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Screening of *Lablab purpureus* (L.) Genotypes against *Alternaria* Leaf Spot Resistance

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ABSTRACT

Background: *Lablab purpureus* (L.) bean research in Uttar Pradesh has given less emphasis on improving quality traits preferred by farmers and focused mostly on developing varieties that are high yielding. Though, there are some released *Lablab purpureus* (L.) varieties farmers mostly prefer to grow their own landrace. Moreover, analysis of farmers' perceptions and views on *Lablab purpureus* (L.) bean diseases and control methods utilized by farmers has been minimal. Farmers' insights and understanding of crop diseases play an important role in sustainable disease management. Therefore, there is a need to assess farmers' priority problems for *Lablab purpureus* (L.) bean production, their variety preference and their perception of the leaf spot disease.

Methods: In our study, the landraces were assessed both in the field and in the greenhouse during 2021-22 with *Alternaria doliconidium* and *Alternaria destruens* isolates.

Result: The highest level of resistance were found Under greenhouse evaluation in VRSEM-757, VRSEM-776, VRSEM-730 and VRSEM-739 to *Alternaria doliconidium* whereas genotypes found to be resistance under field evaluation were VRSEM-799, VRSEM-757 and VRSEM-776. Similarly, genotypes found to be resistance to *Alternaria destruens* were VRSEM- 757 and VRSEM- 702 under greenhouse evaluation whereas, genotypes VRSEM-776 found to be resistance during field evaluation.However, the resistance were moderate. Overall, resistance were highly heritable, suggesting that phenotypic selection can be exploited to improve leaf spot resistance in *lablab* bean varieties.

Key words: *Alternaria destruens, Alternaria doliconidium*, Evaluation, Resistance.

INTRODUCTION

Lablab purpureus (L.) is a legume appropriate to grow in tropical environment as it is adaptable to a wide range of temperature, rainfall and altitudes. It develops well under humid and warm conditions at temperatures ranging between 18°C and 30°C. It is a drought hardy crop grown in humid and semi-arid regions having rainfall between 200 mm-2500 mm (Singhal *et al*., 2012). Green pods, seeds, immature grains, biscuits, leaves and so forth can all be used as food (Davari *et al*., 2018; Habib *et al*., 2017; Rana *et al*., 2021). According to recent research, lablab bean extracts can effectively prevent viral illness infections, which have been dubbed a global pandemic (Liu *et al*., 2020).

Lablab purpureus (L.) is also known as *lablab Purpureus*, L. It belongs to a bean genus in the Fabaceae family which is native to Africa and existed in 1500 BC (Kante and Reddy, 2012). *Lablab purpureus* (L.) Among cultivated plants in the Leguminosae family, sweet is primarily a self-fertile herbaceous forage crop with chromosome number-2n 22 (Kshirsagar *et al*., 2018). It is a drought resistant legume and for some populations in Africa and Asia it is part of the staple foods (Habib *et al*., 2012). It is an important source of relatively cheap dietary proteins for the low income communities and for animal too (Tsuda *et al*., 1994; Nasrin *et al*., 2012; Ramakrishna *et al*., 2007). Food insecurity, vitamin shortages, dietary variety, revenue creation, health advantages, soil conservation and climate-smart cropping are some of the issues that *lablab* helps with (Joshi *et al*., 2020). Genetic, agronomic, culturally eroded, reduced research focus, economic, market, lack of improved varieties, inadequate management practises and improvement and variations in climate patterns have all contributed to the decline in lablab production patterns (Bhatt *et al*., 2019). Numerous investigations on genetic diversity in lab-lab crops have been conducted. These genetic studies cover a wide range of techniques, from traditional

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approaches to the most recent high throughput genotyping techniques, which are exact, accurate and produce a wealth of data on the crop of interest (Yang *et al*., 2020).

In addition, *Lablab purpureus* (L.) is adversely affected by numerous fungal diseases. The major diseases are Dolichos enation mosaic virus (Ramadasan 1966); Anthracnose (*Colletotrichum lindemuthianum*), rust (*Uromyces appendiculatus*), Powdery mildew (*Erysiphe polygoni*), Bacterial blight (*Xanthomonas phaseoli*), leaf spot of l*ablab niger* caused by *Alternaria tenuissima* (Tandon *et al*., 1982).

Currently, some of the available improved genotypes and local landraces in the U.P. are susceptible to *Alternaria* leaf spot and most landraces are associated with undesirable characteristics. Several control measures for Leaf spots are available. These include cultural, chemical, use of resistant varieties and integrated disease management. However, resource-poor farmers cannot afford the use of a chemical which is considered to be one of the most effective control measures. The use of resistant genotypes would be the cheapest and most favourable method for farmers. There is a need to identify and evaluate adapted and new *Lablab purpureus* (L.) bean germplasm for resistance to disease and assess their usefulness in high yielding and resistant variety development.

Occurrence of *Alternaria* was observed seriously in many varieties. Out of various management strategies used, use of resistant varieties is an ideal, the simplest and the cheapest method for avoiding plant disease rather than control. Moreover, it does not disturb field eco-system and avoids hazards of environmental pollution by avoiding fungicides spray to overcome disease. The identification of the source of resistance is a basic need in breeding for disease resistance and hence the present investigation was carried out and the results are reported here.

MATERIALS AND METHODS

Genotypes used

For the screening of *Alternaria* leaf spot, ten genotypes of *Lablab purpureus* (L.) (VRSEM- 847, VRSEM-739, VRSEM-799, VRSEM-757, VRSEM-776, VRSEM-733, VRSEM-730, VRSEM-746, VRSEM-843, VRSEM-702) were procured from Indian Institute of Vegetable Research, Varanasi.

Inoculum preparation and inoculation

Spores of each isolates were produced in mass quantity from a single-conidium of *Alternaria doliconidium* and *Alternaria destruens* using potato dextrose agar media. By single spore technique, the number of spores per ml of suspension was fixed using a Haemocytometer (Bernard *et al*., 2006). The genotypes were artificially inoculated with *Alternaria doliconidium* and *Alternaria destruens* isolates with spore concentration of 2.5×10^5 spores/ml after one month of planting at the seedling stage. Tween-20 were mixed with the suspension to disperse the spores. Inoculation were done using a hand sprayer late in the afternoon and the plants were covered with polyethylene plastic for 24 h to maintain high relative humidity for better infection by the pathogen.

Disease assessment

Disease incidence

For recording the incidence of disease in the field, we randomly selected 4-5 sub plots of 5×5 m^2 and counted the number of diseased and healthy plants. The average disease incidence was calculated by the method given below (Chester, 1950):

Disease incidence = Out of samples collected no. of diseased plants affected in total

$$\% \text{ Disease incidence} = \frac{\text{Total no. of diseased plants}}{\text{Total no. of plants in the field}} \times 100$$

Disease Intensity

From every genotype, 100 affected leaves were selected randomly and arranged in from 0-5 in 6 grades on the basis of percent lamina area affected (James 1971; Chopra and Sharma 1976):

The disease intensity were calculated as below

Disease intensity =

$$\frac{N_1 \times 0 + N_2 \times 1 + N_3 \times 2 + N_4 X_3 + N_5 \times 4 + N_6 \times 5}{N \times 5} \times 100$$

N = Total number of leaves examined.

N_0 to N_6 = Number of leaves in different grades.

Evaluation of *Lablab purpureus* (L.) bean genotype using Isolate *Alternaria doliconidium* and *Alternaria destruens* Under greenhouse evaluation

Four to five seeds per genotypes were planted in pots under greenhouse inoculation for their reaction to pathogen. In this study, seeds of each *Lablab purpureus* (L.) genotype were sown in 25 cm pots with three replications. After 50 days of crop the test genotypes were artificially inoculated with culture of both the isolates. The pots were watered before inoculation to maintain sufficient moisture and were kept in humid chamber for 48-72 hrs and then transferred in open. Observations on the disease intensity were recorded after 20 days of inoculation and genotypes were categorised in terms of leaf area given in Table 1.

Under field evaluation

The genotypes of *Lablab purpureus* (L.) bean were screened against the pathogen under natural conditions. At each site the design were randomized block design in a paired row of 2 m length with three replications. The plots were irrigated to maintain sufficient moisture time to time. At each site in addition to natural inoculum, the test genotypes were artificially inoculated with both the isolates. At flowering, the *lablab* bean genotypes were sprayed with the suspension of isolates using the hand sprayer. To avoid the effects of sunlight on spore viability, Inoculation were done late in the afternoon. The disease intensity were recorded on the basis of per cent leaf area affected at the time of harvest the crop

by randomly selecting 100 leaves from each replications (Chopra and Sharma, 1976). Intensity on leaves were rated using the modified infected leaf area and disease scores were summarised as indicated in Table 1.

Data analysis

The data from the field and lab experiments were subjected to statistical analysis and the genotypes were evaluated based on their disease intensity to the disease. The data were subjected to ANOVA firstly between groups and then within groups to analyse the effect of genotypes and interaction by using the SPSS ver. 20.0 Software. All values were expressed as mean±S.E.M (Standard error of the mean).

RESULTS AND DISCUSSION

Alternaria isolates selection

Isolates of *Alternaria viz.*, *Alternaria doliconidium* and *Alternaria destruens* were isolates and confirmed by sending the samples to NCCS, Pune. *Alternaria doliconidium* were found to be 100% similar to Accession no. MG828864.1 and *Alternaria destruens* were found to be 99-100% similar to Accession no. NR137143.1. Hence, two different isolates *Alternaria doliconidium* and *Alternaria destruens* were selected for further pathological investigations.

Evaluation of *Lablab purpureus* (L.) bean genotypes using *Alternaria doliconidium*

Under greenhouse evaluation

The seeds of different genotypes were screened under artificial conditions of pathogen and the observations on the disease Intensity and disease incidence were summarised in Table 3. The lowest disease intensity were recorded in VRSEM-757 *i.e*. 2.8% while, the highest were recorded in VRSEM-847 *i.e*, 12.8%. Furthur resistant evaluation among genotypes showed that, the genotypes VRSEM-702 had disease Intensity of 9.8% followed by 8.4% for VRSEM-843; 7.4% for VRSEM-746 and 6.2% for VRSEM-799. These were clubbed moderately resistant genotypes. Genotypes found to be resistant had disease intensities of 4.8% for VRSEM-739 followed by 4.2 % for VRSEM-730; 3.8% for VRSEM-776 and 2.8 % for VRSEM-757 whereas disease intensities for moderately susceptible genotypes were 12.8% for VRSEM-847 and 10.2 % for VRSEM-733. It is showing that out of the 10 genotypes evaluated, 40% had disease intensity of moderately resistant and resistant reactions while only 20% showed moderately susceptible reaction. In our study, there were a non- significant effect *i.e.* null hypothesis is accepted at the p>0.05 level for the three conditions F (2, 27) = 0.49; p>0.05 (Table 2).

The disease incidence varied from 10.67% (VRSEM 702) to 34.67% (VRSEM 757). One way ANOVA is used to compare means from two independent groups using the F-distribution. In our study, there were a non-significant effect *i.e*. null hypothesis is accepted at the p>0.05 level for the three conditions F (2, 27) = 2.685; p>0.05.

Under field evaluation

Field evaluation test were conducted for the 10 *Lablab purpureus* (L.) genotypes to determine their potential resistance/tolerance to leaf spot disease. Five genotypes were found moderately resistant, three were found to be resistant and two were found be moderately susceptible (Table 5). The lowest disease intensity were recorded in VRSEM- 757 *i.e*. 3.2% while, the highest were recorded in VRSEM-702 *i.e*. 12.6%. Furthur, resistant evaluation among genotypes showed that the genotypes VRSEM-843 had disease intensity of 5.1% followed by 9.4% for VRSEM-739, 6.2% for VRSEM-746, 7.4% for VRSEM-730 and 5.4% for VRSEM-733. These were clubbed moderately resistant genotypes. Genotype found to be resistant had disease Intensity of 4.4% for VRSEM-799, 3.2% for VRSEM-757 and 2.8% for VRSEM-776 whereas disease intensity for moderately susceptible genotypes were 11.80% for VRSEM-847 and 12.60% for VRSEM-702. In our study, there were a non- significant effect *i.e.* null hypothesis is accepted at the p>0.05 level for the three conditions F (2, 27) = 0.34; p=>0.05 (Table 3).

The mean disease incidence of the ten genotypes were ranged from 8% for VRSEM- 776 to 28% for VRSEM-702.

Table 1: Disease categories on the basis of Leaf area affected.

Per cent leaf area affected	Grade	Number of leaves in grade	Disease ratings	Inference
0	0	N_0	$N_1 \times 0$	Nil
5	1	N_1	$N_2 \times 1$	Resistant
10	2	N_2	$N_3 \times 2$	Moderately resistant
20	3	N_3	$N_4 \times 3$	Moderately susceptible
30	4	N_4	$N_5 \times 4$	Susceptible
40 and above	5	N_5	$N_6 \times 5$	Highly susceptible

Numerical ratings.

0 = No infection.

1 = Upto 5% leaf area infected.

2 = Upto 10% leaf area infected.

3 = Upto 20% leaf area infected.

4 = Upto 30% leaf area infected.

5 = Upto 40% leaf area infected.

The maximum disease incidence of 28% were found in VRSEM-702 followed by 24% for VRSEM-730 and VRSEM-847; 20% in VRSEM-746 and VRSEM- 739; 16% in VRSEM-843 and VRSEM- 733; 12% in VRSEM-799 and VRSEM-757 and 8% in VRSEM- 776. In our study, there were a significant effect *i.e*, null hypothesis is rejected at the p<0.05 level for the three conditions F (2, 27) = 4.74; p =<0.05.

Evaluation of *Lablab purpureus* (L.) bean genotypes using *Alternaria destruens*

Under greenhouse evaluation

The seeds of different genotypes were screened under greenhouse conditions of pathogen for their reaction to pathogen. The lowest disease intensity were recorded in VRSEM-757 *i.e*. 3.2% while, the highest were recorded in VRSEM-730 *i.e*. 12.8%. Furthur, resistant evaluation among genotypes showed that the genotypes VRSEM-730 had disease intensity of 8.66% followed by 8.00% for VRSEM-799; 7.60% for VRSEM-847; 7.46% for VRSEM-739; 7.00% for VRSEM- 843; 6.73% for VRSEM- 733; 5.60% for VRSEM-746 and 5.33% for VRSEM-776. These were clubbed moderately resistant genotypes. Genotypes found to be resistant had disease intensity of 3.86% for VRSEM-702 and 3.20% for VRSEM- 757. Thus, It is showing that out of the 10 genotypes evaluated, 80% had disease intensity of moderately resistant and only 20% found to be resistant. The mean disease incidence varied from 8.00% (VRSEM-843) to 26.33% (VRSEM- 746) with a mean of 20.40% (Table 4).

In our study of disease incidence and disease intensity, there were a non-significant effect *i.e*. null hypothesis is accepted at the p>0.05 level for the three conditions F (2, 27) = 1.52; p>0.05 and F (2, 27) = 0.51 resp.

Under field evaluation

Field evaluation test were conducted for the 10 *Lablab purpureus* (L.) genotypes to determine their potential resistance/tolerance to Leaf spot disease through disease intensity and disease incidence. Eight genotypes were found moderately resistant, one were found to be resistant and

Table 2: Leaf spot disease intensity and disease incidence of *Alternaria doliconidium* on 10 *lablab* genotypes evaluated under greenhouse evaluation.

Genotype	Mean disease incidence (%)	Mean disease intensity (%)	Grade	Disease reaction
VRSEM-843	20.00±2.3	8.4±0.32	2	MR
VRSEM-739	26.67±3.5	4.8±0.70	1	R
VRSEM-799	17.33±3.5	6.2±0.26	2	MR
VRSEM-757	34.67±3.5	2.8±0.20	1	R
VRSEM- 847	22.67±1.33	12.8±0.68	3	MS
VRSEM-746	10.67±1.33	7.4±.36	2	MR
VRSEM-730	18.67±2.67	4.2±0.26	1	R
VRSEM-776	14.67±1.33	3.8±0.47	1	R
VRSEM-733	25.33±3.5	10.2±0.37	3	MS
VRSEM-702	10.67±1.33	9.8±0.73	2	MR
Mean	20.13±1.49	7.07±0.58		
R^2	0.296	0.084		
Std. deviation	8.169	3.177		
Variance	66.74	10.093		

Table 3: Leaf spot disease intensity and disease incidence of *Alternaria doliconidium* on 10 *lablab* genotype evaluated under field evaluation.

Genotype	Mean disease incidence (%)	Mean disease intensity (%)	Grade	Inference
VRSEM-843	18.67±2.6	5.1±0.15	2	MR
VRSEM-739	20.00±2.3	9.4±0.32	2	MR
VRSEM-799	13.33±3.5	4.4±0.20	1	R
VRSEM-757	12.00±2.3	3.2±0.26	1	R
VRSEM- 847	22.66±3.5	11.8±1.4	3	MS
VRSEM-746	15.33±1.6	6.2±0.40	2	MR
VRSEM-730	22.66±1.33	7.4±0.37	2	MR
VRSEM-776	8.00±2.3	2.8±0.26	1	R
VRSEM-733	16.67±4.05	5.4±0.32	2	MR
VRSEM-702	28.00±2.3	12.6±0.92	3	MS
Mean	18.47±1.27	6.83±0.62		
R^2	0.288	0.066		
Std. deviation	7.011	3.411		
Variance	49.016	11.633		

one were found be moderately susceptible. The mean disease intensity ranged from 10.93% for VRSEM-746 to 3.46% for VRSEM-776. The disease intensity for moderately resistant genotypes were 8.53% for VRSEM-833, 7.33% for VRSEM-739 and VRSEM-757, 6.33% for VRSEM-799, 6.26% for VRSEM-739, 6.06% for VRSEM- 847, 5.53% for VRSEM-702 and 5.33% for VRSEM-730. Genotype found to be resistant had disease intensity of 3.46% for VRSEM-776 whereas disease intensity for moderately susceptible Genotypes was 10.93% for VRSEM- 746. The Mean disease incidence of the ten genotypes were ranged from 9.33% for VRSEM-776 to 26.67% for VRSEM- 746 followed by 25.33% for VRSEM- 702, 21.33% for VRSEM-847, 20.00% for VRSEM-730, 17.33% for VRSEM-739, 16.00% for VRSEM-733, 14.67% for VRSEM-843 and VRSEM- 799 and 12.00% for VRSEM-757 (Table 5).

In our study of disease intensity, there were a non-significant effect *i.e*. null hypothesis is accepted at the p>0.05 level for the three conditions F (2, 27) = 4.61; p =>0.05 whereas null hypothesis is rejected *i.e*. significant in case of disease incidence at p<0.05 level for the three conditions F (2, 27) = 0.05; P=<0.05.

No work appears to have been done on the source of resistance to *Lablab purpureus* (L.) bean against *Alternaria destruens* and *Alternaria doliconidium.* However, some work has been done on varietal resistance against *Alternaria alternata* in other beans. Gurha *et al*. (1981) recorded first time the prevalence of pathogenic attack of *Alternaria alternata* on Broad bean (*Vicia faba* L.) and searched the sources of resistance against the pathogen. Maheshwari and Singh (1997) screened 99 genotypes from Uttar Pradesh for their response to the leaf spot caused by *Alternaria*. Cultivars found resistant are JDL 77, Arka Vijay, Rajani and Pusa Early Prolific whereas, cultivars found highly susceptible were Kalyanpur Type 2, 7103, Arka Jay, 81, Culture 6802, HA3, HD1and JDL 85. In our study, the genotypes which were found resistant in both the condition

Table 4: Leaf spot disease intensity and disease incidence of *Alternaria destruens* on ten *lablab* genotype evaluated under green house evaluation.

Genotype	Mean disease incidence (%)	Mean disease intensity (%)	Grade	Disease reaction
VRSEM-843	08.00±2.3	7.00±0.57	2	MR
VRSEM-739	13.33±1.33	7.46±0.33	2	MR
VRSEM-799	18.66±1.33	8.00±0.57	2	MR
VRSEM-757	21.33±1.33	3.20±0.58	1	R
VRSEM- 847	25.33±1.33	7.60±0.33	2	MR
VRSEM-746	26.33±1.33	5.60±0.88	2	MR
VRSEM-730	29.33±3.50	8.66±0.33	2	MR
VRSEM-776	24.00±4.00	5.33±0.58	2	MR
VRSEM-733	21.33±1.33	6.73±0.33	2	MR
VRSEM-702	16.00±4.6	3.86±0.58	1	R
Mean	20.40±1.33	6.33±0.36		
R^2	0.292	0.102		
Std. deviation	7.304	1.953		
Variance	53.352	3.816		

Table 5: Leaf spot disease intensity and disease incidence of *Alternaria destruens* on ten *lablab* genotypes evaluated under field evaluation.

Genotype	Mean disease incidence (%)	Mean disease intensity (%)	Grade	Inference
VRSEM-843	14.67±1.33	7.73±0.57	2	MR
VRSEM-739	17.33±2.66	6.26±0.33	2	MR
VRSEM-799	14.67±2.67	6.33±0.33	2	MR
VRSEM-757	12.00±2.3	7.73±0.57	2	MR
VRSEM- 847	21.33±2.66	6.06±0.57	2	MR
VRSEM-746	26.67±1.33	10.93±0.58	3	MS
VRSEM-730	20.00±4.00	5.33±0.57	2	MR
VRSEM-776	9.33±1.33	3.46±0.33	1	R
VRSEM-733	16.00±4.6	8.53±0.33	2	MR
VRSEM-702	25.33±2.66	5.53±0.34	2	MR
Mean	17.73±1.22	6.80±0.40		
R^2	0.302	0.046		
Std. deviation	6.70	2.203		
Variance	44.89	4.855		

is VRSEM 739 and VRSEM 776 whereas Shivanna and Shetty (1991) screened eight cluster bean varieties and found HG 182 showing the lowest per cent infection of *A. cyamopsidis* in both pot and field trials.

The genotypes which were found Moderately resistant under greenhouse evaluation and field trial against *Alternaria* leaf spot is VRSEM 873 whereas, Bharodia *et al*. (1993) found cluster bean variety GAUG 34, the variety with high gum content as moderately resistant to bacterial blight, *Alternaria* leaf spot and Powdery mildew while Kushwaha *et al*. (1993) reported 12 cultivars of faba bean seedlings being inoculated by *Alternaria alternate* pathogen and sustained under plastic covers with high relative humidity, JV-I and Dholi-II found moderately resistant (6-10% infection). The genotypes which were found Moderately susceptible under both condition against *Alternaria* leaf spot were VRSEM 847 whereas, Pangavhane (2001) screened varieties of cluster bean against blight and leaf spot caused by *Alternaria cyamposidis* and found Pusa Navbahar variety highly susceptible, Neelam-51 as susceptible and Dipali and Sona-51 found to be moderately susceptible. Varieties have been graded by using scale from 0 to 5 as suggested by Mathur *et al*. (1972). On the other hand, Singh and Singh 1998 screened 29 *Phaseouls vulgaris* varieties against *A. alternata* in field and Kentucky wonder, EC- 26392 and PBL-14-1 were found resistant. EC-57080, Sel-9, MB- Radish spotted, sum-1, p-37, sel-48-2-3 and sel48-1-2 gave moderately resistant reaction. MB light brown, Arka Komal, Mbwhite, Pusa Parvati Contender, D- 48, Pusa Hem Lata, Sel- 2, Sel- 4 and SB black were moderately susceptible. Aleme (2022) evaluate the performance of 12 accessions of *Lablab* across various locations of Ethiopia. In this study, lablab accessions are relatively more tolerant to disease than Hidosa *et al*. (2016). According to Kankwatsa (2018), there were *lablab* accessions that displayed higher disease resistance levels in Uganda.

CONCLUSION

Lablab purpureus (L.) genotypes with low sore densities, low illness seriousness and low sporulation might be wellsprings of fractional opposition that may restrain contamination and sore advancement, or might be a consequence of a contrary response. The *Lablab purpureus* (L.) genotypes with evident protection from leaf spot ought to be crossed with high yielding lines and adjusted assortments. The determined populaces will be utilized in reproducing enhanced leaf spot safe lines and for mapping their obstruction qualities. A portion of the chose lablab promotions can possibly give *Lablab purpureus* (L.) leaf spot obstruction qualities that might be valuable for consolidation into business *lablab* genotypes to create leaf spot safe genotypes. The wellsprings of obstruction distinguished in this exploration may give the opposition qualities expected to future advancement of leaf spot safe *lablab* genotypes.

Conflict of interest

The authors declare that they have no conflict of interest.

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