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Genetic Variability Studies on Resistance to Sorghum Downy Mildew Disease in MAGIC Population of Maize (*Zea mays* L.)

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Abstract

Development of the host plant resistance is an effective way to mitigate yield losses and to maintain self-sufficiency in maize. Sorghum downy mildew (SDM) is an important foliar disease in maize and is known to hinder its production potential. Thus, the development and identification of inbred lines resistant to SDM can contribute to minimizing the losses caused by SDM. In this regard, a set of 1331 Multiparent Advanced Generation Intercross (MAGIC) Recombinant Inbred Lines (RILs) were screened for their response to SDM at ZARS, V.C. Farm Mandya during *kharif* 2022. Results from the analysis of variance indicated the presence of a substantial amount of variability for disease response. The disease incidence ranged from 0 to 100 per cent with a mean disease incidence of 78.07 per cent. The genetic estimates PCV, GCV, GAM and broad sense heritability were high indicating the ample scope for identifying and selecting the resistant lines. Skewness and kurtosis values were -0.61 and 2.70, respectively suggesting the involvement of many genes with duplicate gene interaction for resistance. Out of 1331 RILs, 37 were resistant, 47 were moderately resistant, 158 were moderately susceptible and 1089 were susceptible.

Keywords : Maize, Sorghum downy mildew, MAGIC RILs, Resistance, Skewness, Kurtosis

Maize is the third most important staple food crop worldwide contributing to the nation's food and nutritional security. Photosynthetically efficient (C4), day-neutral and highly adaptive nature of the maize makes it suitable for cultivation in most of the agro-climatic regions. This crop is incredibly versatile and finds application in various industries beyond food, including animal feed, ethanol production and as a raw material in the manufacturing of bioplastics (Lohithaswa *et al.,* 2022). India contributes nearly 2.13 per cent of maize to the global food market which is grown in an area of 205.87 million ha (FICCI, 2023). Recently, due to increased area under maize cultivation, the growing of uniform crop varieties, shrinkage in genetic diversity, high density planting and high fertility management made maize highly sensitive to many of the biotic and abiotic production constraints. In addition to this, changing climate makes the crop vulnerable to many of the new emerging pests and diseases (Gazal *et al*., 2018). Annual yield losses in maize caused by diseases (excluding viral) are estimated around 4-14 per cent globally (Zhu *et al*., 2021). Sorghum downy mildew (SDM), caused by an oomycetes fungus [*Peranosclerospora sorghi* (Weston and Uppal) C. G. Shaw], is prevalent in three states of peninsular India *i.e.*, Karnataka, Andhra Pradesh and Tamil Nadu. It is known to cause a yield loss of up to 30-40 per

cent (Rasheed *et al*., 2023). Disease incidence at the early crop stages leads to death of the plants and at the later crop stages plants become sterile. Though SDM can be controlled by the use of fungicides, its effectiveness in controlling the disease is variable (Sumathi *et al.,* 2020). Further, the development of resistance to fungicides and the evolution of different pathotypes of *P. sorghi* make the need for genetic resistance in maize cultivation a mandate. Thus, the development and deployment of the host plant resistance is considered to be an effective, eco-friendly and long-term viable strategy to mitigate the yield losses due to diseases (Nair *et al.,* 2004).

Selection is the most critical step in plant breeding and the effectiveness of selection depends on the extent of variability for the target trait in the base population (Banakara *et al.,* 2022 and Mujjassim *et al.,* 2023). Variability generated through deliberate crossing is the major source to recover the desired recombinants. Biparental crosses, which are produced by crossing two contrasting and complementary parents are successful in analyzing the complex genetic architecture of the traits. However, these bi-parental mapping populations (BMPs) will segregate only for two alleles, have poor mapping resolution and less power of QTLs detection (Mahan *et al*., 2018). Consequently, multi-parental mapping populations were developed to address the pitfalls of BMPs. These populations are renowned for increasing trait variability through a greater number of recombination events by disrupting strong linkages (Huang *et al.,* 2015; Dell Aqua *et al.,* 2015; Mahan *et al.,* 2018 and Desai *et al.,* 2022). Among the multi-parental populations, Multi parental Advanced Generation Inter Crosses (MAGIC) and Nested Association Mapping (NAM) populations are the major ones. MAGIC lines are the fine scale mosaics of the genomic regions from more than two founder parents with maximum trait variability coverage (Mackey and Powell, 2007). Recent studies have shown that multi parental populations can be an excellent source for generating superior inbred lines and can speed up genetic gain. Higher recombination rates with a broad range of variability for target traits will indirectly assist in effective selection. Genetic studies of SDM resistance have shown polygenic inheritance with a predominance of additive gene effects (Sumathi *et al.,* 2020 and Rasheed *et al*., 2023). Thus, the use of recombinant inbred lines derived from complex crosses like MAGIC is known to offer numerous benefits over inbreds derived from crossing two contrasting parents in identifying the resistance source to devastating disease like Sorghum downy mildew. Hence, the present investigation was undertaken to identify the sources of resistance to sorghum downy mildew in a MAGIC RIL population.

Material and Methods

The material used for the study comprised of a set of 1331 MAGIC Recombinant Inbred Lines (RILs) obtained from crossing eight elite founder parents (Table 1). The procedure followed for deriving RILs is given in Fig. 1. The MAGIC RILs were planted in an augmented design (Federer, 1956) with SKV 50

Table 1

List of maize inbred lines used in the development of multi-parental population

Inbred	Pedigree details	Characteristic features
PDM-4341	(Comp8551 × Comp 8527 × Ageti76 × MDR) -9- 4-2-8-7-1-1-2-1-L-1-1	Significant GCA effects for grain yield (kg/ha)
VL109545	[CL-G2501 × CML170]-B-2-3-2-BB-3-BB	Resistant to Northern corn leaf blight (NCLB)
CML451	((NPH-28-1/G25)/NPH-28-1)-1-2-1-1-3-1-B	Drought tolerant
CAL1443	(CTS013008/AMATLC0HS71-1-1-2-1-1-1-B*5/Nei402020)-B*5	Resistant to NCLB

Continued....

Table 1 Continued....

Inbred	Pedigree details	Characteristic features
CM 212	USA/ACC No.2132 (Alm)-3-2-f-#-13-#-bulk	Resistant to Fusarium stalk rot (FSR)
SKV50	SKV-50 (population 147-F_2 # 89-3-2-B-1-B)	Resistant to NCLB, Sorghum downy mildew (SDM), Polysora rust (PR)
CAL1518	SW5-10-B*5-2	Significant GCA effects for test weight
CM 202	C121 (EARLY)	Susceptible to NCLB, SDM but resistant to FSR

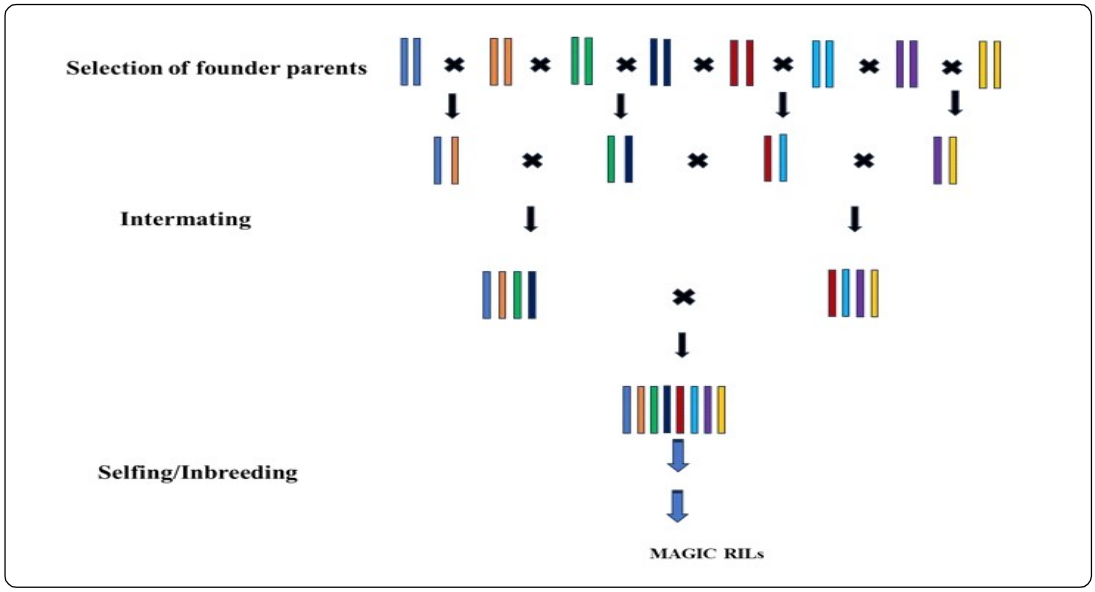

Fig. 1 : Strategy used for deriving Multiparent advanced generation inter cross recombinant inbred lines

and African tall as resistant and susceptible checks, respectively during *kharif* 2022 at ZARS, V.C. Farm, Mandya. The MAGIC RILs were planted in 2 m rows with a spacing of 60 cm between the rows and 20 cm between the plants.

Artificial Inoculation and Data Recording

To ensure the proper disease spread, artificial inoculation was carried out. Disease spread was ascertained by planting spreader rows 15-20 days before taking up sowing of main test entries (Hooda *et al.,* 2018). To ensure uniform disease spread, infected leaves were collected and washed with water and spore suspension thus resulted was sprayed on the 7 days old plants during midnight. Observations were recorded at 45 days after sowing. Per cent disease incidence (PDI) was calculated using the formula given below.

$$PDI = \frac{No.\ of\ infected\ plants}{total\ number\ of\ plants} \times 100$$

Disease scoring was done following the method given by Pupipat (1976) as given below.

Infection rate (%)	Disease reaction
≤ 10	Resistant (R)
10.1-25	Moderately resistant (MR)
25.1-50	Moderately susceptible (MS)
≥ 50	Susceptible (S)

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Statistical Analysis

Recorded data were subjected to arcsine transformation using the PBIB package (Kaur *et al.,* 2017) in R-Studio and subjected to statistical analyses. Analysis of variance was performed using the '*augmented RCBD*' package in R software version 3.3.2. Descriptive statistics *viz*., mean, variance and standard deviation were estimated using the '*psych'* package in R software.

Variability Parameters

Phenotypic and genotypic coefficient of variation (PCV and GCV) were calculated by the formula given by Burton and DeVane (1953).

$$\text{PCV (\%)} = \frac{\sqrt{\sigma_p^2}}{\overline{X}} \times 100$$

$$\text{GCV (\%)} = \frac{\sqrt{\sigma_g^2}}{\overline{X}} \times 100$$

where, $\overline{X}$ = grand mean

σ_g^2 = genotypic variance

σ_p^2 = phenotypic variance

Broad sense heritability (H) was estimated using the following formula (Allard, 1960).

$$H = \frac{\sigma_g^2}{\sigma_p^2} \times 100$$

where,

σ_g^2 = genotypic variance

σ_p^2 = phenotypic variance

Expected Genetic advance (GA) was calculated by the following formula (Allard, 1960).

$$GA = k \times h_b^2 \times \sqrt{\sigma_p^2}$$

where, k = selection differential (2.06) at 5% selection intensity

$\sqrt{\sigma_p^2}$ = phenotypic standard deviation

The genetic advance as a percentage of the mean was estimated as:

$$GAM = \frac{GA}{\overline{X}} \times 100$$

Where, GA = genetic advance; $\overline{X}$ = grand mean

Third- and fourth-degree statistics *viz*., skewness and kurtosis were calculated to know the nature of gene action and number of genes involved in controlling the trait using R software Version 4.1.3 and the package '*moments*'.

Results and Discussion

Results from ANOVA indicated the presence of a substantial amount of genetic variability for the disease response (Table 2). The disease incidence ranged from 0 to 100 per cent with a mean disease incidence of 78.07 per cent. The per cent disease

Table 2

Analysis of variance for Sorghum downy mildew disease severity in Multiparent advanced generation inter cross derived recombinant inbred lines of Maize

Source of variation	Degrees of freedom	Mean Sum of squares	
Genotypes adjusted			
Blocks (ignoring genotypes)	41	0.41	***
Genotypes (eliminating Blocks)	1332	0.17	***
Genotypes : Check	1	9.06	***
Genotypes : Test and Test vs. Check	1331	0.16	***
Block adjusted			
Genotypes (ignoring Blocks)	1332	0.18	***
Genotypes : Check	1	9.07	***
Genotypes : Test	1330	0.16	***
Genotypes : Test vs. Check	1	13.78	***
Block (eliminating genotypes)	41	0.04	
Residuals	41	0.04	
CV (%)		17.77	

CV : Co-efficient of Variation; *** Significant at P = 0.001; ** Significant at P = 0.01; * Significant at P = 0.05

incidence in the resistant (SKV50) and the susceptible check (African tall) were 18.72 and 74.65 per cent, respectively.

Estimates of Genetic Parameters

The observable phenotype is the result of genotype (G), environment (E) and their interaction (G×E). Estimation of phenotypic and genotypic coefficients of variations gives an idea about the trait variability in the material under study. The idea of coefficient of variation mainly GCV pinpoints the reliability of using the breeding material in genetic improvement programmes. Estimated descriptive statistics and genetic parameters are represented in Table 3. Estimated PCV (33.75%) and GCV (28.94%) were high indicating the presence of a substantial amount of trait variability, thus, the selection would be rewarding. However, the magnitude of the phenotypic coefficient of variation was higher than that of the genotypic coefficient of variation, indicating the significant role of the environment in the trait expression (Magar *et al.,* 2021).

Table 3

Descriptive statistics and genetic parameters of Multiparent advanced generation inter cross derived recombinant inbred lines for sorghum downy mildew disease reaction in Maize

Parameters	Values
Minimum	0
Maximum	100
Range (%)	100
Mean (%)	78.07
Mean per cent disease incidence in resistant check (%)	18.72
Mean per cent disease incidence in susceptible check (%)	74.65
Standard deviation	26.61
Skewness	-0.61
Kurtosis	2.70
Phenotypic Coefficient of Variation (%)	33.75
Genotypic Coefficient of Variation (%)	28.94
Heritability (Broad sense) (%)	73.55
Genetic advance over per cent mean (%)	51.21

In addition to PCV and GCV, the estimates of heritability and genetic advance would provide better insight on the reliability of using them as a selection criterion in breeding programmes (Johnson *et al.,* 1955 and Desai *et al.,* 2022). The estimated broad sense heritability was high with higher genetic advance over per cent mean with values 73.55 and 51.21 per cent, respectively. Compared to bi-parental mapping population, multi-parental mapping populations are expected to show increased trait variation and heritability indicating its relevance in resistance breeding (Desai *et al*., 2022). A combination of high heritability and high genetic advance over per cent mean for a trait, implies the role of additive gene effects, suggesting the scope for selection of resistant progenies (Rasheed *et al.,* 2023 and Jhadav *et al.,* 2019).

Skewness and Kurtosis

Skewness is a measure of symmetry of the normal distribution. However, kurtosis is a measure of the peakedness of the distribution. The knowledge about skewness will aid in understanding the genetic basis for variation in the trait (Fisher *et al.,* 1932). In plant breeding information about the skewness and kurtosis will help in understanding the nature of gene action (Fisher *et al.,* 1932) and the number of genes involved in controlling the trait expression (Robson, 1956), respectively. Positive skewness indicates complementary gene interaction while negative skewness indicates duplicate gene interaction (Desai *et al.,* 2022). Whereas, a kurtosis value of <3 indicates the platykurtic curve visualising the involvement of a large number of genes in governing the trait, while kurtosis of >3, is leptokurtic indicating the involvement of the few genes in trait expression. The skewness and kurtosis values were -0.61 and 2.70, respectively (Table 3 and Fig. 2)

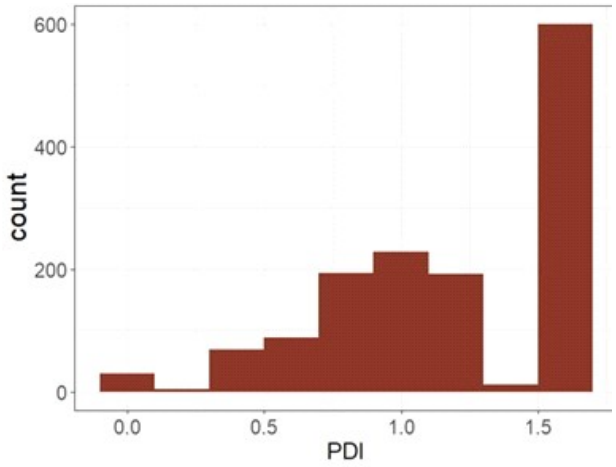
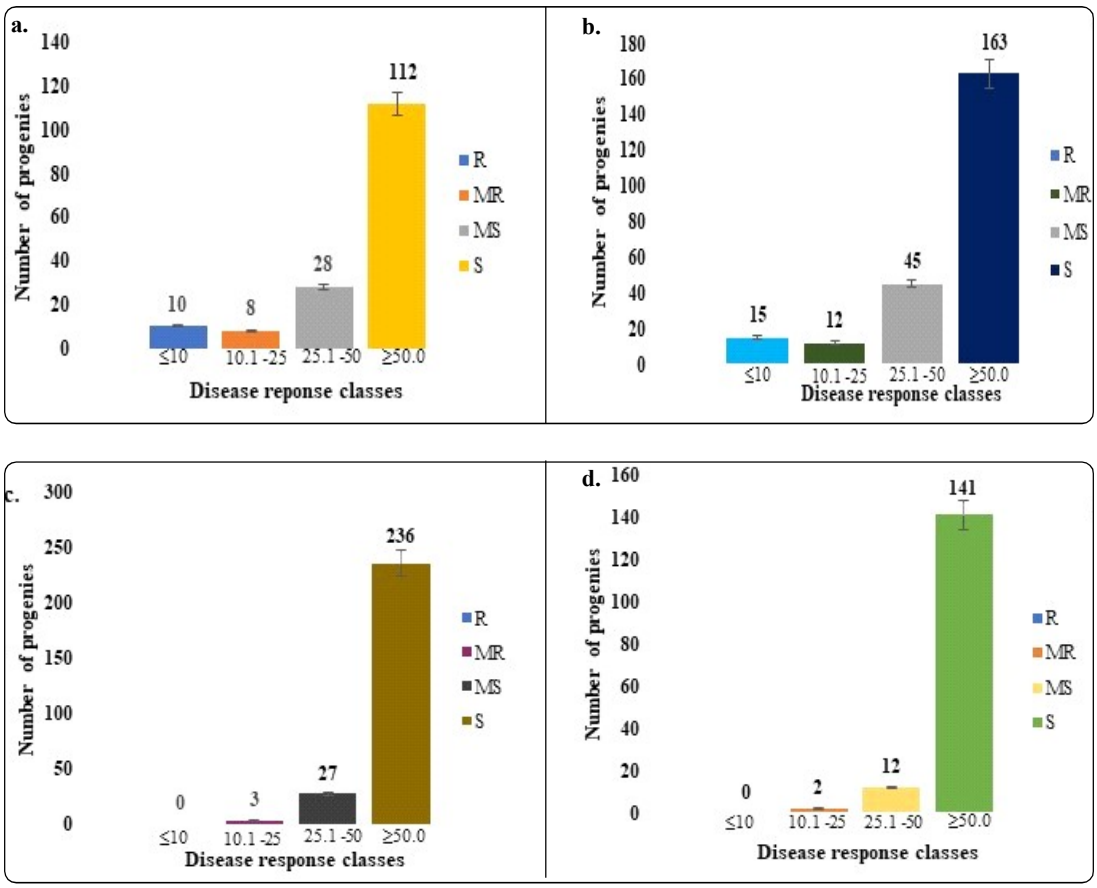
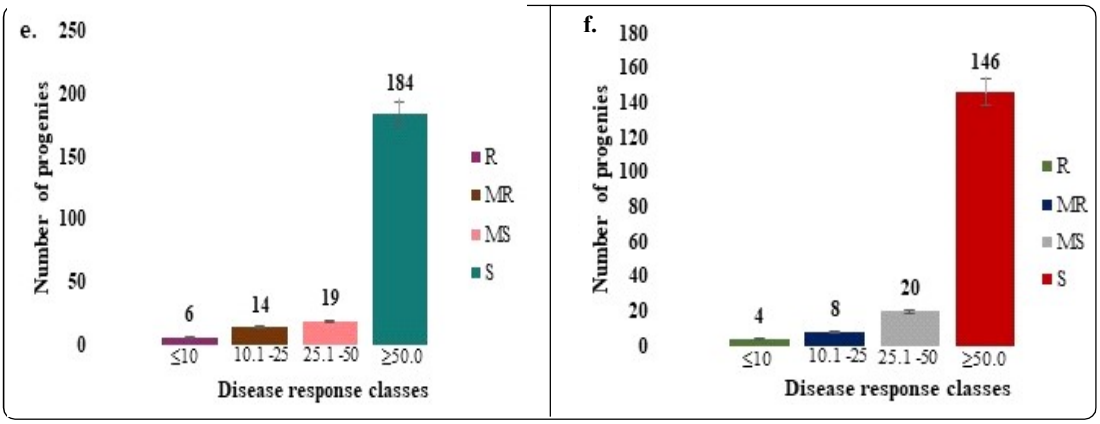


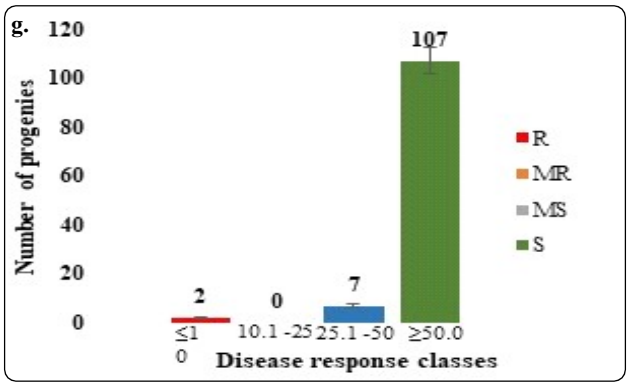
Fig. 2 : Frequency distribution of per cent disease incidence in Multiparent advanced generation inter cross derived recombinant inbred lines into different disease response classes

indicating the presence of polygenes with duplicate gene interaction in regulating the disease resistance. The possible reason for obtaining a negatively skewed distribution for disease response would be that out of eight founder parents used, only one parental genotype (SKV50) was resistant to SDM which might cause the distribution to skew towards susceptibility.

The presence of duplicate gene action indicates the presence of predominantly dispersed alleles at the interacting loci, suggesting the effectiveness of breeding methods for exploiting the non-additive component of gene action (Singh *et al*., 2019). Similar findings were obtained by Jhadhav *et al*. (2019). The phenomenon of duplicate epistasis is unfavourable from the breeder's point of view as the presence of duplicate epistasis would be detrimental to rapid genetic progress. It is difficult to fix genotypes with an increased level of trait expression because the positive effects of one parameter would be cancelled out by the negative effects of another, whereas a complementary type of epistasis has favourable effect in breeding programme (Gunasekar *et al*., 2018).







R - Resistant; MR - Moderately Resistant; MS - Moderately Susceptible and S - Susceptible

Fig. 3 : Response of individual eight-way crosses to (a-first; b-second; c-third; d-fourth; e-fifth; f-sixth and g- seventh eight way cross) disease response

Distribution of different Phenotypic Classes within the Population

The second eight way cross had a greater number of resistant progenies (15) followed by the first (10), fifth (6), sixth (4) and seventh (2) eight-way crosses and the remaining two eight-way crosses did not yield any resistant phenotypes (Fig. 3).

Identification of Lines with Resistance to Sorghum Downy Mildew

Host plant resistance serves as an economic way to mitigate the yield losses due to diseases, because of its durability. Out of 1331 MAGIC RILs evaluated for the response to sorghum downy mildew, 37 were found to be resistant, 47 were moderately resistant, 158 of them were moderately susceptible and 1089 of them showed a susceptible reaction. The increased proportion of susceptible progenies compared to other disease response classes, could be attributed to the parental contribution as out of the eight founder parents used, only one parent SKV 50 is known to be resistant to sorghum downy mildew. The identified resistant lines have to be used in the development of the high yielding hybrids with resistance to sorghum downy mildew.

Higher estimates of phenotypic and genotypic coefficients of variations and heritability with high genetic advance were observed for disease response to sorghum downy mildew in the MAGIC RILs. It suggests the scope for identification and selection of resistant lines. The skewness and kurtosis values indicated the involvement of polygenes with duplicate gene interaction implying the need for few cycles of recombination.

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