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CULTURAL, NUTRITIONAL AND PHYSIOLOGICAL REQUIREMENTS OF COLLETOTRICHUM CURCUMAE CAUSING TURMERIC LEAF SPOT

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ABSTRACT

Turmeric leaf spot caused by *Colletotrichum curcumae* is an important foliar disease, while information on the cultural, nutritional and physiological requirements of the pathogen remains limited. Two representative isolates, CCDI-1 and CCMI-2, were evaluated under in vitro conditions. Ten solid media and five broth media were assessed for radial growth, colony characters and dry mycelial biomass; five carbon and five nitrogen sources were tested in Richard's basal medium; and growth responses were examined at five temperatures (15-35°C) and seven pH levels (4-10). At 9 days after inoculation (DAI), potato dextrose agar supported complete growth (9.00 cm) of both isolates, while oat meal agar supported 9.00 and 8.97 cm growth of CCDI-1 and CCMI-2, respectively. Czapek Dox broth produced the highest biomass (510 and 460 mg). Dextrose was the most favourable carbon source (9.00 and 8.80 cm), whereas urea was the most favourable nitrogen source (8.80 cm in both isolates). CCDI-1 showed maximum growth at 25°C and pH 6, while CCMI-2 showed maximum growth at 30°C and pH 7. Both isolates grew throughout the tested temperature and pH ranges, although growth declined at thermal extremes and under alkaline conditions. The findings identify suitable conditions for routine laboratory cultivation of *C. curcumae* and document quantitative physiological variation between the two isolates.

Key words : Turmeric, *Colletotrichum curcumae*, Culture media, Nutrition, Physiology.

Introduction

Turmeric (*Curcuma longa* L.) is an economically important spice and medicinal crop cultivated throughout tropical and subtropical regions. Foliar diseases caused by *Colletotrichum* spp. reduce functional leaf area and may affect crop performance when weather remains favourable for infection. Leaf spot of turmeric has been associated with different *Colletotrichum* species, including *C. curcumae* and related taxa (Adhipathi *et al.*, 2013, 2014; Li *et al.*, 2016; Guji and Wakjira, 2019; Yadav and Shukla, 2023). Morphological overlap among *Colletotrichum* taxa can complicate routine identification; consequently, standardized cultural observations remain useful for comparative characterization (Damm *et al.*, 2009; Gupta *et al.*, 2017).

Growth characteristics of phytopathogenic fungi are

strongly influenced by the nutritional composition of the substrate and the surrounding physicochemical environment. Culture medium affects colony expansion, pigmentation, sporulation and biomass accumulation, whereas the form of available carbon and nitrogen directly influences metabolic activity. Considerable cultural and physiological variability has been recorded among *Colletotrichum* species and isolates (Hubballi *et al.*, 2011; Marak *et al.*, 2019; Majumdar and Mandal, 2019).

Temperature and pH are additional determinants of fungal growth. Several *Colletotrichum* pathosystems show favourable vegetative growth around 25-30°C and under mildly acidic to neutral conditions, although the precise optimum varies among species and isolates (Shukla *et al.*, 2014; Akhtar *et al.*, 2018; Dev and Somasekhara, 2018). Carbon and nitrogen utilization is

similarly variable (Deshmukh *et al.*, 2012; Vithal *et al.*, 2021). Basic information on these requirements remains comparatively limited for *C. curcumae* from turmeric. Therefore, the present investigation compared two representative isolates of *C. curcumae* for cultural growth, biomass production, carbon and nitrogen utilization, and responses to temperature and pH.

Materials and Methods

Fungal isolates

Two representative isolates of *C. curcumae*, CCDI-1 from Dinahata, Cooch Behar and CCMI-2 from Mondouri, Nadia, West Bengal, India, were used. The isolates were maintained on potato dextrose agar (PDA), and actively growing 7-10-day-old cultures were used as inoculum.

Effect of culture media on radial growth and colony morphology

Ten solid media, viz.; potato dextrose agar (PDA), malt extract agar (MEA), Czapek Dox agar (CZA), oat meal agar (OMA), carrot dextrose agar (CDA), potato carrot agar (PCA), Richard's agar (RA), V8 agar (V8A), Sabouraud agar (SA) and corn meal agar (CMA) were evaluated. Media were prepared according to their respective formulations and autoclaved at 121°C and 15 psi for 15 min. A 5-mm disc from the actively growing margin of a 7–10-day-old culture was placed centrally in each 90-mm Petri plate. Three replicate plates were maintained for each medium and isolate and incubated at 25 ± 2°C. Colony diameter was recorded at 24-h intervals up to 9 DAI; pigmentation, texture, zonation and colony margin were also recorded.

Biomass production in broth media

Potato dextrose broth, malt extract broth, Czapek Dox broth, potato carrot broth and carrot dextrose broth were evaluated. Each flask contained 80 ml of the respective medium and was inoculated with a 5-mm mycelial disc from a 7-day-old culture. Three replicate flasks were maintained for each broth medium and isolate and incubated at 27 ± 1°C. After incubation, the mycelium was recovered by filtration through pre-weighed, oven-dried filter paper, dried at 60°C to constant weight, and weighed. Dry mycelial biomass was calculated after subtracting the weight of the filter paper.

Carbon and nitrogen utilization

Carbon utilization was evaluated in Richard's agar by replacing the sucrose carbon with equivalent amounts of mannitol, maltose, dextrose, lactose or sucrose on an equivalent-carbon basis; a carbon-free medium served as control. Nitrogen utilization was evaluated by replacing

the nitrogen supplied by potassium nitrate with equivalent nitrogen from sodium nitrate, ammonium sulphate, potassium nitrate, urea or ammonium nitrate; a nitrogen-free medium served as control. A 5-mm mycelial disc was placed centrally in each 90-mm Petri plate. Three replications were maintained for each treatment and isolate at 27 ± 1°C and radial growth was recorded at 24-h intervals up to 9 DAI.

Effect of temperature and pH

Temperature response was assessed on PDA at 15, 20, 25, 30 and 35°C in BOD incubators. For the pH experiment, PDA was adjusted to pH 4, 5, 6, 7, 8, 9 and 10 using 0.1 N HCl or 0.1 N NaOH and checked with a digital pH meter before sterilization. The media were autoclaved at 121°C and 15 psi for 15 min. A 5-mm mycelial disc was placed centrally in each plate, three replications were maintained for each treatment and isolate and colony diameter was recorded at 24-h intervals up to 9 DAI.

Statistical analysis

All laboratory experiments were conducted in a completely randomized design (CRD) with three replications. Data from each isolate were analysed separately by analysis of variance (ANOVA). Treatment means were compared using the critical difference (CD) at $P = 0.05$. Standard error of the mean [SEm (±)] and coefficient of variation [CV (%)] were calculated.

Results and Discussion

Effect of culture media on radial growth and colony morphology

Radial growth differed significantly among the ten solid media. At 9 DAI, CCDI-1 attained complete growth on PDA and OMA (9.00 cm), followed by MEA and CDA (8.90 cm). CCMI-2 showed complete growth on PDA (9.00 cm), followed closely by OMA (8.97 cm), SA (8.93 cm) and MEA (8.83 cm). V8A supported the lowest growth of CCMI-2 (7.30 cm), while CMA recorded the lowest value for CCDI-1 (7.70 cm) (Table 1, Fig. 1).

The pronounced medium effect was also evident in colony pigmentation, zonation, texture and margin (Plate 1). Nutrient-rich natural or semi-synthetic media such as PDA and OMA probably favoured rapid vegetative expansion because they provide readily utilizable carbohydrates together with complex nutrients. Hubballi *et al.* (2011), Akhtar *et al.* (2018) and Majumdar and Mandal (2019) similarly reported strong growth of *Colletotrichum* spp. on PDA, while other studies have recorded favourable growth on OMA. The differences observed between CCDI-1 and CCMI-2 indicate

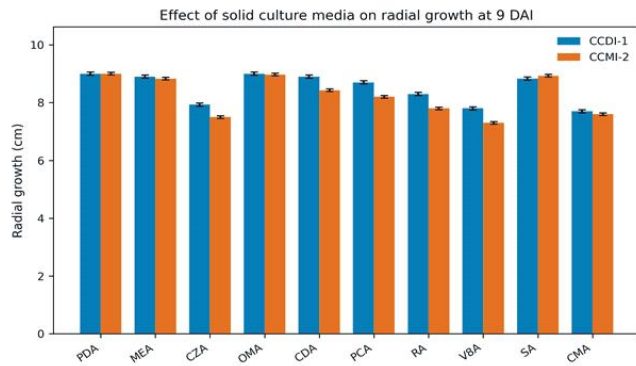


Fig. 1 : Effect of culture media on radial growth of *C. curcumae* isolates at 9 DAI.

Table 1 : Effect of different culture media on radial growth (cm) of *Colletotrichum curcumae* isolates at 9 DAI.

Growth medium	CCDI-1	CCMI-2
PDA	9.00	9.00
MEA	8.90	8.83
CZA	7.93	7.50
OMA	9.00	8.97
CDA	8.90	8.43
PCA	8.70	8.20
RA	8.30	7.80
V8A	7.80	7.30
SA	8.83	8.93
CMA	7.70	7.60
SEm(±)	0.055	0.045
CD (0.05)	0.164	0.135
CV (%)	1.136	0.963

PDA = potato dextrose agar; MEA = malt extract agar; CZA = Czapek Dox agar; OMA = oat meal agar; CDA = carrot dextrose agar; PCA = potato carrot agar; RA = Richard's agar; V8A = V8 agar; SA = Sabouraud agar; CMA = corn meal agar.

quantitative physiological variability, although the present analysis does not test the isolates directly against each other.

Biomass production in broth media

Czapek Dox broth supported the maximum dry mycelial biomass, recording 510 mg in CCDI-1 and 460 mg in CCMI-2. Carrot dextrose broth ranked second, while potato carrot broth produced the least biomass in both isolates (Table 2). The result indicates that rapid radial expansion on solid medium and total biomass accumulation in liquid medium are distinct growth responses.

Effect of carbon sources

Dextrose was the most favourable carbon source for both isolates, producing 9.00 cm growth in CCDI-1 and 8.80 cm in CCMI-2 at 9 DAI. Maltose and sucrose

Table 2 : Effect of different broth media on dry mycelial biomass of *Colletotrichum curcumae* isolates.

Broth medium	CCDI-1 (mg)	CCMI-2 (mg)
Potato dextrose broth	255	270
Malt extract broth	305	295
Czapek Dox broth	510	460
Potato carrot broth	195	188
Carrot dextrose broth	345	355
SEm(±)	7.920	2.788
CD (0.05)	24.957	8.787
CV (%)	4.260	1.542

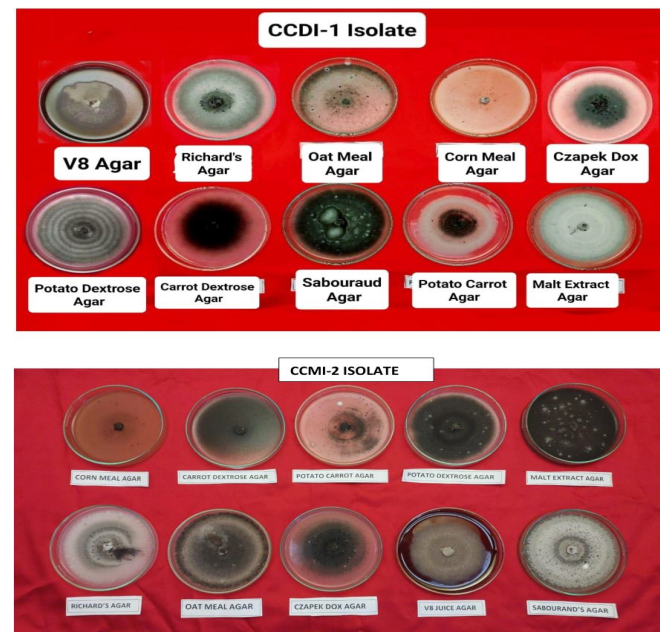


Plate 1 : Colony morphology of CCDI-1 and CCMI-2 isolates of *C. curcumae* on different solid media.

also supported high growth, whereas the carbon-free control recorded the lowest values (6.43 and 6.31 cm) (Table 3, Fig. 2). The significant reduction in the control confirms the importance of an available carbon source for vigorous mycelial development.

Dextrose is a readily assimilable sugar and can enter central carbon metabolism with comparatively little biochemical conversion, which may explain its favourable effect. However, nutritional preference differs among *Colletotrichum* spp. Deshmukh *et al.* (2012) reported strong utilization of other carbon substrates by *C. gloeosporioides*, whereas Vithal *et al.* (2021) observed isolate-dependent responses in *C. capsici*. Thus, the preference recorded here should be regarded as a characteristic of the tested *C. curcumae* isolates.

Effect of nitrogen sources

Urea supported the highest radial growth of both isolates (8.80 cm), followed by nitrate sources. Ammonium

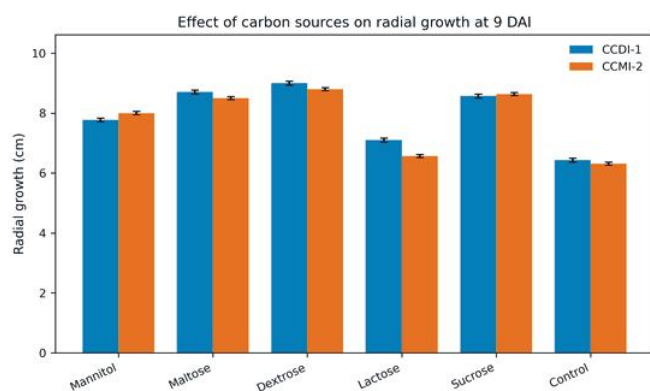


Fig. 2 : Effect of carbon sources on radial growth of *C. curcumae* isolates at 9 DAI.

Table 3 : Effect of different carbon sources on radial growth (cm) of *Colletotrichum curcumae* isolates at 9 DAI.

Carbon source	CCDI-1	CCMI-2
Mannitol	7.77	8.00
Maltose	8.70	8.50
Dextrose	9.00	8.80
Lactose	7.10	6.57
Sucrose	8.57	8.63
Control	6.43	6.31
SEm(±)	0.066	0.053
CD (0.05)	0.205	0.164
CV (%)	1.450	1.179

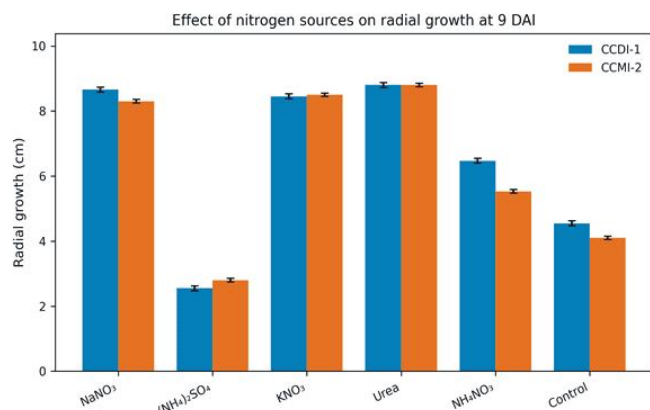


Fig. 3 : Effect of nitrogen sources on radial growth of *C. curcumae* isolates at 9 DAI.

sulphate was distinctly unfavourable, restricting growth to 2.55 cm in CCDI-1 and 2.80 cm in CCMI-2 (Table 4, Fig. 3). The low growth in the nitrogen-free control further demonstrated the importance of nitrogen nutrition.

The response suggests efficient utilization of urea and nitrate nitrogen, whereas ammonium sulphate may have altered the nutritional or ionic environment unfavourably. Deshmukh *et al.* (2012) reported potassium nitrate as favourable for *C. gloeosporioides*, while Vithal *et al.* (2021) observed good growth of several *C. capsici*

Table 4 : Effect of different nitrogen sources on radial growth (cm) of *Colletotrichum curcumae* isolates at 9 DAI.

Nitrogen source	CCDI-1	CCMI-2
Sodium nitrate	8.66	8.30
Ammonium sulphate	2.55	2.80
Potassium nitrate	8.45	8.50
Urea	8.80	8.80
Ammonium nitrate	6.47	5.53
Control	4.55	4.10
SEm(±)	0.076	0.054
CD (0.05)	0.234	0.167
CV (%)	1.999	1.487

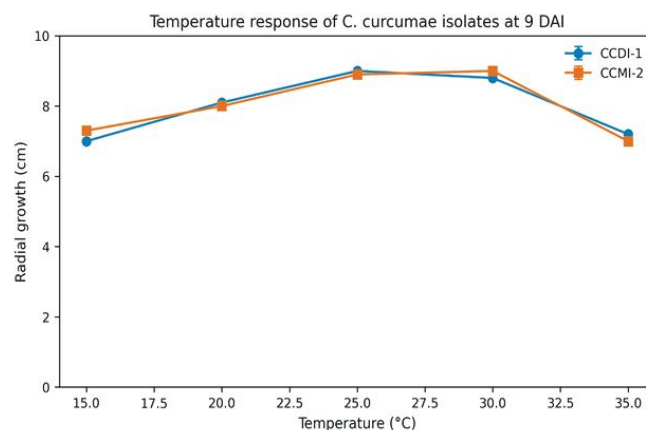


Fig. 4 : Effect of temperature on radial growth of *C. curcumae* isolates at 9 DAI.

Table 5 : Effect of different temperature regimes on radial growth (cm) of *Colletotrichum curcumae* isolates at 9 DAI.

Temperature (°C)	CCDI-1	CCMI-2
15	7.00	7.30
20	8.10	8.00
25	9.00	8.90
30	8.80	9.00
35	7.20	7.00
SEm(±)	0.055	0.044
CD (0.05)	0.175	0.140
CV (%)	1.190	0.960

isolates with urea, emphasizing that nitrogen preference is not uniform within the genus.

Effect of temperature

Temperature markedly affected radial growth. CCDI-1 reached maximum growth at 25°C (9.00 cm), while CCMI-2 reached maximum growth at 30°C (9.00 cm). Growth declined at 15°C and 35°C in both isolates (Table 5, Fig. 4, Plate 2). Thus, the most favourable thermal range was 25-30°C.

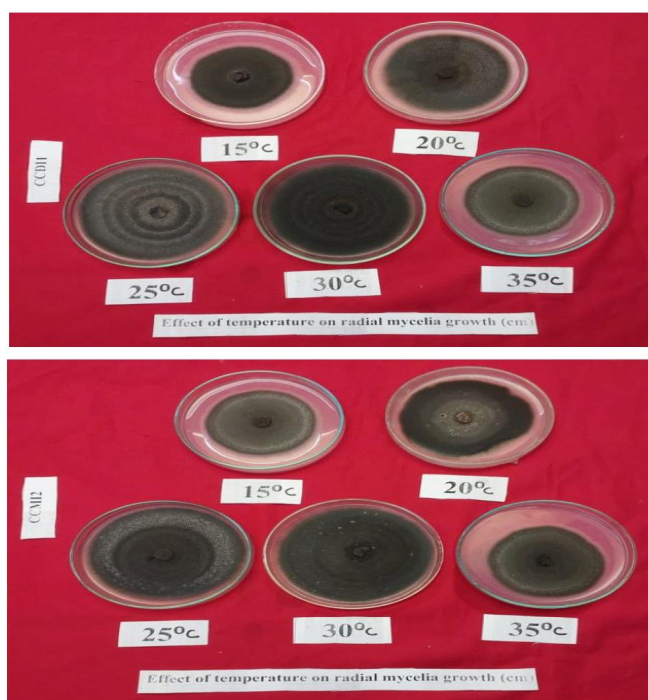


Plate 2 : Radial growth of CCDI-1 and CCMI-2 isolates of *C. curcumae* under different temperature regimes.

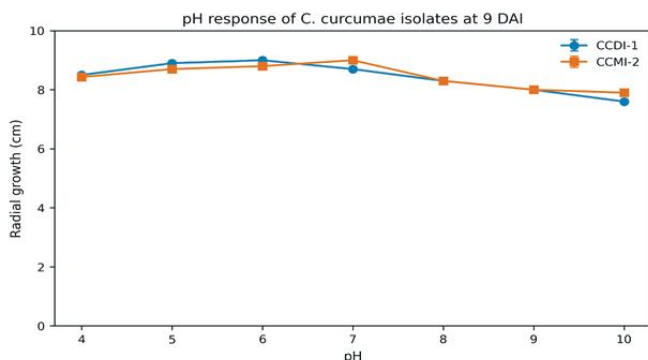


Fig. 5 : Effect of pH on radial growth of *C. curcumae* isolates at 9 DAI.

The optimum range agrees with the general thermal response reported for several *Colletotrichum* species. Hubballi *et al.* (2011) and Shukla *et al.* (2014) recorded favourable growth between 25 and 30°C, while Akhtar *et al.* (2018) reported maximum growth of *C. capsici* at 30°C. The small difference in numerical optimum between the present isolates represents quantitative physiological variation and should not be interpreted as geographic adaptation without a larger isolate population.

Effect of pH

Both isolates grew over a broad pH range. CCDI-1 recorded maximum growth at pH 6 (9.00 cm), whereas CCMI-2 recorded maximum growth at pH 7 (9.00 cm). Growth progressively declined toward alkaline conditions, reaching 7.60 and 7.90 cm at pH 10 (Table 6, Fig. 5, Plate 3).

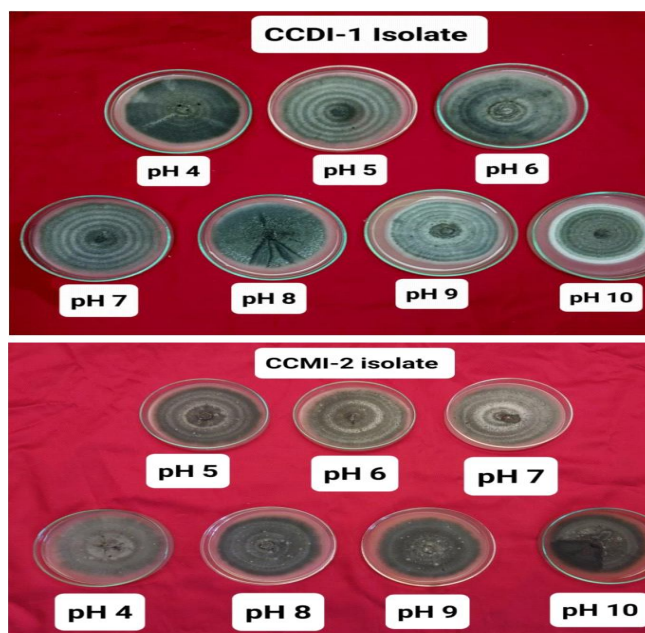


Plate 3 : Radial growth of CCDI-1 and CCMI-2 isolates of *C. curcumae* at different pH levels.

Table 6 : Effect of different pH levels on radial growth (cm) of *Colletotrichum curcumae* isolates at 9 DAI.

pH	CCDI-1	CCMI-2
4	8.50	8.43
5	8.90	8.70
6	9.00	8.80
7	8.70	9.00
8	8.30	8.30
9	8.00	8.00
10	7.60	7.90
SEm(±)	0.058	0.053
CD (0.05)	0.178	0.162
CV (%)	1.205	1.092

The results indicate that mildly acidic to neutral conditions are most favourable, although both isolates tolerated pH 4-10. Comparable optima around pH 6-7 have been reported for *C. gloeosporioides* and *C. truncatum* (Hubballi *et al.*, 2011; Shukla *et al.*, 2014; Dev and Somasekhara, 2018). Changes in pH can influence nutrient availability, membrane transport and extracellular enzyme activity, which collectively affect fungal growth.

Conclusion

The cultural, nutritional and physiological behaviour of *C. curcumae* was strongly influenced by growth substrate, nutrient source, temperature and pH. PDA and OMA supported the most rapid radial growth, whereas Czapek Dox broth produced the highest dry biomass. Dextrose and urea were the most favourable carbon and

nitrogen sources, respectively. The optimum temperature range was 25-30°C, while maximum growth occurred under mildly acidic to neutral conditions (pH 6-7). The two isolates showed broadly similar biological requirements but differed quantitatively in their response to some treatments. These findings provide practical conditions for routine laboratory cultivation and comparative studies of *C. curcuma*.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this study.

Generative AI disclosure

Generative AI disclosure: ChatGPT (OpenAI) was used during manuscript preparation for language editing and improvement of organization and presentation. The authors independently reviewed and verified the scientific content, data, citations and interpretations and take full responsibility for the final manuscript.

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