

Citrus rootstock genotypes response to drought: alternation in morphology, physiology and leaf mineral content

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ABSTRACT

The potted seedlings (18 months old) of seven citrus genotypes were imposed to drought-rewater cycles for every 24 days to study the alterations in morpho-physiological traits and leaf mineral nutrients. The continued growth under drought was observed in Soh sarkar (11.88% more), RLC-7 (10.25% more) and Karna Khatta (10.74% more) as of normal conditions. Moreover, after re-watering, RLC-7 enhanced the plant height to the great extent (17%) as compared to drought. RLC-7, RLC-5 and Soh sarkar maintained a leaf area ratio even under drought condition, while it was inhibited in Jatti khatti and Karna khatta significantly under drought as compared to well-watered conditions. Moreover, reduction in the root volume in most of the rootstocks except RLC-5 and Soh sarkar was noticed. Most of the rootstocks were able to maintain intrinsic water use efficiency after rewatering except RLC-6. Chlorophyll 'b' enhanced under drought in RLC-5, RLC-6 and RLC-7, while it was inhibited in Soh sarkar at the end of the drought. Concomitantly, Soh sarkar and RLC-5 exhibited a noticeable alternation of mineral nutrients, having sharp increase of K (RLC-5), but P, Fe and Zn were relatively stable throughout the experiment as compared to start of drought and after rewater stage. From the study, it could conclude that Soh sarkar and RLC-5 (*Citris jambhiri*) can escape moderate drought. Further, it can also be argued that differential mechanism works for drought tolerance in RLC-5 and Soh sarkar genotypes.

Key words: Chlorophyll, intrinsic water use efficiency, root volume, leaf mineral nutrients.

INTRODUCTION

Citrus is the most important group of fruits, and being cultivated in more than one hundred forty countries of tropical and subtropical regions of the world. Some citrus species are the useful source of potent antioxidants, providing prevention against heart diseases, besides possessing the anticancer, inflammation, antiviral, antibacterial and antifungal activities. Global climate changing scenario and increasing population, demands developing of crops tolerant to abiotic stresses included drought. Drought, a physiological form of water deficit, is one of the most noteworthy environmental factors inhibiting growth, performance, and distribution of plant species worldwide and dropping crop yield (nearly 70%) (Nakashima and Yamaguchi-Shinozaki, 20). Because drought adversely influences the ion translocation and uptake, carbohydrate and nutrient metabolism consequently inhibits cell enlargement and growth, and finally it tends to kill the plants (Jaleel *et al.*,12). The physiological response of plants to drought events includes alterations in photosynthetic metabolism and activity of enzymes involved in the Calvin cycle; or indirect damage the

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photosynthetic apparatus caused by oxidative stress. The behavior of plant response to drought depends upon plant species, drought intensity and duration. In commercial grown citrus fruits, drought tolerance can be achieved by grafting the intended scions onto drought-tolerant citrus rootstocks, making it possible to grow citrus trees under water-limited environments. The previous findings of responses to drought stress of different citrus genotypes have shown the huge differences in their defence mechanism. Hereby, the present experiment aimed at assessment of the drought sustainment of citrus rootstock genotypes for further utilizing in extending the citriculture under drought affected areas.

MATERIALS AND METHODS

The eighteen months old seedlings of citrus genotypes, viz. Jatti khatti (*Citrus jambhiri* Lush), Karna khatta (*C. karna* Raf.), RLC-5 (*C. jambhiri* Lush.), RLC-6 (*C. jambhiri* Lush.), RLC-7 (*C. jambhiri* Lush.), sour orange (*C. aurantium*) and Soh sarkar (*C. karna* Raf) were transplanted into 12-inch plastic pots containing native orchard soil mixed with farmyard manure (1:1). Seedlings were regularly irrigated, supplied once with 20 g NPK (19:19:19). After 3-months of transplanting, the final established seedlings were divided into two sets. In first set,

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50% seedlings of each genotype provided normal irrigation and rest 50% were subjected to drought for 24 days to record root volume of testing genotypes under both conditions. While in the second set, first all seedlings of each genotype were given normal irrigation, then subjected to the irrigation withholding for 24 days, subsequent to 24-days re-water to record physiological traits and leaf mineral contents. Plant height and root volume were recorded after 24 days of drought periods from the first set. The evaluation of physiological traits and mineral nutrients was done in the beginning, at the end of the drought and after rewatering. For morphological evaluation, the plant height was measured with a meter scale, and leaf area ratio (LAR) was calculated on total leaf area/ plant and plant dry weight (DW) and expressed as cm² g⁻¹ (Radford, 21). The root volume was measured on wholly uprooted plants before and after drying (65°C ±1°C) until constant weight. Visual wilt injury of the plant was scored from zero with no sign of wilting or damage, to score 5 for severe injury exhibited by cupping to curling, chlorotic and shedding of leaves at end of the drought. The leaf gas exchange including photosynthetic rate, stomatal conductance to water vapor (g_{i}) and internal CO₂ concentration (Ci) was determined on matured leaves with the help of the infra-red gas analyser (IRGA) (LI-6200, LI-COR Biosciences, Lincoln, NE, USA). Intrinsic water use efficiency was determined from the ratio of A to g_{a} (WUEi, A/g) (dos Santos et al., 4). The leaf chlorophyll a (Chl a) and b (Chl b) were measured based on the method of Hiscox and Isarelstam (9) using DMSO and formulae of Arnon (2). Then ratio of chl a and b was determined. Mineral nutrient concentration was determined by wet di-acid digestion using nitric acid (HNO₂) and perchloric acid (HClO₄) in ratio 9:4 (Jackson, 10). The filtered extractant, was used for the estimation of phosphorus (Chapman and Pratt, 3), potassium with Systronics Flame Photometer 128. For iron (Fe), copper (Cu) and zinc (Zn), atomic absorption spectrophotometer (Model- Analytikjena ZEEnit 760, Germany) was used. The experiment was designed with the completely randomized block with five replications. Data in factorial arrangement were analyzed with Proc GLM procedure in SAS 9.3 version software (SAS, USA INC). At end of drought, gravimetric soil water also determined which ranged between 2.16%-2.77%.

RESULTS AND DISCUSSION

Visual wilting symptoms showed that Soh sarkar scored lowest value (1.33) followed by RLC-5 (3.33) and Karna khatta (3.33), while Jatti khatti scored highest (4.67) out of 5 scores (Fig 1A).The visual wilt symptoms can be used for testing of drought

tolerance because of its tight linear relationships with leaf water status or soil water availability. Wang *et al.* (22) also reported that drought tolerant apple species (*Malus prunifolia*) exhibited the least wilt scores and in reversion from drought sensitive species (*M. hupehensis*).

Almost seizure in plant height was noticed in most of the tested genotypes under drought except Soh sarkar and RLC-5. Similar pattern was observed, while rootstocks were subjected to rewater (Fig 1b). Variable results in respect of plant height have been reported earlier such by Karimi *et al.* (14), who suggested the ability of drought tolerant olive rootstock to maintain its normal physio-biochemical process for progressive plant growth, while Luvaha *et al.* (18) found the restricted plant height in mango rootstock drouhgt under the influence of drought stress.

Leaf area ratio (LAR) reduced significantly in Jatti khatti (18.93%) and Karna khatta (14.84%) under drought as compared to beginning of drought. No alternation in LAR was observed in RLC-5, RLC-6 and Soh sarkar under drought in comparison to the beginning of drought (Fig 1C). Noticeably, an increase of 26.99% in LAR was observed in RLC-7 under drought. An increase of LAR from plant that experienced drought was resulted from newly emerged leaves, which led to the elevation of total leaf area following rewatering. Khoyerdi et al. (15) indicated various trends of changing in LAR under drought stress. Reduction of leaf number was consequence of the leaf senescence and shedding influenced by drought (Jie et al., 13). We noticed stability in LAR in most of the citrus genotypes except RLC-7, wherein it increased. Contrary to this, Al-Absi (1) proved that the stability of LAR resulted from the decline in both total leaf area (LA) and plant dry matter in the same proportion when experienced drought in citrus.

Data pertaining to root volume showed significant increase in the root volume in Soh sarkar (29.15%) and RLC-5 (28.69) at the end of the drought compared to beginning of drought, while other genotypes exhibited seizure in root growth under drought (Fig. 2) Previously, a vigorous adventitious root growth was noticed in drought tolerant Royal Gala (Malus domestica) seedlings for water mining and mineral nutrient absorption (Geng et al., 8), marked increase of root volume was also exhibited in maize (Li et al., 17). Consequently, from our result Soh sarkar and RLC-5 appears to be most drought tolerant among genotypes tested as an increase in root volume could be a consequence of drought avoiding mechanism with the extensive adventitious and taproot growth.

Citrus Rootstock Genotypes Response to Drought

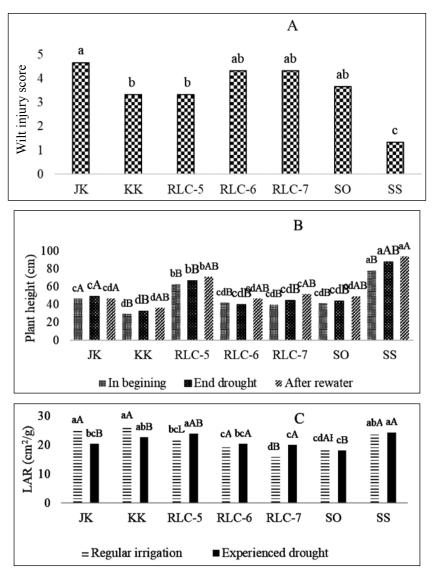


Fig. 1. Score of visual wilt injury (A), plant height (B) and leaf area ratio (LAR) (C) of tested citrus genotypes under regular irrigation and drought of 24 days. Values with the same lower letters are not significantly different in Duncan Multiple Range Test (p<0.05) in rows, however, values with same upper letters are not significantly different between column in t-test (p<0.05).</p>

JK; Jatti khatti, KK;Karna khatta, RLC-5, 6,7; rough lemon collection-5, 6,7, SO; sour orange, SS;Soh sarkar.

During the drought stage, non-significant variation of intracellular CO_2 concentration (C_i) was noticed among tested genotypes. In relation to the beginning of drought, *Ci* rose in almost all genotypes at varying levels between 5.14%-42%. The *Ci* of Jatti khatti was elevated by 42.74% under drought and it further elevated by 59.92% at rewater. The same trend exhibited in RLC-6 with 17.42% and 26.77% increase. While the rest genotypes had statistical stability of *Ci* during drought and recovery periods, resulted in insignificant differences with respective well-watered situation (Table 1). Result of intrinsic

water use efficiency (WUEi) showed a significant increase in sour orange (117.32%) and RLC-5 (62.01%) under drought as of beginning of drought, while a significant reduction in WUE_i was noticed in RLC-6 (44.71%) and Karna khatta (47.07%) as compared to the normal irrigation period. While after rewatering, WUE_i of RLC-5 decreased significantly (28.36%) with no alternation in other rootstocks. dos Santos et al. (5) reported the increase in WUE_i up to 108% under drought in citrus.

It is witnessed from the data (Fig. 3A) that chlorophyll 'a' synthesis was inhibited under drought

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Genotype	Initial	Drought	Rewater	Mean
$C_{i} \ (\mu mol \ H_{2}O \ m^{-2}s^{-1})$				
Jatti khatti	131.01cC	187.00aB	209.50aAB	175.84ab
Karna khatta	143.67abcBC	180.33aAB	170.67abcB	164.89ab
RLC-5	150.00abB	163.33aAB	151.83cB	155.06bc
RLC-6	155.00aBC	182.00aAB	196.50abA	177.83a
RLC-7	145.00abBC	156.83aB	169.00abcAB	156.95abc
Sour orange	155.11aBC	168.67aAB	159.00bcBC	160.93abc
Soh sarkar	139.88bcB	147.07aAB	141.59cB	142.85c
Mean	145.67B	169.32A	171.16A	
<i>WUE</i> , (<i>A/g</i> , µmol C	O ₂ mol ⁻¹ H ₂ O)			
Jatti khatti	170aA	165.67bcA	207.50aA	181.06ab
Karna khatta	233.67aA	123.67cB	169.67aAB	175.67ab
RLC-5	129.31aB	209.50bA	150.08aB	162.96ab
RLC-6	204.67aA	113.17cB	110.08aB	142.64b
RLC-7	155.83aA	172.33bcA	141.33aAB	156.50ab
Sour orange	132.83aB	288.67aA	205.33aAB	208.94a
Soh sarkar	156.56aA	157.00bcA	134.98aAB	149.51b
Mean	168.98A	175.71A	159.85A	

Table 1. Intracellular CO_2 concentration (C_i) and intrinsic water use efficiency (WUE_i) of citrus genotypes in the beginning, end of the drought and after re-water.

Values with the same lower letters are not significantly different in Duncan Multiple Range Test (p<0.05) in rows, however, values with same upper letters are not significantly different between column in t-test (p<0.05).

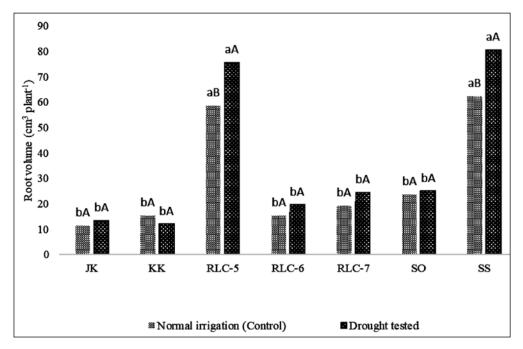


Fig. 2. Root volume of tested citrus genotypes under regular irrigation and drought of 24 days. Values with the same lower letters are not significantly different in Duncan Multiple Range Test (p<0.05) in rows, however, values with same upper letters are not significantly different between column in t-test (p<0.05).

JK; Jatti khatti, KK;Karna khatta, RLC-5, 6,7; rough lemon collection-5, 6,7, SO; sour orange, SS;Soh sarkar.

in all citrus genotypes tested. The magnitude of reduction was significantly higher in Karna khatta (47.01%), RLC-5 (44.93%) and Jatti khatti (36.15%). The lowest inhibition was recorded in Soh sarkar (16.89%) as compared to beginning of drought. After rewatering stage, it significantly up surged in all rootstocks except Jatti khatti and RLC-6. Chlorphyll 'a' was higher in RLC-5 (45.11%), Karna Khatta (41.03%) and Soh sarkar (18.96%) than drought conditions.

During the course of drought, Chl b followed the declining trend in Jatti khatti, Karna khatta, Soh sarkar and sour orange, while increased in RLC-7, RLC-6 and RLC-5 as of beginning of drought (Fig 3B). The highest increase was recorded in RLC-7 (90.91%) and it was lowest in RLC-6 (14.84%). Further, highest declining in chlorophyll 'b' was found in Jatti khatti (35.62%). Notwithstanding, at rewater stage, Chl 'b' maintained higher levels (RLC-7 and

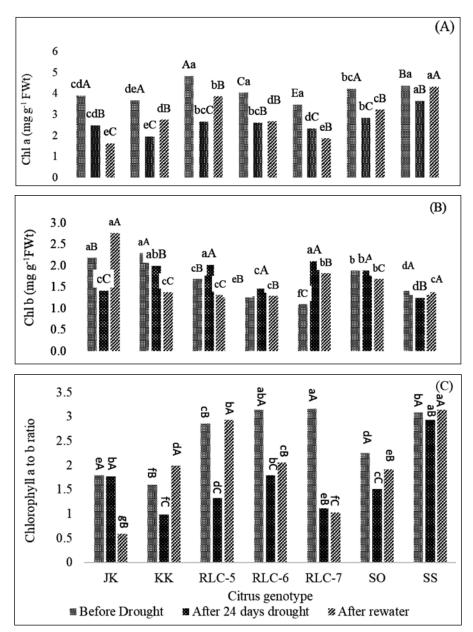


Fig. 3. Content of chlorophyll 'a' (A), chlorophyll 'b' (B), ratio of chlorophyll a to b (C) in beginning, end of drought and after re-water. Values with the same lower letters are not significantly different in Duncan Multiple Range Test (p<0.05) in rows, however, values with same upper letters are not significantly different between column in t-test (p<0.05).

JK; Jatti khatti, KK;Karna khatta, RLC-5, 6,7; rough lemon collection-5, 6,7, SO; sour orange, SS;Soh sarkar.

Jatti khatti). Noticeably, out of tested genotypes, Soh sarkar exhibited no alteration in chlorophyll 'b' under drought compared to the beginning of drought. After rewatering, chlorophyll 'b' was regulated in Jatti khatti (95.74%) and Soh sarkar (10.40%) as compared to drought, while it decreased in rest of the citrus genotypes even on rewatering. Overall rootstock exhibited significant variations in chlorophyll a/b ratio under each condition (Fig 3C). Moreover, a declining trend in chlorophyll a to b ratio was noticed in all citrus genotypes except Jatti khatti at the end of drought compared to beginning of drought. The magnitude of decline in chlorophyll a to b was higher in RLC-7 (64.87%), RLC-6 (42.99%) and RLC-5 (53.85%), while least inhibition was recorded in Soh sarkar (5.18%). After rewatering significant increase in chlorophyll, a to b ratio was observed in most of the citrus genotypes except Jatti khatti and RLC-7. Karna khatta (103%) and RLC-5 (121%) had promoted a to b ratio maximum and least enhancement was noticed Soh sarkar as compared to drought conditions. The variation in Chl a and b, Chl a/b was reported previously under drought. In citrus rootstock, Chlorophyll'a' and Chlorophyll a/b reduced in citrus rootstock (dos Santos et al., 5).

The leaf nutrient status of citrus genotypes as influenced significantly due to drought is given in Table 2. Except Soh sarkar and Jatti khatti, rest of the citrus genotypes exhibited a decline in leaf P content at varying degree under drought. The highest reduction was noticed in RLC-7 (20.00%), while lowest in RLC-6 (11.90%) at end of drought

Table 2. Leaf mineral composition of citrus genotypes in the beginning, end of the drought and after re-water.

Genotype	Initial	Drought	Rewater	Mean
P (%)				
Jatti khatti	0.35bcBC	0.33bC	0.38aAB	0.35b
Karna khatta	0.39abA	0.33bB	0.33bcB	0.35b
RLC-5	0.31cAB	0.26cC	0.29cBC	0.29d
RLC-6	0.42aA	0.37aB	0.38aAB	0.39a
RLC-7	0.40abA	0.32bBC	0.31bcC	0.34bc
Sour orange	0.37abcA	0.30bB	0.30bcB	0.32c
Soh sarkar	0.36bcAB	0.37aB	0.34abB	0.36b
Mean	0.37A	0.33B	0.33B	
K (%)				
Jatti khatti	1.41aA	1.41bcA	1.44aA	1.42a
Karna khatta	1.33abA	1.36bcA	1.35abcA	1.35b
RLC-5	1.35abAB	1.45abA	1.31bcB	1.37ab
RLC-6	1.31abcAB	1.27cB	1.22dB	1.26c
RLC-7	1.22cB	1.34bcA	1.28cdAB	1.28c
Sour orange	1.26bcAB	1.35bcA	1.36abcA	1.32bc
Soh sarkar	1.32abcBC	1.57aA	1.38abB	1.43a
Mean	1.31B	1.39A	1.33B	
Fe (ppm)				
Jatti Khatti	163.33bB	206.67bcA	182.67aAB	184.22cd
Karna khatta	183.33aB	206.67bcAB	188.00aB	192.67bcd
RLC-5	189.67aB	212.00bcAB	190.00aB	197.22bc
RLC-6	187.33aB	262.00aA	195.00aB	214.78c
RLC-7	180.00abB	234.00abA	189.33aB	201.11ab
Sour orange	172.67abB	189.33cAB	180.67aB	180.89d
Soh sarkar	185.33aAB	209.33bcA	182.00aB	192.22bcd
Mean	180.24B	217.14A	186.81B	

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Genotype	Initial	Drought	Rewater	Mean
Cu (ppm)				
Jatti khatti	3.33bA	3.00aB	3.33bA	3.22b
Karna khatta	2.67dA	2.60bAB	2.67dA	2.64c
RLC-5	3.00cA	2.40bcB	3.00cA	2.80c
RLC-6	4.00aA	2.93aB	4.00aA	3.64a
RLC-7	3.00cA	2.33cC	2.67dB	2.67c
Sour orange	3.33bA	2.27cC	2.67dB	2.76c
Soh sarkar	2.67dB	2.47bcBC	3.00cA	2.71c
Mean	3.14A	2.57B	3.05A	
Zn (ppm)				
Jatti khatti	206.67bA	163.33aAB	132.67bB	167.56b
Karna khatta	206.67bA	183.33aAB	138.00abB	176.00b
RLC-5	177.67bAB	172.67aB	140.00abB	163.44b
RLC-6	310.67aA	187.33aB	145.00abBC	214.33a
RLC-7	206.00bA	169.33aAB	139.33abB	171.56b
Sour orange	189.33bAB	153.67aB	130.67bC	157.89b
Soh sarkar	209.33bA	185.33aAB	167.33aABC	187.33a
Mean	215.19A	173.57B	141.86C	

Values with the same lower letters are not significantly different in Duncan Multiple Range Test (p<0.05) in rows, however, values with same upper letters are not significantly different between column in t-test (p<0.05).

compared to beginning of drought. However, most of the genotypes showed stability in leaf P content after rewatering as of drought except Jatti khatti in which it was increased by 15.15% than drought conditions. Though, Jafaria et al. (11) proved that drought tolerant rootstock had a higher P concentration in fig but in our study, we found higher or equal leaf P content in drought sensitive genotypes too. Furthermore, the leaf K content remain unchanged at end of drought in most of the genotypes except RLC-7 and Soh sarkar. The highest increase in leaf K content (30.00%) was found at end of drought in RLC-7 followed by Soh sarkar (12.95%). While comparing drought and rewatering stage, significantly higher reduction was noticed in the leaf K content of RLC-6 (25.57%), RLC-7 (19.00%) and Soh sarkar (13.06%). All the genotypes maintained the level of leaf K after rewatering that was at the beginning of drought. Soh sarkar and RLC-5 maintained higher leaf K content at drought stage (1.45-1.57%). Garcia-Sanchez et al. (7) also found an increase in leaf K in Carrizo citrange and Cleopatra mandarin under drought. As reported that K ion involved in osmoregulation to prevent from ROS in drought tolerance (Mengel and Kirkby, 19), Soh sarkar and RLC-5 could be considered as drought tolerant.

The present results exhibited significant increase of Fe content in Jatti khatti. RLC-6 and RLC-7 and restored at rewater stage (Table 2). While it was statistically stable throughout the experiment in Soh sarkar, but it tended to decrease in leaf Fe content in RLC-6 (25.57%), RLC-7 ((19.09%) and Soh sarkar (13.06%) after rewatering. RLC-5 genotypemaintained leaf Fe content throughout experiment (before the drought, at drought and after watering). Leaf Cu decreased significantly in sour orange (31.83%), RLC-6 (26.75%), RLC-7 (22.33%) and RLC-5 (20.00%) during drought than beginning of drought. Soh sarkar and Karna khatta maintained the level of Cu under drought. Except RLC-7 and sour orange, rest of the genotypes regained a leaf Cu content level after rewatering of beginning of drought. Results on Zn proved the significant decline under drought in RLC-6 (39.70%) as of before the drought stage, however, no modification in leaf Zn content was noticed in the rest of the genotypes under drought. After rewatering, significant decline in leaf Zn content was noticed in most of the rootstocks except Soh sarkar and RLC-5, where it was maintained throughout experiment in all three conditions. Decline of leaf Zn under drought was noticed in sweet cherry rootstocks (Kuçukyumuk et al. 16). The tolerance behaviour of Soh sarkar and RLC-5 under drought might be due to maintaining the level of Zn, Cu and Fe under drought and rewatering stage, which may induce Cu/Zn-SOD and Fe-SOD throughout drought (Filippou *et al.*, 6).

The present results showed that Soh sarkar and RLC-5 maintained leaf Cu, Zn and Fe under drought, which can induce Cu/Zn-SOD and Fe-SOD, hence escaped injury from drought. Besides, a deferential mechanism for drought tolerance exist in Soh sarkar and RLC-5 as RLC-5 maintained leaf K level throughout the experiment, while decline was observed in Soh sarkar. From the results it could be said that Soh sarkar and RLC-5 can be considered drought tolerant citrus genotypes.

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