

Evaluation of Drought Tolerance of the Cumin (*Cuminum cyminum* **L.) Ecotypes in Kerman Province**

E. Moslemi ^a, M.M .Akbarian ^{[a*](#page-0-0)},S. Z. Ravari ^b,M. R. Yavarzadeh ^a, N. Modafeh-Behzadi^a

a. Department of Agriculture, College of Agronomy, Bam Branch, Islamic Azad University, Bam, Iran.

b.Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization (AREEO), Kerman, Iran

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^{*}Corresponding Author: M. M. Akbarian *E-mail address*: akbarian@iaubam.ac.ir

1- Introduction

Due to the side effects of chemical drugs, medicinal plants and natural compounds can be considered suitable alternatives. Consequently, medicinal plants are widely used annually by a significant portion of the population, especially in developing countries (Rasool et al., 2020; Ekor, 2014). These plants are extensively employed in disease treatment due to their synthesis of diverse secondary metabolites (Sureshkumar et al., 2017; Tuttolomondo et al., 2014).

Cumin (*Cuminum cyminum*), an herbaceous annual plant belonging to the Apiaceae family, is one of Iran's most important domesticated medicinal plants. It has broad applications in pharmaceutical, food, cosmetic, and hygiene industries (Ebrahimiyan et al., 2017). Due to its short growth period, low water demand, and high economic value, cumin has been integrated into the cultivation patterns of arid and semi-arid regions (Afshar Karimi et al., 2014). For example, in 2015, Razavi province cultivated 8,100 hectares of irrigated cumin, producing 5,500 tons, and 4,000 hectares of rain-fed cumin, yielding 1,000 tons.

Native to central and southern Asia, cumin is cultivated in countries such as India, Pakistan, Turkey, Iran, and Spain. In Iran, it is grown both as irrigated and rain-fed crops in provinces like South Khorasan, Razavi Khorasan, North Khorasan, Semnan, Yazd, Kerman, Markazi, East Azerbaijan, and Sistan-Baluchestan. Cumin requires appropriate temperatures and sufficient light during its growth. Essential oil content is higher in plants grown in warm, sunny regions. During flowering and fruit development, the plant needs less moisture.

Cumin thrives in medium-textured soils, particularly sandy loam soils. Poor, nutrient-deficient sandy soils are unsuitable as they increase susceptibility to fungal diseases. The ideal soil pH for cumin cultivation ranges from 5.4 to 8.2 (Eswar and Qureshi, 2010). The essential oil content of cumin seeds varies by ecotype: 2.33% in Indian, 1.45% in Iranian, 3.8% in Chinese, and 3.5% in Bulgarian types, influenced by genetic, geographical, and climatic diversity (Saiednia and Gohari, 2011; Hajlaoui et al., 2010).

Drought stress is a major environmental constraint affecting agricultural production, particularly in arid and semi-arid regions (Blum, 2011). It disrupts the balance between reactive oxygen species production and antioxidant defense activities, leading to oxidative stress and reduced plant performance (Soares et al., 2018). The essential oil of cumin has antifungal and insecticidal properties, such as inhibiting aflatoxin production (Khosravi and Haghighi Minooeian, 2014) and controlling storage pests like flour beetles (Khodadoost et al., 2012).

Research on the Apiaceae family indicates that yield components like the number of umbels per plant, seeds per umbel, and thousand-seed weight significantly influence yield (Ehsanipour et al., 2013). Among these, the number of umbels per plant is a critical yield component due to its direct correlation with seed production (Afshar et al., 2016). Ahmadian et al. (2011) reported that drought stress

significantly impacts seed yield, the number of umbels per plant, and seed weight in cumin.

Despite cumin's economic, medicinal, and agricultural significance, the response of medicinal and aromatic plants to water scarcity remains underexplored. Optimizing water use in arid and semi-arid areas can expand cultivation, increase production and farmers' income, and reduce migration from these regions.

 This study aimed to evaluate the combined performance of commonly cultivated cumin ecotypes under drought stress during flowering, assess their responses, and identify the most drought-tolerant ecotypes under Kerman's climatic conditions.

2- Materials and Methods:

 Seeds from five cumin ecotypes were collected from medicinal plant research centers across cumin-producing regions. The study was conducted at the Shahid Zendehrooh Agricultural Research and Natural Resources Station, located 18 km south of Kerman Province. The station is characterized by an average annual rainfall of 150 mm, an elevation of 1,756 meters, and geographical coordinates of 30°01' E and 57°06' N. The experiments were performed in the 1399–1400 and 1400–1401 cropping years.

 Soil samples were collected from a depth of 0–30 cm before land preparation in both years. These samples were analyzed for physical and chemical properties in the soil science laboratory of the Faculty of Agriculture, as shown in Table 1.

Table1.Some physical and chemical characteristics of the farm

soil								
ω Soil Textur	$N($ % $)$	$\left(\frac{\text{m}}{\text{s}}\text{N}\text{g} \right)$	$(\mathrm{mg/kg})$ ے	EC (ds/m)	Carbon(₉₆) rgani	囯	Yea	
Silty	0.07	150	6	2.4	0.34	7.4	2021	
Lom Silty Lom	0.06	154	8	1.8	0.12	7.9	2022	

Kerman is a dry region where most rainfall occurs in autumn and winter, and annual evaporation exceeds average rainfall. Monthly minimum and maximum temperatures during the study period are detailed in Tables 2 and 3.

Tabel 2-Regional meteorological records of study site during the 2021 growing season

The moon	Jan	Feb	Mar	Apr	May	June
MIN	1.3	5.6	10.6	17.5	19.5	23.5
MAX (Mean of temperature $({}^{\circ}c)$	16.1	21.7	24.5	31.5	33.6	36.7
Rainfall (mm)	0.2	0.1	11.5	0.8	7.7	0.1

Tabel 3-Regional meteorological records of study site during the 2022 growing season

 In both cropping years, seeds were soaked in sterile distilled water for 24 hours before planting to ensure uniform germination and seedling establishment while washing away phenolic compounds. To prevent soil-borne diseases, the seeds were disinfected using Mancozeb fungicide.

 The field preparation included plowing (with a moldboard plow), two rounds of disking, cultivating with a cultivator, and leveling with a land leveler. The amount of fertilizer used was based on soil test results and the recommendations of soil science experts, consisting of 80 kg of phosphorus, 30 kg of potassium, and 60 kg of nitrogen, sourced from ammonium phosphate, potassium sulfate, and urea. Phosphorus and potassium fertilizers, along with onethird of the urea, were applied during field preparation, while the remaining two-thirds of urea were applied during the flowering stage. Each experimental replicate included three main plots (irrigation treatments) and five subplots (cumin ecotypes).

 The planting rows were prepared with a spacing of 40 cm using a furrower. The seeds were then sown in furrows at a depth of 1 to 2 cm. Planting was done on both sides of the ridge, with a 20 cm row spacing and a density of 120 plants per meter. Each plot measured 4 meters in length and 3 meters in width, with a 1-meter gap between blocks. In total, there were 45 plots, or 15 treatments with three replications.

 Irrigation was performed depending on rainfall. From planting to germination, irrigation was carried out once a week. To prevent soil crusting, irrigation continued every 15

days from germination to maturity, considering the low water requirements of cumin.

 After that, irrigation was cut off for the plots where drought stress would be applied, at 50% and 100% flowering stages. After the seedlings were fully established, two thinning stages were performed to achieve the desired plant density and maintain row spacing. Extra plants were removed each year.

 Since cumin's growth rate is very slow in the early stages and the seedlings are delicate and weak, the plant cannot compete with weeds, and complete weed control is necessary. If the weeds are not managed, the plant's yield will drastically decrease. Therefore, manual weeding was carried out in two stages (mid-April and mid-May).

 Harvesting occurred on June 15 in both years, performed by workers. To eliminate edge effects, 0.5 meters from the beginning and end of the planting rows, as well as two rows from both sides, were removed, and only the remaining area was harvested.

 In both cropping years, to determine the yield components, 20 plants were randomly selected from each plot at the physiological maturity stage and cut at ground level. The samples were stored in separate bags according to their ecotypes. The seeds were dried using the shadowdrying method to preserve seed quality and essential oil content until they reached a constant weight. The number of sub-branches, number of umbels, number of umbellets, and number of seeds per plant were measured.

 For the measurement of other traits, including seed yield, biological yield, seed weight per plant, straw and stubble weight, harvest index, essential oil percentage, and essential oil yield, the samples were transferred to the laboratory where the measurements were taken.

 The data were analyzed using the backward elimination method, analysis of variance, and Pearson's correlation coefficient between traits. Finally, the data were statistically analyzed using SAS version 9.4 software, and mean comparisons for each trait were performed using Duncan's multiple range test at the 1% probability level. Graphs were drawn using Excel software.

3-Results

The results of the analysis of variance indicated that the effects of ecotype and drought stress (different irrigation levels) were significant both independently and interactively for all the traits studied (Table 4). Given the significant effects of ecotype and drought stress on the measured traits, the results of the mean comparisons for each irrigation level showed that the highest number of sub-branches was found in the Kashmar ecotype under non-stress conditions, while the highest number of sub-branches in the Sabzevar ecotype occurred under the irrigation cutoff after 50% flowering (Tables 5 and 6). The highest number of umbels and seeds per plant were found in the Kohbanan ecotype under nonstress conditions (full irrigation), while the lowest number of umbels and seeds per plant was observed in the Sabzevar ecotype under the irrigation cutoff after 50% flowering (Tables 5 and 6).

Table 4- variance analysis of measured traits of (Cuminum cyminum L.) in various irrigation levels									
S.O.V	df	No. of sub- branches in the bush	No. of umbels in the bush	No. of small umbels in the bush	No. of seeds in the bush	Seed weight per plant	Weight of straw and Stubble	Biological yield	
Year	1	$9.34*$	$20.50*$	94.04^{ns}	1867.78ns	0.219 ^{ns}	0.04 ^{ns}	1284.44 ns	
Year \times Replication (a)	4	0.51 ^{ns}	$5.65*$	14.81 ^{ns}	150.71*	0.049 ^{ns}	0.008 ^{ns}	7704.58*	
stress	$\sqrt{2}$	12.98**	174.70**	837/68**	132030.71**	$0.97*$	$0.25**$	351030.71**	
$Stress \times Year$	$\overline{2}$	0.04 ^{ns}	0.27 ^{ns}	0.14 ^{ns}	107.78 ^{ns}	0.00004 ^{ns}	0.0005 ^{ns}	101.11 ^{ns}	
Stress \times Year \times Replication (b)	8	0.86 ^{ns}	3.57^{ns}	10.99 ^{ns}	196.04 ^{ns}	$0.130*$	0.0028 ^{ns}	220.91 ^{ns}	
Ecotype	$\overline{4}$	$3.85**$	154.34**	1016.68**	6999449**	$0.396**$	$0.499**$	111012.27**	
Year×ecotype	4	0.317^{ns}	0.15 ^{ns}	0.128 ^{ns}	281.67^{ns}	0.00004 ^{ns}	0.0001 ^{ns}	106.67 ^{ns}	
Ecotype× Stress	8	1.325 [*]	3.69 ^{ns}	161.025^{ns}	7173.16**	0.090^{ns}	$0.125**$	21816.27**	
$Ectype\times$ Stress×Year	8	0.142 ^{ns}	0.180 ^{ns}	0.103 ^{ns}	121.67 ^{ns}	0.00004^{ns}	0.0001 ^{ns}	115.00 ^{ns}	
Error(C)	48	0.508	2.07	9.09	415.6	0.048	0.007	2162.83	
$CV(\%)$		11.42	12.27	9.43	10.45	15.86	20.91	13.26	
\varkappa^2 Bartlett	1	0.0268 ^{ns}	0.0621 ^{ns}	0.0105 ^{ns}	0.0158 ^{ns}	0.00054 ^{ns}	0.0478 ^{ns}	0.0359 ^{ns}	
Pr>ChiSq		0.87	0.94	0.92	0.90	0.98	0.83	0.85	
$R2-Adj$		0.53	0.84	0.895	0.94	0.466	0.339	0.87	

**, * and ns: are significant at 1 and 5 probability levels and non-significant, respectively

Table 4- variance analysis of measured traits of (*Cuminum cyminum* **L.) in various irrigation levels**

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Table 5- Comparison of different traits for different ecotypes of cumin								
Ecotype	Number sub- branches in the bush	Number of umbels in the bush	Number of small umbels in the bush	Number of seeds in the bush	Seed weight per plant(g)	Weight of straw and Stubble(kg.ha) Ł	Biological Yield(kg.ha)	
Mahan	9.06 ± 0.168 ab	28.44±0.339b	$100.00 \pm 0.710b$	767.89±4.805b	$2.08 \pm 0.052a$	$1.02 \pm 0.162b$	$1078.78 \pm 10.926a$	
Kouhbanan	8.56 ± 0.168 bc	32.00 ± 0.339 a	$98.34 \pm 0.710h$	876.33±4.805a	$1.78 \pm 0.052c$	1.25±0.162a	$1008.88 \pm 10.962 b$	
Khusf	8.44 ± 0.168 bc	31.89±0.339a	$110.00 \pm 0.710a$	863.44±4.805a	2.01 ± 0.052 ab	1.26 ± 0.162	$936.11 \pm 10.962c$	
Kashmar	9.39 ± 0.168 a	$28.33 \pm 0.339c$	$89.67 \pm 0.710d$	773.89±4.805b	$1.73 \pm 0.052c$	$1.22 + 0.162a$	$929.44 \pm 10.962c$	
Sabzevar	$8.28 \pm 0.168c$	24.94±0.339d	95.00 ± 0.710 ac	736.33±4.805c	1.86 ± 0.052 bc	$1.36 \pm 0.162a$	877.22 ± 10.962 d	

Table 5- Comparison of different traits for different ecotypes of cumin

Means with the same letters within each column are not significantly different at 1% level. Given that the difference between the means of this trait is significant at the 5% level, the comparison of means was also performed at the same level.Due to the non-normality of the data and the need for data transformation, this attribute was analyzed using radical transformation.

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The maximum number of umbellets and relative leaf water content were observed in the Khosuf ecotype under full irrigation, while the minimum number of umbellets and relative leaf water content were recorded in the Kashmar ecotype under the irrigation cutoff after 50% flowering. The maximum straw and stubble weight was recorded in the Sabzevar ecotype under non-stress conditions (normal irrigation), and the minimum straw and stubble weight was observed in the Mahan ecotype under the irrigation cutoff after 50% flowering (Tables 5 and 6).

The highest harvest index, seed yield, and biological yield were found in the Mahan ecotype under full irrigation, while the lowest harvest index, seed yield, and biological yield were observed in the Khosuf ecotype under the irrigation cutoff after 100% flowering, and in the Kohbanan and Sabzevar ecotypes under the irrigation cutoff after 50% flowering (Tables 5 and 6).

The highest essential oil percentage and yield were found in the Khosuf and Mahan ecotypes under the irrigation cutoff after 50% flowering (Tables 5 and 6). The highest water loss occurred in the Kohbanan ecotype under the irrigation cutoff after 100% flowering, and the lowest water loss was observed in the Khosuf ecotype under full irrigation. The highest ion leakage was observed in the Mahan ecotype under the irrigation cutoff after 100% flowering (Tables 5 and 6).

To evaluate the effect of various variables on cumin seed yield, multivariate regression analysis was used. The best method for entering variables into the model was the backward method. Initially, all variables were entered into the model, and then the variables with the least effect were removed one by one. The results of the final step of the model are shown in (Table 7).

a= Constant, $Y = a+b_1X_1+b_2X_2+b_3X_3+b_4X_4+b_5X_5+b_6X_6+b_7X_7$

 X_1 = Number of umbels in the bush, X_2 = Number of seeds in the bush, X_3 = Seed weight per plant

, X4=Essential oil Percentage

 X_5 = Relative water content, X_6 = Electrolyte Leakage

, X_7 = Essencee yield

Y= -51.94-0.6X1-0.59X2-0.71X3+0.80X4-1.14X5+1.95X6+0.66X7

Based on the results of the analysis of variance, the year effect was significant for most traits, except for biological yield, seed yield, and essential oil yield (Table 5). The effects of drought stress and ecotype were also significant for all traits. The significance level for drought stress and ecotype effects, except for the relative leaf water content under different drought stresses, was 1% for all traits, indicating significant changes in the mean values of traits due to the application of different drought stress treatments and clear differences between the studied ecotypes.

Under irrigation cutoff, the number of sub-branches per plant significantly decreased in comparison to full irrigation (non-stress conditions). The results of the studies support this finding, where irrigation cutoff under drought stress significantly reduced the number of sub-branches compared to full irrigation. The Kashmar ecotype had the highest number of sub-branches. Sadeghipour and Aghaei (2012) reported that under drought stress conditions, water flow around growing cells decreases, which stops the elongation of these cells. Under irrigation cutoff, the lower nitrogen uptake reduced the fertility of sub-branches, a finding also supported by Tabatabaie et al. (2014), who studied the effect of drought stress on the height and number of branches in cumin ecotypes.

According to the experimental results, under full irrigation (non-stress), the Kohbanan and Khosuf ecotypes had more umbels and umbellets per plant compared to other ecotypes. In the Sabzevar ecotype, the number of umbels and umbellets decreased due to the increased intensity of drought stress. Since cumin is an indeterminate plant, many of its sub-branches may grow late and not have the opportunity to produce umbels. The number of umbels and umbellets in the plant depends on vegetative growth, and reduced vegetative growth due to stress before flowering led to a decrease in the number of umbels and umbellets. Kafi and Keshmiri (2011) stated that under drought stress conditions, the number of umbels per plant decreases due to flower drop and the abortion of newly formed seeds, which results in reduced umbels, umbellets, and seeds. This finding aligns with the report by Afshar et al. (2016) on cumin.

Since seeds are one of the most important parts of the plant, in addition to their significant role in yield, they are considered one of the most important physiological destinations. The number of seeds per plant in the Sabzevar ecotype significantly decreased, while the Kohbanan and Khosuf ecotypes had higher numbers of seeds per plant. Reduced irrigation water, through disruption in pollination and shortening the pollination period, led to poor fertilization of flowers and a decrease in the number of seeds per plant. The reduction in seed count per plant under drought stress has been reported in various plants. Research by Motamedi-Mirhosseini et al. (2011) showed that the reduction in seed number under drought stress could be due to decreased carbohydrate supply caused by reduced leaf area and photosynthesis during the seed-filling stage.

The results of this study also indicated that the weight of straw and stubble decreased in the Mahan ecotype. Research by Rasam et al. (2014) on drought stress and its effects on the morphological traits of cumin, including straw and stubble weight, showed that increased drought stress causes a reduction in straw and stubble weight in some cumin ecotypes. Their findings indicated that drought stress reduces plant growth, plant height, the number of subbranches, and the number of leaves, especially leaf area, leading to decreased photosynthetic ability and a reduction in dry matter accumulation. According to the results, the highest seed weight was observed in the Mahan ecotype under non-stress conditions. Eftekharinasab et al. (2011) attributed the reduction in seed weight due to drought stress to an increase in the photosynthesis process and stated that the carbohydrates and nitrogen stored during the flowering period determine seed filling, and nitrogen deficiency reduces seed weight by decreasing stored materials.

Biological yield is one of the important indicators used to determine the growth of crop plants. The comparison of mean data based on Duncan's multiple range test at the 1% level showed that the Mahan ecotype and non-stress conditions in the field had the maximum seed yield and biological yield. One of the reasons for increased yield is the stimulation of the plant to increase water absorption and carbon dioxide fixation, which ultimately enhances photosynthesis. This leads to increased production of assimilates, and consequently, seed filling speed, seed weight, and, ultimately, yield increase.

The findings of Farrokhinia et al. (2011) also indicated that drought stress in cumin plants, by reducing leaf water content and causing stomatal closure, leads to a decline in photosynthesis. Additionally, drought stress affects enzymatic activities and related processes, resulting in reduced seed yield.

The results of this study support that after irrigation cutoff at 50% flowering, the Khosuf and Mahan ecotypes had the highest essential oil percentage and yield. Since there is an inverse relationship between the level of photosynthates and the production of secondary metabolites, any factor that reduces growth and the production of photosynthates will increase the percentage of secondary metabolites and essential oil in medicinal plants (Omidi and Jafarzadeh, 2010). Similar results were reported by Ahmadian et al. (2010), who found an increase in essential oil percentage and yield under drought stress in cumin plants. The increase in essential oil content is a defense mechanism and biochemical adaptation to environmental conditions.

Harvest index, which indicates the relative distribution of photosynthetic products between economic sinks and other sinks in the plant, was highest under non-stress conditions. The lowest harvest index was observed under irrigation cutoff after 100% flowering. Among the ecotypes, the Mahan ecotype had the highest harvest index. With drought stress, due to a reduction in seed weight, seed yield decreases, and this reduction is greater than the decrease in biological yield (Farnia et al., 2006).

The analysis of variance (Table 5) showed that the effects of ecotype and drought stress on physiological traits, including relative leaf water content and leaf water loss, were significantly different at the 1% level. This difference is likely due to the genetic structural differences among the ecotypes. The highest relative leaf water content was found in the Khosuf ecotype under non-stress conditions (full irrigation). Changes, especially reductions in relative leaf water content, are important traits for assessing drought resistance in plants. Heidary et al. (2014) reported that under drought stress, the plant enters wilting phase, and its relative water content decreases.

The reduction in relative water content and stomatal closure is the first effect of drought stress, resulting in a decrease in intracellular carbon dioxide and ultimately leading to disruption in photosynthesis and plant metabolism (Unyayar et al., 2004). According to the results, the highest leaf water

4. Conclusions

Overall, the results of the present study indicate that the effects of ecotype and drought stress on all morphological traits were significantly different at the 1% level. This difference is likely due to the genetic structural differences among the ecotypes. The results showed that traits such as seed yield, biological yield, straw and stubble weight, seed weight per plant, number of umbels per plant, number of umbellets, number of seeds per plant, number of subbranches, and harvest index significantly decreased due to drought stress.

loss was observed in the Kohbanan ecotype under irrigation cutoff after 100% flowering.

Drought stress causes changes in cellular water content and osmotic substances within tissues to maximize water absorption from the soil. This results in a reduction of water available for other processes, including cell expansion. Additionally, activities such as reduced root growth and activity, as well as changes in transpiration and evaporation rates in drought-stressed plants, affect the relative water content. The maximum relative leaf water content in ecotypes of drought-resistant cultivars is lower in terms of water loss compared to sensitive cultivars. This reduction is likely due to physiological activities in the plant, such as the timing of stomatal opening and closing and cellular changes within the plant (Karimizadeh and Mohammadi, 2011).

Based on the results of this study, both ecotype and drought stress led to significant changes in the ion leakage of the ecotypes examined. Overall, drought stress increased ion leakage, with the highest ion leakage observed in the Mahan ecotype under irrigation cutoff after 100% flowering. In resistant cultivars, ion leakage was lower than in sensitive cultivars. This is due to the lower stability of the cytoplasmic membrane in these cultivars, as reported by Farshadfar and Javadinia (1997).

According to the regression results, the number of umbels, seeds per plant, seed weight, essential oil percentage, relative leaf water content, ion leakage, and essential oil yield explained 91.3% of the variation in seed yield (Table 8). As shown, the traits of number of umbels, seeds per plant, seed weight per plant, essential oil percentage, relative leaf water content, and ion leakage created a significant multivariate regression model.

As a result, these traits are among the key characteristics that could be considered by plant breeders. However, the yield is positively correlated with the traits of number of umbels per plant, essential oil percentage, essential oil yield, and ion leakage, while it is negatively correlated with the traits of seed number per plant and seed weight. In other similar studies on cumin, traits such as thousand-seed weight, number of umbels, number of seeds per umbel, and number of stem branches were included in the regression model, and these traits were found to be related to seed yield (Afshar et al., 2016).

According to the results of the analysis of variance, the effect of the year was significant for most traits, except for biological yield, seed yield, essential oil yield, number of umbels and umbellets per plant, straw and stubble weight, and seed weight per plant. The effects of drought stress and ecotype were significant for all traits. Under drought stress conditions (irrigation cutoff after 50% flowering), the essential oil percentage and yield increased.

The results of the multivariate regression analysis using the backward method showed that traits such as number of umbels, number of seeds per plant, seed weight, essential oil percentage, relative leaf water content, ion leakage, and essential oil yield significantly contributed to the multivariate regression model. By achieving these results and identifying the traits that most influence economic performance, it is possible to select and utilize the best ecotypes for breeding programs.

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