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Diversity and Genetic Association Analysis of Dry Chillies (*Capsicum annuum* L.) of Northern India for Yield and Biochemical Traits

Garima Verma, Amit Vikram and Meenu Gupta

Dept. of Vegetable Science, Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh (173 230), India

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Corresponding vermagarima405@gmail.com

0000-0002-8220-487X

ABSTRACT

A study was conducted at the Experimental Farm of Department of Vegetable Science, Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (HP), India during the year 2018 (May–October) to screen twenty-one (21) genotypes of chilli for thirteen characters including yield and total capsaicin content. The evaluation was done in a randomized block design with three replications. Sufficient genetic variability was found for all characters. Correlation analysis revealed a positive significant correlation of fresh ripe yield plant^{-1} with the number of red fruits plant^{-1} (0.842*), days to fresh ripe maturity (0.394*), dry yield as percentage of fresh ripe (0.386*), days to 50 % flowering (0.384*), plant height (0.374*) and days to green fruit maturity (0.352*). The number of red fruits plant^{-1} (0.440*) had a positive significant correlation with total capsaicin content. Path analysis revealed a positive direct effect of number of red fruits plant^{-1} (0.698) on fresh ripe yield followed by days to green fruit maturity (0.372), number of seeds fruit^{-1} (0.218), average red fruit weight (0.168), dry yield as percentage of fresh ripe (0.111), plant height (0.111) and red fruit length (0.007) whereas total capsaicin content in dry chilli had the positive direct correlation with days to 50% flowering, days to fresh ripe maturity, number of fruits plant^{-1} and 1000 seed weight. Finally, it was revealed that the selection of genotypes bearing many small-thick fruits would be the most suitable criterion for selecting a population having high capsaicin content and fruit yield.

KEYWORDS: Capsaicin, chilli, correlation, diversity, path analysis

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1. INTRODUCTION

Chilli, also known as hot pepper, is a main vegetable cum spice crop belonging to the family Solanaceae and genus *Capsicum*. Its ability to provide pungency, flavour and colour to food makes it a key element of various cuisines worldwide (Bahurupe et al., 2013). Till date, the existence of thirty-five *Capsicum* species was reported (Carrizo et al., 2016) but, only five of them, namely *C. annuum*, *C. baccatum*, *C. chinense*, *C. frutescens*, and *C. pubescens* were brought into cultivation and were eventually domesticated independently (Andrews, 1995). Among these well-known species of *Capsicum*, *Capsicum annuum* is one of the relevant valuable agricultural commodities. It originated in the American tropics. Portuguese introduced chilli into Southern parts of India (Rego et al., 2011). By the end of the 19th century, it spread throughout the country. This long history of cultivation and selection within the Indian subcontinent resulted in the genesis of sufficient genetic variability within the crop in terms of growth and yield characteristics, such as fruit shape, fruit weight, fruit colour, pungency, flower colour, plant height and maturity, which can be potentially exploited for breeding purposes (Chattopadhyay et al., 2011, Krishnamurthy et al., 2013, Bijalwan and Mishra, 2016, Pidigam et al., 2019, Pidigam et al., 2021, Saisupriya et al., 2022).

Chilli peppers are reported to possess various nutritional compounds; including carotenoids, flavonoids, essential mineral elements (Lee et al., 2005), antioxidants and vitamins; including A, C and E (Howard et al., 2000, Farhad et al., 2010, El-Ghoraba et al., 2013, Chakrabarty et al., 2017) and other important chemical compounds such as fatty acids and sterols (Matthaus and Ozcan, 2009). These compounds are well-known for their preventive action against tumors, heart diseases and cancer.

The red fruit colour of chilli is due to the presence of carotenoids; particularly capsanthin and capsorubin which exhibit strong oxygen-scavenging properties, confirming their antioxidant properties (Nishino et al., 2016). And, the pungency of peppers is due to the presence of capsaicinoids. Capsaicinoids include a family of up to 25 related alkaloid which are secreted as secondary metabolites from the glandular epidermal cells of the placenta and accumulates along the epidermis to form the blister in peppers (Nishino et al., 2016, Stewart et al., 2007). Capsaicin and dihydrocapsaicin are the predominant compounds, accounting for almost 90% of capsaicinoids, in which concentrations of capsaicin are generally higher than those of dihydrocapsaicin (Antonious et al., 2009). These compounds are reported to possess anticarcinogenic, anti-inflammatory, antimicrobial and antifungal properties (Luo et al., 2011). The ability of capsaicinoids to desensitize pain receptors has led to their successful application as a topical analgesic (Caterina et al., 2000).

India is the major producer, consumer and exporter of chilli (dry-red and green chilli). However, the productivity of the chilli crop in the past few years has been constrained due to the lack of availability of suitable genotypes (Krishna et al., 2007, Tirupathamma et al., 2021,). Thus, the present study was undertaken to evaluate the genetic diversity in 21 chilli genotypes based on their yield and yield contributing characteristics to determine the selection criterion for future chilli hybridization programs. However, to bring about improvement, the knowledge of its association with other characters is very important because the selection of one trait invariably affects several other associated characters (Vikram et al., 2014, Pujar et al., 2017, Lata and Sharma, 2022). Therefore, correlation and path coefficient among these characters were estimated to make the selection more reliable.

2. MATERIALS AND METHODS

A genetic stock of 21 genotypes (Table 1) was arranged and grown in a randomized block design with three replications at the Experimental Farm of Department of Vegetable Science, Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (HP), India in the year 2018 (May–October) under open conditions with a plot size of 2.25×1.80 m^2 at a spacing of 45×45 cm^2 accommodating twenty plants per plot. The experimental site was located at 35°5' N latitude and 77°11' E longitude at an elevation of 1270 m above mean sea level, about 13 km away from the South East of Solan city (HP). It falls in the sub-humid, sub-temperate and mid-hill zone of Himachal Pradesh. The mean temperature during the cropping season; May, 2018 to October, 2018 varied from 16.86 to 24.83°C while the relative humidity varied from 42.60% to 79.60%.

Morpho-metric data were recorded for 13 economic traits, namely days to 50 % flowering, days to green fruit maturity, days to fresh ripe maturity, plant height (cm), number of red fruits plant^{-1}, average red fruit weight (g), red fruit length (cm), red fruit breadth at the middle (cm), fresh ripe yield plant^{-1} (g), dry yield as percentage of fresh ripe, number of seeds fruit^{-1}, 1000 seed weight (g), total capsaicin content (%) in dry chilli.

2.1. *Total capsaicin content*

The total capsaicin content in the red dried chilli fruits was determined by the calorimetric method as described by Sadasivam and Manickam (1996). The capsaicin concentration was observed from the standard curve plotted by using a standard capsaicin solution (Fluka AG).

2.1.1. *Harvesting of fruits for estimating capsaicin content*

For estimating capsaicin content, hundreds of grams of red

Table 1: List of the genotypes

Sl. No.	Genotype	Source	Sl. No.	Genotype	Source
1.	UHF-CHI-1	YSP UHF, Nauni	12.	Gundu-2	PJTSAU, Hyderabad
2.	UHF-CHI-2	YSP UHF, Nauni	13.	UHF-CHI-13	YSP UHF, Nauni
3.	UHF-CHI-3	YSP UHF, Nauni	14.	UHF-CHI-14	YSP UHF, Nauni
4.	UHF-CHI-5	YSP UHF, Nauni	15.	UHF-CHI-15	YSP UHF, Nauni
5.	UHF-CHI-7	YSP UHF, Nauni	16.	UHF-CHI-16	YSP UHF, Nauni
6.	UHF-CHI-8	YSP UHF, Nauni	17.	UHF-CHI-17	YSP UHF, Nauni
7.	UHF-CHI-9	YSP UHF, Nauni	18.	UHF-CHI-18	YSP UHF, Nauni
8.	UHF-CHI-10	YSP UHF, Nauni	19.	UHF-CHI-19	YSP UHF, Nauni
9.	UHF-CHI-11	YSP UHF, Nauni	20.	UHF Selection 4	YSP UHF, Nauni
10.	UHF-CHI-12	YSP UHF, Nauni	21.	DKC-8 (check)	YSP UHF, Nauni
11.	Gundu-1	PJTSAU, Hyderabad			

YSP UHF, Nauni: Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan; PJTSAU, Hyderabad: Professor Jayashankar Telangana State Agricultural University, Hyderabad, Telangana

marketable fruits of each genotype were collected randomly during the second harvest from each replication. These fruits were then dried in a hot air oven at 65±1°C till the constant dry weight was achieved followed by grinding using a lab grinder.

2.1.2. Reagents

N/10 NaOH and 3% Phosphomolybdic Acid

2.1.3. Procedure

Four grams of grounded chilli fruits weighed using electronic balance were taken in glass-stopper test tubes of 50 ml capacity. 20 ml of acetone was pipette out and thoroughly mixed with the above-grounded sample using a vortex mixer for 10 mins and allowed to stand for 3 hrs. After 3 hrs, the extract solution was filtered using laboratory filter paper and the filtrate was mixed with quick charcoal. Test tubes containing solution were then subjected to motion for 15-30 mins (varied according to genotypes) using a vortex mixer to enable the efficient removal of contamination and colour of the solution. Quick Charcoal was allowed to settle down followed by filtration. The clear supernatant of 5 ml was pipette out into the Borosilicate glass beaker and allowed to evaporate to dryness in the SSU Laboratory Hot Water Bath. The dry residue was dissolved in 5 ml of N/10 NaOH (prepared beforehand). To this solution, 3 ml of 3 % Phosphomolybdic acid solution was added, thoroughly mixed and allowed to stand for 1 hr. A blank solution was also prepared along with test samples. After 1 hr, the blue-coloured solution was transferred into the cuvette one by one (first blank followed by test samples) and the optical density was read at 650 nm. The concentration of the capsaicin was calculated from the standard curve and finally converted into per cent.

2.1.4. Preparation of standard curve

10 mg of pure capsaicin was taken in 100 ml N/10 NaOH in the volumetric flask. From this stock solution 0.00, 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 3.50, 4.00, 4.50 and 5.00 ml were taken out and volumes were raised to 5 ml using N/10 NaOH. To each of these solutions, 3 ml of 3 % Phosphomolybdic acid was added and allowed to stand for 1 hr. After 1 hr, their optical densities were measured at 650 nm and the standard curve was prepared.

2.2. Statistical analysis

The observations were recorded on five randomly selected plants of each genotype in every replication for every trait. The mean values of all thirteen traits were subjected to statistical analysis using SPSS 16.0 software. Various statistical parameters like ANOVA (Gomez and Gomez, 1984), correlation analysis (Al-Jibouri et al., 1958) and path analysis (Dewey and Lu, 1959) were computed.

3. RESULTS AND DISCUSSION

3.1. Analysis of variance

Selection is considered an important step in all crop improvement programmes. However, for selection to succeed, a greater extent of genetic variability is required in the population for the concerned character. In this study, the presence of sufficient genetic variability is clear from the ANOVA, where variations were highly significant for all the traits under study (Table 2). Krishnamurthy et al. (2013), Amit et al. (2014) and Bijalwan and Mishra (2016) were in agreement with our results for the traits under study.

3.2. Correlation analysis

The ultimate response of selection in any improvement

Table 2: ANOVA (analysis of variance) for morphological and quality characters of chilli germplasm

Source of variation	Characters						
	df	X_1	X_2	X_3	X_4	X_5	X_6
Replication	2	35.254	60.143	32.587	3.075	9.986	0.007
Treatment	20	236.92*	380.45*	284.94*	490.024*	3991.11*	4.908*
Error	40	5.687	5.61	4.187	0.976	2.417	0.005

Table 2: Continue...

Source of variation	Characters							
	df	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}
Replication	2	7.609	0.088	0.002	0.391	0.005	1.696	0.00003
Treatment	20	13619.04*	26.034*	0.579*	2297.09*	2.113*	26.609*	0.02395*
Error	40	6.999	0.107	0.002	1.229	0.007	0.585	0.00005

X_1: Days to 50% Flowering; X_2: Days to green fruit maturity; X_3: Days to fresh ripe maturity; X_4: Number of red fruits plant^{-1}, X_5: Average red fruit weight (g); X_6: Fresh ripe yield plant^{-1}, X_7: Red fruit length (cm); X_8: Red fruit breadth at the middle (cm); X_9: Number of seeds fruit^{-1}; X_{10}: 1000 seed weight (g), X_{11}: Dry yield as percentage of fresh ripe; X_{12}: Plant height (cm); X_{13}: Total capsaicin content in dry chilli (%)

programme is determined by the magnitude and direction of the genetic correlation among and between the various traits. For that estimates of the correlation coefficient at phenotypic and genotypic levels, were worked out in all possible combinations (Table 3).

The correlation coefficient at the genotypic level was higher than the phenotypic level indicating towards strong inherent association among these characters under study. According to Nandpuri et al. (1973), this might be related to the environment's masking or modifying impact on trait expression.

Fresh ripe yield plant^{-1} is considered an economic trait in chilli. The fresh ripe yield plant^{-1} had a positive significant correlation with days to 50 % flowering, days to green fruit maturity, days to fresh ripe maturity, the number of red fruits plant^{-1}, dry yield as percentage of fresh ripe and plant height. Red fruit breadth at the middle and 1000 seed weight was found to have a negative yet significant correlation with fresh ripe yield. Chattopadhyay et al. (2011) and Krishnamurthy et al. (2013) reported similar findings where Chattopadhyay et al. (2011) discovered a significant positive relationship between dry (red) fruit yield plant^{-1} and days to 50% flowering and number of fruits per day and Krishnamurthy et al. (2013) between fresh ripe yield and number of fruits plant^{-1}. Kumar et al. (2012) discovered a significant positive association between fruit yield per plant and fruit number per plant. Total capsaicin content in dry chilli showed a positive significant correlation with the number of red fruits plant^{-1}, days to 50% flowering and days to green and fresh ripe maturity and the number of fruits plant^{-1} whereas, it showed a negative significant correlation with average red fruit weight, red fruit length and 1000 seed weight suggesting that selection for genotypes with a greater number of small-sized fruits would be a most suitable selection criterion for high pungent fruits. These results were in harmony with those of Gupta and Singh (1992), Kumar et al. (2003), Krishnamurthy et al. (2013) and Amit et al. (2014). Days to 50 % flowering had a positive and significant correlation with days to green fruit maturity and days to fresh ripe maturity. The result of Vikram et al. 2014 was in favour of our findings and suggested that the selection of genotypes having early flowering would be most appropriate to fetch early produce. Average red fruit weight also showed a positive significant correlation with red fruit length, the number of seeds fruit^{-1} and 1000 seed weight. In previous investigations, Bijalwan and Mishra (2016) have also found a positive significant relationship between average fruit weight and fruit length. However, Pujar et al. (2017) discovered a significant positive relationship between average fruit weight and the number of seeds fruit^{-1} and 1000 seed weight.

3.3. Path analysis

Selection merely based on a correlation study only reveals the nature of association which alone doesn't provide precise insight into the relative influence of every trait on yield. At such point, path coefficient analysis is required which provides the actual cause of such associations by partitioning the genotypic coefficient of correlation into direct and indirect effects. A positive direct effect of number of red fruits plant^{-1} on fresh ripe yield was revealed from the path analysis (Table 4) followed by days to green fruit maturity, number of seeds fruit^{-1}, average red fruit weight,

Table 3: Genotypic and phenotypic coefficients of correlation among different characters in red chilli germplasm

		X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}
X_2	G	0.854*											
	P	0.826*											
X_3	G	0.791*	0.890*										
	P	0.761*	0.876*										
X_4	G	0.576	0.556*	0.567*									
	P	0.557*	0.544*	0.554*									
X_5	G	-0.514*	-0.535*	-0.558*	-0.502*								
	P	-0.497*	-0.524*	-0.547*	-0.500*								
X_6	G	0.384*	0.352*	0.394*	0.842*	-0.205							
	P	0.369*	0.343*	0.386*	0.841*	-0.204							
X_7	G	-0.25	-0.191	-0.233	0.025	0.562*	0.21						
	P	-0.241	-0.186	-0.23	0.025	0.559*	0.209						
X_8	G	-0.542*	-0.506*	-0.643*	-0.654*	0.192	-0.657*	-0.320*					
	P	-0.517*	-0.487*	-0.624*	-0.650*	0.19	-0.654*	-0.316*					
X_9	G	-0.622*	-0.639*	-0.586*	-0.562	0.375*	-0.430*	-0.222	0.716*				
	P	-0.600*	-0.622*	-0.573*	-0.561	0.374*	-0.429*	-0.22	0.712*				
X_{10}	G	-0.484*	-0.282*	-0.494*	-0.522	0.452*	-0.542*	0.422*	0.298*	0.142			
	P	-0.466*	-0.277*	-0.480*	-0.52	0.448*	-0.538*	0.418*	0.293*	0.14			
X_{11}	G	0.283*	0.342*	0.283*	0.188	-0.066	0.386*	0.046	-0.313*	-0.393*	-0.322*		
	P	0.270*	0.330*	0.262*	0.183	-0.065	0.375*	0.051	-0.297*	-0.382*	-0.308*		
X_{12}	G	-0.058	0.176	-0.025	0.333*	-0.241	0.374*	0.273*	-0.024	-0.299*	0.015	0.297*	
	P	-0.054	0.17	-0.027	0.332*	-0.239	0.374*	0.271*	-0.023	-0.299*	0.015	0.293*	
X_{13}	G	0.542*	0.375*	0.333*	0.440*	-0.504*	0.228	-0.578*	-0.037	-0.05	-0.545*	0.046	-0.128
	P	0.525*	0.365*	0.327*	0.439*	-0.502*	0.226	-0.576*	-0.037	-0.049	-0.540*	0.039	-0.126

P: Phenotypic correlation coefficient; G: Genotypic correlation coefficient

Table 4: Direct and indirect effect of 12 characters on fresh ripe yield plant^{-1} at genotypic level

Ch.	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	GCC
X_1	-0.114	0.318	-0.359	0.402	-0.087	-0.0017	0.222	-0.136	0.178	0.031	-0.006	-0.061	0.384*
X_2	-0.097	0.372	-0.404	0.388	-0.090	-0.0013	0.207	-0.139	0.104	0.038	0.020	-0.042	0.352*
X_3	-0.090	0.331	-0.454	0.396	-0.094	-0.0016	0.263	-0.128	0.181	0.031	-0.003	-0.037	0.395*
X_4	-0.066	0.207	-0.258	0.698	-0.084	0.0002	0.268	-0.122	0.192	0.021	0.037	-0.049	0.842*
X_5	0.059	-0.199	0.253	-0.350	0.168	0.0038	-0.079	0.082	-0.166	-0.007	-0.027	0.057	-0.205
X_6	0.028	-0.071	0.106	0.017	0.095	0.0067	0.131	-0.048	-0.155	0.005	0.030	0.065	0.210
X_7	0.062	-0.188	0.292	-0.456	0.032	-0.0022	-0.409	0.156	-0.109	-0.035	-0.003	0.004	-0.657*
X_8	0.071	-0.238	0.266	-0.392	0.063	-0.0015	-0.293	0.218	-0.052	-0.044	-0.033	0.006	-0.430
X_9	0.055	-0.105	0.224	-0.364	0.076	0.0029	-0.122	0.031	-0.367	-0.036	0.002	0.061	-0.542
X_{10}	-0.032	0.128	-0.128	0.131	-0.011	0.0003	0.128	-0.086	0.118	0.111	0.033	-0.005	0.386*
X_{11}	0.007	0.066	0.011	0.232	-0.041	0.0018	0.010	-0.065	-0.006	0.033	0.111	0.014	0.374*
X_{12}	-0.062	0.140	-0.151	0.307	-0.085	-0.0039	0.015	-0.011	0.200	0.005	-0.014	-0.112	0.228

GCC: Genotypic correlation coefficient; * Signifiant at (p=0.05) level of significane, Residual = 0.104

dry yield as percentage of fresh ripe, plant height and red fruit length indicating towards their direct selection for improving total yield. Whereas, negative direct effect was observed for days to fresh ripe maturity, red fruit breadth at the middle, 1000 seed weight, days to 50% flowering and total capsaicin content in dry chilli. Apart from the negative direct effect, total capsaicin content in dry chilli showed a negative indirect effect on fresh ripe yield via days to 50 % flowering, days to fresh ripe yield, average red fruit weight and the number of seeds fruit^{-1} whereas, it showed positive indirect effect of number of red fruits plant^{-1}, red fruit breadth at the middle and dry yield as percentage of fresh ripe. The residual effect (0.104) was found low which signified the relevance of selected characters in representing total genetic variability.

For the total capsaicin content in dry chilli a positive direct effect of days to 50% flowering, days to fresh ripe maturity, number of fruits plant^{-1} and 1000 seed weight was observed which contributes towards the positive correlation between these characters (Table 5) whereas, red fruit length, days to green fruit maturity showed a negative direct effect so, indirect selection on these traits may lead to improvement of the respective characters and finally the total capsaicin content in dry chilli.

The residual value was found to be 0.3 indicating that 70% of the variability in total capsaicin content in dry chilli was governed by the characters which are included in this analysis. The previous studies by Chattopadhyay et al. (2011), Vikram et al. (2014) and Hasan et al. (2016) agree with the present report.

Table 5: Direct and indirect effect of 12 characters on total capsaicin content in dry chilli at genotypic level

Ch.	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	GCC
X_1	2.605	-3.616	2.350	-0.041	0.699	-0.211	-0.016	0.173	-0.569	-0.265	-0.708	0.139	0.542**
X_2	2.257	-4.174	2.649	0.125	0.675	-0.220	-0.015	0.132	-0.530	-0.272	-0.416	0.163	0.375**
X_3	2.084	-3.763	2.938	-0.018	0.689	-0.228	-0.016	0.161	-0.678	-0.249	-0.721	0.136	0.334**
X_4	-0.149	-0.731	-0.074	0.712	0.403	-0.099	-0.016	-0.189	-0.023	-0.127	0.022	0.142	-0.128
X_5	1.502	-2.321	1.668	0.237	1.213	-0.206	-0.035	-0.017	-0.687	-0.239	-0.764	0.089	0.440**
X_6	-1.341	2.237	-1.637	-0.171	-0.609	0.410	0.008	-0.389	0.199	0.160	0.661	-0.031	-0.504**
X_7	1.000	-1.468	1.158	0.267	1.021	-0.084	-0.041	-0.146	-0.690	-0.182	-0.794	0.186	0.227
X_8	-0.653	0.797	-0.684	0.194	0.030	0.230	-0.009	-0.692	-0.338	-0.094	0.618	0.022	-0.578**
X_9	-1.405	2.095	-1.888	-0.016	-0.789	0.077	0.027	0.222	1.055	0.304	0.432	-0.148	-0.035
X_{10}	-1.620	2.664	-1.722	-0.213	-0.681	0.154	0.018	0.154	0.753	0.425	0.208	-0.188	-0.05
X_{11}	-1.260	1.187	-1.446	0.011	-0.633	0.185	0.022	-0.292	0.311	0.060	1.464	-0.155	-0.545**
X_{12}	0.753	-1.417	0.830	0.210	0.225	-0.027	-0.016	-0.031	-0.326	-0.167	-0.471	0.481	0.046

GCC: Genotypic correlation coefficient; *: Signifiant at (p=0.05) level of significane, Residual =0.3

4. CONCLUSION

ANOVA demonstrated the significant genetic variability among genotypes for all the traits, which can be further exploited in crop improvement programmes. Further, the correlation and path analysis study revealed the importance of all horticultural traits in influencing a genotype's yield and capsaicin content from which it was suggested that selecting genotypes bearing a large number of small-thick fruits with early maturity would be the best criterion for selecting a population with high capsaicin content and fruit yield.

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