DOI: 10.5965/223811712312024001

Revista de Ciências Agroveterinárias 23 (1): 2024
Universidade do Estado de Santa Catarina

First report of wheat stripe mosaic virus in wheat in Santa Catarina, Brazil

Primeiro relato de wheat stripe mosaic virus em trigo no estado de Santa Catarina, Brasil

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Submission: 08/08/2023 | Acceptance: 20/10/2023

ABSTRACT

The soil-borne wheat mosaic disease (SBWMD) significantly impacts wheat crops. In Brazil, this disease is caused by the wheat stripe mosaic virus (WhSMV). Typical symptoms of SBWMD were observed in a commercial wheat field in Campos Novos, Santa Catarina, Brazil. This study aimed to elucidate the etiological agent of SBWMD in wheat crop in the state of Santa Catarina. Total RNA was extracted from symptomatic sample using TRIzol, following subsequent RT-PCR. Specific primer pairs were used to amplify genomic regions of 641 bp and 872 bp, corresponding to the coat protein (CP) and partial replicase genes, respectively. Amplicons were sequenced and the resulting nucleotide sequences were compared with WhSMV sequences from GenBank using a BLASTn search. The SDT program was used to determine nucleotide identity, and phylogenetic relationships were established using the MEGA11 software. Analyses were performed on a global dataset that included sequences obtained in this study and sequences previously characterized in South Africa, Paraguay and Brazil. The nucleotide sequences showed an identity ranging from 94% to 100% for the two analyzed regions when subjected to paired analysis. Based on specific primers amplification and aminoacid sequence of CP identity higher than 90% when compared to other isolates, the viral isolate characterized in this study is WhSMV. Phylogenetic analyses revealed two distinct clades. Notably no demarcation was observed between the WhSMV isolate characterized in this study and Brazilian isolates previously characterized, indicating a close relationship between them. This is the first report of WhSMV associated with wheat mosaic disease in Santa Catarina, Brazil.

KEYWORDS: Winter cereal; viruses; *Triticum aestivum*; soil-borne wheat mosaic disease.

RESUMO

O mosaico do trigo, conhecido como (SBWMD), causa impacto significativo nas lavouras de trigo. No Brasil esta doença é causada pelo wheat stripe mosaic virus (WhSMV). Sintomas típicos de SBWMD foram observados em uma lavoura comercial de trigo em Campos Novos, Santa Catarina, Brasil. Este estudo objetivou elucidar o agente etiológico do SBWMD em trigo no estado de Santa Catarina. O RNA total foi extraído de amostras sintomáticas usando TRIzol e, posteriormente, submetido à RT-PCR. Foram utilizados iniciadores específicos para amplificar uma região genômica de 641 bp e 872 bp, correspondentes aos genes da capa proteica (CP) e da replicase parcial, respectivamente. Os amplicons foram sequenciados e as sequências de nucleotídeos resultantes foram comparadas com sequências de WhSMV do banco de dados do GenBank usando a ferramenta BLASTn. A identidade de nucleotídeos foi determinada usando o programa SDT, e as relações filogenéticas foram estabelecidas usando o software MEGA11. As análises foram realizadas para um conjunto de dados globais, incluindo as sequências obtidas neste estudo e sequências já caracterizadas na África do Sul, no Paraguai e no Brasil. O conjunto de sequências de nucleotídeos quando submetido a análise pareada exibiu identidade variando de 94% a 100% para as duas regiões analisadas. Com base nas amplificações utilizando iniciadores específicos e na identidade de aminoácidos da CP maior que 90% quando comparada aos outros isolados, o isolado viral caracterizado neste estudo é WhSMV. As análises filogenéticas revelaram dois clados distintos, notavelmente sem demarcação entre o isolado WhSMV caracterizado neste estudo e os isolados brasileiros caracterizados anteriormente, demonstrando estreita relação entre os mesmos. Este é o primeiro relato de WhSMV associado à doença do mosaico do trigo em Santa Catarina, Brasil.

PALAVRAS-CHAVE: Cereal de inverno; viroses; *Triticum aestivum*; Vírus do mosaico do trigo.

INTRODUCTION

Viral diseases can cause serious damage to wheat crops. In Brazil, particulary in the southern region, the soil-borne wheat mosaic disease (SBWMD) can result in productivity losses of up to 50% (LAU 2014, MACIEL et al. 2020). For the past 40 years, this disease has been attributed to the soil-borne wheat mosaic virus (SBWMV), which belongs to the *Virgaviridae* family. However, recent genome sequencing revealed that, in Brazil, SBWMD is caused by a new species tentatively named wheat stripe mosaic virus (WhSMV), belonging to the *Benyviridae* family (VALENTE et al. 2019).

WhSMV has been found widely disseminated throughout the states of Rio Grande do Sul and Paraná (VALENTE et al. 2019) and has also been reported in Paraguay and South Africa (FARIÑA et al. 2019, TEREFE et al. 2019). Typical symptoms of SBWMD appear as irregular stains in the field and include characteristic yellow mosaics on leaves and stems. Affected plants may also exhibit stunting or rosette (TEREFE et al. 2021, STEMPKOWSKI et al. 2022). Viruses from the *Benyviridae* family, such as WhSMV and rice stripe necrosis virus (RSNV) are transmitted by the root-infecting, obligatory parasite, *Polymyxa graminis* Ledingham (KANYUKA et al. 2003, GILMER et al. 2017, VALENTE et al. 2019). Since chemical control methods are inefficient, management measures for SBWMD typically consist of genetic resistance combined with crop rotation (STEMPKOWSKI et al. 2020).

In 2022, wheat plants of the cultivar Anak exhibiting typical mosaic symptoms were observed in a commercial area in Campos Novos, a municipality of the state of Santa Catarina (Southern Brazil) (27°21'40"S and 51°15'02"W). This study aimed to characterize the virus associated with SBWMD in wheat sample from Santa Catarina state.

MATERIAL AND METHODS

Total RNA was extracted from the leaves of a symptomatic wheat plant (Figure 1) using TRIzol® reagent (Invitrogen, USA), following the manufacturer's instructions. The cDNA synthesis was performed using M-MLV reverse transcriptase and oligo dT primer (Promega, USA), in accordance with the manufacturer's instructions, from 1 µg of extracted total RNA. PCR was then performed using specific primers for two genomic regions of the WhSMV. The primer pairs Beny_CP (F) (5'-AAGTGTCGCAAGCTTCGCG-3') and Beny_CP (5'-ATCGCACCGACGTAAGAACT-3') were used to amplify a genomic region of 641 bp corresponding to the complete coat protein (CP) gene. The primer pairs RdRp_WhSMV (F) (5'-TAGGTAGACGCGCTAAAG-3') and RdRp_WhSMV (5' GTAGCCACGGAACGAAATAG-3') were used to amplify a genomic region of 872 bp corresponding to the partial replicase gene (VALENTE et al. 2019). The PCR mixture contained 5 µL cDNA (*c*. 1 µg), 10 µL of 5× PCR buffer, 2 µL $MgCl_2$ (25 mm), 1 µL of dNTP (10 mm), 1 µL (0.25 U μL^{-1}) of Go *Taq* DNA polymerase (5 U μL^{-1}; Promega, USA), and ultrapure water to a final volume of 50 µL. Reaction conditions were set at 94°C for 2 min, followed by 34 cycles of 94 °C for 1 min, 54 °C for 1 min, and 72°C for 1 min, and a final extension at 72°C for 10 min (VALENTE et al. 2019). The amplified fragments were subjected to 1% agarose gel electrophoresis, stained with GelRed (Biotium), visualized under a UV light transilluminator, and photographed. All amplifications showing the expected size were sequenced in both directions (sense and antisense) at ACTGene (Alvorada, RS) using the aforementioned primer pairs.

The nucleotide and amino acid identities was determined using the SDT program version 1.2 (SDTv1.2) (MUHIRE et al. 2014), and the phylogenetic relationship was verified using the maximum likelihood method implemented in the MEGA11 v.11 program (TAMURA et al. 2021). The phylogenetic relationship was inferred using the maximum likelihood method, Kimura 2-parameter model KIMURA (1980), and proportion of invariable sites (I) in both analyses. Bootstrap values of 10,000 and 9,816 to the complete coat protein (CP) and partial replicase genes, respectively, were used. In addition to the sequences obtained in this study, CP and partial replicase nucleotide sequences from previously characterized isolates in Brazil, Paraguay, and South Africa were used for analysis.

RESULTS AND DISCUSSION

The RT-PCR amplification of the complete coat protein (CP) and partial replicase genes confirmed the presence of WhSMV infection in the collected plant material (Figure 2). This result corroborates the effectiveness of using the primer pairs described in previous studies for the diagnosis of WhSMV (VALENTE et al. 2019, STEMPKOWSKI et al. 2020).

Figure 1. Symptoms of stripe mosaic on the leaves of wheat in Campos Novos municipality (SC). The geographic coordinates of the sample collection site are indicated on the map.

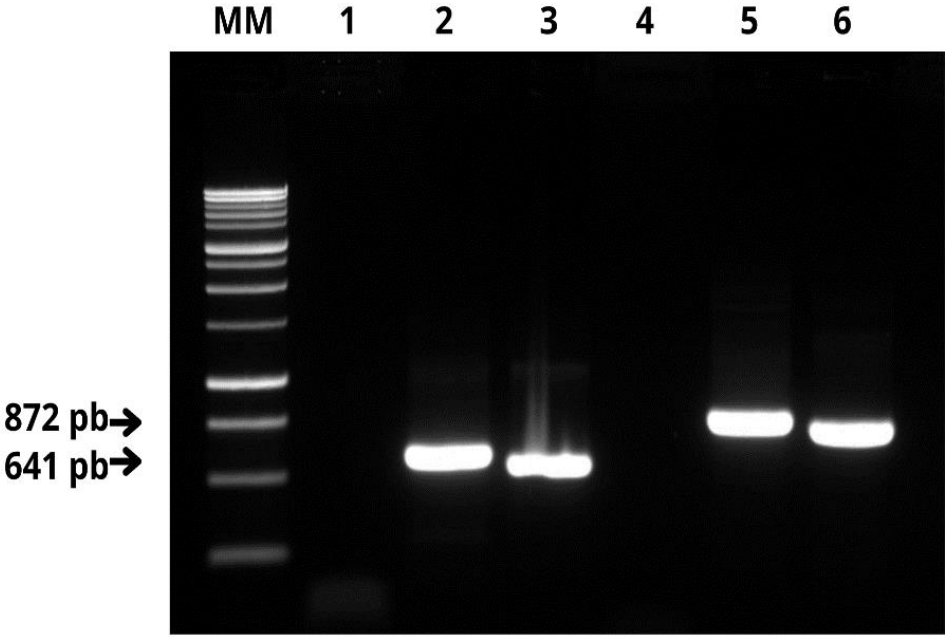


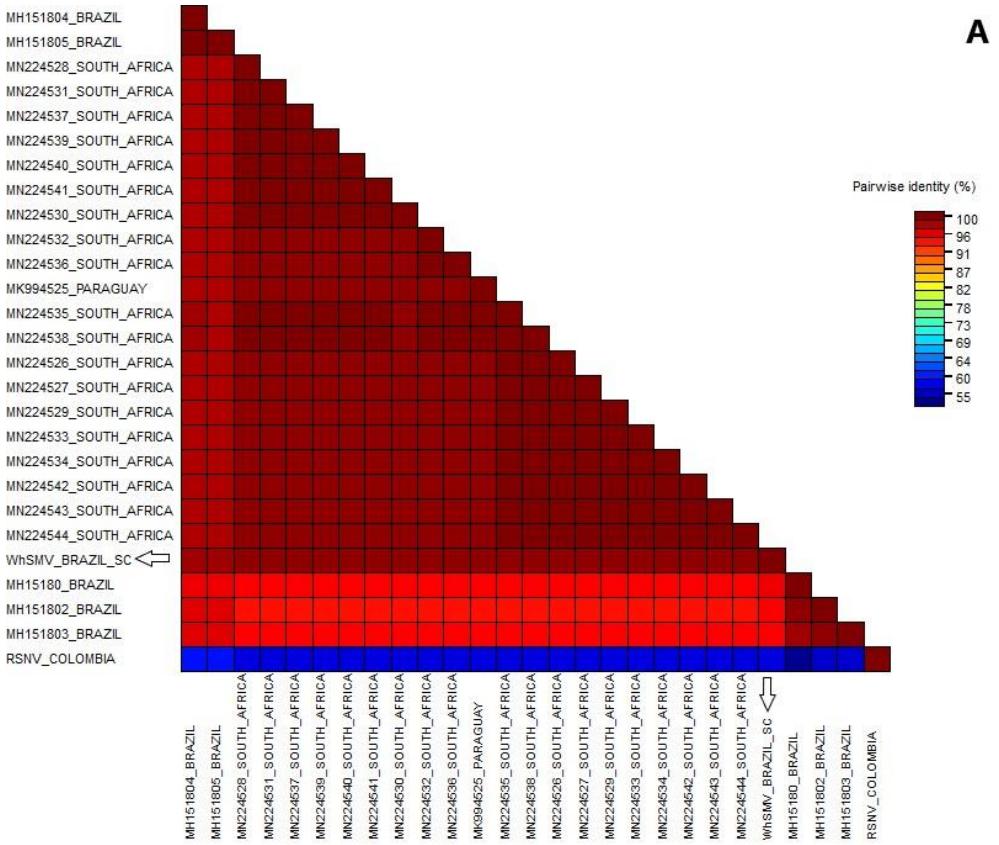
Figure 2. Detection of the complete coat protein (CP) and partial replicase genes of WhSMV using RT-PCR. MM is the molecular marker of 1 kb DNA ladder. 1 and 4 are negative controls of RT-PCR for CP and partial replicase genes, respectively. 2 and 5 are positive controls of RT-PCR for CP and partial replicase genes, respectively. 3 and 6 are the amplifications of the CP and partial replicase genes, respectively.

The nucleotide sequences of CP and partial replicase of the viral isolate characterized in this study showed an identity of 94%−100% and 97%−100%, respectively, with sequences from the same genes of the WhSMV previously characterized in 2019 (Figure 3) (VALENTE et al. 2019). When compared to the global population, the nucleotide sequences of CP and partial replicase genes showed identities ranging from 94% to 100% in both cases, indicating a high similarity between the isolate from Santa Catarina and isolates previously characterized. TEREFE et al. (2021) found nucleotide identity of 96% for partial replicase gene and between 90%−92% for CP gene when comparing Brazilian and South African isolates.

The Brazilian population showed high nucleotide identity, with values greater than 98% for replicase and above 97% for CP segments. Similarly, when comparing the Santa Catarina isolates obtained in this study with other Brazilian isolates already characterized, values above 97% for replicase and above 94% for CP were observed. The taxonomic criterion used for species demarcation in the genus *Benyvirus* is amino acid sequence identity of CP less than 90% (GILMER et al. 2017, ICTV 2022). In the comparison of the amino acid sequence of the CP from the characterized isolate in this study with other WhSMV isolates, identity greater than 97% was observed (Figure 3).

Therefore, based on specific primers amplification and the nucleotide and amino acid sequences identified in the viral isolate described in this study, it can be considered a species of the family *Benyviridae* named wheat stripe mosaic virus.

The phylogenetic analysis, as shown in Figure 4, indicates the formation of two clades. However, some of the Brazilian isolates are still related to the African and Paraguayan WhSMV isolates, demonstrating that despite the geographic separation of the two populations, there is low variability. The high relationship observed for the CP sequences is due to this region is conserved and the encoded protein is structural (MOURA et al. 2012, CHEN et al. 2023). Phylogenetic analysis based on CP nucleotide sequence revealed two strongly supported clades (bootstrap value > 88), with the isolate characterized in this study grouped with the South African isolates. When the partial replicase coding region was analyzed, the isolate from Santa Catarina was found to be grouped with the other Brazilian isolates. This topological incongruence could be due to a recombination event.



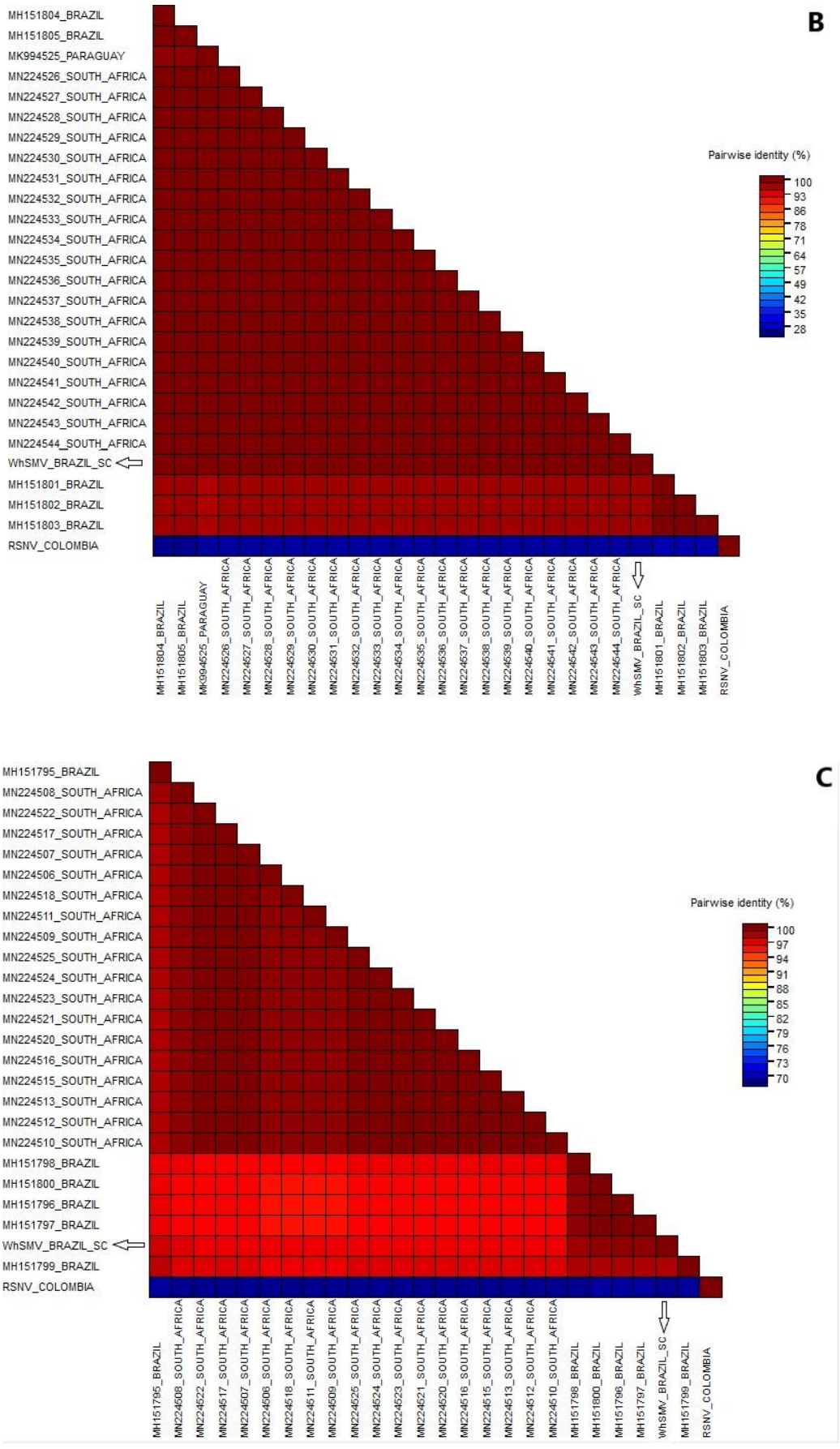


Figure 3. Nucleotide and amino acid identity of the CP (A and B, respectively) and nucleotide identity of the partial replicase (C) of the isolate, when compared to the Brazilian and global populations. Arrow indicate the isolate characterized in this study.

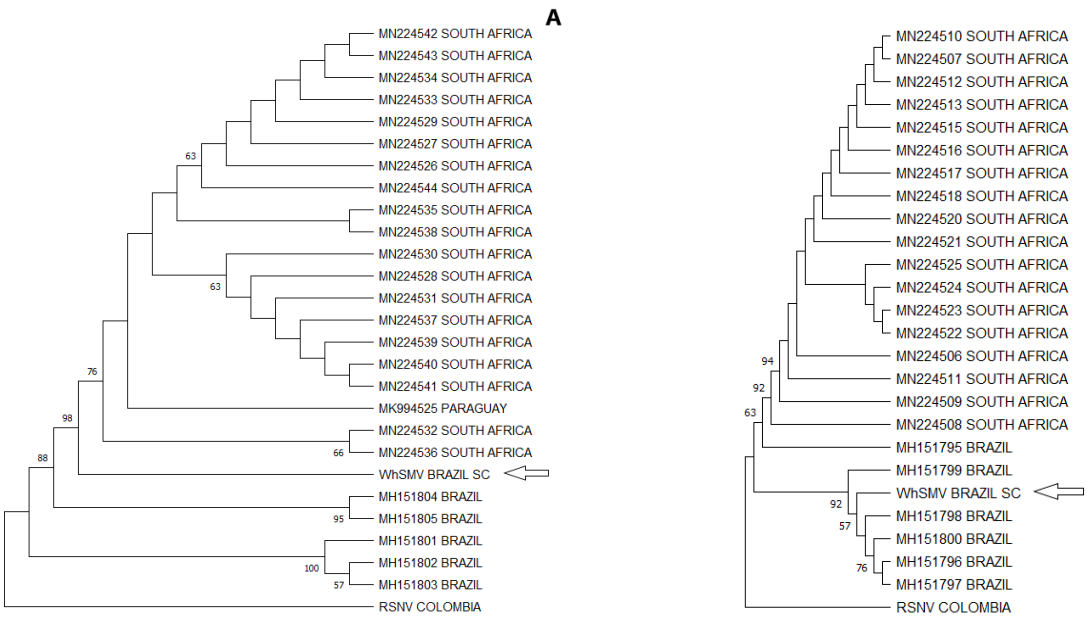


Figure 4. Phylogenetic relationships based on the aligned nucleotide sequences of CP (A) and partial Replicase (B) of the WhSMV isolate from Santa Catarina state and isolates previously characterized using the maximum likelihood method. Numbers on branches indicate bootstrap values. Alignments were performed with muscle. Arrow indicate the isolate characterized in this study.

CONCLUSION

This is the first report on WhSMV associated with SBWMD in Santa Catarina, Brazil. Moreover, the analysis of nucleotide and amino acid identities, and phylogenetic analysis conducted with the isolate characterized in this study, characterized its proximity to other previously characterized Brazilian WhSMV isolates.

ACKNOWLEDGEMENTS

This work was funded by Fundação de Amparo à Pesquisa e Inovação de Santa Catarina and State University of Santa Catarina (FAPESC).

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