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Changes in growth, biochemical, and chemical characteristics and alteration of the antioxidant defense system in the leaves of tea clones (*Camellia sinensis* L.) under drought stress

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ABSTRACT

In this study, we measured the morphological, biochemical, and chemical responses to soil drying in nine tea clones [*Camellia sinensis* (L.) O. Kuntze] grown in a field. Thirteen-year-old tea plants at Shahid Eftekhari Fashalam Experimental Station, Tea Research Center of Iran, were subject to drought stress by withholding water for 40 days. The control group of the clones was regularly watered. The soil moisture content of the non-irrigated and irrigated plants was monitored throughout the experiment. The effects of drought stress were measured by studying physiological (Relative Water Content), biochemical (Proline and Total Sugar Content), and antioxidant activities (Catalase and Peroxidase) after 20 days and 40 days of drought imposition. Green leaf yield and chemical parameters included total polyphenol, caffeine, water extract, and total ash were measured after 40 days of drought stress. Drought stress resulted in a decrease in total polyphenol, water extract, and total ash and an increase in proline, total sugar concentration and, in CAT and POD activities, as a consequence of reduced RWC of the leaves. Thus, drought stress caused a range of biochemical, physiological, and chemical variations, resulting in membrane damage and loss in the functions of the cell and finally a decrease in the tea growth as one of the most important economic crops. The results of grouping the clones under irrigation and drought stress conditions and comparing them with the results of mean comparison of the traits showed that in all cases, clones 276, 100, 285, and 277 were in the group that can be identified as the drought-tolerant group. Also, the results showed that in most cases, clones 278 and 74 were placed in a group that had low values for all the traits and could be considered as a group that is susceptible to drought stress. Overall, these findings provide new insight into the mechanisms of tolerance to drought in tea plants.

1. Introduction

Drought stress limits plant growth and survival. The drought stress is a major environmental agent limiting the productivity of plants in many natural places. This phenomenon delays metabolic plant processes, thereby retarding yield and growth (Araus et al., 2002). Tea is an important drink all over the world, partly due to its medicinal characteristics (Vyas and Kumar, 2005). The tea industry is important to the local economy in Guilan and Mazandaran provinces of Iran. In these provinces, despite sufficient rainfall due to monsoons, seasonal drought is common mainly during the months of June, July, and August, because of annual irregular distribution. In addition, since tea plants in these areas are grown only under rain-fed conditions, drought has become one of the most important restrictions to cultivate tea plants. It has been estimated that more than 20,000 ha of tea plants in Iran are affected by different levels of seasonal water stress. Consequently, maintaining the stability of quality and yield of tea under drought and identifying tea germplasm that can tolerate low soil water content are critical factors for producing tea in this area (Majd Salimi et al., 2010).

It has been reported that the supply of water directly affects production of tea (Chen et al., 2010) and drought could decrease the yield of tea by 40 % (Handique and Manivel (1986); Barua, 1989; Satyanarayana and Cox, 1994; Marimuthu and Kumar, 1998; Sharma and Kumar, 2005; Kigalu, 2007). Drought causes oxidative damage in the tea plants and influences antioxidative systems. Consequently, it changes different biochemical and physiological processes (Upadhyaya and Panda, 2004; Jeyaramraja et al. (2005)), leading to serious crop

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losses. Several morphological and physiological mechanisms for tolerance to drought have been recognized, including increase of proline (Rajasekar et al., 1988; Singh and Handique, 1993; Puthur et al., 1996; Maritim et al., 2015; Upadhyaya et al., 2016) and total sugar (Liu et al., 2015) contents. Furthermore, antioxidative efficiency differs in different clonal tea varieties (Upadhyaya and Panda, 2004), leading to a difference in the responses to drought in various tea clones (Chakraborty et al., 2002).

The tea plant can adapt to diverse stress types. However, the tolerance level varies according to cultivars (Chakraborty et al., 2000). The differences between various tea clones in response to the drought stress have been shown by Carr (1977); Othieno (1978), and Maritim et al. (2015) in Kenya, Nyirenda (1988) in Malawi, Chen et al. (2010) in China, and Upadhyaya et al. (2008); Netto et al. (2010), and Rawat et al. (2017) in India. Thomas et al. (2004) concluded that physiological processes in plants and their response to the environment are necessary factors for exploiting the germplasm of tea to develop drought-tolerant plant material. The effects of drought stress may be lowered by using plants that are tolerant to drought (Jeyaramraja et al., 2003). Considering that extensive studies have not been conducted on drought tolerance of clones which exist in germplasm collection of tea research center of Iran, and also according to the importance of this issue, identification of tolerant clones and use them in breeding programs, this research was designed.

The present research was conducted to specify the yield, biochemical, physiological, antioxidative and chemical responses of nine tea clones to drought stress during the growing season (40 days before summer harvest) for their drought tolerance potential to cultivate in Guilan and Mazandaran provinces of Iran.

2. Materials and methods

2.1. Plant material and stress condition

The study was carried out at the Shahid Eftekhari Fashalam Experimental Station, Tea Research Center, Guilan Province. Nine national tea clones (included 272, 277, 100, 285, 74, 399, 276, 278 and 269) that were 13 years old were grown at 70 cm × 100 cm spacing in the same environment, managed in the same way, and slightly pruned in March 2019. It is noteworthy that the clones were selected based on clonal selection method from different gardens in the west of Guilan province in 2007 and were suitable for yield or quality. The experimental design was used in this project is split plot based on randomized complete block design with three separate replications. The main plot – 15 m^2 (5 m×3 m) – consists of nine tea clones and the sub-plot consists of two irrigation treatments (irrigation and drought stress). The tea plants were periodically watered up to field capacity of soil until applying the stress. Afterward, for non-irrigation treatment (drought stress) the experimental plants (one row) were placed under drought stress during the period of May 22 to June 30, 2019 (summer harvest). In fact, this period was applied after the first plucking, approximately the late of May, when the rainfall is much lower than the water requirement of the tea bushes. During this period there was no irrigation or rainfall. In irrigation treatment, the control plants (one row) were regularly watered using mist spraying irrigation system. Ample distance (one row) was kept between the rows (non-irrigated and irrigated) (Fig. 1).

The time of irrigation for control plants were based on used available moisture or allowable depletion (Maximum Allowable Depletion = 0.4) (Majd Salimi et al., 2010). Thus, in per plot the soil moisture content was measured by gravimetric method from the 0 − 30 cm soil layer every 3 days. To calculate the volume of irrigation water, the depth of water needed to bring the soil moisture content back to field capacity was calculated as follows:

$$d = (P_{fc} - P_i).\ D.\ B_d$$

P_{fc}: moisture content (in terms of weight percentage) at field capacity, P_i: moisture content (in terms of weight percentage) at the time before irrigation, B_d: bulk specific gravity (g/cm^3), D: effective depth rooting (cm) (Kovda et al., 1973).

After calculating the depth of water, the total volume of irrigation water was obtained based on the plot area. The duration of irrigation was calculated by computing the outlet water discharge by the water meter. At each stage, irrigation was carried out in such a way that the moisture content in the soil is not higher than the moisture content at field capacity.

Physical and chemical tests of soil up to 30 cm depth revealed that the soil texture is sandy loam consisting of 16.67 % clay, 12.5 % silt, and 71.33 % sand. Volumetric values of mean-field capacity, permanent wilting point, and bulk density were 24.4 %, 8.5 %, and 1.06 g/cm^3, respectively. Rainfall and other climatic factors were determined on a daily basis in the weather station in approximately 1000 m from the place. The average temperature range and monthly rainfall during the experiment period were recorded as 25.4°C and 0 mm, respectively. The soil water content of the non-irrigated and irrigated plants was monitored throughout the experiment.

Various physiological and biochemical parameters including relative water content (RWC), proline, total sugar, and CAT and POD activities were measured on the 20^{th} (two to the three-leaf stage) and 40^{th} days (four to the five-leaf stage) of drought stress imposition and normal condition. Green leaf yield and chemical parameters like polyphenol, caffeine, water extract, and total ash were measured after 40 days of drought stress (five-leaf stage) and under normal conditions, the parameters were measured when they reached the five-leaf stage. All the leaf samplings were done during morning hours between 9 a.m. and 10 a.m. For each experiment, four plants were used for each point and each experiment was performed in triplicate.

2.2. Soil moisture content

Soil moisture was measured by mentioning the differences between the dry and fresh soil mass, expressed as percent by the use of the gravimetric method (Gupta, 1999). The volume of soil was taken from the 0 − 30 cm soil layer and dried at 105°C for 48 h in an oven. The gravimetric moisture content was measured as the difference between dry and fresh soil masses and expressed as the volumetric percentage.

2.3. Green leaf yield and RWC

To determine the green leaf yield, the tea shoots were harvested in the standard form (two leaves and a bud) from the experimental plots and their weight was measured by a precision scale and expressed as g/m^2 (IPGRI, 1997). Green leaf yield was measured in triplicate for each experiment. The method proposed by Barrs and Weatherley (1962) was employed to measure the RWC. The third leaf was used to measure relative water content.

2.4. Proline content

The proline content in the tea leaf was determined following the procedure proposed by Bates et al. (1973). We homogenized the sample of the leaf (0.5 g) by 5 mL of 3 % sulfosalicylic acid using a mortar and a pestle and then filtered by the Whatman No. 1. The filtrate volume was increased by 10 mL using the sulfosalicylic acid. Then, 2 mL of the filtrate was incubated by 2 mL of ninhydrin and 2 mL of glacial acetic acid and boiled in a water bath at a 100°C for 30 min. In the next step, the mixture of the reaction was cooled down and 6 mL of toluene was added to the mixture. The mixture was cyclomixed and the amount of absorbance was recorded at a wavelength of 570 nm. The third leaf was used to measure proline content.

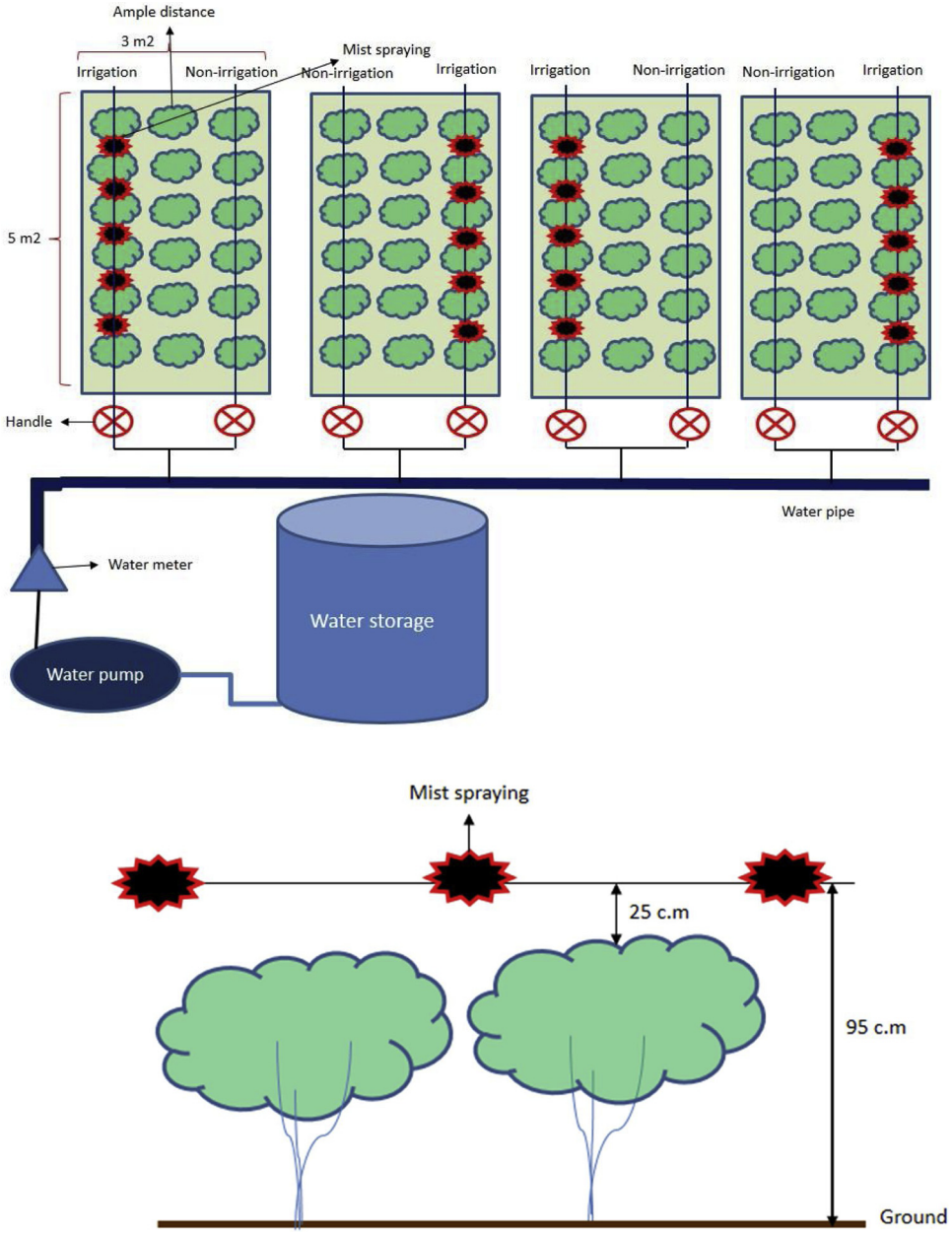

Fig. 1. Schematic figure of the irrigation system.

2.5. Total sugar content

The total sugar was extracted from the tea third leaf in ethanol (80 % (v/v)). Aliquots of the ethanol extract (80 %) were taken by Anthrone reagent to estimate the total sugar (Yoshida et al. (1971)).

2.6. Extraction and assay of antioxidant enzymes

To determine the activity of the enzyme, we ground 100 mg tissues of the third leaf by the use of liquid nitrogen and then re-suspended in 1 mL of buffer solution (50 mMTris − HCl (pH = 7.8) fortified 1 % PVP). We centrifuged homogenates at 12,000 rpm for 20 min at 4°C. The supernatant was utilized to determine the CAT and POD activities. The activity of CAT was measured by reading the absorbance at 240 (Upadhyaya and Panda, 2004). The activity of POD was measured according to Chance and Maehly (1995).

2.7. Determination of total polyphenol, caffeine, water extract, and total ash

The plant tissue was sampled after 40 days of water treatment to determinate total polyphenols, caffeine, water extract, and total ash. In each of the experimental units, approximately 100 g of the fresh shoots (with one bud and two leaves) were plucked. Then, the samples were placed inside the labeled paper bags and dried at 70°C for 24 h. The dried samples were blended, placed inside the paper bags in dry and dark conditions until laboratory analysis.

2.7.1. Polyphenol and caffeine determination

Total polyphenol was estimated as per the ISO TC 34/SC 8/WG (2003) using the Folin-Ciocalteu phenol reagent. Caffeine was extracted from 0.5 g of the ground shoots of tea (one bud and two leaves) using 50 mL chloroform (four times and each time 12.5 mL) with 2.5 mL of ammonium solution. Next, the caffeine content was determined using the UV/VIS spectrophotometer at 276 nm. The caffeine content was

determined using the standard curve of caffeine (Lakin, 1989).

2.7.2. Water extract determination

To determine the water extract content, the soluble matter from 2 g of the ground shoots of tea (one bud and two leaves) was mixed with boiling water under refluxing, filtering, washing, drying, and weighing the insoluble residue in hot water, and calculating water extract (International Organization for Standardization (1994)).

2.7.3. Total ash determination

To measure the total ash, the organic matter of 2 g of ground shoots of tea (one bud and two leaves) was destructed and heated at 525 ± 25°C to a constant mass with a furnace (International Organization for Standardization (1987)).

2.8. Statistical analyses

The SAS software (version 9.4) was used to analyze the data. To test the effects of irrigation treatments, clones, and their interaction on the different measured factors, the data were subjected to ANOVA of a split-plot design. Tukey's test was utilized to compare the means at a 5 % level. The standardized data of the clones were used for cluster and principal component analysis. A Pearson correlation matrix was used to perform PCA analysis. Cluster analysis was done using the ward method in the SPSS software and PCA in the PAST software.

3. Results and discussion

3.1. Soil moisture content and the appearance of the bushes demonstrate the degree of drought stress

Water stress was gradually induced for 40 days until the soil moisture content decreased to 62.83 % of that of the control group (level of normal moisture) (Fig. 2). After 40 days of drought stress, most of the leaves of susceptible clones were reddish-brown, curled, and withered due to a decline in the water availability, while for tolerant clones, only the yellow patches and needle spots were observed (Fig. 3).

3.2. Impact of drought stress on the quality and productivity of tea

The results of ANOVA in Table 1 showed that clones had significant effects on green leaf yield, total polyphenol, caffeine, water extract, and total ash. There were also significant effects of the irrigation treatment on green leaf yield, total polyphenol, caffeine, water extract and total ash, and significant effects of clone×irrigation treatment on green leaf yield, caffeine, total polyphenol, water extract, and total ash. In the present research, 13-year-old tea plants responded to the severe drought with a reduction in green leaf yield. Also, tea quality showed a significant reduction in the total polyphenol (Table 2).

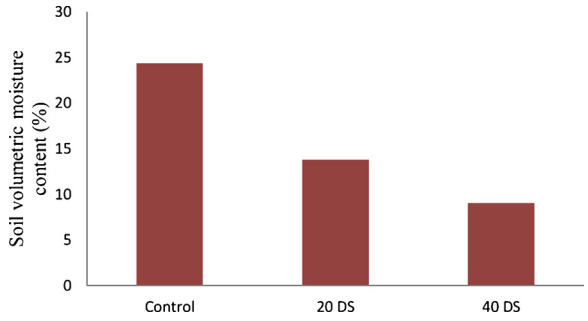

Fig. 2. Effect of drought stress on mean soil moisture content; well-watered (control), 20 DS (after 20 days of drought stress imposition), and 40 DS (after 40 days of drought stress imposition). The values of soil moisture content are the mean values of 9 tea clones. DS: drought stress.

3.2.1. Green leaf yield

Green leaf yield decreased with prolonging the drought stress (Table 2). A decrease in green leaf yield was highest in the clones 278 (68.53 %) and 399 (64.23 %), whereas clones 276 (27.27 %), 100 (33.84 %), and 285 (35.41 %) showed less decrease over control after 40 days of stress. The results of the present research are consistent with the findings of Netto et al. (2010); Waheed et al. (2012); Lipiec et al. (2013); Cirillo et al. (2014); Maritim et al. (2015); Upadhyaya et al. (2016); Rawat et al. (2017); Rahimi et al. (2019). These studies showed that tea production was directly influenced by the water supply.

3.2.2. Chemical composition

Polyphenolic compounds of tea in the leaf are major functional components detrimental to the health of humans. Table 2 indicates that the total polyphenol content in the leaf of 13-year-old tea plants was significantly greater in the irrigation treatment than drought stress. The reduction in the contents of polyphenol compared to the control was maximum in clone 74 (25.59 %), followed by clone 277 (23.63 %), whereas clone 399 (0.48 %) and clone 276 (0.96 %) indicated the minimum reduction after 40 days of drought stress. Phenolic compounds in plants are mostly produced to keep plants safe from ROS, stress, UV light, wounds, herbivores, and disease (Dixon and Paiva, 1995).

The water content of extract and total ash were significantly higher in irrigation than drought stress except for three cases (Table 2). The caffeine content had the reverse trend from total polyphenol except for two items (Table 2). Under the control and drought conditions, the clone 100 had the highest caffeine level. These findings showed that the total polyphenol was generally reduced when drought stress was prolonged, but caffeine content apparently increased.

3.3. Rwc

All nine tea clones utilized in the present research responded to a decrease in soil moisture content by reducing their relative water content, as can be seen in Table 4. The mean RWC levels of the leaf significantly differed ($P \leq 0.01$) between clones (Table 3). The reduction in RWC of the leaf can be related to the lower content of soil water. The minimum reduction in RWC was observed in clones 100 (3.17 %), 276 (4.61 %), and 277 (4.92 %) after 40 days of imposition of drought compared with the control group, while clones 74 (26.41 %), 269 (26.03 %), and 278 (22.90 %) showed a high decrease (Table 4). It has been reported that the maintenance of high RWC in the cultivars resistant to drought conditions is a sort of adaptation to drought in tea plants (Chakraborty et al., 2002; Upadhyaya et al., 2008; Waheed et al., 2012; Maritim et al., 2015; Upadhyaya et al., 2016; Rawat et al., 2017).

3.4. Changes in proline and total sugar content

As moisture stress days proceeded, the proline content as the characteristic feature of plants acclimatizing under stress increased in all the clones. Leaf proline contents differed significantly with the changes in the days of subjecting to drought (Table 4). Accumulation of proline was maximum in clone 276 (50.13 %) after 40 days of drought stress imposition, whereas clone 278 (1.54 %) indicated the least increase compared with the control plants (Table 4). Proline is an osmoprotectant during water stress. In this regard, a high proline accumulation in clone 276 showed that this clone had genotypic tolerance to drought, because the accumulation of proline contributes to the maintenance of the water relations, acts as a hydroxyl radical scavenger, and prevents distortion of membrane (Yoshiba et al., 1997; Matysik et al., 2002). Netto et al. (2010) observed higher proline accumulation in clones UPASI-2, TTL-1, and TTL-6 after 30 days of drought stress, which indicated their tolerance to drought. Maritim et al. (2015) stated that certain tea cultivars with various levels of resistance to drought differed in their capacity for the accumulation of

Fig. 3. Leaf morphology of the tea clones under drought stress and irrigation: (A) After 40 days of drought stress and (B) Normal condition.

proline. Moreover, under the water stress, the resistant-tolerant cultivars accumulated higher levels of proline compared to the susceptible cultivars. Screening of 14 tea genotypes for drought tolerance subjected to 30 days without irrigation reported the highest accumulation of proline in the genotypes tolerant to drought (100 and 399) (Rahimi et al., 2019). All these results indicated that the proline accumulation under stress was indicative of particular drought-tolerant varieties.

As shown in Table 3, the total sugar shows a drought-dependent behavior such that it peaked at 40 days of drought. After 20 days of drought, the clones showed an increase in the total sugar level (35.45 % for the clone 276 and 33.60 % for the clone 399) compared with control. After 40 days of drought stress, the total sugar content increased by 52.20 % and 52.08 % for the clone 285 and 399, respectively, compared to the control. Under the water stress, especially severe or prolonged drought stress, plants optimally utilized soluble sugar to keep growth (Ruan et al., 2010). An increase in the content of the total sugar with desiccation is one of the positive characteristics of the drought-tolerant tea plant (Liu et al., 2015).

Several studies have shown that proline and soluble sugar have a key role in signal transduction and osmotic adjustment simultaneously, and signaling of proline could interact with the signaling pathway of soluble sugar (Moustakas et al., 2011; Sperdouli and Moustakas, 2012; Mohammadkhani and Heidari, 2008). Sperdouli and Moustakas (2012) have suggested that activation of specific scavenging systems of ROS causes to protect proline and soluble sugar against oxidant stress. In addition, the findings of the present study showed that the

Table 1
Mean of squares and significance levels of ANOVA for clones and treatment effects on green leaf yield, total polyphenol, water extract, total ash and caffeine of tea leaves.

Source	df	Green leaf yield(g/m^2)	Total polyphenol (%)	Water extract (%)	Total ash (%)	Caffeine (%)
Clone	8	142448.05**	4.94**	18.77**	0.64**	0.49**
Treatments	1	464562.49**	33.13**	1.94**	0.05**	0.13**
Clone×treatments	8	14202.43**	2.43**	5.73**	0.43	0.11**
C.V (%)		14.08	1.95	0.67	1.70	2.42

Treatments include: irrigation (normal condition) and 40 days of drought stress.
** Significant differences at 1 % probability level.

Table 2
Changes in green leaf yield, total polyphenol, water extract, total ash, and caffeine content in nine clonal varieties of C. *sinensis* subjected to 40 days of drought stress.

Clones	Treatments	Green leaf yield (g/m^2)	Total polyphenol (%)	Water extract (%)	Total ash (%)	Caffeine (%)
272	Control	131.86^{hi} ± 1.85	14.21^{ab} ± 0.50	40.40^{ab} ± 0.25	5.97^{hi} ± 0.15	2.15^{fg} ± 0.11
	40 DS	79.18^{j} ± 1.08	12.46^{f} ± 0.26	38.58^{de} ± 0.41	5.81^{i} ± 0.09	2.21^{fg} ± 0.14
277	Control	216.19^{fg} ± 1.94	14.17^{ab} ± 0.09	37.90^{e} ± 0.18	6.69^{c-e} ± 0.07	2.56^{de} ± 0.13
	40 DS	105.90^{ij} ± 1.11	10.82^{g} ± 0.15	40.67^{ab} ± 0.48	6.43^{e-g} ± 0.11	2.90^{a-c} ± 0.08
100	Control	609.66^{b} ± 1.70	14.21^{ab} ± 0.09	40.83^{a} ± 0.28	6.21^{gh} ± 0.33	3.01^{ab} ± 0.09
	40 DS	403.33^{c} ± 1.96	13.74^{ab} ± 0.04	40.28^{ab} ± 0.10	5.85^{i} ± 0.12	3.10^{a} ± 0.06
285	Control	671.00^{a} ± 1.96	14.50^{a} ± 0.10	39.10^{cd} ± 0.12	6.85^{bc} ± 0.12	2.36^{ef} ± 0.13
	40 DS	403.33^{c} ± 2.25	12.91^{c-f} ± 0.37	36.79^{f} ± 0.16	6.58^{c-f} ± 0.10	2.91^{a-c} ± 0.12
74	Control	311.66^{de} ± 0.98	13.77^{ab} ± 0.10	38.60^{de} ± 0.12	6.25^{f-h} ± 0.12	2.87^{bc} ± 0.14
	40 DS	123.66^{hi} ± 1.41	10.25^{g} ± 0.12	40.40^{ab} ± 0.18	7.08^{ab} ± 0.09	3^{ab} ± 0.11
399	Control	590.00^{b} ± 2.96	13.69^{a-c} ± 0.1	40.31^{ab} ± 0.23	6.80^{b-d} ± 0.25	2.85^{bc} ± 0.14
	40 DS	211^{g} ± 1.96	13.62^{b-d} ± 0.13	37.80^{de} ± 0.34	6.25^{f-h} ± 0.17	2.76^{cd} ± 0.18
276	Control	348.33^{de} ± 1.86	12.80^{d-f} ± 0.22	36.60^{f} ± 0.46	5.90^{i} ± 0.25	2.74^{cd} ± 0.19
	40 DS	253.33^{f} ± 1.86	12.68^{ef} ± 0.12	35.31^{g} ± 0.42	6.38^{e-g} ± 0.22	2.91^{a-c} ± 0.15
278	Control	350.66^{d} ± 1.83	12.17^{f} ± 0.18	36.40^{f} ± 0.18	7.23^{a} ± 0.15	2.88^{bc} ± 0.11
	40 DS	110.33^{h-j} ± 1.87	10.13^{g} ± 0.12	34.23^{h} ± 0.29	6.45^{d-g} ± 0.20	2.95^{a-c} ± 0.07
269	Control	310.33^{e} ± 1.78	13.49^{b-e} ± 0.18	38.16^{e} ± 0.22	6.13^{g-i} ± 0.14	2.56^{de} ± 0.11
	40 DS	150^{h} ± 1.55	12.32^{f} ± 0.24	39.92^{bc} ± 0.15	6.63^{c-e} ± 0.06	2.13^{g} ± 0.15

Data are means ± standard error. The data followed by different letters in the same column were significantly different according to the Tukey test at the p = 0.05 level.
Control plants were watered, 40 DS indicates 40 days of drought stress imposition.

accumulation of proline and total sugar in the leaf of the tea plant under water-deficit stress was dependent upon the duration and intensity of stress and the cultivar.

3.5. Changes to antioxidant activity

When the tea plants are exposed to drought stress, antioxidative genes are expressed, antioxidant system activities are meant for ROS scavenging, and the ROS level is increased. These factors lead to drought tolerance (Mano, 2002). Although conditions of stress result in increasing the total foliar antioxidants, little is known about the control and coordination of different activities of antioxidative enzymes in plants, particularly tea, under water deficit stress.

The prolonged period of drought stress significantly increased the activities of CAT in most of the clones (Table 4). Under normal conditions, clone 277 had higher CAT activities compared to other clones. After 20 days of drought stress, the activities of CAT increased by 30.63 % and 26.10 % compared to the control group for the clone 269 and 277, respectively. After 40 days of drought stress, the clone 100 had a peak and a significant increase in the activities of CAT compared to control. After 40 days of drought, the decrease in the activities of CAT was observed for the clones 399, 277, and 74, compared to 20 days of drought stress.

In the current research, CAT seemed to be an important enzyme against drought imposed oxidative stress because the activities of CAT have been increased in the plants under stress. An increase in the activity of CAT with an increase in stress in the tea clones shows that the enzyme could be the first defense line during the process of adaptation to drought. Since tea is a C_3 plant, the higher activity of CAT could scavenge hydrogen peroxide created in the pathway of photorespiratory and thus reduce the photorespiration rate (Jeyaramraja et al., 2003). Changes in the activities of the antioxidative enzyme under drought stress depend on the species of plant and intensity of stress (Gill and Tuteja, 2010). Our results were in accordance with those of Upadhyaya et al. (2008) and Liu et al. (2015), who stated that the activities of CAT were higher in the tea plants tolerant to drought than the genotypes susceptible to drought.

The activity of POD increased in the plants under stress compared with the control group after 20 and 40 days of dehydration, where the maximum activity was observed in clone 285 (57.38 %) and clone 276 (53.11 %) after 40 days of the imposition of stress compared with the control group, while POD activity was lower in clones 269 and 74 (Table 4). The increase in POD activity in almost all the clones under stress could be an acclimatization step for overcoming the stress (Upadhyaya et al., 2008)

Changes in the physiological and morphological traits of leaves could more accurately show the level of stress (Hayano-Kanashiro et al., 2009; Sperdouli and Moustakas, 2012). The results showed that with the prolonged drought stress, the levels of proline and total sugar significantly increased and the CAT and POD activities increased, as well. The findings were in line with the literature (Upadhyaya and Panda, 2004; Liu et al., 2010; Netto et al., 2010; Liu et al., 2015), which showed that the responses of tea plants to drought stress were severely influenced by the intensity and duration of water deficit stress.

3.6. Cluster analysis for clones under normal and drought stress

Cluster analysis classified the clones into 4 groups under normal conditions (Fig. 5A). The cluster III contained clones 285, 399 and 100 which was better for most traits and can be introduced as superior clones for normal conditions.

Nine tea clones were grouped into 3 clusters based on various traits

Table 3
Mean of squares and significant levels of ANOVA for clones and treatment effects on relative water content, proline, total sugar, CAT and POD activity of tea leaves.

Source	df	Relative water content	Proline	Total sugar	CAT activity	POD activity
Clone	8	89.63**	19.78**	1.26**	82.42**	8.88**
treatment	2	552.46**	67.25**	4.00**	85.94**	20.84**
Clone×treatment	16	45.58**	9.05**	0.13**	8.14**	1.93**
C.V (%)		3.46	5.53	6.84	8.63	9.16

Treatments include: irrigation (normal condition), 20 and 40 days of drought stress.
** Significant differences at 1 % probability level.

Table 4
Changes in relative water content, proline, total sugar content, CAT and POD activities in nine clonal varieties of *C. sinensis* subjected to irrigation (control), 20 days of drought stress (20 DS) and 40 days of drought stress (40 DS) imposition.

Clones	Treatments	Relative Water Content (%)	Proline (mg/g FW)	Total sugar (mg/g FW)	Catalase (μmol hydrogen peroxide/min/mg protein)	Peroxidase (μmol hydrogen peroxide/min/mg protein)
272	Control	$61.77^{a-e} \pm 0.72$	$5.31^{k} \pm 0.46$	$1.14^{h-k} \pm 0.14$	$9.93^{g-i} \pm 0.69$	$5.22^{b-g} \pm 0.30$
	20 DS	$58.70^{de} \pm 0.74$	$7.15^{ij} \pm 0.42$	$1.29^{g-j} \pm 0.15$	$10.14^{g-i} \pm 0.42$	$5.82^{a-d} \pm 0.31$
	40 DS	$57.14^{ef} \pm 0.40$	$7.29^{hj} \pm 0.53$	$1.30^{g-j} \pm 0.16$	$10.95^{f-i} \pm 0.63$	$6.47^{a-c} \pm 0.51$
277	Control	$67.80^{a} \pm 0.42$	$8.38^{e-i} \pm 0.13$	$1.62^{e-g} \pm 0.22$	$14.86^{c-e} \pm 0.61$	$3.46^{i-k} \pm 0.27$
	20 DS	$67.53^{ab} \pm 0.51$	$8.83^{e-h} \pm 0.19$	$1.84^{de} \pm 0.15$	$20.11^{a} \pm 0.69$	$6.80^{a} \pm 0.26$
	40 DS	$64.46^{a-d} \pm 0.69$	$10.97^{cd} \pm 0.28$	$2.37^{a-c} \pm 0.12$	$18.73^{ab} \pm 0.75$	$5.64^{a-d} \pm 0.59$
100	Control	$63.16^{a-e} \pm 0.46$	$6.15^{jk} \pm 0.15$	$1.05^{i-k} \pm 0.18$	$7.85^{i} \pm 0.64$	$3.52^{i-k} \pm 0.32$
	20 DS	$61.39^{a-e} \pm 0.47$	$7.92^{g-i} \pm 0.15$	$1.35^{f-i} \pm 0.12$	$10.44^{g-i} \pm 0.19$	$5.15^{c-h} \pm 0.11$
	40 DS	$61.16^{a-e} \pm 0.58$	$9.04^{e-g} \pm 0.22$	$1.66^{ef} \pm 0.07$	$15.95^{b-d} \pm 0.68$	$6.91^{a} \pm 0.34$
285	Control	$63.05^{a-e} \pm 0.33$	$8.25^{e-i} \pm 0.30$	$1.13^{h-k} \pm 0.17$	$9.76^{g-i} \pm 0.20$	$2.21^{k} \pm 0.09$
	20 DS	$63.03^{a-e} \pm 0.47$	$8.34^{e-i} \pm 0.36$	$1.44^{f-h} \pm 0.16$	$11.54^{e-h} \pm 0.37$	$4.11^{e-j} \pm 0.18$
	40 DS	$57.48^{ef} \pm 1.19$	$13.96^{b} \pm 0.17$	$2.37^{a-c} \pm 0.15$	$14.79^{c-e} \pm 0.54$	$5.20^{b-g} \pm 0.35$
74	Control	$67.76^{ab} \pm 0.59$	$7.66^{g-j} \pm 0.62$	$1.54^{e-g} \pm 0.11$	$8.47^{hi} \pm 0.14$	$3.74^{ij} \pm 0.45$
	20 DS	$60.53^{c-e} \pm 1.17$	$9.62^{d-f} \pm 0.41$	$2.04^{cd} \pm 0.15$	$10.53^{g-i} \pm 0.18$	$3.85^{h-j} \pm 0.24$
	40 DS	$49.86^{g} \pm 1.27$	$9.27^{e-g} \pm 0.13$	$2.43^{ab} \pm 0.25$	$9.73^{g-i} \pm 0.14$	$4.07^{f-j} \pm 0.44$
399	Control	$61.62^{a-e} \pm 0.33$	$8.37^{e-i} \pm 0.29$	$1.03^{i-k} \pm 0.09$	$11.03^{f-i} \pm 0.70$	$3.02^{jk} \pm 0.51$
	20 DS	$60.90^{b-e} \pm 0.54$	$8.49^{e-i} \pm 0.31$	$1.56^{e-g} \pm 0.11$	$14.12^{c-f} \pm 0.28$	$3.91^{h-j} \pm 0.24$
	40 DS	$50.2^{g} \pm 0.80$	$11.73^{c} \pm 0.18$	$2.16^{a-c} \pm 0.24$	$11.43^{e-h} \pm 0.53$	$3.84^{g-j} \pm 0.44$
276	Control	$64.56^{a-d} \pm 0.64$	$8.67^{e-i} \pm 0.35$	$1.38^{f-i} \pm 0.11$	$14.06^{c-f} \pm 1.18$	$3.06^{jk} \pm 0.13$
	20 DS	$64.47^{a-d} \pm 0.65$	$8.78^{e-h} \pm 0.43$	$2.14^{b-d} \pm 0.29$	$16.30^{bc} \pm 0.44$	$5.25^{b-f} \pm 0.35$
	40 DS	$61.58^{a-e} \pm 0.62$	$17.40^{a} \pm 0.63$	$2.66^{a} \pm 0.23$	$18.96^{ab} \pm 0.33$	$6.52^{ab} \pm 0.27$
278	Control	$65.74^{a-c} \pm 0.54$	$8.27^{e-i} \pm 0.14$	$0.83^{k} \pm 0.15$	$8.33^{hi} \pm 0.19$	$2.38^{k} \pm 0.24$
	20 DS	$51.82^{fg} \pm 0.85$	$9.72^{de} \pm 0.31$	$0.96^{ik} \pm 0.11$	$8.61^{hi} \pm 0.33$	$2.91^{jk} \pm 0.21$
	40 DS	$50.68^{fg} \pm 0.39$	$8.40^{e-i} \pm 0.36$	$1.18^{h-j} \pm 0.15$	$9.35^{g-i} \pm 0.21$	$2.96^{jk} \pm 0.38$
269	Control	$67.70^{ab} \pm 0.73$	$7.41^{h-j} \pm 0.18$	$1.11^{h-k} \pm 0.12$	$8.69^{hi} \pm 0.57$	$4.58^{d-i} \pm 0.35$
	20 DS	$64.97^{a-d} \pm 1.08$	$7.98^{g-i} \pm 0.42$	$1.27^{g-j} \pm 0.19$	$12.53^{d-g} \pm 0.42$	$5.43^{b-e} \pm 0.26$
	40 DS	$50.07^{g} \pm 0.21$	$8.10^{f-i} \pm 0.28$	$1.59^{e-g} \pm 0.17$	$14.55^{c-e} \pm 0.47$	$4.52^{d-i} \pm 0.27$

Data are means ± standard error. The data followed by different letters in the same column were significantly different according to the Tukey test at the p = 0.05 level.
Control plants were watered, 20 DS indicates 20 days of drought stress imposition and 40 DS indicates 40 days of drought stress imposition.

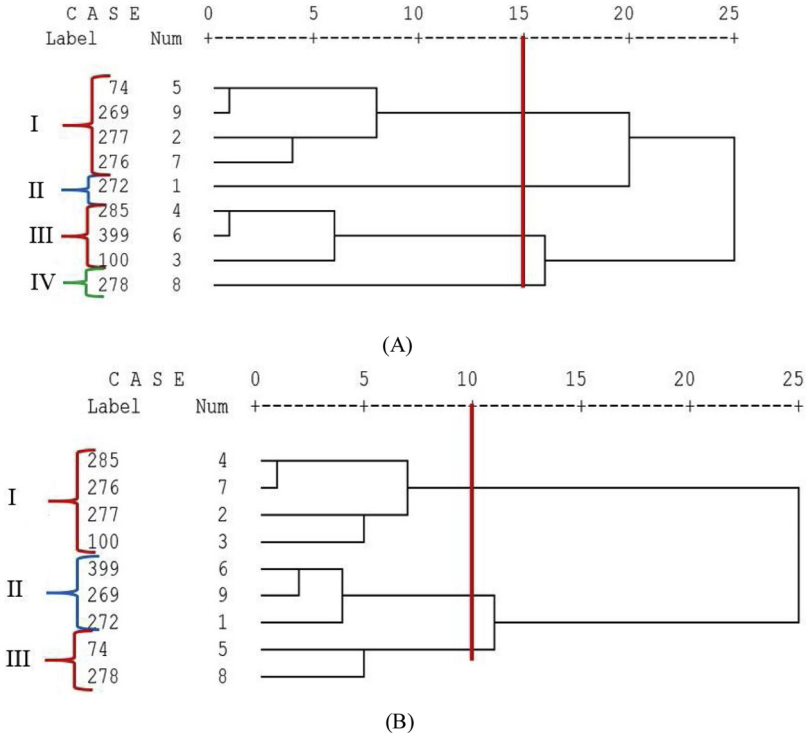

Fig. 4. Grouping tea clones in normal condition (A) and grouping tea clones in 40 days of drought stress (B).

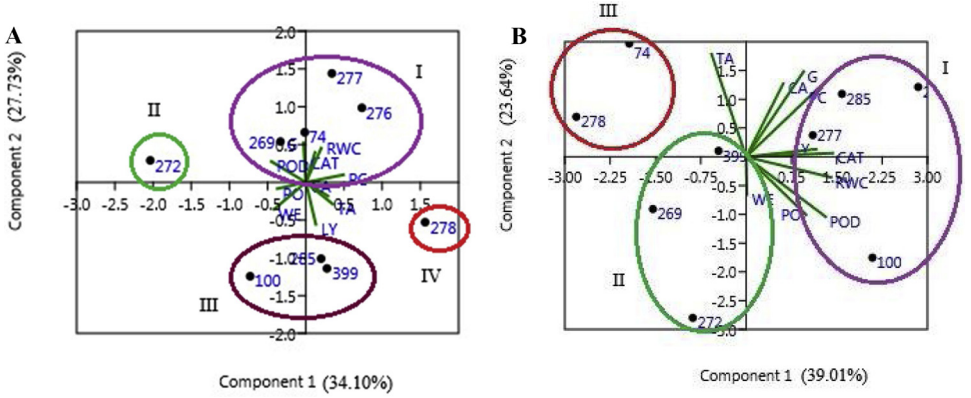

Fig. 5. A clone × trait biplot based on 10 traits. The first two principal components are plotted under normal condition (A) and 40 days of drought stress (B), each accounting for a proportion of the variance in the original dataset, shown in parentheses. Clones are plotted according to scores on each principal component, and traits are plotted on the basis of the eigenvectors on each principal component. Clones are grouped according to cluster analysis. LY = Leaf yield, PO = polyphenol, CA = caffeine, WE = water extract, TA = total ash, PC = proline content, TS = total sugar, CAT = catalase, POD = peroxidase, RWC = relative water content.

under 40 days of drought stress imposition. Cluster analysis showed that cluster I comprised of four clones, cluster II of three while cluster III contained two clones (Fig. 4). Cluster I included clones 285, 276, 277 and 100 with the highest values for green leaf yield, proline, total sugar, RWC, CAT and POD. Hence the members of this group could be utilized for breeding tea genotypes with water stress tolerance. The cluster II comprised of clones 399, 269 and 272 with higher polyphenol content and water extract. The members of cluster III (74 and 278) were characterized by the lowest green leaf yield, polyphenol, water extract, proline, total sugar, RWC, CAT and POD but higher caffeine and total ash that could be considered as sensitive group. The parameters reflecting drought tolerance which could be incorporated into a drought screening procedure were selected in this study. Green leaf yield, proline, total sugar, relative water content, CAT and POD activity could be successfully used to screen the different tea clones for drought tolerance. Rahimi et al. (2019) also reported among the studied clones, clone 100 was better for the most traits and considered as drought-tolerant clone after 30 days of drought stress.

3.7. Principal component analysis (PCA) for clones under normal and drought stress

The PCA provides good information about the association between parameters and classification of tested clones based on their tolerance/sensitive to drought stress. Since it is not sufficient to consider one of the yield-correlated traits as indicative for stress tolerance, we considered physiological, biochemical, chemical parameters, and antioxidant activities all together as indicators for drought tolerance in tea.

PCA for 10 traits and nine clones at two irrigation treatments (normal and drought stress) was performed to identify the principal components of measured parameters that best described the response to irrigation treatments and, thus, to identify tolerant and sensitive clones (Fig. 5 A and 5B). The results showed that the first three principal components contributed 77.15 % and 86.31 % to the total variation among clones at normal and drought stress conditions, respectively (Table 5).

Table 5
Eigenvectors for the principal components obtained for 10 parameters measured on tea clones.

Traits	Irrigation			40 days of drought stress		
	PC1	PC2	PC3	PC1	PC2	PC3
LY	0.127	−0.491	0.288	0.346	0.040	−0.391
PO	−0.365	−0.073	0.498	0.296	−0.301	−0.332
CA	0.283	−0.160	−0.211	0.183	0.381	−0.121
WE	−0.407	−0.314	0.199	0.004	−0.196	0.638
TA	0.344	−0.253	0.093	−0.171	0.534	0.232
PC	0.483	0.088	0.270	0.361	0.361	−0.183
TS	−0.030	0.460	0.384	0.284	0.444	0.243
CAT	0.117	0.350	0.495	0.428	0.019	0.271
POD	−0.443	0.244	−0.261	0.394	−0.312	0.184
RWC	0.197	0.401	−0.198	0.420	−0.106	0.241
Eigenvalue	3.41	2.77	1.53	3.90	2.36	1.35
Contribution%	34.10	27.73	15.31	39.01	23.64	13.56

LY = leaf yield, PO = polyphenol, CA = caffeine, WE = water extract, TA = total ash, PC = proline content, TS = total sugar, CAT = catalase, POD = peroxidase, RWC = relative water content.

In normal condition, the first component accounted for 34.10 % of variation and showed a highly positive and significant correlation with caffeine, total ash and proline content. Since the high values of these parameters are desirable and with respect to the positive and high correlation of the first component with these indicators, if the first component is selected high, so, the highly quality clones will be selected in normal condition. Also, the second main component accounted 27.73 % of existing variation and has a positive and high correlation with total sugar, POD and RWC. The third component has a positive and high correlation with green leaf yield, polyphenol, water extract and CAT activity (Table 5).

In drought stress condition, the first component accounted for 39.01 % of variation and showed a highly positive and significant correlation with green leaf yield, polyphenol, proline content, relative water content, CAT and POD. So, this first component can be called a drought tolerant component. Since higher and positive values for these parameters will lead to the identification of tolerant clones, this component can be named the drought tolerant component. This revealed that this group of clones could be exploited for improvement in drought tolerance. The second main component accounted 23.64 % of existing variation and has a positive and high correlation with caffeine, total ash and total sugar. The third component has a positive and high correlation with water extract (Table 5).

Parameters which significantly correlated with the first three components were the traits with the greatest variability. The factor loadings refer to be coefficients in each principle component or the correlation between the component and the traits. A high correlation between the first component and a trait indicates that the trait is associated with the direction of the maximum amount of variation in the data set. The partitioning of total variance into its components aids for conservation and practical exploitation of genetic resources. It also facilitates planning for utilization of appropriate germplasms in crop improvement for particular plant characters (Sneath and Sokal, 1973).

A clone × triat biplot was constructed from a two-way matrix of 10 traits and nine clones (Fig. 5). The plot condenses the information from this matrix into principal components. Clones were classified into four groups under normal and three groups under drought stress conditions. The plot shows the relationship between traits. The cosine of the angle between vectors connecting traits to the origin is proportional to the correlation coefficient between those traits (Yan and Kang, 2003). Thus, traits on opposite sides of the origin are negatively correlated, traits near each other are positively correlated, and traits at 90° to each other

with respect to the origin are not correlated. For example, under drought stress condition, it was shown that total ash and green leaf yield were negatively correlated (Fig. 5B). Traits like CAT, POD and RWC had high correlation with each other because of close adjacent of their vectors (Fig. 5B) and clones in the same direction of the respective vectors had the higher value for these traits (de la Vega et al., 2001), so clones 276 as well as 100, 285 and 277 at the same direction of most traits and grouped in one cluster (Fig. 5B). These clones characterized by higher green leaf yield, polyphenol, proline content, RWC, CAT, POD, and low total ash. These clones were identified as tolerant clones under drought stress condition (Fig. 5B). Clones 272, 269 and 399 were grouped as moderately sensitive (Fig. 5B). The clones in the reverse direction of green leaf yield, proline content, RWC, CAT and POD were differentiated with low values of these traits and were drought sensitive clones (74 and 278) (Fig. 5B). The last two groups of clones were separated and located in the reverse side of PCA biplot (Fig. 5B) which represents efficiency of PCA as a powerful tool for identifying of drought sensitive and tolerant clones.

4. Conclusion

The findings of the present research show that the tea clones more tolerant of drought had a highly-integrated system, with the leaf morphology resistant to water stress and effective antioxidant defense systems allowing reduction of drought damages under drought stress. This work will contribute to understanding the potential of drought tolerance of different clones of tea plants. In this process, some of the clones can be advised to grow in the areas prone to drought and, especially, for benefiting the large-scale tea industry.

In summary, based on cluster and PCA analysis, clones 276, 100, 285 and 277 characterized by higher green leaf yield, polyphenol, proline content, RWC, CAT, POD, and low total ash under drought stress condition. These clones were identified as tolerant clones under drought stress condition Clones 272, 269 and 399 were grouped as moderately sensitive. The clones 74 and 278 with lowest value for green leaf yield, proline content, RWC, CAT and POD were identified as sensitive clones

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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