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Morphological and Pathogenic Variability among *Sclerotium rolfsii* Sacc isolates, the Causal Agent of Wheat Foot Rot Disease

Sindhu M.M.[1]* P.V. Patil[2], Kiran B.M.[1] and Supriya M.L.[1]
[1]Research Scholar, Department of Plant Pathology, UAS, Dharwad, (Karnataka), India.
[2]Professor, Department of Plant Pathology, UAS, Dharwad, (Karnataka), India.

(Corresponding author: Sindhu M.M.)*
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ABSTRACT: **The current study intends to evaluate the morpho-pathological variability among *S. rolfsii* isolates of wheat due to their ability to rapidly adapt and evolve, which will aid in the breeding of foot rot resistance. For this study, Ten isolates of *Sclerotium rolfsii* Sacc. collected from different wheat growing areas of Northern Karnataka were subjected for isolation from foot rot infected samples and these isolates showed variability with respect to cultural and morphological characters, toxin production and pathogenic ability. Isolates varied in mycelial characters like colony margin, mycelial growth and texture. With respect to sclerotial characters *viz.*, variation in shape, colour, diameter, test weight, number of sclerotial bodies per cm^2 and number of days taken to form sclerotial bodies were recorded. Oxalic acid production varied from 1.17 to 2.23 mg/ml. On the basis of pathogenicity, Pathotype-I consists of eight isolates, Pathotype II and III consists of only one isolate *Sr* DWR4 and *Sr* DWR1, respectively. Based on overall variability study, the *S. rolfsii* isolates were classified into two groups. Group I consist of four virulent isolates *viz.*, *Sr* DWR2, *Sr* DWR5, *Sr* BGM2and *Sr* BGT2 and group II consists remaining six isolates which are less virulent. This study provides information on the genetic divergence and occurrence of pathogenic races and also it will be helpful for the development of effective disease management strategies.**

Keywords: Wheat, *Sclerotium rolfsii*, Isolates and Variability.

INTRODUCTION

Wheat is an important cereal crop and a staple food of vast majority of the human population. It plays an important role in the cropping programme of Karnataka and it has already been proved to be the best component crop under multiple cropping system of the state. Similarly, there are good possibilities of increasing the area due to change in food habits of the people of Karnataka. Northern Karnataka being the main wheat belt, accounts to about two - third of the total wheat area. Wheat in Karnataka is susceptible to foot rot, brown rust and leaf blight. Foot rot of wheat is caused by *Sclerotium rolfsii* Sacc. has become a matter of interest to research workers due to variability in causal organism from place to place and it is a serious problem mainly in the rainfed area.

Sclerotium rolfsii Sacc. is a well-known ubiquitous soil inhabiting and most destructive soil borne pathogen and it was primarily described by Rolfs (1892) on tomato. It is predominantly distributed throughout tropical and subtropical regions where the temperature reaches higher levels during the *rabi* season. *S. rolfsii* having a saprophytic activity in soil and can survive in soil for many years by producing resting structures called sclerotial bodies (Weber, 1931). These sclerotia act as primary inoculum of the pathogen and mainly for survival and dispersal of the fungus under various adverse environmental conditions. This pathogen is soil borne and damaging wheat crop by causing pre and post emergence death of the seedlings (Kalappanavar and Patil 2000).

Studies on variability within the population in a geographical region are important because these also document the changes occurring in the population and it will helpful in foot rot resistant breeding. Variation in the production of oxalic acid in culture filtrate of *S. rolfsii* isolates is one of the reasons for change in the virulence pattern of isolates of *S. rolfsii* on wheat genotypes. So, it is necessary to study the variation in production of oxalic acid by different isolates and this information is use full in foot rot resistance breeding. Foot rot in wheat has become a hot research topic due to the variability of isolates from different locations. This organism has primarily been studied in terms of survey and management. As a result, this study was done to identify the pathogen responsible for foot rot disease in wheat, as well as their virulence, using morphological characteristics, oxalic acid production, and pathogenic variability study.

MATERIAL AND METHODS

A. Cultural and morphological variability

This experiment was conducted at Department of Plant Pathology, College of Agriculture, Dharwad during 2018-19. The cultural characters of all the isolates of *S. rolfsii* were studied on Potato Dextrose Agar (PDA) medium by pouring 15 ml of PDA into 90 mm diameter petri plates. These plates were inoculated at the center with fungal discs of 5 mm diameter of each isolate from

the periphery of seven-day old pure culture and incubated at 26 ±1ºC for a period till full growth appeared in any one of the isolates whichever is early. Three replications were maintained for each isolate. Then cultural and morphological characters of all the isolates of *S. rolfsii viz*., colony morphology (color and growth behavior), sclerotial formation period, sclerotial characters like colour, size, shape and test weight were noted down.

B. Biochemical variability

All the isolates of *S. rolfsii* were tested for oxalic acid production in culture filtrates. For this, each isolate was grown separately in potato dextrose broth. Fifty ml of potato dextrose broth was poured into 250 ml flasks and sterilized at 121°C for 15 min. Each flask was inoculated individually with five mm mycelial disc of seven-day old culture grown on PDA plates and incubated at 27 ± 1°C for ten days. The oxalic acid present in the culture filtrate was isolated using procedure given by Mahadevan and Sridhar (1986) and the amount of oxalic acid present in culture filtrate was calculated as 1 ml of 0.02 N potassium permanganate reacted with 1.265 mg of oxalic acid. Three replications were made for each isolate and amount (mg/ml) of oxalic acid produced by each isolate of *S. rolfsii* was noted down.

C. Pathogenic variability

The pathogenic variability in 10 isolates of *S. rolfsii* was studied by inoculating each isolate on a set of wheat genotypes. The reaction of each isolate on each genotype was noted down.

Wheat genotypes: Eleven genotypes (Bijaga yellow, Lal bahadur, Amrut, UAS446, UAS347, UAS 375, DWR 2006, Kalyansona, Sonalika, HD 2189 and Lerma Roja) were used to study the pathogenic variability in *S. rolfsii* under green house in pot culture experiment.

Pathogenic variability among the isolates was known by inoculating each isolate of *S. rolfsii* on a set of wheat genotypes exhibiting different degree of resistance/ susceptibility to *S. rolfsii* under greenhouse condition in pot culture. The sterilized sand, soil and FYM was mixed in 1:1:0.5 proportion on W/W basis and filled in 10cm diameter plastic cups. Four per cent mass multiplied inoculum of *S. rolfsii* was added to the soil in pots and mixed thoroughly. Apparently healthy seeds of wheat genotypes were sown at the rate of seven seeds per pot separately. The pot devoid of inoculum serve as control. Each treatment was replicated twice. Watering was done to maintain soil moisture at field capacity. Observation on seedling stand was recorded 21st day after sowing. The post emergence seedling mortality was calculated and reaction of the genotypes to isolates was categorized as follows (Anon., 2018):

Sr. No.	Per cent disease incidence	Category
1.	Up to 10	Resistant
2.	10.1 - 20	Moderately resistant
3.	20.1 – 30	Moderately Susceptible
4.	>30	Susceptible

RESULT AND DISCUSSION

The ten isolates of *S. rolfsii* isolated from the foot rot infected samples collected from different parts of northern Karnataka during survey were subjected for cultural and morphological characters *viz.*, colony diameter, type of margin, mycelial growth and texture. The study revealed that mycelial characters like colony margin varied from irregular to regular, mycelial growth from flat to raised and texture from smooth to coarse. Raised mycelial growth was observed in *Sr* VJR, *Sr* DWR3, *Sr* DWR4 and remaining seven isolates showed flat type of mycelial growth. Colony margin was regular in 8 isolates (*Sr* DWR1, *Sr* BGT1, *Sr* BGM1, *Sr* DWR2, *Sr* DWR4, *Sr* DWR5, *Sr* BGM2, *Sr* BGT2) and it was irregular in remaining (SrVJR and *Sr* DWR3) isolates (Fig. 1). No variation was observed with respect to colony diameter in all the isolates and recorded maximum of 85 mm (Table 1). Similar type of results was recorded by Manu *et al.* (2018) in the isolates of *S. rolfsii* causes foot and collar rot in different crops of southern Karnataka. Prabhu (2003) reported that colony diameter varies among the isolates of *S. rolfsii* (collar rot of soybean) with the range of 84.00 mm to 90.00 mm. The results of this study were comparable with the findings of Hegde *et al*. (2021), they reported that mycelium of the *s. rolfsii* isolates of wheat showed fluffy white growth on PDA medium. The mycelial growth was coarse in two isolates (Sr BGL and Sr BVG), and the remaining three isolates (Sr DWR, Sr MUL and Sr UGK) showed a smooth texture.

A. Sclerotial characters

All ten isolates showed variation with respect to sclerotial characters like sclerotial shape varied from round to oval, colour from light brown to dark brown, diameter from 0.68 to 1.05 mm, test weight from 56.81 to 160.24 mg, number of sclerotial bodies per cm^2 varied from 3. 33 to 9.45, and number of days taken to form sclerotial bodies varied from 9 to 15.

With regard to sclerotial colour, four isolates *viz.*, *Sr* DWR1, *Sr* BGT1, *Sr* BGM1and *Sr* DWR4produced light brown sclerotia while remaining isolates produced dark brown coloured sclerotia. *Sr* DWR2 and SrDWR5 isolate were oval in shape whereas, others were round (Table 1). *Sr* BGM1 isolate produced biggest sclerotia with mean diameter of 1.05 mm followed by *Sr* DWR3 isolate (1.04 mm) whereas, *Sr* BGT2 isolate produced smallest sclerotia with mean diameter of 0.78 mm. Four isolates *viz.*, *Sr* DWR2, *Sr* DWR5, *Sr* BGM2 *and Sr* BGT2 which have taken 8 to 10 days for sclerotial initiation and remaining six isolates *viz.*, *Sr* DWR1, *Sr* VJR, *Sr* BGT1, *Sr* BGM1, *Sr* DWR3 and *Sr* DWR4 have taken 11 to 13 days. *Sr* BGT2 and *Sr* DWR4 produced maximum number of 9.45 and 9.21 sclerotia per cm^2 of culture, whereas, *Sr* DWR2 and *Sr* DWR5 produces minimum number of 3.33 and 3.95 sclerotia per cm^2 of culture.

Isolates showed variation with respect to test weight of sclerotial bodies. Group I consists of five isolates (SrVJR, *Sr* DWR3, *Sr* DWR4, *Sr* BGM2, *Sr* BGT2) with test weight ranging from 50-100 mg. Group II

consists of remaining five isolates (*Sr*DWR1, *Sr* BGT1, *Sr* BGM1, *Sr* DWR2, *Sr* DWR5) with test weight ranging from 100.1 - 200 mg. The results are in accordance with the findings of Prabhu (2003); Shwetha (2011); Manu (2018) who reported that, variation existed in sclerotial weight among the isolates of *S. rolfsii* and it was in the range of 69.20 to 147.50 mg (collar rot of soybean), 140.00 to 800.00 mg (wilt of stevia) and 40.00 to 71.00 mg (collar and stem rot in different crops), respectively.

B. Biochemical variability

Maximum oxalic acid production of 2.23mg/mlwas recorded in *Sr* BGT2 isolate and it was on par with *Sr* BGM2 (2.18 mg/ml), *Sr* DWR5 (2.10 mg/ml) and *Sr* DWR4 (1.98 mg/ml) isolates. However, least oxalic acid production (1.17 mg/ml) was observed in *Sr* DWR3isolate and it was significantly differ from rest of the isolates subjected for oxalic acid production (Table 2).

Gawande *et al.* (2013) reported that production of oxalic acid in the culture filtrate of *S. rolfsii* infecting different crops varies in the range of 1.04 to 2.87 mg/ ml. Oxalic acid, a metabolite of *S. rolfsii* is known to play a very important role in pathogenesis of this fungus (Kirtzman *et al.,* 1977). Oxalic acid sequesters the calcium in the host cell wall there by favouring the pectic enzymes secreted by the pathogen to hydrolyse the pectate in the middle lamella more rapidly to cause the collar rot/root rot symptoms. Punja and Jenkins (1984) reported that the isolates of *S. rolfsii* varied in oxalic acid production in the culture filtrate. Ferrar and Walker (1993) postulated that the action of oxalic acid enhances the success of the pathogen by suppressing the host defence mechanisms. Ansari and Agnihotri (2000) have found positive correlation between oxalic acid production and virulence of the isolates of *S. rolfsii*.

C. Pathogenic variability

Pathogenic variability by pot culture experiment revealed that similar and different reaction on wheat genotypes to ten isolates of *S. rolfsii*. The isolates of *S. rolfsii* from northern Karnataka showed variation in their pathogenicity. Some were pathogenic to all the genotypes and others were pathogenic to single genotype of wheat.

Based on the reaction of eleven wheat genotypes, all the ten isolates could be categorized into three pathotypes. Pathotype-I consists of eight isolates (*Sr* VJR, *Sr* BGT1, *Sr* BGM1, *Sr* DWR2, *Sr* DWR3, *Sr* DWR5, *Sr* BGM2and *Sr* BGT2) which were showed susceptible reaction on all eleven genotypes. Pathotype II consists of only one isolate (SrDWR4) which was showed moderately resistant reaction on HD 2189 and susceptible reaction on rest of the genotypes (Bijaga yellow, Lal bahadur, Amrut, UAS 446, UAS 347, UAS 375, DWR 2006, Kalyansona, Sonalika and Lerma Roja). Pathotype III consists of only one isolates (*Sr* DWR1) which was showed moderately susceptible reaction on Bijaga yellow and Lerma Roja, moderately resistant reaction on Amrut but susceptible reaction on Lal bahadur, UAS 446, UAS 347, UAS 375, DWR 2006, Kalyansona, Sonalika, and HD 2189 (Table 3a and 3b).

Differences in severity of the disease was observed between different isolates across different sunflower cultivars suggest a form of physiological specialization in this pathogen. Variation in virulence has been correlated with synthesis of oxalic acid and of enzymes secreted by the pathogen (Ansari and Agnihotri 2000) and also environment. Some of the earlier workers reported that there was a difference in virulence of isolates from same host as well as from various hosts (Punja, 1985).

D. Grouping of S. rolfsii isolates based on different characters

The results of the study indicated that, virulence was related to the size of the sclerotial bodies, number of days taken to form sclerotial bodies, reaction on all wheat genotypes and oxalic acid production.

Based on above mentioned characters, the isolates of *S. rolfsii* were classified in to two groups. Group I consist of four virulent isolates *viz.*, *Sr* DWR2, *Sr* DWR5, *Sr* BGM2and *Sr* BGT2 with sclerotial size varied from 0.68 to 0.98 mm, number of days taken to form sclerotial bodies varied from 8 to 10, susceptible reaction on all eleven wheat genotypes and oxalic acid production varied from 2.18 to 2.23 mg/ml. Group II consists of six less virulent isolates *viz.*, *Sr* DWR1, *Sr* VJR, *Sr* BGT1, *Sr* BGM1, *Sr* DWR3 and *Sr* DWR4with sclerotial size varied from 1.01 to 1.05 mm, number of days taken to form sclerotial bodies varied from 11 to 15, susceptible to resistant reaction on different wheat genotypes and oxalic acid production varied from 1.17 to 2.23 mg/ml (Table 4). These results were compared with the studies conducted by Palaiah (2002); Sarma *et al.* (2002), they have observed variation in the isolates of *S. rolfsii* and which depends on soil type, host crop and the environmental factors. The overall variability studies indicated that, the variation among the isolates of *S. rolfsii* may be depend on the geographical location from which isolates were collected.

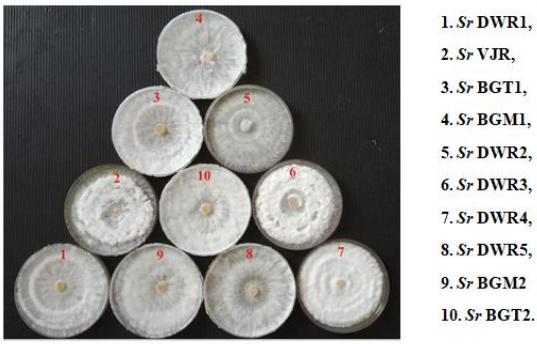

Fig. 1. Morphological variability among the isolates of *Sclerotium rolfsii* Sac.

Table 1: Cultural and morphological variability in the isolates of *Sclerotium rolfsii* S.

Isolate	Colony diameter (mm)	Type of margin	Mycelial growth	Texture	Morphology of sclerotia					
					Colour	Shape	Number of sclerotia per cm^2 of culture	Number of days taken to form sclerotial bodies	Weight of 100 sclerotial bodies (mg)	Diameter (mm)
Sr DWR1	85	Regular	Flat	Smooth	Light brown	Round	5.13	14	134.10	1.01
Sr VJR	85	Irregular	Raised	Coarse	Dark brown	Round	8.14	11	75.13	1.02
Sr BGT1	85	Regular	Flat	Smooth	Light brown	Round	4.30	11	139.00	1.03
Sr BGM1	85	Regular	Flat	Smooth	Light brown	Round	4.02	15	142.12	1.05
Sr DWR2	85	Regular	Flat	Smooth	Dark brown	Oval	3.33	09	160.24	0.80
Sr DWR3	85	Irregular	Raised	Coarse	Dark brown	Round	8.84	12	56.81	1.04
Sr DWR4	85	Regular	Raised	Smooth	Light brown	Round	9.21	13	80.23	1.02
Sr DWR5	85	Regular	Flat	Smooth	Dark brown	Oval	3.95	10	136.25	0.98
Sr BGM2	85	Regular	Flat	Smooth	Dark brown	Round	7.37	09	81.45	0.82
Sr BGT2	85	Regular	Flat	Smooth	Dark brown	Round	9.45	08	76.27	0.78

Table 2: Oxalic acid production by isolates of *Sclerotium rolfsii* Sacc. word.

Sr. No.	Isolates	Oxalic acid (mg/ml)
1.	*Sr* DWR1	1.49
2.	*Sr VJR*	2.08
3.	*Sr* BGT1	1.92
4.	*Sr* BGM1	1.58
5.	*Sr* DWR2	2.20
6.	*Sr* DWR3	1.17
7.	*Sr* DWR4	1.98
8.	*Sr* DWR5	2.10
9.	*Sr* BGM2	2.18
10.	*Sr* BGT2	2.23
S. Em. ±		**0.07**
C. D. at 1%		**0.29**

Table 3a: Per cent disease incidence in eleven wheat genotypes as influenced by ten isolates of *Sclerotium rolfsii* Sacc.

Sr. No.	Isolates	Bijaga yellow	Lal bahadur	Amrut	UAS 446	UAS 347	UAS 375	DWR 2006	Kalyansona	Sonalika	Lerma Roja	HD 2189	Mean
1.	*Sr* DWR1	23.08 (MS)	100 (S)	14.29 (MR)	35.71 (S)	35.74 (S)	0.00 (S)	92.31 (S)	38.46 (S)	46.15 (S)	21.43 (MS)	46.15 (S)	**41.21 (S)**
2.	*Sr* VJR	100 (S)	92.86 (S)	100 (S)	100 (S)	92.86 (S)	92.31 (S)	92.34 (S)	100 (S)	46.15 (S)	50.00 (S)	76.92 (S)	**85.77 (S)**
3.	*Sr* BGT1	100 (S)	85.71 (S)	100 (S)	85.71 (S)	85.74 (S)	100 (S)	92.31 (S)	100 (S)	76.92 (S)	64.29 (S)	61.54 (S)	**86.56 (S)**
4.	*Sr* BGM1	100 (S)	100 (S)	71.40 (S)	64.29 (S)	57.14 (S)	38.46 (S)	76.92 (S)	84.62 (S)	46.15 (S)	42.86 (S)	61.54 (S)	**67.58 (S)**
5.	*Sr* DWR2	92.31 (S)	78.57 (S)	100 (S)	100 (S)	100 (S)	100 (S)	84.62 (S)	100 (S)	92.31 (S)	85.71 (S)	92.31 (S)	**93.25 (S)**
6.	*Sr* DWR3	100 (S)	85.71 (S)	78.57 (S)	100 (S)	78.57 (S)	69.23 (S)	100 (S)	92.31 (S)	46.15 (S)	64.29 (S)	53.85 (S)	**78.97 (S)**
7.	*Sr* DWR4	76.92 (S)	100 (S)	78.57 (S)	71.43 (S)	57.14 (S)	30.77 (S)	76.92 (S)	61.54 (S)	76.92 (S)	78.57 (S)	15.38 (S)	**65.83 (S)**
8.	*Sr* DWR5	100 (S)	92.86 (S)	100 (S)	100 (S)	85.71 (S)	92.31 (S)	92.34 (S)	100 (S)	69.23 (S)	71.43 (S)	46.15 (S)	**86.36 (S)**
9.	*Sr* BGM2	100 (S)	85.71 (S)	92.86 (S)	92.88 (S)	71.43 (S)	100 (S)	100 (S)	100 (S)	84.62 (S)	92.86 (S)	92.31 (S)	**92.06 (S)**
10.	*Sr* BGT2	84.62 (S)	100 (S)	100 (S)	92.86 (S)	92.87 (S)	100 (S)	100 (S)	100 (S)	92.31 (S)	92.86 (S)	84.62 (S)	**94.55 (S)**

S = Susceptible, **MS** = Moderately susceptible, **MR** = Moderately resistant.

Table 3b: Reaction of eleven wheat genotypes to ten isolates of *Sclerotium rolfsii* Sacc.

Sr. No.	Isolates	Bijaga yellow	Lal bahadur	Amrut	UAS 446	UAS 347	UAS 375	DWR 2006	Kalyansona	Sonalika	Lerma Roja	HD 2189	Pathotype designation
1.	*Sr* DWR1	MS	S	MR	S	S	S	S	S	S	MS	S	III
2.	*Sr VJR*	S	S	S	S	S	S	S	S	S	S	S	I
3.	*Sr* BGT1	S	S	S	S	S	S	S	S	S	S	S	I
4.	*Sr* BGM1	S	S	S	S	S	S	S	S	S	S	S	I
5.	*Sr* DWR2	S	S	S	S	S	S	S	S	S	S	S	I
6.	*Sr* DWR3	S	S	S	S	S	S	S	S	S	S	S	I
7.	*Sr* DWR4	S	S	S	S	S	S	S	S	S	S	MR	II
8.	*Sr* DWR5	S	S	S	S	S	S	S	S	S	S	S	I
9.	*Sr* BGM2	S	S	S	S	S	S	S	S	S	S	S	I
10.	*Sr* BGT2	S	S	S	S	S	S	S	S	S	S	S	I

S = Susceptible, **MS** = Moderately susceptible, **MR** = Moderately resistant.

Table 4: Grouping of *Sclerotium rolfsii* Sacc. isolates based on different characters.

Group	Isolate	Sclerotial colour	Oxalic acid (mg/ml)	Sclerotial size (mm)	Number of days taken to form sclerotial bodies	Pathotype designation	Pathogenic reaction
Group I	*Sr* DWR2	Dark brown	2.20	0.80	09	I	Virulent
	Sr DWR5	Dark brown	2.10	0.98	10	I	
	Sr BGM2	Dark brown	2.18	0.82	09	I	
	Sr BGT2	Dark brown	2.23	0.78	08	I	
Group II	*Sr* DWR1	Light brown	1.49	1.01	14	III	Less virulent
	Sr BJR	Dark brown	2.08	1.02	11	I	
	Sr BGT1	Light brown	1.92	1.03	11	I	
	Sr BGM1	Light brown	1.58	1.05	15	I	
	Sr DWR3	Dark brown	1.17	1.04	12	I	
	Sr DWR4	Light brown	1.98	1.02	13	II	

CONCLUSIONS

Ten isolates of *Sclerotium rolfsii* Sacc. collected from different wheat growing areas of Northern Karnataka were subjected for isolation from foot rot infected samples and these isolates showed variability with respect to cultural and morphological characters, toxin production and pathogenic ability. Studies on variability within the population in a geographical region are important because these also document the changes occurring in the population. Variation in oxalic acid production in culture filtrate of *S. rolfsii* isolates is one of the impediments responsible for degrading the host tissue. So, it is necessary to study the variation in production of oxalic acid by different isolates and this information is use full in foot rot resistance breeding.

FUTURE SCOPE

Variability studies can provide insights into the genetic diversity, population dynamics, and evolutionary processes of plant pathogenic fungi. Understanding how these fungi adapt to different environments, host plants, and management practices can aid in predicting their future behavior and developing effective control strategies.

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Conflict of Interest. None.

Ethical Approval. This article does not contain any studies with human participants or animals performed by any of the authors.

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