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EFFECT OF INBREEDING ON SEED YIELD IN FORAGE WATERMELON (*CITRULLUS LANATUS* VAR. *CITROIDES*)

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SUMMARY

The purpose of this study was to determine the effects of inbreeding on seed set in forage watermelon genotypes obtained from different sources. The study, conducted in 2017, used watermelon genotypes from different sources under Konya ecological conditions. The planting of seeds in pots in the greenhouse produced seedlings, further planted in the trial area with a 200 cm × 100 cm spacing in May 2017. From 66 forage watermelon genotypes used in the study came fruits from both inbred and open pollinated genotypes flowers on the same plant. In 40 of these genotypes, the seed yields obtained in inbreeding appeared higher than the seed yields obtained in the open pollination. In the study, the seed yield (127.6 g fruit^{-1}) acquired from inbreeding was 16.3% higher than the average seed yield (109.7 g fruit^{-1}) from open pollination. This rate showed the success rate in inbreeding was high, with no self-incompatible in the forage watermelon genotypes used in the study, and inbreeding had no negative impacts on pollination, fertilization, fruit set, and seed yield.

Keywords: Fodder watermelon, genotypes, inbreed, pistillate flower, inbreeding, staminate flower

Key findings: The maximum receptivity period of the staminate flower was between 6:30 and 9:30 a.m. in field conditions. No self-incompatibility occurred in the forage watermelon genotypes used in the study, and inbreeding had no negative impacts on pollination, fertilization, fruit set, and seed yield. Sufficient seeds can result in forage watermelon with controlled self-pollination made in accordance with the technique.

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INTRODUCTION

Forage watermelon (*Citrullus lanatus var. citroides*) is an annual plant of the Cucurbitaceae family. The genus and species of forage watermelon and edible watermelon are the same, but they have different varieties (Özköse and Acar, 2022). Forage watermelon has several names mentioned in literatures, such as, forage watermelon, fodder melon, horse watermelon, preserving melon, stock melon, citron, citron melon, and tsamma melon (Robinson and Decker-Walters, 1997; Laghetti and Hammer, 2007; Santos *et al.,* 2017; Shaik *et al.,* 2017; Ngwepe *et al.,* 2019; Ribeiro *et al.,* 2022; Stephens, 2023).

The fruit skin is hard, thick, and durable. The fruit flesh is yellow-white or greenish in forage watermelons, and the flesh is firm and retains its juice for longer durations after ripening (Acar *et al.,* 2015). The amount of water-soluble dry matter (Brix) in the fruit is low (Özköse and Acar, 2022) and the amount of pectin is high (Acar *et al.,* 2019). Its structure does not break easily due to its elastic structure and hard shell, changing from oval to cylindrical (Acar *et al.,* 2019). The background color (main color) of the outer shell of the fruit is non-lined and non-patterned or very slightly patterned.

One of the most important characteristics of forage watermelon is the long storage life of its fruits. Kavut *et al.* (2014) reported the storage of ripe fruits can last for more than six months without any decay and significant loss of nutritional quality. With the unit area yield being high, it is a source of roughage, which can have longer storage durations without spoiling, making the forage watermelon a good alternative to water-rich roughage (Tokat *et al.,* 2020).

The cultivation of forage watermelon is quite uncommon and prevalent in specific countries and areas. The spread of forage watermelon agriculture is possible by improving its varieties and producing sufficient seeds. The genus and species of forage watermelon and edible watermelon are the same; but, they have different varieties (Özköse and Acar, 2022). The breeding methods used in edible watermelon can also be applicable in forage watermelon breeding. The male and female flowers are on the same plant but in different places in forage watermelon. A high rate of foreign pollination is possible because of open pollination. Pollination occurs through insects. Inbreeding is necessary to obtain pure lines and continue their availability in forage watermelon breeding.

The effect of inbreeding on the seed yield of forage watermelon genotypes is unknown because of insufficient breeding studies on forage watermelon genotypes. For this reason, the presented study aimed to determine the effects of inbreeding on seed set in forage watermelon genotypes obtained from different sources.

MATERIALS AND METHODS

This study proceeded at the Faculty of Agriculture, Selçuk University, during the 2017 cultivation season (May-September). The Konya province where the study transpired has a continental climate. The altitude above sea level is about 1100 meters. Konya sits in the southern part of the Central Anatolian Region and the continental climate is predominantly harsh, cold, and snowy winters, and hot and dry summers. During the trial in 2017, the relative humidity was 45.02%, the average temperature was 21.54 °C, and the total precipitation was 91.8 mm. In the long-term average, the relative humidity was 46.88%, the average temperature was 20.28 °C, and the total precipitation was 96.3 mm. The climate data of the long-term average and the trial year are almost similar (Table 1). The soil sample, taken from a depth of 0–30 cm, underwent analysis. According to soil analysis, the trial field's soil is loamy-clayey and alkaline (pH = 7.7). In addition, the organic matter, EC, P_2O_5, K_2O, Na, Ca, Zn, Mn, Cu, and Fe contents of the soil appeared as 1.08%, 190 µS cm^{-1}, 10.58, 219.13, 65.37, 5700, 2.22, 4.90, 0.79, and 1.33 ppm, respectively.

In this study, a total of 286 forage or forage watermelon hybrid genotypes belonging to 32 populations, originating from Turkey and Turkmenistan, were sample materials. The seeds of these genotypes, sown in the

Table 1. Climate data of Konya Province for 2017 and long-term (LT) average.*

Month	Precipitation (mm)		Air Temperature °C		Relative Humidity (%)	
	2017	LT	2017	LT	2017	LT
May	43.7	44.4	15.4	15.7	57.9	56.2
Jun	25.4	24.8	20.4	20.1	54.6	49.0
Jul	0.0	6.9	25.2	23.5	35.6	41.3
Aug	19.4	6.7	24.3	23.3	45.3	41.0
Sep	3.3	13.5	22.4	18.8	31.7	46.9
Total	91.8	96.3	-	-	-	-
Mean	-	-	21.54	20.28	45.02	46.88

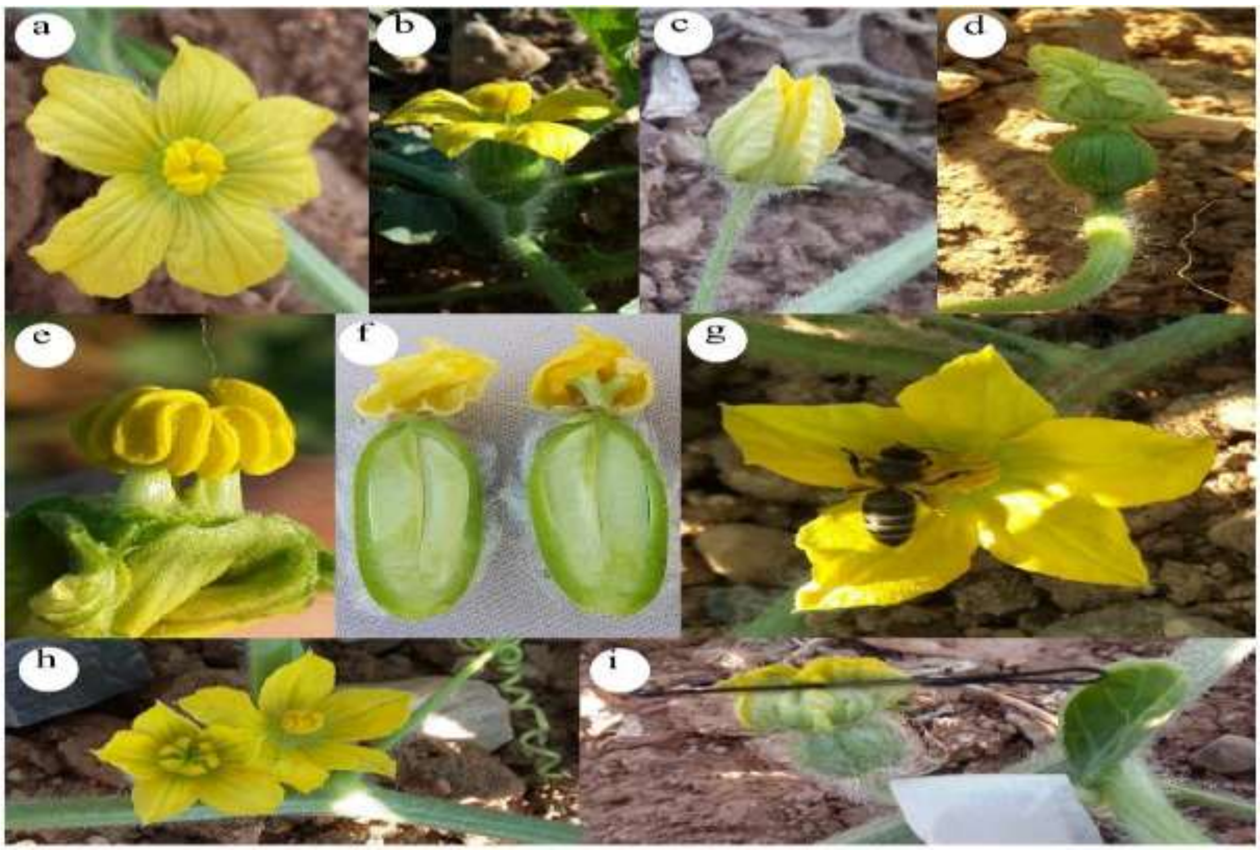

Figure 1. Flowers in forage watermelon (*Citrullus lanatus var. citroides*) varieties: a) staminate flower (♂), b) pistillate flower (♀), c) staminate flower one day after anthesis, d) pistillate flower one day after anthesis e) staminate flower (♂), f) pistillate flower (♀), g) pollinator bee, h) staminate flower (♂), pistillate flower (♀), and i) pistillate flower (♀) after hand-pollinated for inbreeding.

greenhouse in early April, produced seedlings. Afterward, transplanting the seedlings continued in the trial field on May 22, 2017, at intervals of 200 cm × 100 cm (row spacing × plant spacing). After planting, applying cultural practices, such as, hoeing, throat filling, irrigation, and fertilization ensued for weed control according to the development of plants. Harvest followed on September 21, 2017. The Single Selection Method intended for foreign-pollinated plants was the applied technique in the presented study. Male and female flowers are prevalent in different places (monoecious) on the same plant (Figure 1a, b, c, d, e, f, g,

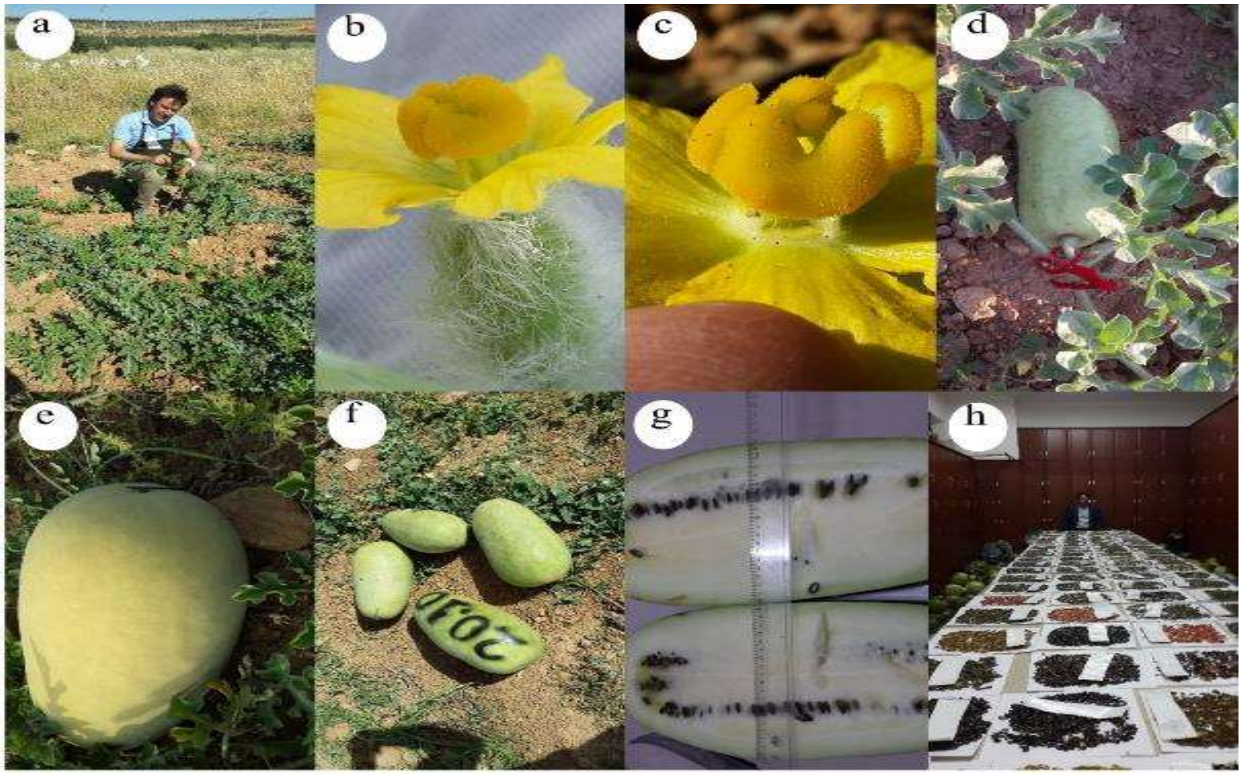

Figure 2. Inbreeding-study in forage watermelon (*Citrullus lanatus var. citroides*) varieties: a) inbreeding, b) the anthesis of pistillate flower (♀), c) pollen grains on the stigma of the pistillate flower (♀) d) forage watermelon obtained as a result of inbreeding, e) ripe forage watermelon obtained as a result of self-fertilization, f) harvest period g) cross-section of forage watermelon fruit, and h) seeds of different forage watermelon genotypes.

h) in many of the watermelon genotypes (Zhang, 1998). For this reason, cross-pollination is visible at a high rate (Wehner, 2008; Kumar *et al.,* 2013; Ngwepe *et al.,* 2019). Furthermore, the plants' inbreeding helped obtain pure lines from the genotypes for selection. The study had fruits both obtained from open-pollinated and self-pollinated flowers in 66 of 286 genotypes. Inbreed seeds will serve in the production of selected genotypes in further stages of breeding.

The inbreeding of plants sought to obtain a pure line from the watermelon genotypes for selection. Balkaya *et al.* (2008) method for zucchini became in the inbreeding process used, as mentioned below. Applying flower isolation in inbreeding aided in taking the seeds of each plant separately. One day before inbreeding (1 day before anthesis), male and female flowers of the same plant, which were determined to open (to be receptive) 1 day later, underwent isolation with the help of clips (kirby grip) to prevent open pollination (Figure 1i). The next day, inbreeding application with these flowers occurred between 6:00 and 9:30 in the morning (in the following hours, the receptivity of the female flower decreases and for this reason, fruit set rarely occurs) (Figure 2a). After the inbreeding, the female flower underwent further isolation, preventing foreign pollen entry externally. During the inbreeding, the anthers taken from the male flower together with its stem, which was about to spread its pollen, underwent pollination with light movements. This caused the stigma of the female flower, with fully opened petals, reached complete pollen covering (Figure 2b, c).

The fruits, determined for pollination and fertilization two days after the inbreeding and started to grow, bore labels and data recording (Figure 2d). Inbreeding performance was in such a way as to allow only one inbreeding fruit for each plant. Inbreeding seeds will serve as materials in the production of the selected genotypes in further stages of selection.

From each plant, harvesting of inbred and open-pollinated fruits occurred separately, with their seeds removed (Figure 2e, f, g, h). They also underwent comparison to determine the effects of inbreeding on seed yield. The seed yield of the fruit, obtained from the open-pollinated flower of the same genotype, was 100. The seed yield of the watermelon obtained from the inbred flower was proportionate, and calculating the change in % was by multiplying by 100. In doing so, the effect of inbreeding on seed yield (%) reached an estimation.

RESULTS AND DISCUSSION

In the presented study, applying inbreeding in each genotype occurred; however, it revealed unsuccessful for some genotypes. Genotypes with successful inbreeding in the same plant and fruit obtained from open pollination incurred comparison in the study. Inbreeding and non-inbreeding fruits from each plant, harvested separately, had their seeds removed. In the same plant, the seed yield of the watermelon obtained from the inbred flower was proportionate to the seed yield of the watermelon obtained from the free-pollinated flower, determining the change in percentage by multiplying by 100 (Table 2).

Table 2. Effect of inbreeding on seed yield in watermelon genotypes.

Genotype No.	Seed yield (g fruit^{-1}) free pollination	Seed yield (g fruit^{-1}) selfie	Effect of inbreeding (%)	Genotype No.	Seed yield (g fruit^{-1}) free pollination	Seed yield (g fruit^{-1}) selfie	Effect of inbreeding (%)
1	122.09	188.57	154.5	161	110.90	108.30	97.7
4	95.79	223.81	233.6	172	31.94	158.07	494.9
12	126.99	110.36	86.9	173	138.25	163.40	118.2
17	143.26	162.12	113.2	177	177.14	216.34	122.1
22	76.30	148.80	195.0	188	112.56	92.50	82.2
25	73.25	54.84	74.9	193	97.43	124.27	127.5
27	66.50	67.90	102.1	196	124.71	104.88	84.1
29	182.11	164.81	90.5	198	87.18	113.07	129.7
39	109.10	131.12	120.2	200	99.74	133.04	133.4
43	102.52	90.11	87.9	202	91.73	77.11	84.1
45	121.20	127.86	105.5	204	75.58	98.69	130.6
49	94.63	114.25	120.7	206	128.23	67.22	52.4
58	85.65	177.60	207.4	218	83.80	110.80	132.2
59	61.39	143.75	234.2	224	107.02	105.85	98.9
61	73.29	185.54	253.2	228	93.34	61.97	66.4
68	232.66	196.35	84.4	232	49.79	43.76	87.9
70	154.80	166.16	107.3	234	65.77	66.90	101.7
71	203.78	219.23	107.6	238	170.50	235.60	138.2
72	184.19	202.26	109.8	241	212.79	200.94	94.4
73	157.52	260.70	165.5	246	164.24	154.24	93.9
84	96.23	46.17	48.0	247	182.15	140.65	77.2
89	79.49	104.82	131.9	251	90.61	132.94	146.7
90	37.60	27.89	74.2	254	197.87	168.88	85.3
96	82.29	95.74	116.3	257	77.30	68.93	89.2
104	80.31	95.24	118.6	259	112.30	109.16	97.2
111	76.78	63.05	82.1	262	115.67	40.08	34.7
118	141.34	166.98	118.1	277	11.21	156.20	1393.4
126	26.09	67.34	258.1	278	210.94	246.73	117.0
130	59.94	84.03	140.2	279	195.24	205.64	105.3
135	36.13	34.77	96.2	280	104.98	103.53	98.6
142	79.28	99.80	125.9	282	82.96	189.28	228.2
153	97.35	107.86	110.8	283	106.50	79.90	75.0
156	49.20	80.58	163.8	284	120.60	129.35	107.3
Mean					109.7	127.6	116.3

Obtaining fruits and seeds from both open-pollinated and self-pollinated flowers ensued in 66 of the genotypes. The seeds obtained from fruits consisted of inbred flowers in 40 genotypes, appeared to be higher than the seed yield coming from the fruit consisting of open-pollinated flowers in the same genotype. Higher seeds resulted in 26 genotypes from the fruits consisting of open-pollinated flowers.

When the average seed yields (109.7 g fruit^{-1}) obtained in the open pollination of 66 plants underwent comparing with the seed yields obtained in inbreeding (127.6 g fruit^{-1}), it was 16.3% lower than seed yields from inbreeding.

The average seed yield from inbreeding was 127.6 g in the watermelon genotypes. These incurred further study. Although, the inbreeding proceeded manually in one step, sufficient pollen and vitality transferred to the female flower, achieving the pollination, fertilization, fruit, fruit set, and seed production successfully.

The success of inbreeding in watermelon also correlated with what time of day its application transpired. In the early morning hours (before the sun rises), the watermelon flower petals are still closed. In the study, the flowers started to open after 6:30 a.m. every day, which points to the time when to begin inbreeding during working hours. Bomfim *et al.* (2015) observed the corolla movement of male and female flowers in watermelon under controlled conditions and determined the flowers started to open at 5:20 a.m. and closed at 2:00 p.m., with flowers fully opened between 6:00–10:00 a.m. Kwon *et al.* (2005) reported the maximum receptivity period of the stigma was between 6:00–10:30 a.m. Similarly, Parris (1949) found the highest percentage of fruit set occurred when depositing pollen grains on the stigma between 09:00–10:00 a.m. in seeded cultivars. Although it varies among regions, it will be more effective to do it between 7:00 and 11:00 a.m. for hand pollination (inbreeding) to be successful and for fruit set and seed yield to be high.

Keeping enough seeds in open pollination requires sufficient pollinator activity between these hours. Pollinator insects need to visit both male and female flowers. Pollen-collecting insects may visit female flowers less frequently because there is no pollen. For this reason, it may be possible to carry more pollen in manual pollination. Pollination occurs in the morning hours by bees (Mu, 1992). Pollination mediation come from honeybees (Apis mellifera L.) in watermelon and bumblebees (Bombus impatiens Cresson), which come to flowers to collect more pollen and nectar (Bomfim *et al.,* 2015). According to study observations, while the researchers were in the field for inbreeding, honeybees were more effective in pollination.

The bees visit both flowers because they collect nectar from both male and female flowers. However, male flowers produce more nectar than female flowers (Taha and Bayoumi, 2009). When pollinators come to take this nectar, they carry the pollen. However, there must be sufficient live pollen for pollination. Stanghellini and Schultheis (2005) reported the number of pollen grains varied at 26,000–38,000 in a flower and 134.206–264.589 in a watermelon. Freeman *et al.* (2008) reported over 95% of the pollen grains were viable in watermelon varieties. Multiple flower visits by pollinators are necessary to ensure fruit set (Wijesinghe *et al.,* 2020). The approximately 500–1000 live pollen need transporting to the female flower for normal size fruit formation and seed production (Free, 1993; Adlerz, 1996). Researchers stated 6–8 bee (Adlerz, 1996; Stanghellini *et al.,* 1997) or roughly 10–60 pollinator (Winfree *et al.,* (2007) visits are essential for fruit set in watermelon.

The activities of honeybees as pollinators were evident in our field studies. In the presented study, the average seed yield of the fruits obtained from the open-pollinated flowers was lower than the inbreeding average. One of the reasons for the low pollination may be the low pollinator bee activity because, besides fruit set and fruit size, the number of pollinator visits in open pollination also affects the number of seeds (Guerra Sanz and

Serrano, 2008). Additionally, climatic factors, such as, temperature and humidity during flowering, and flowering plant species, aside from watermelon attracting pollinator insects, influenced pollination, fertilization, and seed yield.

The use of more than one male flower in breeding will provide more fertile pollen grains and increase the success rate. It is a disadvantage in some cases because a limited number of fertile male flowers blooming will exist at the same time as the female flower on the same plant. Sex expression in cucurbits, besides being genetically controlled, also has high alterations from environment (temperature, humidity, light, and nutrition) (Kumar and Wehner, 2011). In watermelon trials, a higher day temperature (32 °C) reduces female flower number, while a lower temperature (27 °C) increases it (Rudich and Peles, 1976). This may decrease the success rate in inbreeding. However, pollinator bees are more likely to carry pollen from fertile male flowers on different plants because of unlimited open pollination. Here, the main thing is no incompatibility of the plant with itself appeared, as the inbreeding will be done manually (Badami *et al.,* 2020; Mohosina *et al.,* 2020; Marveldani *et al.,* 2023).

Seed yields may differ among genotypes. When the seed yields obtained in open pollination of the 68 genotypes studied reached evaluation, the seed yield was below 20 g in 20 genotypes, while the seed yield was above 200 g in 10 genotypes. For example, the fruit obtained from the open-pollinated flower yielded 232.66 g in genotype 68, and the fruit obtained from the inbred flower yielded 196.35 g of seeds. According to this result, one can confirm the seed yield ability of genotype 68 was high.

CONCLUSIONS

In forage watermelon, many factors are effective in pollination and fertilization. The combination of these factors determines the yield. The impacts of some of these factors on yield were rational in the scope of this study. When evaluating the study results in general, it is possible to argue sufficient seeds can come from inbreeding in the watermelon genotypes used in the study, and there was no self-incompatibility. Conducting new studies is necessary in this field, which will contribute to better understand the subject. Sufficient seeds can emerge in forage watermelon, with controlled self-pollination made in accordance with the technique.

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