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Cultural, Morphological and Pathogenic Variability in *Alternaria cyamopsidis* Causing Alternaria Blight of Clusterbean in Rajasthan

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ABSTRACT

Background: *Alternaria cyamopsidis* (Rang. and Rao) causes Alternaria blight of clusterbean and it is one of the significant disease of clusterbean. Studies were conducted to compare the Cultural, morphological and pathogenic variability among ten isolates of *Alternaria cyamopsidis* from clusterbean, in five districts of Rajasthan *viz.,* Bikaner, Barmer, Churu, Hanumangarh and Jaipur.

Methods: During 2016-17 exhaustive survey was conducted in clusterbean growing areas of Rajasthan and collected diseased samples of clusterbean caused by *Alternaria.* All the samples were processed for isolation, purification, and their pathogenicity was proved in cagehouse and laboratory and standard methods were adopted for cultural and morphological variability study.

Result: All the isolates showed variation in their morphological characters, *i.e.*, colony color and shape; conidial number, size, width, length, shape and septation on PDA. Out of ten isolates two isolate, *viz*., AlcyJp1 and AlcyJp2 showed maximum colony diameter 89.50 and 86.30 mm, respectively. All the isolates varied in their spore length and width and virulent on the tested variety of clusterbean for virulence. AlcyJp1 was the most virulent and produced maximum (65.50%) disease intensity, followed by AlcyJp2 isolate (61.22%).

Key words: Alternaria blight, *Alternaria cyamopsidis,* Clusterbean, Morphological, Pathogenic variability.

INTRODUCTION

In India, pulses have been described as a 'Poor man's meat and rich man's vegetable.' Clusterbean [*Cyamopsis tetragonaloba* (L.) Taub.], commonly called "Guar," is an important arid legume crop. Clusterbean is being grown in India since ancient times. However, it is believed to be of African origin (Vavilov, 1951). Traditionally, clusterbean is produced for different purposes *viz*., vegetable (pods), green fodder, green manure, straw and seed production.

Tendergreen guar pods are an essential source of nutrition for human beings and animals. India's contribution is around 75-80 per cent ofthe world's total production, followed by Pakistan and others (Tripathy and Das, 2013). India stands in the first position in the world concerning the area and production of guar.In India, this crop is mainly grown in the states of Rajasthan, Haryana, Gujarat, Punjab, U.P. and M.P. In Rajasthan, it is primarily grown in Bikaner, Jaisalmer, Barmer, Churu, Hanumangarh, Sriganganagar, Jodhpur, Nagaur, Sikar, Jhunjhunu and Jaipur districts. The production of clusterbean in terms of grain and fodder is limited primarily due to the Alternaria blight disease of clusterbean in Northern India and Rajasthan (Rangaswami and Rao, 1957; Meena *et al.,* 2012). The maximum severity of the blight takes place between bloom and pod set. Higher yield losses (43-78%) were recorded when leaves were infected at the seedling stage than at the old stage (Sharma, 1981 and Anonymous, 2013-14). Very little information on morphological and pathogenic variability in *Alternaria cyamopsidis* of clusterbean is available from India. Variability studies give an idea about pathogenic variability among *Alternaria cyamopsidis* and such information will be helpful to develop resistant cultivars to the disease. Keeping in view of the variations in disease intensity of different areas, studies have been conducted to ascertain the cultural, morphological and pathogenic variations among different isolates of *A. cyamopsidis* from different regions of Rajasthan.

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MATERIALS AND METHODS

Survey and collection

Survey of major clusterbean growing areas of Bikaner, Hanumangarh, Barmer, Churu and Jaipurdistricts of Rajasthan was conducted at the pod formation stage of the crop to record *Alternaria* blight intensity and to collect disease samples. The diseased samples collected during the survey were brought to the laboratory in paper bags for further studies.

Isolation, purification and identification

To ascertain the variability among the isolates of *A. cyamopsidis,* cultural and morphological studies were conducted on potato dextrose agar (PDA) medium. Isolations were made from the infected plants showing typical symptoms (Concentric rings) of Alternaria blight. Ten isolates of *A. cyamopsidis* isolated from clusterbean leaves, collected from different locations of Bikaner, Barmer, Churu, Hanumangarh, and Jaipur districts of Rajasthan and designated as AlcyBk (*A. cyamopsidis* Bikaner isolate), AlcyBr (*A. cyamopsidis* Barmer isolate), AlcyCh (*A. cyamopsidis* Churu isolate), AlcyHg (*A. cyamopsidis* Hanumangarh isolate) and AlcyJp (*A. cyamopsidis* Jaipur isolate), respectively. Small pieces of the leaves of the clusterbean plant were cut from the diseased portion along with some healthy tissues, surface-sterilized for 1-2 minutes in 1.0 per cent Sodium hypochlorite solution with three piles of washing by sterilized distilled water. These bits were transferred aseptically to potato dextrose agar in Petri plates separately. Incubation was done at 25±1°C for seven days. Sub-culturing from uncontaminated peripheral growth was made on PDA slants.

For the purification of the fungus, a single spore isolation technique was used. After sporulation, the conidial suspension was made in sterile water. The dilution was adjusted so that in one loop full, 20-25 conidia could be counted under the low power objective. One such loop full was mixed with 20 ml melted and sterilized agar (2%) and poured in sterile Petri plates. After 12 hours of incubation at 25±1°C, the single germinating conidium was cut with the help of a dummy objective and transferred to PDA slants. They were subsequently allowed to grow and sporulate. Monoconidial culture established in this way was maintained by periodical transfer on PDA slants. After purification, the fungus was allowed to sporulate. The pure sporulating culture was identified based on morphological characters and literature basis.

Cultural and morphological variability

Single spore cultures of different isolates established and maintained (Table 1) on potato dextrose agar (PDA) were studied for their cultural and morphological characters. Seven days old culture of each isolate was inoculated (5 mm diameter disc) separately on PDA and incubated at 25±1°C. After seven days of incubation, radial growth of fungal mycelium, colony characters of each isolate, such as colony growth and colony colour. The measurements of the size (length and width) of conidia and septation were taken with the help of fluorescence microscopy.

Pathogenic variability

To test the pathogenic variability among ten isolates, apparently healthy surface-sterilized cluster bean seeds (Variety RGC-936) were taken. Seeds were sown at five cm depth in micro plots with three replications of each isolate. Spore suspension of each isolate was prepared in sterilized distilled water separately by blending seven days old fungal culture in pestle and mortar and filtered through cheesecloth, spore suspension was further diluted to $1x10^5$ spores/ml and 20 days old clusterbean plant were separately inoculated with each isolate and observations on disease intensity were recorded after 20 days of inoculation as per 0-5 rating scale (Table 2 and Plate 1). Randomly selected ten plants from each field were rated as per the following description and per cent disease intensity (PDI) on foliage was calculated using the formula of McKinney (1923) and Meena *et al.* (2012).

Alternaria blight disease intensity (%) =

$$\frac{\text{Sum of all numerical ratings}}{\text{Total number of plants observed} \times \text{Maximum disease grade}} \times 100$$

RESULTS AND DISCUSSION

Survey and collection

A roving survey was conducted during *Kharif* 2016-17 in five clusterbean growing districts of Rajasthan *viz.,* Bikaner, Barmer, Churu, Hanumangarh and Jaipur. The survey was carried out in two villages from one tehsil of each district. During the survey, discussions were held with the farmers concerned about the disease's occurrence and incidence. During the survey,the disease intensity of Alternaria blight of clusterbean was recorded from 21.17 to 40.17 per cent in all surveyed areas of Rajasthan.

Isolation, purification and identification

Isolation of the pathogen from diseased plants of clusterbean collected from farmers' fields was done on potato dextrose agar (PDA) medium. After seven days of incubation at 25±1°C, the growth of the fungus was obtained. Pure culture of the pathogen, obtained by single sporing on water agar, yielded *Alternaria cyamopsidis*. The colonies were profused, dark-blackish brown or greyish black velvety. Hyphae were branched, septate, hyaline at first, later turn brown-black to olivaceous - black or brown in colour. Hypha is 2.81 to 4.45 µ width. Conidiophores were arising in groups, greyish olive in color, septate, geniculate, and prominent scar at each geniculation.

Table 1: The coding for each isolates were given as under.

District	Tehsil	Village	Isolate no.
Bikaner	Bikaner	Palana	AlcyBk1
Bikaner	Bikaner	Rashisar	AlcyBK2
Barmer	Pachpadra	Budhiwara	AlcyBr1
Barmer	Pachpadra	Jhagsa	AlcyBr2
Churu	Rajgarh	Daderwa	AlcyCh1
Churu	Rajgarh	Chandkhoti	AlcyCH2
Hanumangarh	Bhadra	Bhadra	AlcyHg1
Hanumangarh	Bhadra	Gogamedi	AlcyHg2
Jaipur	Phulera	Tyod	AlcyJp1
Jaipur	Phulera	Khatwadi	AlcyJp2

Conidia were smooth to echinulated, greyish olive coloured, straight, some time with a curved beak, obclavate, borne singly or in chains of upto three conidia, muriform with measuring length 95.433 - 210.791 µ (Av. 173.402 µ) x width 16.135 - 26.543 (Av. 20.682 µ). The conidial body tapers abruptly to the junction with the beak. The conidial beak is filiform, septate, lighter in colour as compared to the conidial body, 0-3 septate. The beaks were usually long, varying ½ to 1 ½ times the length of conidium. The beak is bulbous at the base and rapidly narrowing towards the apex. On the basis of measurement and other morphological characters with the help of fluorescence microscopy and

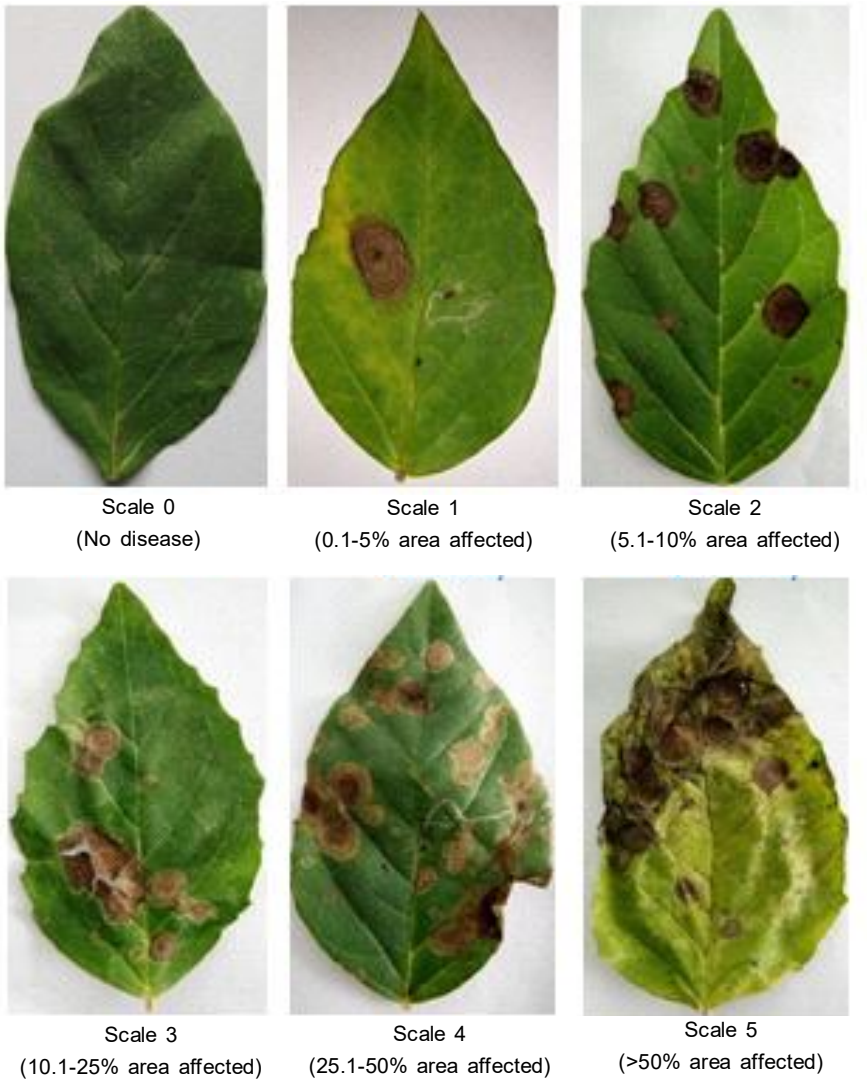

Plate 1: Alternaria blight disease rating scale on clusterbean.

Table 2: Alternaria blight disease rating scale on clusterbean.

Disease rating /grade	Per cent leaf area affected	Description
0	-	Healthy
1	0.1-5.0	One or two scattered lesion or spots on leaves
2	5.1-10.0	moderate number of lesions or spots on leaves
3	10.1-25.0	Moderate infection, abundant lesions or spots on leaves
4	25.1-50.0	Heavy infection, abundant lesions or spots on leaves
5	>50.0	Very heavy infection, lesions or spots abundant on leaves, plant prematury, dry or killed by the disease

based on literature, the pathogen was identified as *Alternaria cyamopsidis* Rang. and (Rangaswami and Rao, 1957 and Orellana and Simmons, 1965). Similarly, isolation of pathogen on PDA was reported by Singh and Prasad (1973). The cultural characters of *Alternaria cyamopsidis* normal longitudinal and transverse septa. The findings are in confirmation with those of Rangaswami and Rao (1957) and Orellana and Simmons (1965).

Cultural and morphologicalvariability

The cultural characteristics such as shape, colour and size of the colony and morphological features such as the size of conidia and septation were recorded for different isolates of *A. cyamopsidis* by growing them on PDA medium.

The results showed that isolates of *A. cyamopsidis* differ in their colony characters, colony colour and colony diameter (Table 3). Out of ten isolates, AlcyJp1 (collected from Tyod village of Phulera tehsil of Jaipur district) showed maximum colony diameter (89.50 followed by AlcyJp2 (86.30 mm), AlcyCh1 (85.60 mm), AlcyHg2 (83.50 mm), AlcyHg1 (82.30 mm), AlcyCh2 (80.10 mm), AlcyBr1 (76.30 mm), AlcyBk1 (74.50 mm) and AlcyBk2 (65.80 mm) while isolate AlcyBr2 showed minimum colony diameter (60.50 mm) after seven days of incubation.Isolate AlcyJp1 showed greyish white colony colour, fluffy white growth with a smooth greyish margin. Isolate AlcyJp2 showed gray center, blackish gray velvety growth with smooth grayish margin but isolate AlcyBr2 showed grayish centre, white, black cottony growth with the white periphery.

Morphological observations of each isolate revealed that all the isolates vary in their spore length and width. In general, spore length and width were found in between 95.433 to 210.791 µ and 16.135 to 26.543 µ, respectively. In contrast, the number of horizontal and vertical septa varied between 6 to 8 and 2 to 5, respectively (Table 2). The AlcyJp1 isolate showed maximum length and width of conidia 210.791 µ and 26.543 µ, respectively.whereas isolate AlcyBk2 showed minimum length and width of conidia 95.433 µ and 16.135 µ, respectively.

The maximum number of horizontal septa (8) were observed in AlcyJp1, AlcyJp2, AlcyHg1, AlcyHg2 and AlcyCh2 isolates and minimum number in AlcyBk2 and AlcyCh1 isolates, respectively. Vertical septa were maximum (5) in AlcyJp1 isolate and minimum (2) in AlcyBk1, AlcyBk2, AlcyBr1 and AlcyCh1 isolates.

Pathogenic variability

Results presented in Table 4 revealed that all the isolates were pathogenic to clusterbean and produced characteristic symptoms of the disease. The mean per cent disease intensity was highest (63.38%) with Jaipur isolates followed by Hanumangarh isolates (56.50%), Barmer isolates (44.52%) and Churu isolates (38.74%). The minimum mean per cent disease intensity was in Bikaner (36.51%) isolates. The overall mean disease intensity of the five districts was 47.93 per cent.

Table 3: Cultural and morphological variability of different isolates of *Alternaria cymopsidis*.

Isolate no.	Colony characters	Colony diameter (mm)	Length × width of conidia (µ)	Septation	
				Horizontal	Vertical
AlcyBk1	Grey and black centre, greyish growth with black white round periphery	74.50	163.019 × 19.113	7	2
AlcyBk2	Greenish white centre, greenish grey velvety growth with white smooth margin	65.80	95.433 × 16.135	6	2
AlcyBr1	Greenish white centre, velvety growth with white periphery	76.30	174.028 × 16.809	7	2
AlcyBr2	Greyish centre, white black cottony growth with white periphery	60.50	195.328 × 21.708	7	3
AlcyCh1	Dark black centre, greyish black growth with greenish periphery	85.60	143.051 × 20.511	6	2
AlcyCh2	White black centre, greyish black growth with white round periphery	80.10	175.416 × 17.835	8	3
AlcyHg1	Dark black centre, greyish white cottony growth with irregular margin	82.30	195.713 × 21.633	8	4
AlcyHg2	Black centre with dark black cottony growth with irregular margin	83.50	180.064 × 22.518	8	3
AlcyJp1	Dark black centre, greyish white fluffy growth with greyish smooth margin	89.50	210.791 × 26.543	8	5
AlcyJp2	Grey centre, blackish grey velvety growth with greyish smooth margin	86.30	201.113 × 24.015	8	4

Among these isolates AlcyJp1 was found to be most virulent and produced maximum (65.50%) disease intensity followed by AlcyJp2 isolate (61.22%), AlcyHg1 isolate (58.66%), AlcyHg2 isolate (54.33%), AlcyBr2 isolate (46.71%), AlcyBr1 isolate (42.33%), AlcyCh2 isolate (40.31%), AlcyBk1 isolate (38.33%) and AlcyCh1 isolate (37.17%) whereas AlcyBk2 isolate was found less virulent with minimum disease intensity (34.69%).

Phenotypic, Cultural and pathogenic variability among isolates of different species of *Alternaria* has also been reported by several workers (Sharma and Pandey, 2012, Shekhawat*et al.,* 2013, Meena *et al.,* 2014, Singh *et al.,* 2014and Nikam*et al*., 2015).

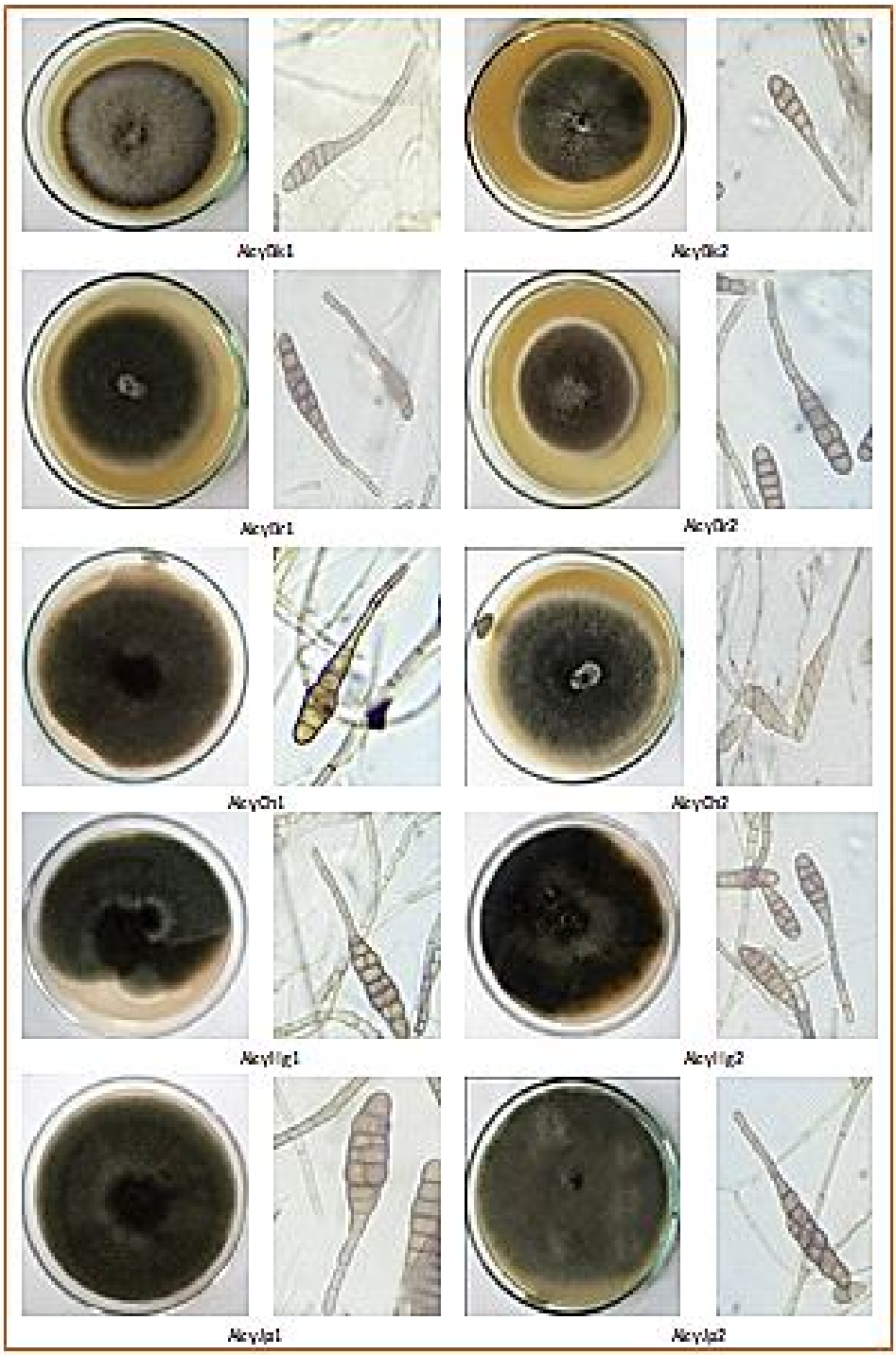

Plate 2: Cultural amd morphological variability of different isolates of *Alternaria cyamopsidis*.

Table 4: Pathogenic variability of different isolates of *Alternaria. cymopsidis.*

Isolate No.	District	Tehsil	Village	Disease intensity (%)
AlcyBk1	Bikaner	Bikaner	Palana	38.33
AlcyBk2	Bikaner	Bikaner	Rashisar	34.69
			Mean	**36.51**
AlcyBr1	Barmer	Pachpadra	Budhiwara	42.33
AlcyBr2	Barmer	Pachpadra	Jhagsa	46.71
			Mean	**44.52**
AlcyCh1	Churu	Rajgarh	Daderwa	37.17
AlcyCh2	Churu	Rajgarh	Chandkhoti	40.31
			Mean	**38.74**
AlcyHg1	Hanumangarh	Bhadra	Bhadra	58.66
AlcyHg2	Hanumangarh	Bhadra	Gogamedi	54.33
			Mean	**56.50**
AlcyJp1	Jaipur	Phulera	Tyod	65.50
AlcyJp2	Jaipur	Phulera	Khatwadi	61.22
			Mean	**63.38**
			Overall mean	**47.93**

CONCLUSION

The results on cultural, morphological and pathogenic variability among different isolates of *A. cyamposidis*, collected from various locations in five districts of Rajasthan, showed that isolates differed in their colony characters, diameter and septation.

Isolate (AlcyJp1) from Jaipur district showed maximum mycelial growth (89.50 mm), maximum length x width of conidia (210.791X26.543 µ),the diameter of conidia LxW (5595.026 µ), top horizontal (8) and vertical (5) septa. It was found most virulent and produced maximum (65.50%) disease intensity.

Isolate (AlcyBr2) from the Barmer district showed minimum mycelial growth with a colony diameter of 60.50 mm. In general conidial length and width were observed in between 95.433 to 210.791 µ and 16.135 to 26.543 µ, respectively. All the isolates were found pathogenic to clusterbean and produced characteristic symptoms of the disease. Among these isolates, AlcyJp1 was found to be most virulent and had maximum (65.50%) disease intensity, followed by AlcyJp2 isolate (61.22%). The highest mean per cent disease intensity was with AlcyJp1 isolate (63.38%) and lowest with $AlcyBk_2$ (34.69%).

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