene. (Mackill, 1981; Maestri *et al.*, 2002). According to Mackill and Ni (2001), several genes have an impact on the recessive genetic regulation of excessive pollen shedding in rice. Yoshida *et al.* (1981), in contrast, noted that the majority of the genetic variation related to pollen shedding is additive. Their results showed significant broad sense and narrow sense heritabilities of 76 and 71%, respectively, while finding a high correlation between spikelet fertility and pollen shedding.

#### Evaluation of germplasm lines under control (S1) and high temperature conditions (S2 and S3)

Each growth phase of the rice plant is primarily defined by its cultivars, although the plant's growth environment also influences the source-sink dynamics of the plant as a whole. The grain yield per unit area is used to evaluate the performance of various cultivars in rice. This is a significant trait, but other indicators, such as stability and efficiency, are also becoming more significant in considering climate change and the impending energy and water shortages (Saikia *et al.*, 2022).

#### Heat tolerance index

Heat tolerance indices were calculated under stress with relation to performance under control and presented in Table 2. A358 recorded minimum value for heat tolerant index of 0.02. The germplasm line A424 recorded higher HTI values of 40.21 under stress condition. Among the

germplasm lines mean value recorded of 6.07 and it was significantly exceeded by sixty nine genotypes.

#### Heat susceptibility index

Heat susceptibility index were calculated for stress condition and presented in Table 2. A358 (0.004) recorded the minimum HSI. A424 recorded lower HSI values of 2.88. One sixty nine genotypes exceeded the general mean (0.93) significantly. Out of 293 genotypes, sixty nine genotypes significantly exceeded for both HTI and HS I (Fig 1).

Every year, the temperature rises and experiences significant changes, which pose a huge challenge to agricultural productivity. Only genotypes that are physiologically efficient or resistant can be used to produce crops under such conditions (Saikia *et al.*, 2022). Hence it may be possible to combine single plant yield and heat susceptibility index by specific breeding programme for high temperature tolerance in rice.

#### Correlation among yield components

The nature and extent of association that existed between the single plant yield and other yield component traits and also the association among the high temperature stress components were studied through correlation analysis. Character correlation studies enable clarify the strength and scope of character association in a crop. The inter relationship of component characters of yield provided the information about the consequences of selection for simultaneous improvement of desirable characters under selection.

The present study indicated that the single plant yield had highly significant and positive association with the traits viz., panicle exertion, number of total tillers per plant, number of productive tillers, panicle length, number of filled grains per panicle and total dry matter production (Table 3). Nor *et al.* (2014) for number of productive tillers and number of total tillers per plant, Rao *et al.* (2014) for panicle length

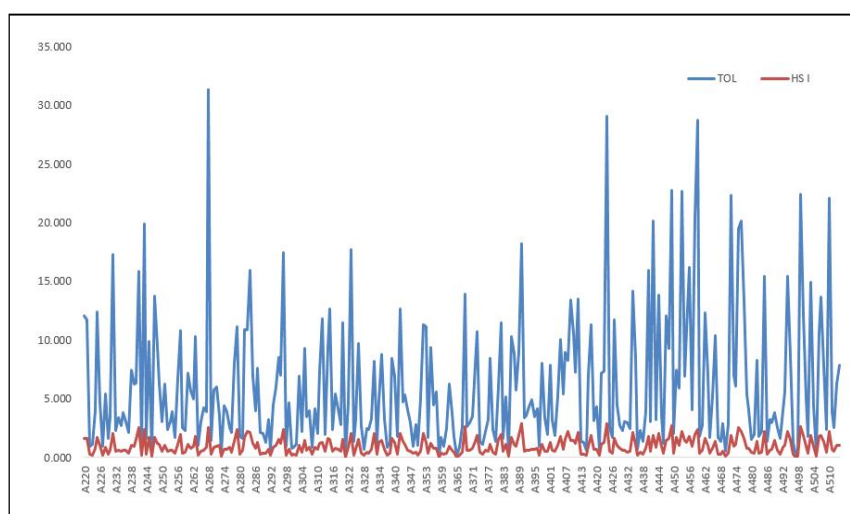


Fig 1: Relationship between TOL and HSI.

**Table 2:** Estimation of Heat tolerance Indices (TOL and HSI) of 293 germplasm lines.

Genotype	Yield mean		Genotype	Yield mean		Genotype	Yield mean	
	TOL	HSI		TOL	HSI		TOL	HSI
A220	12.019	1.552	A270	5.742	0.859	A320	11.414	1.523
A221	11.727	1.585	A271	5.966	0.918	A321	0.074	0.023
A222	0.908	0.228	A272	3.534	1.010	A322	4.594	0.818
A223	1.046	0.166	A273	0.412	0.066	A323	17.669	2.013
A224	3.980	0.785	A274	4.353	0.635	A324	1.303	0.190
A225	12.331	1.666	A275	3.885	0.676	A325	3.170	0.655
A226	4.670	0.963	A276	2.558	0.839	A326	9.655	1.534
A227	0.923	0.149	A277	2.114	0.411	A327	2.660	0.362
A228	5.400	0.802	A278	7.941	1.347	A328	0.685	0.155
A229	1.560	0.223	A279	11.135	2.328	A329	2.400	0.396
A230	3.360	0.571	A280	1.728	0.265	A330	2.323	0.338
A231	17.220	2.027	A281	1.620	0.545	A331	3.288	0.648
A232	2.240	0.457	A282	10.882	1.668	A332	8.177	2.030
A233	3.382	0.545	A283	10.811	2.144	A333	1.266	0.255
A234	2.650	0.531	A284	15.888	2.102	A334	4.710	1.263
A235	3.810	0.621	A285	6.894	1.134	A335	8.770	1.415
A236	3.058	0.559	A286	3.961	0.790	A336	3.294	0.660
A237	2.123	0.346	A287	7.530	1.259	A337	0.868	0.157
A238	7.366	1.003	A288	2.075	0.250	A338	1.230	0.294
A239	6.179	0.944	A289	2.028	0.351	A339	8.436	1.555
A240	6.300	1.502	A290	1.275	0.321	A340	6.914	1.234
A241	15.802	2.552	A291	3.216	0.633	A341	1.765	0.274
A242	0.864	0.195	A292	0.610	0.126	A342	12.634	2.015
A243	19.860	2.360	A293	4.449	0.806	A343	4.693	1.368
A244	1.580	0.260	A294	5.985	0.995	A345	5.335	1.094
A245	9.858	1.706	A295	8.500	1.490	A346	4.040	0.615
A246	0.311	0.062	A296	6.952	1.172	A347	2.974	0.518
A247	13.673	1.696	A297	17.377	2.346	A348	0.940	0.332
A248	9.469	1.155	A298	0.951	0.134	A349	2.750	0.429
A249	6.310	1.055	A299	4.638	0.675	A350	1.040	0.167
A250	3.010	0.504	A300	0.778	0.130	A351	4.926	0.497
A251	6.188	1.025	A301	0.796	0.203	A352	11.239	1.976
A252	2.316	0.534	A302	1.090	0.192	A353	11.126	1.230
A253	2.985	0.595	A303	6.926	0.976	A354	1.670	0.319
A254	3.838	0.605	A304	2.180	0.424	A355	9.328	1.209
A255	1.643	0.309	A305	9.228	1.455	A356	4.410	0.712
A256	6.640	1.106	A306	3.427	0.596	A357	5.586	0.759
A257	10.726	1.886	A307	3.960	0.829	A358	0.018	0.004
A258	2.508	0.357	A308	0.846	0.235	A359	1.648	0.346
A259	2.230	0.409	A309	4.097	0.811	A360	0.937	0.215
A260	7.124	1.071	A310	2.015	0.679	A361	2.471	0.364
A261	5.655	0.744	A311	7.271	1.171	A362	6.190	0.951
A262	4.920	0.901	A312	11.814	1.264	A363	3.802	0.551
A263	10.245	1.738	A313	1.901	0.47	A364	1.794	0.409
A264	1.005	0.190	A314	9.268	1.583	A365	0.131	0.025
A265	2.983	0.485	A315	12.590	1.507	A366	0.845	0.182
A266	4.207	0.622	A316	2.334	0.458	A367	2.588	0.388
A267	3.830	0.824	A317	5.406	0.788	A368	13.855	2.615
A268	31.296	2.527	A318	4.149	0.645	A369	2.640	0.550
A269	1.432	0.217	A319	2.807	0.487	A370	2.962	0.600

Table 2: Continue...

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A371	3.439	0.745	A420	4.278	0.675	A468	1.371	0.226
A372	7.815	1.358	A421	0.705	0.130	A469	2.864	0.477
A373	10.644	1.852	A422	7.161	1.082	A470	0.484	0.111
A374	1.318	0.397	A423	7.333	1.228	A471	2.761	0.366
A375	1.108	0.250	A424	40.210	2.878	A472	22.278	1.878
A376	2.155	0.578	A425	2.523	0.457	A473	6.968	0.911
A377	3.221	0.512	A426	1.665	0.287	A474	6.026	1.060
A378	8.433	1.107	A427	11.685	1.612	A475	19.444	2.482
A379	2.383	0.383	A428	4.442	0.975	A476	20.131	2.209
A380	1.376	0.209	A429	2.644	0.708	A477	13.406	1.594
A381	5.978	1.512	A430	2.287	0.622	A478	5.418	0.752
A382	11.458	1.892	A431	3.062	0.611	A479	4.03375	0.72732
A383	1.622	0.514	A432	2.937	0.435	A479	4.034	0.727
A384	5.102	1.116	A433	2.416	0.53	A480	1.531	0.426
A385	0.507	0.073	A434	14.109	2.073	A481	1.917	0.364
A386	10.249	1.639	A435	8.516	1.051	A482	8.233	1.313
A387	8.830	1.115	A436	0.673	0.142	A483	1.794	0.329
A388	5.724	0.939	A437	2.258	0.374	A484	2.195	0.416
A389	8.921	1.811	A438	1.347	0.222	A485	15.432	2.146
A390	18.194	2.860	A439	3.829	0.728	A486	1.323	0.253
A391	3.372	0.503	A440	15.861	1.782	A487	3.183	0.611
A392	3.485	0.621	A441	2.996	0.484	A488	2.919	0.686
A393	4.281	0.619	A442	20.082	1.866	A489	3.812	1.438
A394	4.831	0.662	A443	3.178	0.839	A490	2.540	0.545
A395	3.426	0.676	A444	13.828	2.031	A491	1.558	0.249
A396	4.154	0.769	A445	3.521	0.952	A492	3.968	0.818
A397	0.900	0.193	A446	1.202	0.346	A493	5.582	0.964
A398	8.025	1.119	A447	12.008	1.436	A494	15.403	2.219
A399	3.349	0.507	A448	9.227	1.572	A495	8.643	1.526
A400	1.889	0.536	A449	22.734	2.689	A496	1.251	0.251
A401	7.830	1.283	A450	2.006	0.293	A497	0.131	0.033
A402	3.172	0.563	A451	7.400	1.682	A498	0.444	0.073
A403	1.844	0.477	A452	5.857	1.031	A499	22.399	2.579
A404	5.115	0.993	A453	22.644	2.140	A500	12.215	1.823
A405	10.021	1.777	A454	6.933	1.445	A501	5.032	0.910
A406	5.393	0.685	A455	10.787	1.252	A502	1.789	0.352
A407	8.900	1.495	A456	16.191	1.789	A503	14.906	1.847
A408	8.240	2.193	A457	4.026	0.896	A504	4.406	0.901
A409	13.342	1.392	A458	20.293	1.965	A505	0.370	0.068
A410	10.634	1.436	A459	28.696	2.377	A506	10.477	1.752
A411	7.238	1.162	A460	1.784	0.309	A507	13.618	1.808
A412	13.446	2.075	A461	2.664	0.560	A508	7.781	1.314
A413	1.299	0.272	A462	12.298	1.564	A509	2.353	0.370
A414	1.250	0.281	A463	7.042	0.980	A510	22.026	2.218
A415	0.599	0.143	A464	1.697	0.351	A511	3.847	0.628
A416	6.967	0.892	A465	5.159	0.773	A512	2.523	0.459
A417	11.299	1.814	A466	10.339	1.347	A513	6.192	0.968
A419	3.078	0.660	A467	1.668	0.282	A514	7.795	0.963
Mean		6.501	0.964		2SE		4.120	0.077

**Table 3:** Genotypic correlation coefficient among component traits and high temperature stress indices.

Variables	PH (cm)	DFF (days)	NTT (No.)	NPT (No.)	PL (cm)	NFG (No.)	DM (days)	HGW (g)	SS (%)	TDMP (g)	GL (mm)	GB (mm)	GLBR	GFR (g/day)	HTI	HSI	SPY (g)
PE (days)	0.46**	0.45**	-0.14**	-0.18**	0.95**	0.40**	0.45**	0.04	0.25**	0.39**	0.24**	-0.07	0.19**	0.02	0.14**	0.08	0.21**
PH (cm)	1	0.36**	-0.11	-0.15*	0.48**	0.23**	0.35**	0.00	0.14*	0.49**	-0.15**	0.26**	-0.27**	-0.06	0.10	0.10	0.05
DFF (days)		1	-0.17**	-0.21**	0.47**	0.23**	0.98**	-0.03	0.36**	0.37**	0.12*	-0.07	0.10	0.01	0.19**	0.22**	0.06
NTT (No.)			1	0.97**	-0.13*	-0.20**	-0.16**	-0.25**	-0.02	0.38**	-0.07	-0.15**	0.04	-0.01	-0.08	-0.11*	0.16**
NPT (No.)				1	-0.16**	-0.22**	-0.21**	-0.27**	-0.05	0.32**	-0.11	-0.15**	0.03	-0.03	-0.08	-0.12*	0.15**
PL (cm)					1	0.41**	0.47**	0.05	0.29**	0.43**	0.27**	-0.07	0.20**	0.00	0.15**	0.09	0.22**
NFG (No.)						1	0.23**	-0.11*	-0.00	0.30**	-0.12*	-0.11	0.02	-0.12*	0.01	-0.09	0.39**
DM (days)							1	-0.05	0.37**	0.37**	0.12*	-0.08	0.11	0.01	0.18**	0.22**	0.05
HGW (g)								1	-0.11	-0.07	0.34**	0.44**	-0.12*	0.21**	0.06	0.03	0.03
SS (%)									1	0.32**	0.14*	-0.16**	0.18**	0.09	0.17**	0.24**	-0.14*
TDMP (g)										1	-0.03	0.01	-0.05	0.06	0.05	-0.02	0.43**
GL (mm)											1	-0.33**	0.75**	0.15**	0.15**	0.15**	0.00
GB (mm)												1	-0.85**	0.03	-0.01	-0.04	0.02
GLBR													1	0.05	0.09	0.10	-0.01
GFR (g/day)														1	-0.01	-0.01	-0.01
HTI															1	0.91**	0.08
HSI																1	-0.37**

\*,\*\*Significant at 5% and 1% level respectively.



and for total dry matter production was reported by Venkanna *et al.* (2014). Spikelet sterility and heat susceptibility index had negative and significant correlation with single plant yield. Rice plants at the reproductive stage, including the processes of panicle initiation, male and female gametophyte development, anthesis, pollination and fertilization, are more susceptible to heat stress than at the vegetative stage (Xu *et al.*, 2021). Hence it may be possible to combine single plant yield and heat susceptibility index by specific breeding programme for high temperature tolerance in rice. The information on the correlation among the yield components shows the nature and extent of relationship with each other. This will help in the simultaneous improvement of high temperature tolerance traits along with single plant yield in the breeding programmes.

Number of productive tillers had significant and positive correlation with total dry matter production. Panicle length, number of filled grains per panicle, days to maturity, hundred grain weight, grain breadth and heat susceptibility index had significant and negative correlation. Lakshmi *et al.* (2014) reported that the correlation between number of productive tillers per plant and panicle length was negatively correlated. Grain breadth recorded significant and negative correlation with grain length breadth ratio. Similar findings were reported by Venkanna *et al.* (2014) and Kiran *et al.*, (2016). Spikelet sterility had negative and significant association with grain breadth.

## CONCLUSION

From the foregoing discussion, it can be concluded that the traits such as panicle exertion, number of total tillers per plant, number of productive tillers per plant, panicle length, number of filled grains per panicle, total dry matter production, heat tolerance index and heat susceptibility index are the major yield component traits with positive effect on single plant yield and the improvement of these traits would result in increased yield with high temperature tolerance in rice. It suggests that, increase in spikelet sterility will reduce the single plant yield. We can get desirable genotypes with high temperature tolerance and yield components from the present set of experimental materials.

## Conflict of interest

All Authors declare that they have no conflict of interest.

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