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Proceeding Paper

Characterization and Agronomic Evaluation of Chia Germplasm in La Plata, Buenos Aires, Argentina †

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Abstract: Chia (*Salvia hispanica* L.), an ancestral crop currently revalued for its nutritional properties, is one of the main sources of omega-3 fatty acids. It is a short-day plant and sensitive to frost. The objective of this study was to assess the possibility of growing chia in La Plata (Buenos Aires, Argentina) (34°54′29″ S, 58°2′25″ W) by analyzing interpopulation variability between four accessions of this species. Chia was sown on February 11 in a randomized complete block design (three replicates). Ten uniform and representative plants per plot were labeled and monitored throughout the crop cycle. Phenological stages and morphological characteristics (plant height, width of the main stem, number of pairs of folded leaves and number of pairs of side shoots, and length of central inflorescence) were recorded from seedling emergence to harvest. The emergence and the first pair of unfolded leaves were recorded 3 and 10 days after sowing, respectively. The beginning of verticillaster emergence was detected after 50 days and the beginning of flowering after 66 days of sowing. The highest growth rates were achieved in CMP and EMP. After 77 days of sowing, CMP presented the highest values for the width of the main stem (10.4 mm) and height (90.89 cm), which were statistically different from EMP. The lowest variability between populations was found for the number of pairs of unfolded leaves and side shoots. The observed variability is promising for plant breeding to obtain cultivars capable of completing their cycle at this latitude.

Keywords: crop; phenology; *Salvia hispanica* L.; variability

1. Introduction

Salvia hispanica L., known as chia, is an annual herbaceous plant belonging to the *Lamiaceae* family. This species is native to southern Mexico and northern Guatemala. Currently, its cultivation is carried out commercially in Argentina, Australia, Bolivia, Colombia, Ecuador, Guatemala, Mexico, Nicaragua, Paraguay, Peru, and Southeast Asia [1,2]. Chia is originally a short-day plant, beginning the reproductive phase when the shortening of the day exceeds a certain threshold (10.5–12 h) [1,3]. It is a frost-sensitive crop. It develops optimally in tropical and subtropical climates, growing appropriately at latitudes ranging from approximately 20° N to 25° S. At higher latitudes, their growth cycle is not completed as the plants die due to frost damage [4]. Cultivars with different responses to photoperiod, capable of flowering in places where the length of the day is longer than 12.5 h, have been developed. This fact has allowed the development of its cultivation in central Europe and southern Germany [5]. In general, the grain yield is around 1400–2200 kg/ha. In addition, the content of lipids, protein, fiber, and ashes of chia fruits (nutlets, commonly named "seeds") varies between 25–32%, 19–29%, 27–29%, and 4–5%, respectively, in addition to the presence of tocopherols and polyphenols (myricetin, chlorogenic and caffeic acids, kaempferol, and quercetin) [6]. These attributes make it a crop with outstanding potential for its introduction into the horticultural systems of Gran La Plata (Buenos Aires, Argentina, 34°54′29′′ S, 58°2′25′′ W). This productive area has a total area of 8600 ha for horticultural production—including field and greenhouse crops—and is one of the main horticultural belts in Argentina. Artichoke (*Cynara scolymus* L.), tomato (*Solanum lycopersicum* L.), pepper (*Capsicum annuum* L.), celery (*Apium graveolens*), and lettuce (*Lactuca sativa* L.) are the main crops grown in this region [7]. Thus, the objectives of this work are to evaluate the potential of incorporating chia crop into the horticultural production systems of Gran La Plata and to analyze the interpopulation variability of four accessions of chia from our germplasm work collection.

2. Materials and Methods

2.1. Vegetal Material

The vegetal material consisted of four genotypes (populations): a commercial population cultivated by growers of Salta with mixed seeds (white, beige, and grayish brown) (CMP); one population bred by mass selection in the Estación Experimental Agroindustrial Obispo Colombres (Tucumán) (EWP1); and two populations provided by EEA INTA Salta, with white seeds (EWP2) and mixed seeds (EMP).

2.2. Edaphoclimatic Conditions

The assay was performed in the Estación Experimental MDA Gorina, located in La Plata, Buenos Aires, Argentina (34°54′29′′ S, 58°2′25′′ W), during the 2022 season (austral summer). In this area, the weather is of the mild-wet type, with annual rainfall of 1079 mm/year, relative humidity of around 77%, and a medium temperature of 15 °C. According to hydric balance, the weather in the region is B2C"2"r"a"(Thornthwaite system), corresponding to humid weather (B2), and microthermal (C2), indicating evapotranspiration values higher than 570 mm, with null or little water deficiency ("r") [8]. Figure 1a,b shows the mean, minimum, and maximum temperatures and the solar radiation in La Plata, respectively, during the crop cycle of chia (February to June) of 2022. These data were recorded by Weather Station OMXH®.

The soils of Gorina's series are taxonomically typical Hapludert. They are deep soils, moderately well drained, with slow or very slow permeability. They have a dark horizon of 20–30 cm in thickness, with a silty clay loam texture and clay tenors of 32–40%. The Bt horizon has a thickness between 80 and 100 cm with a clay content of 50–60%. They are fertile soils from the chemical point of view, generally provided by organic matter and nitrogen, with a slightly acidic reaction on their surface and are lightly alkaline in depth, with no interchangeable sodium harmful tenors or soluble salts [9].

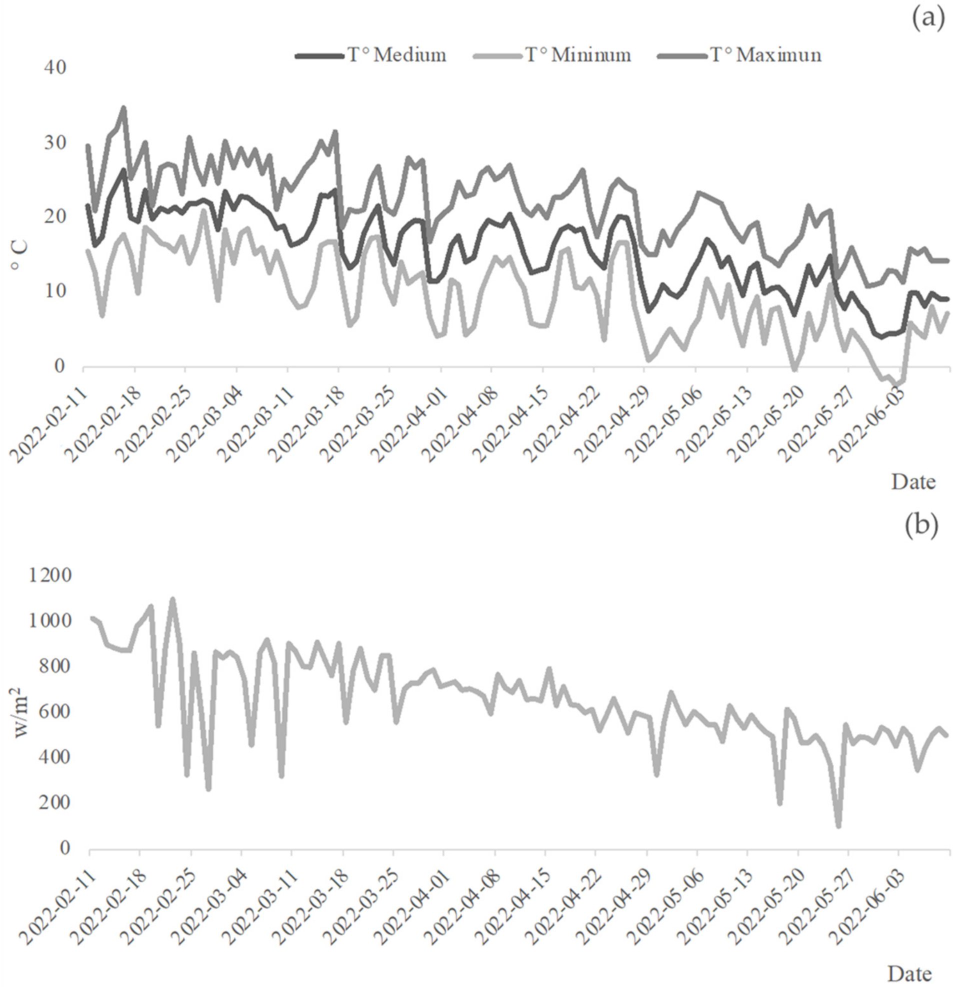

Figure 1. (**a**) Temperature and (**b**) solar radiation in La Plata (Buenos Aires, Argentina) during the chia crop cycle.

2.3. Field Experiments

The experiment was conducted on a natural field in a randomized completed block design with three replicates. The sowing date was on February 11, 2022. After 15 and 30 days of emergence, plots (four rows, 0.70 m apart, and 6 m in length) were over-seeded and hand-thinned to a final density of 8 plants per m^{-2}. To complement natural rainfall and avoid water deficits, plots were drip-irrigated for the whole of the growing seasons. The control of weeds, insects, and fungi was enhanced under organic management practices. Spontaneous species were hand removed throughout the growing season. A garlic extract solution was applied as a preventive treatment. After emergence, ten uniform plants from the central lines of each plot were labeled and monitored twice a week throughout the crop cycle.

The quantitative characteristics were measured at 33, 45, 60, 69, 76, and 83 days after sowing, recording: plant height, main stem width (at the height of the first leaf node), number of unfolded leaves on the main stem, number of pairs of side shoots, and central inflorescence length. In each plot, the days from sowing to each of the following phenological stages were also recorded: emergency, first pair of unfolded leaves, fourth pair of unfolded leaves, side shoot appearance, verticillaster appearance, flowering initiation, and full flowering. The phenological scale corresponded to the Biologische Bundesanstalt, Bundessortenamt and Chemical Industry (BBCH) systems.

2.4. Statistical Analysis

Experiments were performed following a randomized block experimental design as previously described. Data were subjected to analysis of variance (ANOVA) at a 95% confidence level ($p \leq 0.05$). Significantly different datasets were classified after post-hoc comparison tests using Tukey´s honestly significant difference test (HSD = $p \leq 0.05$). The statistical analyses were performed using the InfoStat software version 2020I (Universidad Nacional de Córdoba, Córdoba, Argentina) [10].

3. Results and Discussion

Table 1 shows the evolution of the plant height and the main stem width along the crop cycle. The CMP genotype evidenced the highest growth, recording a height of 92.15 cm after 83 days from the sowing, which was significantly different ($p \leq 0.05$) from EMP and EWP2. Additionally, CMP presented a stem width significantly higher than EMP, recording a value of 10.79 mm at the same time. The genotype EWP1 showed an intermediate behavior between EMP and CMP, with a height of 90.69 cm and a stem width of 10.44 mm after 83 days of sowing. The EMP and EWP2 populations presented a final height of 85.57 and 85.35 cm, respectively, without significant differences ($p > 0.05$) between them. Regarding the main stem width, the recorded values were 9.48 and 10.53 mm for EMP and EWP2, respectively (Table 1).

Table 1. Height of the plant (cm) and main stem width (mm) of four populations of chia (*Salvia hispanica* L.) evaluated along the crop cycle.

Character	Days after Sowing	EMP	CMP	EWP1	EWP2
Plant height (cm)	33	15.58 ^{A} ± 2.52	17.43 ^{AB} ± 2.48	18.07 ^{B} ± 3.29	18.38 ^{B} ± 3.38
	45	25.62 ^{A} ± 4.38	28.43 ^{AB} ± 4.01	28.98 ^{B} ± 5.02	29.00 ^{B} ± 5.64
	60	49.37 ^{A} ± 8.16	55.15 ^{B} ± 6.56	55.72 ^{B} ± 8.03	52.43 ^{AB} ± 9.49
	69	68.92 ^{A} ± 8.94	76.16 ^{B} ± 7.82	74.24 ^{AB} ± 9.40	70.97 ^{AB} ± 10.61
	76	83.37 ^{A} ± 8.99	90.89 ^{B} ± 9.01	87.85 ^{AB} ± 7.79	83.67 ^{A} ± 8.95
	83	85.57 ^{A} ± 9.50	92.15 ^{B} ± 9.09	90.69 ^{AB} ± 3.38	85.35 ^{A} ± 10.10
Main stem width (mm)	33	3.33 ^{A} ± 0.78	3.76 ^{AB} ± 0.76	3.45 ^{A} ± 0.75	4.15 ^{B} ± 1.49
	45	5.61 ^{A} ± 0.92	6.34 ^{B} ± 1.19	5.85 ^{AB} ± 0.91	6.28 ^{AB} ± 1.27
	60	7.55 ^{A} ± 1.34	8.41 ^{B} ± 0.60	7.74 ^{AB} ± 0.94	8.18 ^{AB} ± 1.52
	69	8.38 ^{A} ± 1.80	9.06 ^{A} ± 1.16	8.77 ^{A} ± 1.13	9.06 ^{A} ± 1.57
	76	9.25 ^{A} ± 1.46	10.40 ^{B} ± 1.41	9.85 ^{AB} ± 0.74	9.99 ^{AB} ± 1.83
	83	9.48 ^{A} ± 1.63	10.79 ^{B} ± 1.27	10.44 ^{AB} ± 1.04	10.53 ^{AB} ± 2.23

Different letters in each row indicate significant statistical differences ($p \leq 0.05$) between genotypes.

Tables 2 and 3 show the number of unfolded leaves on the main stem and the number of pairs of side shoots. These characteristics were the most stable, with no significant differences ($p > 0.05$) between the four populations studied. After 69 days of sowing, the final numbers of unfolded leaves and side shoots were 10 and 9, respectively. It is possible to observe from Tables 2 and 3 that the highest increase in these two characteristics was recorded between 33 and 45 days after sowing.

Table 2. Number of unfolded leaves on the main stem of four populations of chia (*Salvia hispanica* L.) evaluated along the crop cycle.

Days after Sowing	EMP	CMP	EWP1	EWP2
33	4.43 ^{AB} ± 0.50	4.73 ^{B} ± 0.52	4.27 ^{A} ± 0.45	4.73 ^{B} ± 0.78
45	7.02 ^{B} ± 0.65	7.27 ^{B} ± 0.55	6.50 ^{A} ± 0.51	7.23 ^{B} ± 0.54
60	9.07 ^{A} ± 0.65	9.75 ^{BC} ± 0.58	9.18 ^{AB} ± 0.59	9.76 ^{C} ± 0.73
69	10.20 ^{A} ± 0.77	10.33 ^{A} ± 0.62	9.87 ^{A} ± 0.64	10.33 ^{A} ± 0.72

Different letters in each row indicate significant statistical differences ($p \leq 0.05$) between genotypes.

Table 3. Number of pairs of side shoots of four populations of chia (*Salvia hispanica* L.) evaluated along the crop cycle.

Days after Sowing	EMP	CMP	EWP1	EWP2
33	1.93 ^{AB} ± 1.11	2.53 ^{B} ± 0.86	1.60 ^{A} ± 1.16	2.57 ^{B} ± 1.22
45	5.25 ^{A} ± 0.79	5.45 ^{A} ± 0.60	5.12 ^{A} ± 0.62	5.49 ^{A} ± 0.69
60	7.45 ^{AB} ± 0.51	7.97 ^{BC} ± 0.62	7.33 ^{A} ± 0.49	8.29 ^{C} ± 0.84
69	8.73 ^{AB} ± 0.70	9.27 ^{B} ± 0.70	8.53 ^{A} ± 0.64	8.80 ^{AB} ± 0.56
76	8.83 ^{A} ± 0.71	9.27 ^{A} ± 0.70	8.87 ^{A} ± 0.74	9.10 ^{A} ± 0.83

Different letters in each row indicate significant statistical differences ($p \leq 0.05$) between genotypes.

Finally, regarding the reproductive stage, the length of the main inflorescence had no significant differences ($p > 0.05$) between the populations studied, increasing from 18.04–20.42 mm to 138.11–156.63 mm, from 69 to 97 days after sowing (Table 4).

Table 4. Central inflorescence length (mm) from four populations of chia (*Salvia hispanica* L.) evaluated along the crop cycle.

Days after Sowing	EMP	CMP	EWP1	EWP2
69	18.04 ^{A} ± 4.66	20.42 ^{A} ± 3.74	18.20 ^{A} ± 4.06	20.08 ^{A} ± 6.10
74	35.41 ^{A} ± 8.09	36.69 ^{A} ± 9.83	33.49 ^{A} ± 8.01	36.69 ^{A} ± 13.40
76	45.21 ^{A} ± 11.62	46.86 ^{A} ± 10.47	39.71 ^{A} ± 10.78	45.70 ^{A} ± 17.16
83	59.30 ^{A} ± 18.19	64.27 ^{A} ± 16.37	55.53 ^{A} ± 18.55	63.81 ^{A} ± 27.99
90	116.31 ^{A} ±35.87	118.08 ^{A} ± 26.01	108.70 ^{A} ± 38.89	110.35 ^{A} ± 38.91
97	156.63 ^{A} ±32.76	138.11 ^{A} ± 36.71	155.08 ^{A} ± 34.58	141.05 ^{A} ± 32.74

Different letters in each row indicate significant statistical differences ($p \leq 0.05$) between genotypes.

Regarding the principal growth stages, six of the ten stages described in the BBCH scale were achieved by the chia crop in the Gran La Plata region under field conditions: germination (stage 0), leaf appearance (stage 1), shoot appearance (stage 2), stem elongation (stage 3), inflorescence growth on the main stem verticillaster (stage 5), and flowering (stage 6).

Figure 2 shows the evolution of the chia crop, indicating the phenological stage according to the scale proposed by the Biologische Bundesanstalt, Bundessortenamt and Chemical Industry (BBCH) and applied by Brandán et al. [11].

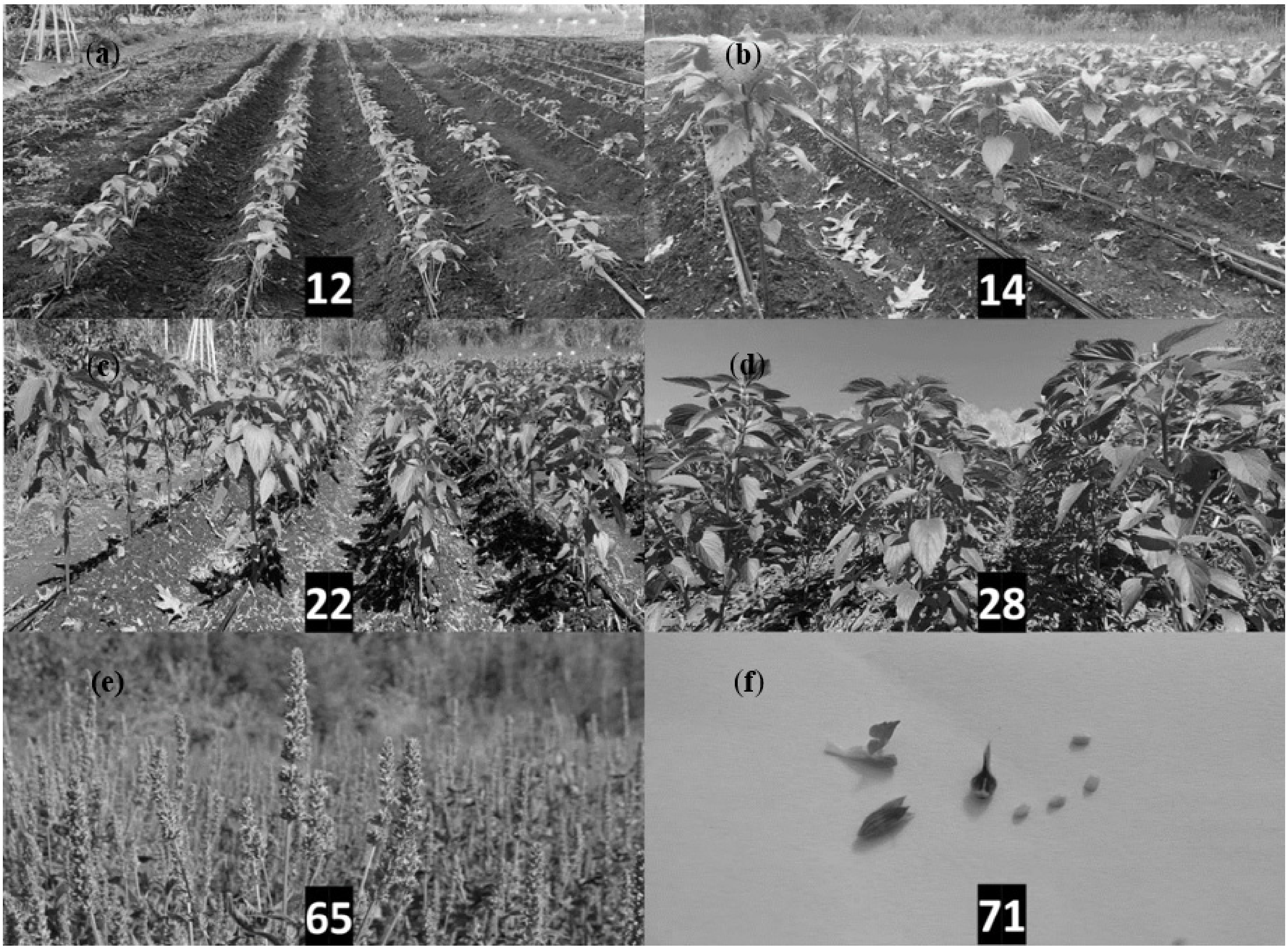

Figure 2. Crop evolution along the vegetative and reproductive cycles of chia. Numbers indicate phenological stages according to the Biologische Bundesanstalt, Bundessortenamt and Chemical Industry (BBCH) system. (**a**) growth stage 12, second pair (four leaves) unfolded; (**b**) growth stage 14, fourth pair (eight leaves) unfolded; (**c**) growth stage 22, four side shoots detectable; (**d**) growth stage 28, eight side shoots detectable; (**e**) growth stage 65, full flowering: at least one open flower in the apical-third of the verticillaster; and (**f**) growth stage 71, grains of the basal-third of the verticillaster with milk texture.

Overall, the emergence, recorded when cotyledons emerge over the soil surface (stage 09 of the BBCH scale), was verified 3 days after sowing. This stage is relevant in crop establishment as any environmental adversity could generate a low plant stand [11]. The first pair of leaves (stage 11) was recorded after 10 days from sowing. Figure 2a,b show stages 12 and 14, respectively, which correspond to the second (four leaves) and four pairs (eight leaves) of unfolded leaves. In this trial, the final number of unfolded leaves was about 10–11 for all the genotypes. Stage 14 was recorded 31 days after sowing, which coincided with the appearing of the first pair of side shoots (stage 21), which present an acropetal growth.

The number of days to differentiate verticillaster from leaves (stage 51) was 53 days from sowing when plants presented 10–11 unfolded pairs of leaves (stages 110 and 11), and 8–9 pairs of side shoots (stages 28 and 29).

The beginning of flowering (stage 60) was 66 days after sowing, when at least one flower in the basal-third of the verticillaster had opened. Full flowering (stage 65) was achieved in CMP and EMP at 76 days after sowing, whereas EWP1 and EWP2 took 86 days, indicating that the first two genotypes greater were earlier than the last two.

4. Conclusions

The main differences between the assayed populations were detected for the height and the stem diameter; the number of leaves and shoots were stable regardless of the population evaluated. The plants that first reached the flowering stage corresponded to genotypes with mixed seeds, thus being the earliest ones. The first frost recorded in the area was on April 29 (Figure 1a). After that date, the medium temperatures dropped along with solar radiation (Figure 1b), hindering the favorable evolution because it is a frost-sensitive crop. The assay was finished 100 days after sowing with plants highly affected by low temperatures, and genotypes could not complete the cycle under field conditions. However, it is relevant to note that the populations were also evaluated under greenhouse conditions (data not shown). Under these conditions, they did not show cold damage and were in grain filling after 160 days from the sowing date.

The introduction of genotypes less sensitive to the photoperiod is necessary for the adoption of this crop in the Gran La Plata area if it is to be produced in the field. However, future research on the results obtained under greenhouse conditions may be potentially favorable for incorporating the crop into covered horticultural systems.

Variability was found among the studied populations, which is promising for future genetic breeding plans.

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