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Physiological Analysis of Calyx Growth and Yield Analysis in Roselle (*Hibiscus subdariffa* L.)

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ABSTRACT

The study conducted during the month of July to November, 2022 aimed to evaluate the roselle (*Hibiscus subdariffa*) genotypes suitable for calyx yield. Five roselle genotypes were evaluated in four replications for physiological analysis of calyx growth and it's biochemical status under rainfed condition. The actual and effective growth and lag periods for calyx growth ranged between 33 and 37, 15.00 and 21.82, 11.93 and 22.36 days, respectively. Genotype AHC-1 required lower actual growth period, lag period and longer effective growth period. The genotype, HSLC-1 recorded minimum plant height, higher number of leaves, number of branches and number of fruits and maintained higher fresh and dry weight of calyx productivity. Genotype, AHC-1 was superior for maintaining fresh and dry weight of calyx, volume of calyx per flower bud, higher absolute growth rate of calyx, higher phenolic and anthocyanin content in calyx. The genotypes, HSLC-1 and AHC-1 required maximum growing degree days and maintained higher heat use efficiency, helio thermal units and heliothermal use efficiency. Considering physiological and biochemical variation in calyx growth and yield influenced by roselle genotypes, genotype AHC-1 with highest fresh weight of calyx per flower bud, highest phenolic content and anthocyanin content having high medicinal value could be suggested to farmers. In earliness point of view and overall calyx productivity the genotype HSLC-1 could be suggested to farmers.

KEYWORDS: Phenology, growth, calyx, growth rates, phenolics, heat units

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1. INTRODUCTION

Roselle (*Hibiscus sabdariffa* L.) is popularly recognized as 'mesta' or 'chukur' in Indian subcontinent including Bangladesh. Roselle is locally known by different names in different countries (Ismail et al., 2008). It belongs to the family of Malvaceae which is originated from West Africa and commonly available in the tropics especially in the African countries (Elsadig et al., 2013). It is widely cultivated in Tropical Africa, Sudan, Mali, Nigeria, Chad, India, Indonesia, Phillipines, Malaysia, Brazil, Australia, Mexico, Hawai and Florida of USA. In the Indian subcontinent (especially in Ganges Delta region), roselle is cultivated for vegetable fibres. Every fraction of roselle plants including leaves, fruits, roots and seeds are utilized in various foods. Among them, red fleshy calyces are employed for making fresh beverage tastes like Ribena, juice, jam, jelly, syrup, gelatin, pudding, wine, cakes, ice-cream and flavors and also dried and brewed into tea (Pacome et al., 2014; Delgado and Parcedes, 2003). The bright red color coupled with exceptional flavor and other organoleptic attributes make them valuable food products such as wine, syrup, ice cream, pies, snakes, tarts and other desserts. The typically red calyx, consists of 5 large sepals with a collar (epicalyx) of 8–12 slim, pointed bracts (or bracteole) around the base, they begin to enlarge at the end of the day, 3.2–5.7 cm long and fully enclose the fruit (Mahadevan et al., 2009).

Roselle is a multipurpose plant and all above ground parts of roselle is used as traditional medicine for the treatment of several diseases in Africa, Senegal, India, Thailand and Mexico. Many medicinal applications of the plant parts of roselle have been reported in different countries of the world (Lin et al., 2011; Fullerton et al., 2011). Several reports listed which affirm the traditional health benefits of roselle extract. The drink contains vitamin C and anthocyanins which act as antioxidants. Anthocyanins present in roselle are dephinidin 3-sambubioside, cyaniding 3-sambubioside, delphinidin 3-glucoside and cyanidin 3-glucoside. Because of its commercial potential as a natural food and coloring agent roselle has drawn interest of manufacturers of food, beverage and pharmaceutical. Roselle seeds are used to produce biodiesel and also used as animal feed as the seeds contain 17.8 to 21% non-edible oil and 20% cent protein (Duarte-Valenzuela et al., 2016). Temperature is an important environmental factor that influences the growth and development, phenology and yield of crops (Bishnoi et al., 1995). Hence, it becomes imperative to have the knowledge of exact duration of various phenological stages of crop in a particular growing environment and their impact on its yield. Crop phenology is an essential component of crop growth and yield and it can be used to estimate the most appropriate date and time of specific development process. The duration of each phenophase determines the dry matter accumulation and its partitioning into different organs (Okosun et al., 2006). Fleshy calyces are a good source of natural food colorants because of their high pigment content (Bridle and Timberlake, 1997). Moreover, the dried calyces are consumed worldwide in hot infusions and in cold drinks (Hernández and Goni, 2012). Besides its extended consumption as a beverage and its uses in the food industry, Roselle is also used in animal feed, nutraceuticals, cosmetics, and pharmaceuticals (Wang et al., 2012; Jabeur et al., 2017). Therefore, an experiment was conducted to to study the physiological and biochemical variation in calyx growth and to determine the phenology and heat unit requirement of promising roselle genotypes.

2. MATERIALS AND METHODS

2.1. Experimental site

The experiment was conducted at All India Network Project on Jute and Allied Fibres, MPKV, Rahuri, Dist.: Ahmednagar during the main cropping season in *kharif* (July–November, 2022) under rainfed condition. The description of the test environments are shown in Table 1.

Table 1: Description of the test environments testing location

Testing location	Altitude (m.a.s.l)	Latitude	Longitude	Annual Rainfall (mm)	Min. annual Temp.(°C)	Max. annual Temp.(°C)
MPKV, Rahuri	657 meters	19°-48° N and 19°-57° N	74°-32°E and 76°-19° E	520 mm	17.98oC	35.12oC

2.2. Experimental materials and design

Five genotypes of roselle viz. AHC-1, HSLC-1, HSLC-2, AMV-5 and HS-4288 were evaluated in four replications for Physiological and biochemical variation for calyx growth and yield. The gross and net plot sizes were 4.50×2.70 m^2. The spacing was 45×15 cm^2. The plot was manured by 5 tons of FYM ha^{-1} during land preparation and incorporated well with harrowing. The half dose Nitrogen (30 kg ha^{-1}) and full dose of P_2O_5 and K^2O (30 kg ha^{-1}) applied at the time of sowing. The remaining dose of Nitrogen was applied at 35 DAS and 55 days after sowing.

2.3. Data collection

The observations on growth and calyx yield parameters were recorded on days to initiation of flowering, 50% flowering calyx maturity, Plant height (cm), Number of leaves plant^{-1}, Number of branches plant^{-1}, Number of fruits plant^{-1}, Fresh weight of calyx plant^{-1} (g), Fresh weight of calyx plot^{-1} (kg), Dry weight of calyx plant^{-1} (g) and Dry weight of calyx plot^{-1} (kg). Around 500 flower buds were tagged in each plot to record the observations on calyx growth and observations were recorded on fresh weight, dry weight and volume of the calyx periodically at 4 days interval until calyx maturity.

2.4. Data analysis

The data analyzed for growth rates as per the formulae;

Absolute growth rate (AGR)=(Final weight-Initial weight)/ Time interval (days), Radford (1967)

Relative growth rate (RGR)= [Log_e(Final weight)-Log_e(Initial weight)]/Time interval (days), Radford (1967)

Effective growth period(EGP)=(Final weight/Mean dry matter), Daynard and Kannenberg (1976)

Lag period=(Actual growth period-Effective growth period), Daynard and Kannenberg (1976)

Growing degree days (GDD)=(T_{max}+T_{min}/2)–T_{base}, Girijesh et al. (2011)

Heat Use Efficiency (HUE)=(Dry matter/GDD), Girijesh et al. (2011)

Heliothermal Units (HTU)=(GDD×Actual bright sunshine hours), Girijesh et al. (2011)

Heliothermal Use Efficiency (HTUE)= (Dry matter/HTU), Girijesh et al. (2011)

Estimation of Phenols and Anthocyanin content was carried out with Folin-Ciocalteu reagent by method of Bray and Thorpe (1954) and Anthocyanin reagent by method of Swain and Hillis (1959), respectively.

The data was statistical analyzed by standard methods for Randomized Block Design as suggested by Panse and Sukhatme (1985).

3. RESULTS AND DISCUSSION

3.1. Crop phenology

The data on crop phenology are presented in Table 2. The differences among the genotypes were statistically significant for phonological characters. In the present investigation, the genotype HS 4288 required maximum number of days to initiation of flowering (134.75), 50% flowering (141.50) and calyx maturity (178.50), whereas HSLC was earlier for days to initiation of flowering (106.50), 50% flowering (115.00) and calyx maturity (149.25). On an average, the crop initiate flowering on 121.95 days and complete 50% flowering on 129.55 days. The calyx maturity completes on 164.80 days. It revealed that, the calyces can mature within 40–50 days after initiation of flowering. Susanto et al. (2013) reported that the criteria of calyxes that are ready to be harvested are already reached the age for harvesting, which is 60 days after blooming.

Table 2: Crop phenology influenced by roselle genotypes

Genotype	Days to initiation of flowering	Days to 50 % flowering	Days to calyx maturity	Actual growth period (days)	Effective growth period (days)	Lag period (days)
AHC-1	113.00	119.75	153.50	33.75	21.82	11.93
HSLC-1	106.50	115.00	149.25	34.25	21.03	13.22
HSLC-2	125.00	132.00	165.25	35.25	16.85	18.40
AMV 5	130.50	139.50	177.50	38.00	15.64	22.36
HS 4288	134.75	141.50	178.50	37.00	15.00	22.00
Mean	121.95	129.55	164.80	35.65	18.07	17.58
SEm±	4.57	5.85	6.56	2.17	0.80	1.00
CD (p=0.05)	14.23	18.23	20.43	6.42	2.484	3.12

The actual growth period (AGP) for calyx growth ranged between 33 and 37 days, whereas, effective growth period (EGP) ranged between 15.00–21.82 days. Genotype AHC-1 (33.75 days) required lowest actual growth period followed by HSLC-1 (34.25 days), HSLC-2 (35.25 days), HS 4288 (37.00 days) and AMV-5 (38.00 days). The genotype HS-4288 (15.00 days) recorded the lowest effective growth period followed by AMV-5 (15.64 days), HSLC-2 (16.85 days), HSLC-1 (21.03 days) and AHC-1 (21.82 days). The lag period for calyx growth ranged between 11.93 and 22.36 days. The genotype AHC-1 (11.93 days) maintained the lowest lag period followed by HSLC-1 (13.22 days), HSLC-2 (18.40 days), HS-4288 (22.00 days) and AMV-5 (22.36 days). Although AGP and EGP were both correlated with yield, all three variables were also correlated with date of flowering and the possibility existed that the former

correlations were simply artifacts of the latter (Daynard and Kannenberg, 1976).

3.2. Growth and yield contributing characters

Growth is a characteristic of a living organism. It is a permanent change which increases the size of the plant. Yield is defined as the harvested or harvestable accumulated increment per unit area. In the present investigation, the differences among the genotypes were statistically significant for growth and yield contributing characters (Table 3).

The genotype, HSLC-1 recorded the minimum plant height (191.25 cm), whereas, it maintained maximum number of leaves plant^{-1} (110.92), number of branches plant^{-1} (21.35), fruits plant^{-1} (78.25), fresh weight of calyx plant^{-1} (136.65 g), fresh weight of calyx plot^{-1} (10.93 kg), dry weight of calyx plant^{-1} (14.02 g) and dry weight of calyx plot^{-1} (1.121 kg). However HS 4288 recorded maximum plant height (325.00 cm), whereas, recorded the minimum number of leaves plant^{-1} (64.32), number of branches plant^{-1} (7.15), fruits plant^{-1} (40.05), fresh weight of calyx plant^{-1} (57.40 g), fresh weight of calyx plot^{-1} (4.59 kg), dry weight of calyx plant^{-1} (6.20 g) and dry weight of calyx plot^{-1} (0.496 kg). Shuhaimi et al. (2018) observed that crops grown in started to show a sharp increment in plant height after 85 days after sowing. The production of leaves was indicative of the plant development and it is independent of plant growth. Saidaiah et al.(2021) reported a wide range of genetic variability was associated plant height, followed by number of fruits per plant and average fruit weight.

Table 3: Growth and yield contributing characters influenced by roselle genotypes

Genotype	Plant height (cm)	No. of leaves plant^{-1}	No. of branches plant^{-1}	No. of fruits plant^{-1}	Fresh weight of calyx plant^{-1} (g)	Fresh weight of calyx plot^{-1} (kg)	Dry weight of calyx plant^{-1} (g)	Dry weight of calyx plot^{-1} (kg)
AHC-1	220.00	95.92	17.22	62.40	120.05	9.60	11.73	0.934
HSLC-1	191.25	110.92	21.35	78.25	136.65	10.93	14.02	1.121
HSLC-2	286.25	82.90	11.95	44.10	89.20	7.14	7.23	0.778
AMV 5	282.50	80.20	9.72	43.70	78.80	6.30	8.49	0.679
HS 4288	325.00	64.32	7.15	40.05	57.40	4.59	6.20	0.496
Mean	261.00	86.85	13.48	53.70	96.42	7.71	9.53	0.800
SEm±	9.824	3.49	1.27	1.77	4.36	0.63	0.91	0.04
CD (p=0.05)	30.606	10.86	3.95	5.52	13.58	1.90	2.83	0.13

3.3. Calyx growth

The differences among the genotypes for fresh weight of calyx were statistically significant at various stages of growth. The fresh weight of calyx increased progressively with advancing age of the crop (Table 4). The growth was rather slow upto 12 DAF, thereafter it was rapid upto 28 DAF and slower towards maturity. The genotype, AHC-1 maintained higher fresh weight of calyx at 4th (0.53 g), 8th (0.478 g), 12th (0.726 g), 16th (1.361 g), 20th (1.163 g), 24th (2.911 g), 28th (3.368 g) and 32nd (3.508 g) days after flowering followed by HSLC-1, HSLC-2, HS-4288 and AMV-5. Haryati et al. (2018) observed that the calyxes increased in fresh weight, lengths and diameters, with the most rapid growth from 12 days to 24 days after anthesis. Fakir et al. (2012) observed that calyx grew larger with aging.

The differences among the genotypes for absolute growth

Table 4: Fresh weight of calyx (g flower bud^{-1}) influenced by roselle genotypes at various stages of growth

Genotype	4 DAF	8 DAF	12 DAF	16 DAF	20 DAF	24 DAF	28 DAF	32 DAF
AHC-1	0.356	0.478	0.726	1.361	2.163	2.911	3.368	3.508
HSLC-1	0.196	0.290	0.350	0.677	1.048	1.623	2.081	2.204
HSLC-2	0.128	0.149	0.189	0.270	0.526	0.938	1.486	1.708
AMV 5	0.063	0.113	0.177	0.208	0.267	0.738	1.140	1.212
HS 4288	0.128	0.172	0.240	0.397	0.714	1.092	1.680	1.810
Mean	0.174	0.240	0.336	0.583	0.944	1.460	1.951	2.088
SEm±	0.007	0.007	0.012	0.017	0.031	0.038	0.057	0.056
CD (p=0.05)	0.021	0.021	0.038	0.052	0.097	0.119	0.179	0.174

rate (AGR) for calyx growth were statistically significant at various stages of growth. The absolute growth rate for calyx growth increased progressively upto 24–28th DAF, thereafter it was declined towards maturity (Table 5). The genotype, AHC-1 maintained higher AGR during 4–8 (0.034 g day^{-1}), 8-12 (0.063 g day^{-1}), 12-16 (0.157 g day^{-1}), 16-20 (0.201 g day^{-1}) and 20-24 DAF (0.187 g day^{-1}), whereas, HS 4288 during 24-28 DAF (0.147 g day^{-1}) and HSLC-2 during 28-32 DAF (0.055 g day^{-1}) recorded higher AGR.

The differences among the genotypes for relative growth rate (RGR) for calyx growth were statistically significant at various stages of growth. The relative growth rate for calyx growth increased progressively upto 12–16th DAF in AHC-1 and HSLC-1, however, it was increased upto 16–20th DAF in HSLC-2 and HS-4288 and 20-24th DAF in AMV-5 (Table 5). The genotypes, AMV-5 at 4-8 DAF (0.056 g g^{-1} day^{-1}), 8–12 DAF (0.051 g g^{-1} day^{-1}), 20–24 DAF (0.111 g g^{-1} day^{-1}) and 28.32 DAF (0.020 g g^{-1} day^{-1}),

Table 5: Absolute growth rate (AGR) for calyx growth (g day^{-1}) influenced by roselle genotypes at various stages of growth

Genotype	4-8 DAF	8-12 DAF	12-16 DAF	16-20 DAF	20-24 DAF	24-28 DAF	28-32 DAF
AHC-1	0.034	0.063	0.157	0.201	0.187	0.115	0.024
HSLC-1	0.024	0.014	0.078	0.093	0.144	0.109	0.024
HSLC-2	0.005	0.010	0.020	0.064	0.103	0.137	0.055
AMV 5	0.013	0.016	0.008	0.015	0.118	0.100	0.017
HS 4288	0.011	0.017	0.039	0.079	0.095	0.147	0.029
Mean	0.017	0.024	0.060	0.090	0.129	0.122	0.030
SEm±	0.001	0.001	0.003	0.005	0.008	0.009	0.002
CD (p=0.05)	0.003	0.003	0.009	0.015	0.024	0.027	0.006

HSLC-1 at 12-16 DAF (0.070 g g^{-1} day^{-1}), HSLC-2 at 16-20 DAF (0.075 g g^{-1} day^{-1}), HS-4288 at 24-28 DAF (0.049 g g^{-1} day^{-1}) recorded higher relative growth rate for calyx growth (Table 6).

The differences among the genotypes for dry weight of calyx were statistically significant at various stages of growth. The dry matter accumulation in calyx increased progressively with advancing age of the crop (Table 7).

Table 6: Relative growth rate (RGR) for calyx growth (g day^{-1}) influenced by roselle genotypes at various stages of growth

Genotype	4-8 DAF	8-12 DAF	12-16 DAF	16-20 DAF	20-24 DAF	24-28 DAF	28-32 DAF
AHC-1	0.038	0.047	0.068	0.050	0.032	0.016	0.004
HSLC-1	0.041	0.022	0.070	0.050	0.048	0.026	0.017
HSLC-2	0.017	0.025	0.038	0.075	0.063	0.050	0.015
AMV 5	0.056	0.051	0.020	0.025	0.111	0.047	0.020
HS 4288	0.032	0.033	0.058	0.063	0.046	0.049	0.008
Mean	0.037	0.036	0.051	0.053	0.060	0.038	0.013
SEm±	0.002	0.002	0.003	0.002	0.004	0.002	0.001
CD (p=0.05)	0.006	0.006	0.010	0.006	0.012	0.006	0.003

Table 7: Dry weight of calyx (g flower bud^{-1}) influenced by roselle genotypes at various stages of growth

Genotype	4 DAF	8 DAF	12 DAF	16 DAF	20 DAF	24 DAF	28 DAF	32 DAF
AHC-1	0.035	0.048	0.074	0.137	0.271	0.312	0.359	0.457
HSLC-1	0.020	0.025	0.037	0.069	0.108	0.159	0.215	0.289
HSLC-2	0.012	0.015	0.020	0.029	0.054	0.095	0.151	0.168
AMV 5	0.007	0.011	0.017	0.020	0.029	0.075	0.113	0.145
HS 4288	0.011	0.013	0.026	0.033	0.073	0.110	0.168	0.193
Mean	0.017	0.022	0.035	0.058	0.107	0.150	0.201	0.250
SEm±	0.001	0.001	0.001	0.003	0.006	0.005	0.007	0.007
CD (p=0.05)	0.003	0.003	0.003	0.009	0.018	0.015	0.023	0.021

The rate of increase of dry weight was rather slow between 4 to 12 DAF, thereafter rate was rapid upto 28 DAF. The genotype AHC-1 maintained higher dry weight of calyx at various stages of growth followed by HSLC-1 and HS 4288. At harvesting, AHC-1 (0.457 g) and AMV 5 (0.145 g) recorded the highest and lowest dry weight of calyx, respectively.

The differences among the genotypes for volume of calyx were statistically significant at various stages of growth. The volume of calyx increased progressively with advancing age of the crop in all the genotypes. The rate of increase volume of calyx was rather slow between 4 to 12 DAF, thereafter rate was rapid upto 28 DAF (Table 8). The genotypes, AHC-1 (3.708 ml) and HSLC-2 (1.608 ml) recorded the maximum and minimum volume at harvesting.

Table 8: Volume of calyx (ml flower bud^{-1}) influenced by roselle genotypes at various stages of growth

Genotype	4 DAF	8 DAF	12 DAF	16 DAF	20 DAF	24 DAF	28 DAF	32 DAF
AHC-1	0.320	0.473	0.665	1.280	2.060	2.800	3.290	3.708
HSLC-1	0.180	0.255	0.305	0.610	0.985	1.520	2.025	2.300
HSLC-2	0.095	0.140	0.169	0.345	0.488	0.865	1.420	1.608
AMV 5	0.045	0.089	0.152	0.170	0.240	0.690	1.040	1.193
HS 4288	0.095	0.171	0.229	0.283	0.665	1.050	1.625	1.835
Mean	0.147	0.226	0.304	0.538	0.888	1.385	1.880	2.129
SEm±	0.012	0.007	0.010	0.041	0.033	0.047	0.057	0.060
CD (p=0.05)	0.038	0.023	0.030	0.129	0.102	0.145	0.178	0.187

3.4. Biochemical parameter and heat units

The differences among the genotypes for phenolic and anthocynin content and parameters of heat units were statistically significant (Table 9). The genotype AHC-1 recorded maximum phenolic (42.375 mg gm GAE^{-1}) and anthocynin (339.500 mg 100 g^{-1}) content followed by HSLC-1 (38.625 mg g^{-1} GAE^{-1}; 315.750 mg 100 g^{-1}) and HSLC-2 (25.750 mg g^{-1} GAE; 213.750 mg 100 g^{-1}). However, HSLC-1 (441.5°C) and AHC-1 (430.7°C) required maximum growing degree days. Similarly, HSLC-1 maintained higher heat use efficiency (32 g°C/day×10^{-3}), Helio thermal units (3576.2 g°C day hours^{-1}) and Heliothermal use efficiency (0.438 g°C / hrs×10^{-3}) followed by AHC-1 (27 g°C/day×10^{-3}; 3488.7 g°C day hours^{-1}; 0.405 g°C/hrs×10^{-3}). Anokwuru et al. (2011) studied the polyphenolic content and antioxidant activity of *Hibiscus sabdariffa* calyx in methanol, ethanol, acetone and water extract. The Total Phenolic Content (TPC) was determined using folin Ciocalteu method while the Total Flavonoid Content (TFC) was determined using aluminum chloride method. Cisse et al. (2012) concluded that roselle calyces are a good source of anthocyanin with several potential applications in the food, pharmaceutical and cosmetic industries as far as anthocyanin extract is concerned. Susanto et al. (2013) reported 0.37 to 0.41 mmol g^{-1} anthocyanin content in Apex pruning treated calyxes of roselle, while on unpruned plants it was only 0.32 mmol g^{-1}.

Table 9: Biochemical parameter and heat units influenced by roselle genotypes

Genotype	Phenolic content (mg gm GAE^{-1})	Anthocynin content (mg 100 g^{-1})	GDD (°C)	Heat use efficiency (HUE) (g °C/day×10^{-3})	Helio- thermal units (HTU) (g °C day hours^{-1})	Helio-thermal use efficiency (HTUE) (g °C/hrs×10^{-3})
AHC-1	0.320	0.473	0.665	1.280	2.060	2.800
HSLC-1	0.180	0.255	0.305	0.610	0.985	1.520
HSLC-2	0.095	0.140	0.169	0.345	0.488	0.865
AMV 5	0.045	0.089	0.152	0.170	0.240	0.690
HS 4288	0.095	0.171	0.229	0.283	0.665	1.050
Mean	0.147	0.226	0.304	0.538	0.888	1.385
SEm±	0.012	0.007	0.010	0.041	0.033	0.047
CD (p=0.05)	0.038	0.023	0.030	0.129	0.102	0.145

4. CONCLUSION

The genotype HSLC-1 maintained lowest plant height, highest leaf number, branches, fruits plant^{-1}, earlier flowering and calyx maturity, higher GDD, heat use efficiency and heliothermal use efficiency during calyx growth. Genotype, AHC-1 was superior for fresh and dry weight of calyx, volume of calyx, higher absolute growth rate, higher effective growth period, lower lag period, higher phenolic and anthocyanin content in calyx. Therefore, the genotype HSLC-1 was superior for yield and yield contributing characters and AHC-1 was better for quality.

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