10.22092/cbj.2020.351375.1057

Responses of some Iranian tea [*Camellia sinensis* (L.) O. Kuntze] clones to drought stress

S. Safaei Chaeikar[1*], Sh. Roofigari Haghighat[2] and S. Marzvan[3], R. Azadi[1]

[1] and [2] Tea Research Center, Horticultural Science Research Institute, Agricultural Research, Education and Extension Organization, Lahijan, Iran.
[3] University of Zanjan, Zanjan, Iran.
*Corresponding author's Email address: s.safaie@areeo.ac.ir

Received: March 2019
Accepted: August 2019

ABSTRACT

Safaei Chaeikar, S., Roofigari Haghighat, Sh., Marzvan, S. and Azadi, R. 2019. Responses of some Iranian tea [*Camellia sinensis* (L.) O. Kuntze] clones to drought stress. **Crop Breeding Journal 9 (1 & 2):** 45-60

Drought is one of the most important environmental stresses affecting tea plantation productivity in tea growing areas. Nine field-grown tea [*Camellia sinensis* (L.) O. Kuntze] clones in the north of Iran were subjected to drought stress by withholding irrigation for 50 days. The effects of drought stress were measured by studying growth and morphological (shoot number, shoot length, shoot fresh weight, length of 5th leaf, width of 5th leaf, green leaf yield), physiological (relative water content), biochemical (proline and total sugar content), and chemical (polyphenol) attributes after 50 days from the time drought stress was imposed. Drought stress resulted in decrease in growth and morphological characteristics, polyphenol content, and an increase in proline and total sugar concentration, that was attributed to reduction of RWC of leaves. Grouping of clones showed that clones 276, 100, and 285 formed drought-tolerant group. These tea clones can be used in the national tea breeding programs for improvement of drought tolerance.

Keywords: tea, antioxidant activity, proline, polyphenol, relative water content, total sugar, water stress index.

INTRODUCTION

Tea [*Camellia sinensis* (L.) O. Kuntze] is a perennial and woody plant. It is one of the most important drinks all over the world (Sharma and Kumar, 2005). Drought as an abiotic stress imposes oxidative damage on tea plants and depresses anti-oxidative systems. Consequently, it changes different biochemical and physiological processes (Upadhyaya and Panda, 2004; Upadhyaya *et al*., 2008; Upadhyaya *et al*., 2010) leading to serious crop losses.

Decrease in yield of tea plantation has been reported under drought stress (Handique and Manivel, 1986; Satyanarayana and Cox, 1994; Marimuthu and Kumar, 1998; Kigalu, 2007). Severity and longevity of drought periods have been increased in most tea-growing areas which have led to decreased production levels (Wijeratne and Fordham, 1996) and increased pests' invasion (Kamunya *et al.*, 2008; Sudoi *et al.*, 2011). Drought stress could decrease the yield of tea by 33% (Cheruiyot *et al.*, 2011). Additionally, it increases mortality of plants by about 19% (Cheruiyot *et al.*, 2010). Generally, responses of plants rely upon the duration and severity of stress, specific developmental phase, and interaction with environmental factors (Achuo *et al.*, 2006; Ramegowda and Senthil-Kumar, 2015).

Tea plants can adapt to diverse types of stress. However, the tolerance level varies in different cultivars (Chakraborty *et al.*, 2000). Different responses of various tea clones to 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, Upadhyaya *et al.* (2008), Netto *et al.* (2010) and Rawat *et al.* (2017) in India. Thomas *et al.* (2004) reported that understanding of physiological processes in plants and their responses to the environment are necessary for using tea germplasm to develop drought-tolerant tea plant material.

Severe drought stress can result in cell death. The effect of drought stress may be mitigated by using drought-tolerant plants (Jeyaramraja *et al.*, 2003).

Biochemical and physiological reactions are often revealed in response of plant to stress. This can set up the groundwork for screening germplasm and selection of stress tolerant varieties. For example, in response to stress, plants accumulate organic osmolytes including; proline, non-reducing sugars, glycine betaine, and polyols (Sabry *et al.*, 1995; Hare *et al.*, 1998). The role of these compounds that are specific to plant species, is not well known. However, these compounds can improve stress tolerance in plants (Hare *et al.*, 1998; Slama *et al.*, 2006).

Research on response to drought stress in different tea clones showed that under prolonged stress, proline content in all clones increased, and drought-tolerant clones had significantly higher relative water content and proline (Singh and Handique, 1993; Rajasekar *et al.*, 1998; Maritim *et al.*, 2015; Upadhyaya *et al.*, 2016). Most organic compounds related to stress are plant's secondary metabolites, and by the way, tea contains considerable contents of polyphenols, especially the flavonol class. Some polyphenol derivatives have been utilized to determine the quality of black tea (Singh *et al.*, 1999) and fruits (Stewart *et al.*, 2001). Cheruiyot *et al.* (2007) studied the role of polyphenols under water deficit conditions and stated that these compounds are suitable as indices of tolerance to desiccation in tea plant.

High-quality tea is planted in hilly areas in Guilan and Mazandaran provinces in the north of Iran. Since tea plants in these areas are usually grown under rainfed conditions, drought has become one of the most important limiting factors for its production. In addition, unbalanced distribution of rainfall throughout the year in tea growing areas, seasonal drought stress is common. It has been estimated that more than 20,000 hectares of tea plantations in Iran, are affected by different levels of seasonal drought stress. Therefore, for improving yield and quality of tea under such a conditions characterization of germplasms that can adapt to low soil water content is important.

The main objective of this research was to study responses of different tea clones to drought stress by withholding of irrigation for 50 days before summer harvest. Changes in morphological, biochemical, chemical, physiological, and growth attributes of tea clones were used to identify tolerant clones.

MATERIALS AND METHODS

Plant material and treatments

This study was conducted in Shahid Eftekhari Fashalam experimental station, Tea Research Center, Lahijan, Guilan province (Latitude 37°15′54″ N, Longitude 38°45′49″ E, and -10 m AMSL). Nine clones of twelve-year-old tea plants (included 272, 277, 100, 285, 74, 399, 276, 278, and 269) (Table 1) were arranged as split-plot in randomized complete block design with three replications.

The above clones were selected based on clonal selection method from different tea plantations (open pollinated population) in western part of Guilan province in 2007, and are desirable in terms of yield quantity and quality of the product. Clones were assigned to the main-plot. Every main-plot [15 m^2 (5 m × 3 m)] had three rows (the middle row was as buffer) and six plants per row, with between and within row spacing of 70 and 100 cm, respectively. The two irrigation Tow irrigation levels (irrigation and drought stress) were randomized in sub-plots (Fig. 1).

Table 1. Code, type, origin, yield and quality of tea clones

Clone	Type	Origin	Yield	Quality
272	Chinese	West of Guilan	Medium	Medium quality
277	Chinese	West of Guilan	Medium	Medium quality
100	Chinese	West of Guilan	High	High quality
285	Chinese	West of Guilan	High	Good quality
74	Chinese	West of Guilan	Medium	Medium quality
399	Chinese	West of Guilan	Medium	Medium quality
276	Chinese	West of Guilan	Medium	Good quality
278	Chinese	West of Guilan	Medium	Medium quality

269 Chinese West of Guilan Medium Medium quality

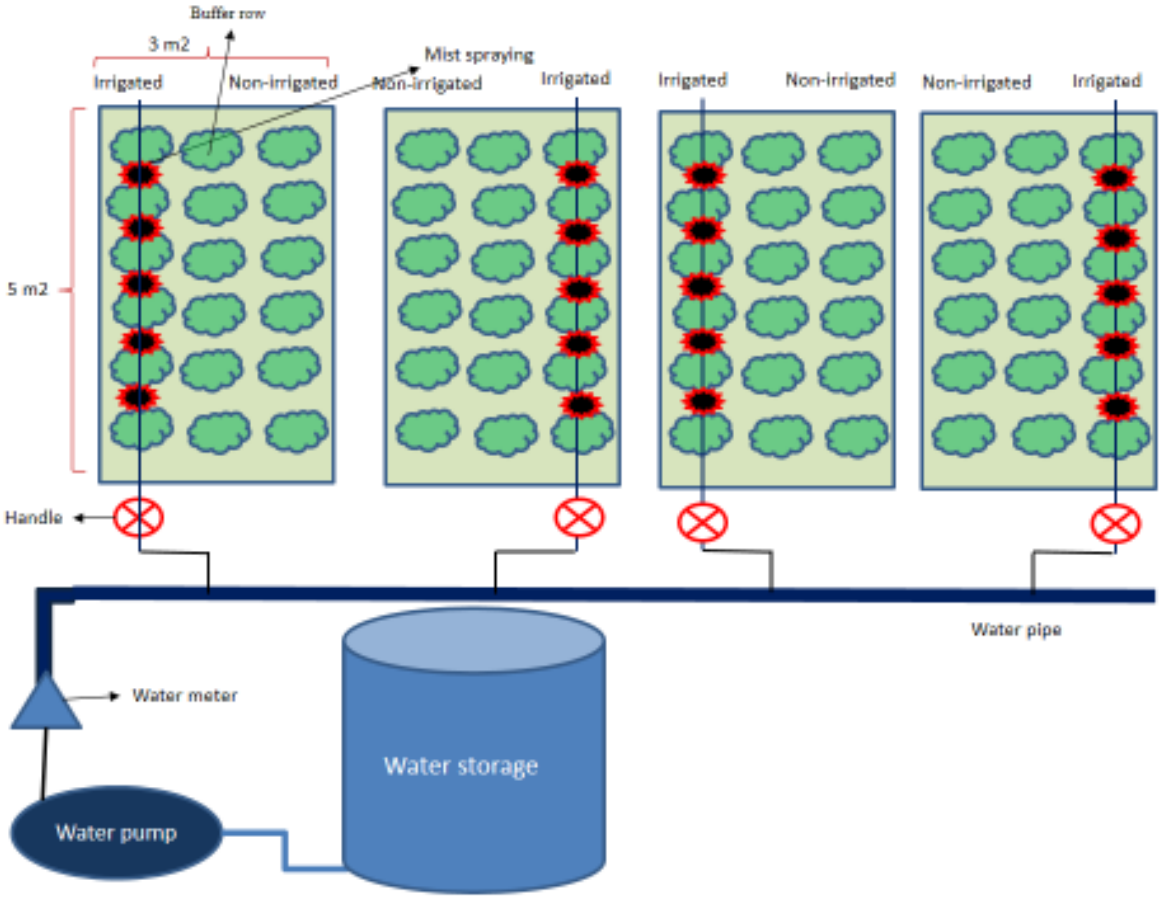


Fig. 1. Schematic of the irrigation system

Physical and chemical soil properties test of 0-30 cm depth showed that the soil texture was sandy loam consisting of 16.17% 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.

Tea clones were irrigated regularly (every 3 days) up to field capacity of the soil, before application of withholding of water in drought stress plots. Then, for non-irrigated treatment (drought stress) from May 30 to July 18, 2018 (summer harvest), plants were subjected to drought stress. This period was in coincidence with the first plucking, approximately in late May, when rainfall is much less than water requirement of tea bushes. During this period there was no irrigation. In irrigated treatment, control plants (one row) were regularly irrigated using mist spraying irrigation system. There was a large gap (one row) between the rows (non-irrigated and irrigated).

Irrigation timing for control plots was determined based on used available soil moisture or allowable depletion (Maximum Allowable Depletion = 0.4) (Majd Salimi *et al.*, 2010). Thus, in each plot, soil moisture content was monitored by the gravimetric method in 0-30 cm soil layer every 3 days. The depth of water needed to bring the soil moisture content back to the field capacity was calculated following Kovda *et al.*, (1973):

$d = (p_{fc} - p_i).\ D.B_d$

where, 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, Bd: bulk specific gravity (g cm^{-3}), D: effective depth rooting (cm).

The required total volume of irrigation water was obtained based on the plot area and the calculated depth of water. The duration of irrigation was calculated by computing the outlet

water discharge by a water meter. At each irrigation time, the soil moisture content was lower than the field capacity.

Rainfall and other meteorological parameters were obtained from meteorological station located approximately 1,000 m from the experimental site. The average of minimum and maximum temperatures and total rainfall during our experimental period were 20.52 °C, 30.95 °C, and 32.8 mm, respectively.

The effect of drought stress on tea plants was observed by morphological, physiological, and biochemical traits and growth characteristics such as shoot number, shoot length, shoot fresh weight, length of 5th leaf, width of 5th leaf, green leaf yield, relative water content (RWC), total soluble sugars, proline and total polyphenol in the non-irrigated plants compared to irrigated samples. The observation and measurements were recorded on the 50th day (four to the five-leaf stage) of the experiment. All leaf sampling was taken between 9 and 10 a.m. For each experiment, four plants were used for each sampling, and each laboratory experiment was performed in triplicate.

Soil moisture content

Soil moisture content was measured by the use of the gravimetric method (Gupta, 1999). The soil samples were taken from the 0-30 cm soil layer and dried at 105 °C for 48 hours in an oven. The gravimetric moisture content was measured as the difference between dry and moist soil masses and expressed as the volumetric percentage (Gupta, 1999).

Morphological and growth characteristics, and RWC

The number and fresh weight of plucking shoots were measured in three replications by using shoots (two leaves and a bud) that collected from 25 × 25 cm frame (randomly located in three locations per plot) and expressed as number m^{-2} and g m^{-2} for number and fresh weight of shoots, respectively (IPGRI, 1997). Shoot length was measured from the beginning of the shoot growth to the terminal bud (IPGRI, 1997). As for the length and width of leaves, the longest and widest part of the 5th mature leaf was measured (IPGRI, 1997). To determine the green leaf yield, the tea shoots were plucked 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).

The third leaf was used to measure relative water content (RWC). Barrs and Weatherly (1962) method was followed to measure RWC:

$$RWC\ (\%) = [(FW-DW) / (TW-DW)] \times 100$$

Where, FW, TW, and TW are fresh weight (g), turgid weight (g), and dry weight (g), respectively.

Water stress index (WSI)

The water stress index was measured based on leaf yield following Cheruiyot *et al.* (2007):

$$WSI = (Y_{actual} / Y_{max})$$

This equation (with minor changes) was to assess the level of water stress in the tea plant under drought stress. Where, Y_{actual} is green leaf yield in a given soil water content (green leaf yield under drought stress); Y_{max} is the maximum green leaf yield obtained under non-drought stress conditions. This index is presented between 0 and 1. A value close to 0 means low tolerance to drought stress and a value close to 1 means tolerance to drought stress.

Biochemical and chemical attributes

For biochemical analysis, the leaves were frozen in the liquid N (leaves sampled 50 days after drought stress) and stored at -80 °C. The proline content of tea leaf was measured according to Bates *et al.*, (1973). The leaf sample (0.5 g) was homogenized by 5 ml of 3% sulfosalicylic acid with using a mortar and a pestle, and then filtered by the Whatman No.1 filter paper. The filtrate volume was increased up to 10 ml by sulfosalicylic acid. Then, 2 ml of the filtrated substance were incubated by 2 ml of ninhydrin and 2 ml of glacial acetic acid and boiled in a water bath at 100 °C for 30 minutes. Then, the reaction mixture 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 570 nm wavelength.

The total sugar was extracted from the tea leaves in 80% (v/v) ethanol. Aliquots of the ethanol extract (80%) were taken by Anthrone reagent to estimate the total sugar (Yoshida *et al.*, 1972).

The plant tissue was sampled after 50 days of application of water treatments to determine

total polyphenols. In each experimental units, approximately 100 g of the fresh shoots (one bud and two leaves) were plucked. Then, the samples were placed inside the labeled paper bags and dried at a 70 ˚C for 24 hours. The dried samples were blended and placed inside the paper bags in dry and dark conditions until laboratory analysis.

The ISO (2003) procedure was used for the analysis of the total polyphenols. Ground shoot samples (0.2 g) were weighed into 10-ml extraction tubes. Five milliliter of 70% v/v methanol (hot methanol/water extraction mixture) was added to every extraction tube. Then, a vortex mixer was used for shaking the tubes. The tubes were placed in a water bath for 10 minutes. Then, the tubes were allowed to be cooled at room temperature. Then, the extracts were centrifuged (3500 rpm, 10 minutes). The supernatant was poured into 10-ml tubes. Then, a cold ethanol/water mixture was added in order to reach 10 ml.

One milliliter of the extract was poured into a 100-ml flask to further dilute, and then, water was added to reach the mark. Standard solutions of gallic acid (1 ml) corresponding to 10, 20, 30, 40, and 50 µg of anhydrous gallic acid, and a similar quantity of water for the reagent blanks, were poured in duplicate into the separate tubes.

One milliliter of the diluted sample extract was poured into the separate tubes and 5 ml of the reagent of Folin-Ciocalteu phenol were added to each of the tubes and mixed. Four milliliter of sodium carbonate solution, about 5 minutes after adding the Folin-Ciocalteu phenol reagent, were added to each of the tubes and allowed to remain for 60 minutes at the room temperature.

By using a 10-mm cell on a spectrophotometer set, optical densities were calculated at a wavelength of 765 nm. Polyphenol contents in the tested samples were measured by a standard curve made by gallic acid and defined as the contents of gallic acid equivalent. By using the mass of the standards of anhydrous gallic acid, the graph of the best-fit linear calibration was drawn in comparison with the standard optical densities of gallic acid, and the content of the total polyphenol, expressed as a percent by the mass on the basis of the sample dry matter, as measured by the ISO (ISO, 2003) procedure

Statistical analysis

Data were analyzed using SAS 9.4 version. Mean comparison was performed using Tukey's test at the 5% probability 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.

RESULTS AND DISCUSSION

Soil moisture content

By continuing water stress, soil moisture content decreased in non-irrigated plots, while the level of soil moisture content remained constant in irrigated plots. Due to dehydration, soil moisture content decreased to 9.20% and 9.12% after 40 and 50 days of stress imposition, respectively, compared to the control (24.54% and 24.50%) (Table 2).

Table 2. Soil moisture content (%) recorded in irrigated and non-irrigated (after 50 days of water stress imposition) plots and ambient environmental conditions

		Days after drought stress imposition					
		0 (2018.05.30)	10 (2018.06.08)	20 (2018.06.18)	30 (2018.06.28)	40 (2018.07.08)	50 (2018.07.18)
SMC[a]	Non-Irrigated	24.54	24.51	24.49	24.44	24.54	24.50
	Irrigated	24.51	19.29	11.90	9.46	9.20	9.12
Minimum temperature (°C)		15.1	16.98	18.8	19.98	23.48	24.02
Maximum temperature (°C)		21.5	27	28.3	31.32	35.42	33.46
Minimum relative humidity (%)		64	59.9	57.4	44.9	48	55
Maximum relative humidity (%)		97	96.5	95.1	91.2	88.77	93.44
Rainfal		0	0.97	1.2	11.1	0	0

a: Soil moisture content (SMC) are mean of nine tea clones, 2. Minimum temperature (°C).
Each record is the mean of 10 days.

Morphological and growth characteristics

Analysis of variance showed that clones, irrigation treatments, and the interactions of clone× irrigation treatment had significant effects ($P \leq 0.05$) on the number of shoots, shoot fresh weight, shoot length, length and width of 5th leaf, and green leaf yield (Table 3 and Table 4).

Table 3. Analysis of variance for clone and irrigation treatment effects on growth and morphological characteristics of tea clones.

		Mean Squares					
S.O.V.	df	Shoot no. m^{-2}	Shoot length (cm)	Shoot fresh weight (g m^{-2})	Length of 5th leaf (cm)	Width of 5th leaf (cm)	Green leaf yield (g m^{-2})
Block	2	61.40	0.12	21.91	0.28	0.06	70.84
Clone	8	9097.97**	2.84**	4802.92**	3.64**	0.40**	158594.00**
Error a	16	40.57	0.45	27.38	0.49	0.09	100.01
Irrigation	1	70272.29**	78.74**	28665.44**	91.33**	16.18**	609995.97**
Clone × treatment	8	3273.33**	1.52**	1419.15**	4.36**	0.65**	23010.86**
Error b	18	45.77	0.20	31.71	0.25	0.08	30.95
C.V. (%)		6.02	7.36	8.71	8.28	11.77	12.09

**: Significant at the 1% probability level.

Table 4. Mean comparison of effects of clones and irrigation treatments on growth characteristics of tea.

	Shoot no. m^{-2}	Shoot length (cm)	Shoot fresh weight (g m^{-2})	Length of 5th leaf (cm)	Width of 5th leaf (cm)	Green leaf yield (g m^{-2})
			Clone			
272	68.66g	6.69a-c	33.72g	6.83a-c	2.32a-c	122.40g
277	107.50d	5.94b-d	55.80ef	6.04b-d	2.24c	150.93f
100	82.83ef	6.77ab	68.33cd	6.66a-c	2.86ab	513.26b
285	179a	7.41a	124.16a	5.11d	2.28bc	540.10a
74	97.66de	5.73cd	45.20fg	5.34d	2.27bc	199.20e
399	161.66b	6.20b-d	90.08b	6.02cd	2.40a-c	367.58c
276	82.83f	6.20b-d	39.78g	7.12ab	2.54a-c	115.60g
278	92ef	5.38d	60.18de	6.46a-c	2.66a-c	244.47d
269	144.66c	7.27a	77.67c	7.43a	2.91a	203.44e
			Irrigation			
Irrigated	149.22a	7.61a	89.14a	7.63a	3.05a	379.29a
Non-Irrigated	77.07b	5.19b	43.06b	5.03b	1.95b	166.72b

Means, in each column and for each factor, followed by at least one letter in common are not significantly different at the 5% probability level-using Tukey's test.

Under drought stress conditions, all nine tea clones indicated a significant decrease in shoot number. The maximum decrease in shoots number was related to the clone 399 (64.13%) after 50 days of drought stress, whereas clone 276 (18.72%) showed less decrease (Table 5). Drought stress affected the length of the shoot. All nine tea clones showed shorter shoot length under drought stress (Table 5). Under irrigated conditions, clones 272 and 285 had the longest shoots, while clone 278 indicated the shortest shoot length. Clone 285 recorded the longest shoot under drought stress (Table 5).

Table 5. Mean comparison of clone × irrigation treatment interaction effect of drought stress on morphological and growth characteristic of tea.

Clone	Treatment	Shoot no. m^{-2}	Shoot length (cm)	Shoot fresh weight (g m^{-2})	Length of 5^{th} leaf (cm)	Width of 5^{th} leaf (cm)	Green leaf yield (g m^{-2})
272	Irrigated	86.6f-h	8.37a	41.08g-i	8.38ab	3.12a-c	157.00g
	Non-Irrigated	50.66i	5.02g-i	26.37i	5.27f-h	1.52fg	87.80k
277	Irrigated	141.66c	7.72a-d	83.05b-d	7.83a-c	3.00a-d	218.80f
	Non-Irrigated	73.33g-i	4.16i	28.54hi	4.25hi	1.47g	83.16k
100	Irrigated	102.66e-f	8.16ab	73.58c-e	7.28a-e	3.36ab	647.73b
	Non-Irrigated	66.00hi	5.38 f-i	63.08ef	6.04d-g	2.35c-g	378.80d
285	Irrigated	231.33ab	8.27a	155.54 a	5.65e-h	2.50b-f	705.33a
	Non-Irrigated	126.66cd	6.55c-g	92.78bc	4.57g-i	2.05d-g	374.88d
74	Irrigated	125.33c-e	7.64a-d	66.04de	6.47c-f	2.61a-e	297.42e
	Non-Irrigated	70.00g-i	3.83i	24.37i	4.22 hi	1.93e-g	100.98jk
399	Irrigated	238.00a	7.19a-e	141.37a	8.76a	3.36ab	583.33c
	Non-Irrigated	85.33f-h	5.21f-i	38.78g-i	3.27i	1.45g	151.83g
276	Irrigated	91.33fg	6.64b-f	45.80f-h	7.41ad	2.51b-e	143.71gh
	Non-Irrigated	74.33gh	5.77e-h	33.76hi	6.82b-f	2.57a-e	87.50k
278	Irrigated	116.00de	6.44d-g	95.45b	8.77a	3.54a	379.25d
	Non-Irrigated	68.00hi	4.32hi	24.90i	4.16hi	1.78e-g	109.69ij
269	Irrigated	210.00b	8.05a-c	100.36b	8.16a-c	3.39 ab	281.04e
	Non-Irrigated	79.33gh	5.66e-h	54.97e-g	6.71b-f	2.43b-g	125.84hi

Means, in each column, followed by at least one letter in common are not significantly different at the 5% probability level-using Tukey's test.

Significant differences in the shoot length between clones showed that the effects of drought stress depended on genotype. The overall decrease in shoot length under drought stress may be attributed to the reduction in a cyclin-dependent kinase activity which led to the slower cell division rate (Mahajan and Tujeta, 2005). Growth is the most drought-sensitive physiological process in plants because of some reduction in the turgor pressure. Daughter cell production by meristematic cell division, and subsequently young cell expansion results in shoot growth. However, cell elongation is inhibited by a water-flow interruption from the xylem to the elongating cells, under severe drought conditions. Therefore, drought impairs mitosis, cell elongation, and expansion, leading to a decrease in plant growth (Anjum *et al.*, 2011).

The fresh weight of shoot in non-irrigated plants was less than the irrigated ones (Table 5). There was a significant decrease in the fresh weight of shoot in all clones. Clone 278 (73.94%) showed the greatest change in shoot weight after 50 days non-irrigated treatment compared to the irrigated conditions followed by clone 399 (72.55%), while clones 100 (13.68%) and 276 (25.94%) recorded the least. Fresh shoot weight decrease under drought stress conditions indicated a photosynthetic arrest, however it was not capable of causing permanent damage to the photosynthesis system (Xu and Zhou, 2007). A similar decrease in the shoot weight has been reported in different plant species under drought stress (Mengel and Kirkby, 1996; Thomas *et al.*, 2004; Upadhyaya *et al.*, 2008; Netto *et al.*, 2010). When the water content in the soil is negligible and transpiration is high, negative water balance takes place. Consequently, water loss will be higher than its uptake by the plant.

Furthermore, this revealed that under water stress, the turgor pressure in plant's cells reduced as well as cell expansion. Therefore, the reduction in cell-size strongly correlates with water stress levels in plant tissues. Growth is indirectly affected by water stress. In other words, water stress affects assimilates allocation and mineral uptake (Puthur, 2000); thereby it decreases the fresh weight. Fresh shoot weight was relatively higher in the clones 100 and 276 under drought stress conditions which may be due to the capacity of these clones to maintain superior water status compared to the clones 278, 399, 277 and 74 (Table 5).

In this research, the length and width of the 5^{th} leaf decreased in all tea clones under drought stress conditions. Under irrigated conditions, clone 278 showed the highest width and length

of the 5th leaf, while clone 285 had the lowest. However, clone 276 had the highest length and width of the 5th leaf under drought stress conditions (Table 5). For photosynthesis and dry matter yield, optimal leaf area is vital. Drought stress notably decreased leaf growth as well as its length and width. The effect of drought stress on leaf growth can be considered as an adaptive mechanism to such conditions for decreasing the transpiration rate (Lu and Neumaan, 1998), to maintain the supply of water in the root media, and to increase plant chances for survival (Passioura, 2002). This mechanism, by which under water stress, plant leaf area is reduced, operates through a reduction in the cell elongation as well as the cell size, thereby reducing the leaf area (Schuppler *et al.*, 1998).

All nine tea clones showed a significant decrease in green leaf yield under drought stress conditions (Table 5). The maximum decrease in green leaf yield was related to clone 399 (73.96%), followed by clone 278 (71.07%), whereas it was low in clones 100 (41.52%) and 276 (39.10%) (Table 5). Water stress is a severe limiting factor of plant growth. Many researchers have demonstrated that water stress results in decrease in growth which may be observed in leaf area, height of plant, dry and fresh weights, yield, and other growth charcteristics (Netto *et al.*, 2010; Lipiec *et al.*, 2013; Cirillo *et al.*, 2014; Maritim *et al.*, 2015; Upadhyaya *et al.*, 2016; Rawat *et al.*, 2017; Rahimi *et al.*, 2019).

Relative water content (RWC)

Analysis of variance of data showed that the effect of clone, irrigation treatment and their interaction was significant ($P \leq 0.01$) on RWC, total sugar content, proline content and total polyphenol (Table 6.). In non-irrigated conditions, the consistent reduction was observed for RWC in nine tea clones as compared to the control (Fig. 2). The maximum reduction in RWC was related to clone 278 (20.31%) after 50 days of application of drought stress compared to the control, while clone 276 (6.63%) showed a less decrease (Fig. 2).

Table 6. Analysis of variance for clone and irrigation treatment effects on physiological, biochemical and chemical attributes of tea .

		Mean Squares			
S.O.V.	df	Relative water content (%)	Total sugar content (mg g^{-1}FW)	Proline (mg g^{-1}FW)	Total polyphenol (%)
Block	2	4.99	0.0002	0.22	0.07
Clone	8	43.40**	1.0400**	9.95**	3.04**
Error a	16	5.74	0.0005	0.10	0.14
Irrigation	1	1608.29**	0.1600**	38.99**	30.91**
Clone × treatment	8	26.44**	0.0030**	3.02**	2.05**
Error b	18	3.69	0.0004	0.09	0.10
C.V. (%)		2.57	1.23	10.00	2.51

**: Significant at the 1% probability level.

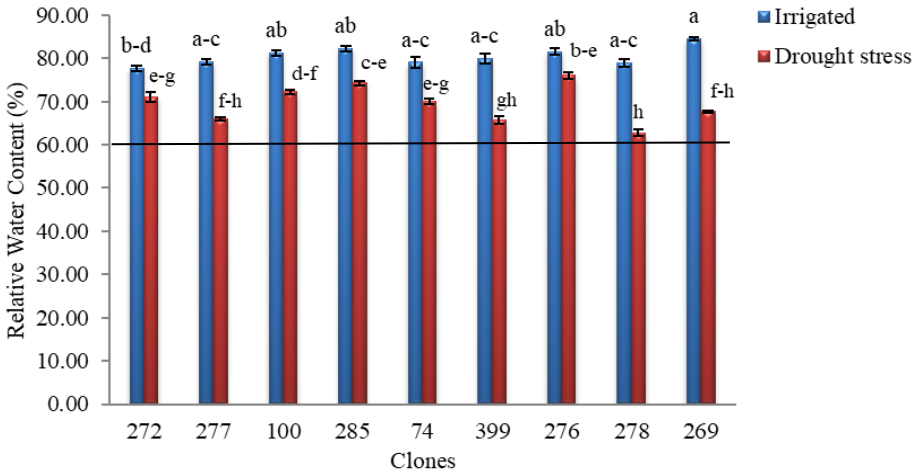


Fig. 2. Relative water content (RWC) as affected by clone × irrigation treatment interaction effect. Bars with

at least one letter in common indicate significant differences at the 5% probability level- using Tukey's test

The reduction in RWC can be attributed to the deficiency in available soil moisture, hence, plant root tissue could not uptake water. Results of this study is in agreement with findings of other researchers (Upadhyaya *et al.*, 2008; Maritim *et al.*, 2015; Upadhyaya *et al.*, 2016; Rawat *et al.*, 2017). The minimal reduction in leaf relative water content serves as an adaptive strategy in tea plants (Chakraborty *et al.*, 2002).

Biochemical and chemical indices

The total soluble sugar increased under drought stress. The soluble sugar content of clone 276 and 100 increased by 7.91% and 7.69%, respectively, under non-irrigated conditions as compared to the control (Fig. 3A) .The accumulation of intracellular soluble sugars plays critical role in osmotic regulation, helping to reduce the cellular water potential and maintain turgor pressure inside the cell under drought stress conditions (Sato *et al.*, 2004). An increase in the total soluble sugar content under desiccation is one of the positive characteristics of drought-tolerant tea plants (Liu *et al.*, 2015).

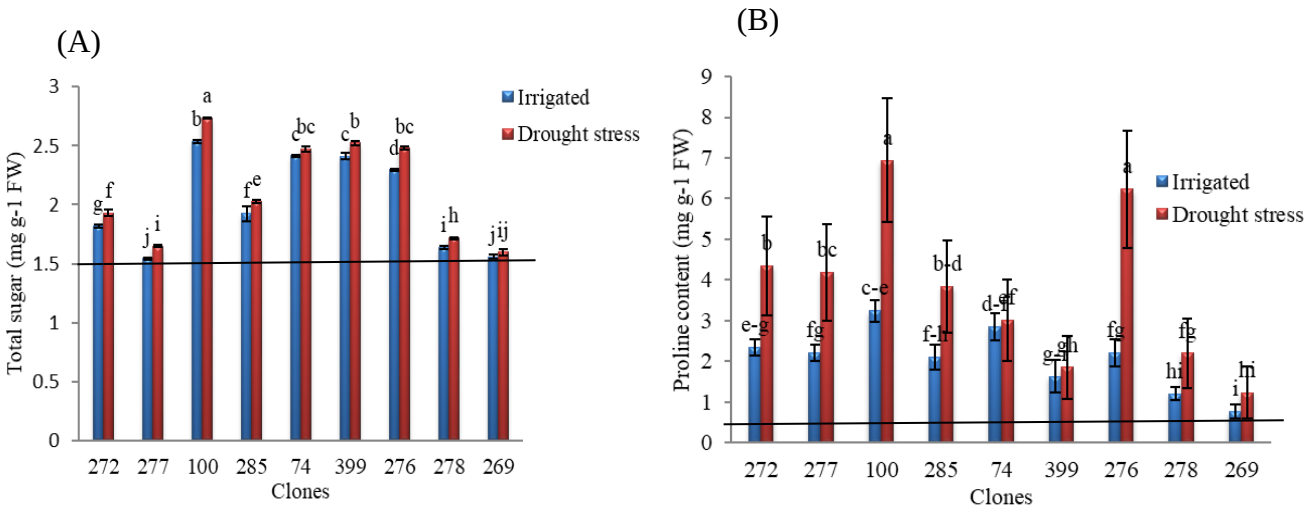


Fig. 3. Total soluble sugar content (A) and proline content (B) as affected by clone × irrigation treatment interaction effect. Bars with at least one letter in common indicate significant differences at the 5% probability level- using Tukey's test

All nine tea clones accumulated more proline under non-irrigated conditions. However, concentration of proline significantly differed among tea clones (Fig. 3B). Clones 100 and 276 showed the highest proline under non-irrigated conditions, while clone 269 showed the lowest proline content (Fig. 3B). Variation in proline accumulation among clones can be related to their different responses to drought stress.

Proline as an organic solute is accumulated in plants under abiotic stresses, including salinity, temperature, drought stress (Saraswathy *et al.*, 1992; Saradhi *et al.*, 1995; Matysik *et al.*, 2002; Puthur and Rajan, 2006; Szabados and Savoure, 2010). Rajasekar *et al*. (1988) found out that under prolonged drought stress, in clones UPASI-2, UPASI-9, and UPASI-10, higher concentrations of proline accumulated in plants which indicated their tolerant to drought stress. Furthermore, Chakraborty *et al.* (2001) investigated biochemical changes in young tea leaves that were exposed to drought. They found out that in all varieties, there was a sharp increase in proline content accumulation after 7 days of drought stress compared to the control.

Proline is related to multiple functions including; maintenance of protein stability, osmotic adjustment, nitrogen and carbon storage for overcoming the stress (Andrade *et al.*, 1995; Netto *et al.*, 2010). Proline can send signals to modulate the mitochondrial functions, affect cell divisions or death and

start the expression of specific gene(s), which may be necessary for plant recovery from stresses (Szabados and Savoure, 2010).

Netto *et al.* (2010) screened 7 tea clones for drought tolerance after 30 days of withholding irrigation and reported the highest accumulation of proline observed in drought-tolerant clones TTL-1, TTL-6 and UPASI-2. Similarly, Maritim *et al.* (2015) stated that specific tea cultivars with different levels of resistance differed capacity for the accumulation of proline. Moreover, under water-stress conditions, the drought-tolerant cultivars of TRFK 306, TRFCA SFS150 and EPK TN14/3 accumulated higher proline concentrations as compared to susceptible cultivars. Rahimi *et al.* (2019) studied 14 tea clones under drought stress conditions of 30 days without irrigation and reported that clones 100 and 399 accumulated the highest proline content under this condition.

The total polyphenol content in tea leaves decreased under drought stress. The reduction in polyphenol content was maximum in clone 277 (24.43%) followed by clone 74 (23.91%), whereas clone 100 (2.51%) and clone 276 (1.27%) had minimum reduction over the control after 50 days of drought stress (Fig. 4)

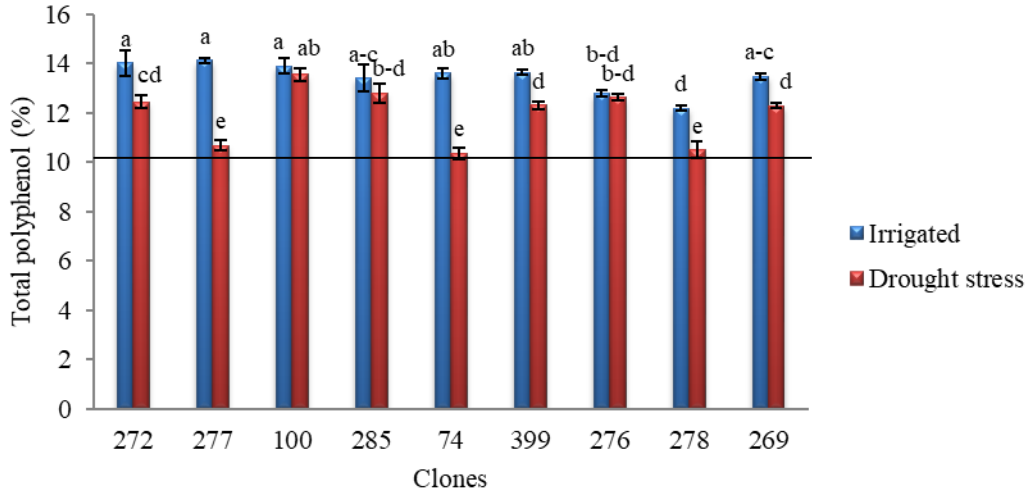


Fig. 4. Total polyphenol as affected by clone × irrigation treatment interaction effect. Bars with at least one letter in common indicate significant differences at the 5% probability level- using Tukey's test

The catechins of tea green leaf, and their oxidation products (theaflavins and thearubigins) have been identified as the important biochemical to determine the quality of tea. These are the most essential properties of plain black tea. Tea is a potent health beverage because its polyphenols are mostly catechins with potential antioxidant properties. Researchers have shown the reduction in the total phenolic compounds in tea cultivars under drought stress conditions, and the simultaneous decrease in ascorbate and glutathione contents showed gradual loss of protection in tea seedlings to overcome oxidative damage resulting from drought and the reduction in growth and quality of tea in drought-prone regions (Dixon and Steele, 1999; Battle and Munne Bosch, 2003).

Cheruiyot *et al.* (2007) showed that polyphenols level could be a potential indicator of drought tolerance in tea plants and could speed up adaptation of cultivars to water stress. In their research, they found that there was fluctuations in the polyphenol level with changes in soil water content. Water is essential in the photosynthetic processes of plants and directly influences the synthesis of secondary metabolites. Tea cultivars with stable polyphenols are tolerant to water stress (Cheruiyot *et al.*, 2007). Cheruiyot *et al.* (2008) also reported that the level of shoot catechins in tea responded to the level of soil

water content. However, different catechins in tea were not influenced uniformly by water stress and the diversity was attributed to their chemical variation.

Water stress index (WSI)

Results showed that under non-irrigated conditions, all clones suffered. Water stress index for clones 278 and 399 approached 0.3 by 50 days after withholding irrigation, while in clones 276 and 100, WSI reached 0.6, suggesting relatively high tolerance to drought stress as compared to others (Fig. 5).

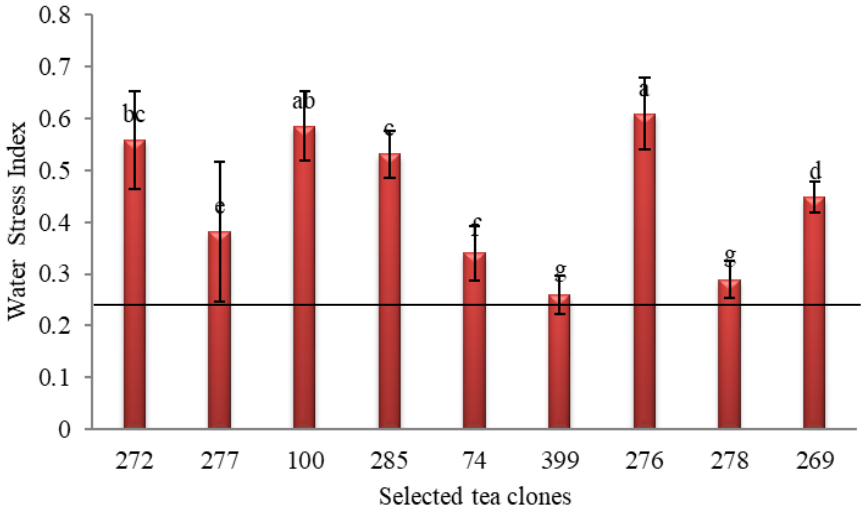


Figure 5. Water stress index for nine tea clones

Principal component analysis (PCA)

The association between parameters and grouping of examined clones based on their tolerance to drought stress can be studied by PCA. For this purpose, we considered morphological, growth, biochemical and chemical parameters altogether as indicators for drought stress tolerance in tea.

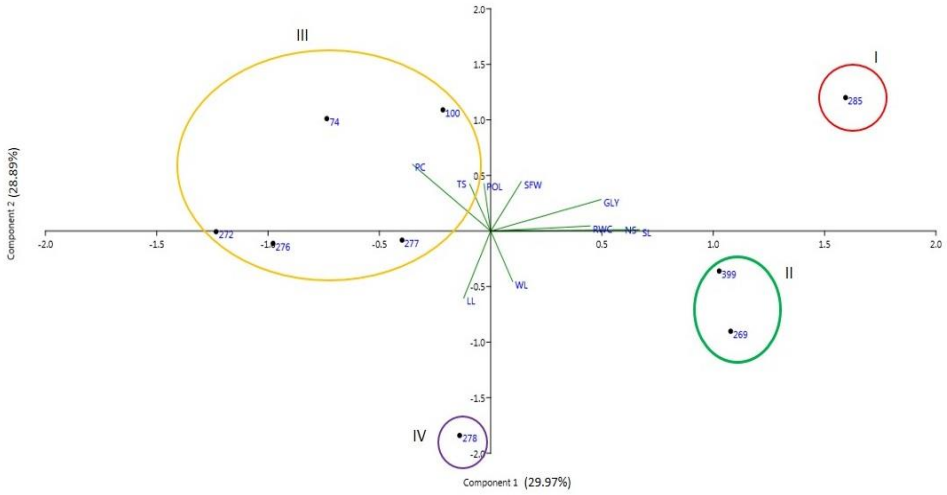
PCA for 10 traits and nine clones at two irrigation treatments was performed to identify the principal components of measured parameters that best describe the response to irrigation treatments thus, to identify tolerant clones (Fig. 6A and Fig. 6B). Results showed that the first three principal components contributed 74.82% and 84.16% to the total variation among clones under irrigated and non-irrigated conditions, respectively (Table 7).

Table 7. Eigenvectors of principal components obtained for 10 traits measured for tea clones

	Irrigated			Non-Irrigated		
Trait	PC1	PC2	PC3	PC1	PC2	PC3
NS	0.54	0.006	0.05	0.21	-0.53	0.07
SFW	0.11	0.35	0.55	0.36	-0.21	0.33
SL	0.54	0.008	0.06	0.36	0.37	0.03
LL	-0.09	0.48	0.26	0.27	0.35	-0.49
WL	0.08	-0.36	0.35	0.32	0.19	-0.33
GLY	0.40	0.22	-0.10	0.33	0.24	0.33
RWC	0.36	0.03	-0.10	0.36	0.22	0.09
PC	0.28	0.47	-0.02	0.24	0.42	0.30
TS	-0.07	0.33	0.32	0.18	0.26	0.55
POL	0.02	0.34	0.60	0.39	0.04	0.04
Eigenvalue	2.99	2.88	1.58	4.81	2.14	1.45
Contribution (%)	29.97	28.89	15.86	48.16	21.47	14.52

NS = number of shoot, SFW = shoot fresh weight, SL = shoot length, LL = length of the 5th leaf, WL = width of the 5th leaf, GLY = green leaf yield, RWC = relative water content, PC = proline content, TS = total sugar, POL = polyphenol.

(A)



(B)

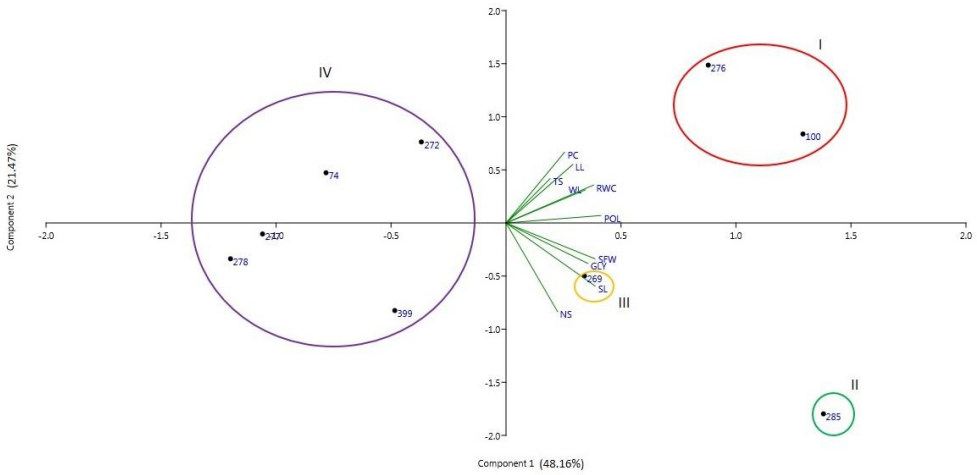


Fig. 6. Biplot of clone × trait based on 10 traits. The first two principal components are plotted under irrigated conditions (A) and non-irrigated conditions (B), each accounted for a proportion of the variance in the original dataset shown in parentheses. Clones are plotted according to scores on each principal component, and traits are plotted on the basis of the eigenvectors on each principal component. NS = number of shoot, SFW = shoot fresh weight, SL = shoot length, LL = length of the 5th leaf, WL = width of the 5th leaf, GLY = green leaf yield, RWC = relative water content, PC = proline content, TS = total sugar, POL = polyphenol

In irrigated conditions, the first component accounted for 29.97% of observed variation and showed a highly positive and significant correlation with the number of shoots, shoots length, green leaf yield and RWC. These relationships would facilitated to select desirable tea clones for irrigated conditions. Also, the second-main component accounted for 28.89% of observed variation and had positive and high correlations with proline content and total sugar. The third component had positive and high correlations with shoot fresh weight, length, and width of the 5th leaf and polyphenol content (Table 7).

In non-irrigated conditions, the first component accounted for 48.16% of observed variation and showed highly positive and significant correlation with the number of shoots, shoot fresh weight, shoots length, the width of the 5th leaf, green leaf yield, relative water content and polyphenol content. The second main component accounted for 21.47% of observed variation and had a positive and high correlation with the length of the 5th leaf and proline content. The third component has a positive and high correlation with total sugar (Table 7).

Biplot of clone × triat was constructed from a two-way matrix of 10 traits and nine clones (Fig. 6). Clones were grouped into four groups under irrigated and non-irrigated conditions. The plot shows the relationship between traits. Traits like proline content, total sugar and length of the 5th leaf had high correlation with each other because of close adjacency of their vectors (Fig. 6B), and clones in the same direction of the respective vectors had the higher value for these traits (de la Vega *et al.*, 2001).

Therefore, clone 276 as well as clone 100 were at the same direction for most traits and were grouped in one cluster (Fig. 6B). These clones were identified by higher polyphenol, proline content, total sugar, RWC, length and width of the 5th leaf. Also clone 285 was characterized by higher number of shoot, shoot fresh weight, green leaf yield and shoot length. These clones (276, 100 and 285) were identified as tolerant under drought stress conditions (Fig. 6B). Clone 269 was grouped as moderately sensitive (Fig. 6B). Clones in reverse direction of all the studied traits were differentiated with low values of these traits and were drought-sensitive clones (272, 74, 277, 399 and 278) (Fig. 6B). The last group of clones was separated and placed on the reverse side of the PCA biplot (Fig. 6B), which represents the efficiency of PCA as a powerful tool for identifying drought tolerant and susceptible clones.

CONCLUSION

According to the results of the present study, drought stress had significant effect on growth and morphological characteristics (shoot number, shoot length, shoot fresh weight, length, and width of the 5th leaf, green leaf yield) all tea clones. It also led to an increase in accumulation of osmolytes, especially proline. Furthermore, our results showed that proline accumulation in leaves was significantly higher in drought tolerant clones and can be used as a marker in selection processes.

Finally, based on PCA analyses, clones 276, 100, and 285 had relatively higher number of shoot, shoot fresh weight, green leaf yield, length and width of the 5th leaf, relative water content, proline content, total soluble sugar contents and also high polyphenol content, implying of these drought tolerance of these clones. Clones 277, 278, 74, 272, and 399 were identified as sensitive.

ACKNOWLEDGEMENTS

This research was financially supported by the Tea Research Center, Horticultural Sciences Research Institute through research project no. 2-21-33008970057.

REFERENCES

Achuo, E. A., Prinsen, E. and Höfte, M. 2006. Influence of drought, salt stress and abscisic acid on the resistance of tomato to *Botrytis cinerea* and *Oidiumneoly copersici*. Plant Pathol. 55 (2): 178-86.

Andrade, J. L., Larque-Saavedra, A. and Trejo, C. L. 1995. Proline accumulation in leaves of four cultivars of *Phaseolus vulgaris* L. with different drought resistance. Phyton-International J. Exp. Bot. 57 (2): 149-58.

Anjum, S. A., Xie, X. Y., Wang, L. C., Saleem, M. F., Man, C. and Lei, W. 2011. Morphological, physiological and biochemical responses of plants to drought stress. Afr. J. Agric. Res. 6 (9): 2026-32.

Barrs, H. D. and Weatherley, P. E. 1962. A re-examination of the relative turgidity technique for estimating water deficits in leaves. Aust. J. Biol. Sci. 15 (3): 413-28.

Bates, L. S., Waldren, R. P. and Teare, I. D. 1973. Rapid determination of free proline for water-stress studies. Plant Soil. 39 (1): 205-7.

Battle, L. A. and Munne-Bosch, S. 2003. Regulation of plant response to drought.

Function of plant hormones and antioxidants. pp. 267-285. In: Hemantaranjan, A. (ed.) Adv. Plant Physiol. Vol 5. Scientific Publishers (India). Jodhpur.

Carr, M. K. 1977. Changes in the water status of tea clones during dry weather in Kenya. J. Agric. Sci. 89 (2): 297-307.

Chakraborty, U., Dutta, S. and Chakraborty, B. N. 2000. Changes in biochemical constituents of tea leaves induced by temperature stress. pp. 246-50. In: Muraleedharan N., Kumar R. R. (eds.) Recent advances in plantation crops research. New Delhi: Allied Publishers Limited.

Chakraborty, U., Dutta, S. and Chakraborty, B. N. 2002. Response of tea plants to water stress. Biol. Plant. 45 (4): 557-62.

Chen, X. H., Zhuang, C. G., He, Y. F., Wang, L., Han, G. Q., Chen, C. and He, H. Q. 2010. Photosynthesis, yield, and chemical composition of Tieguanyin tea plants (*Camellia sinensis* (L.) O. Kuntze) in response to irrigation treatments. Agric. Water Manag. 97 (3): 419-25.

Cheruiyot, E. K., Mumera, L. M., Ng'etich, W. K., Hassanali, A. and Wachira, F. 2007. Polyphenols as potential indicators for drought tolerance in tea (*Camellia sinensis*L.). Biosci. Biotechnol. Biochem. 71 (9): 2190–2197.

Cheruiyot, E. K., Mumera, L. M., Ng'etich, W. K., Hassanali, A., Wachira, F. and Wanyoko, J. K. 2008. Shoot epicatechin and epigallocatechin contents respond to water stress in tea [*Camellia sinensis* (L.) O. Kuntze]. Biosci, Biotechnol. Biochem. 72 (5): 1219-1226.

Cheruiyot, E. K., Mumera, L. M., Ng'etich, W. K., Hassanali, A. and Wachira, F. N. 2010. High fertilizer rates increase susceptibility of tea to water stress. J. Plant. Nutr. 33 (1): 115-129.

Cirillo, C., Rouphael, Y., Caputo, R., Raimondi, G. and De Pascale, S. 2014. The influence of deficit irrigation on growth, ornamental quality, and water use efficiency of three potted *Bougainvillea* genotypes grown in two shapes. HortSci. 49 (10): 1284-1291.

de la Vega, A., Chapman, S. C. and Hall, A. J. 2001. Genotype by environment interaction and indirect selection for yield in sunflower I. Two-mode pattern analysis of oil yield and biomass yield across environments in Argentina. Field Crops Res. 27: 17-38.

Dixon, R. A. and Steele, C. L. 1999. Flavonoids and is a flavonoids–a gold mine for metabolic engineering. Trends Plant Sci. 4 (10): 394-400.

Gupta, P. K.1999. Estimation of soil moisture and soil moisture constant. pp. 16-49. In: Soil plant, water, and fertilizer analysis. 2nd Edition. Agro-Botanica Publishers & Distributor. Bikanar, India.

Handique, A. C. and Manivel, L. 1990. Selection criteria for drought tolerance in tea. Assam Rev. Tea News 79: 18-21.

Hare, P. D., Cress, W. A. and Van Staden, J. 1998. Dissecting the roles of osmolyte accumulation during stress. Plant Cell Environ. 21 (6): 535-553.

IPGRI. 1997. Descriptors for Tea (*Camellia sinensis*). International Plant Genetic Resources Institute.Rome, Italy. 50 pp.

ISO TC 34/SC 8/WG. 2003. Tea: methods for determination of substances characteristics of green and black tea. I. Determination of total polyphenols in tea: colorimetric method using Folin-Ciocalteu reagent. ISO. Genva.

Jeyaramraja, P. R., Jayakumar, D., Pius, P. K. and Kumar, R. R. 2003. Relation of altered protein expression with chlorophyll fluorescence in tea under water stress. Indian J. Plant Physiol. 8 (3): 214-218.

Kamunya, S. M., Wachira, F. N., Lang'at, J., Otieno, W. and Sudoi, V. 2008. Integrated management of root knot nematode (*Meloidogyne* spp.) in tea (*Camellia sinensis*) in Kenya. Int. J. Pest. Manag. 54 (2): 129-136.

Kigalu, J. M. 2007. Effects of planting density and drought on the productivity of tea clones (*Camellia sinensis* L.): Yield responses. Phys. Chem. Earth Parts A/B/C 32(15-18): 1098-1106.

Lipiec, J., Doussan, C., Nosalewicz, A. and Kondracka, K. 2013. Effect of drought and heat stresses on plant growth and yield: a review. Int. Agrophys. 27 (4): 463-477.

Liu, S. C., Yao, M. Z., Ma, C. L., Jin, J. Q., Ma, J. Q., Li, C. F. and Chen, L. 2015. Physiological changes and differential gene expression of tea plant under dehydration and rehydration conditions. Sci. Hortic. 184: 129-

141.

Lu, Z. and Neumann, P. M. 1998. Water-stressed maize, barley and rice seedlings show species diversity in mechanisms of leaf growth inhibition. J. Exp. Bot. 49 (329): 1945-1952.

Mahajan, S. and Tuteja, N. 2005. Cold, salinity and drought stresses: an overview. Arch. Biochem. Biophys. 444 (2): 139-158.

Majd Salimi, K., Bagheri, F. and Salavatian, S. B. 2010. The economical assessment of irrigation interval on water producing and quality of tea. J. Water and Soil. 24 (5): 845-854.

Marimuthu, S. and Kumar, R. R. 1998. Drought management in tea: a physiological approach. Bull UPASI Tea Science Department 51: 16-18.

Maritim, T. K., Kamunya, S. M., Mireji, P., Mwendia, C., Muoki, R. C.,Cheruiyot, E. K. and Wachira, F. N. 2015. Physiological and biochemical response of tea [*Camellia sinensis* (L.) O. Kuntze] to water-deficit stress. J. Hortic. Sci. Biotechnol. 90 (4): 395-400.

Matysik, J., Alia Bhalu, B. and Mohanty, P. 2002. Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. Curr. Sci. 82 (5): 525-532.

Mengel, K. and Kirkby, E. A. 1996. Principals of plant nutrition. 4th Edition. Panima, New Delhi. 485 pp.

Netto, L. A., Jayaram, K. M. and Puthur, J. T. 2010. Clonal variation of tea [*Camellia sinensis* (L.) O. Kuntze] in countering water deficiency. Physiol. Mol. Biol. Plants 16 (4): 359-367.

Nyirenda, H. E. 1988. Performance of new clones. Tea Res. Found Cent. Afr. Q. Newsl. 91: 4-11.

Othieno, C. O. 1978. Supplementary irrigation of young clonal tea in Kenya. II. Internal water status. Exp. Agric. 14 (4): 309-316.

Passioura, J. B. 2002. Environmental biology and crop improvement. Funct. Plant Biol. 29 (5): 537-546.

Puthur J. T. 2000. Photosynthetic events in *Sesbania sesban* (L.) Merrill in relation to osmotic stress during different developmental stages. Ph. D. thesis. Jamia Millia Islamia University. New Delhi, India.

Puthur, J. T. and Rajan, P. 2006. Photosynthetic characteristics and antioxidant mechanism in Vanilla planifolia and exposed to varying intensities of solar radiation. J. Plant. Crops 34 (3): 606-6.

Rahimi, M., Kordrostami, M. and Mortezavi, M. 2019. Evaluation of tea (*Camellia sinensis* L.) biochemical traits in normal and drought stress conditions to identify drought tolerant clones. Physiol. Mol. Biol. Plants 25 (1): 59-69.

Rajasekar, R., Cox, S. and Satyanarayana, N. 1988. Evaluation of certain morphological and physiological factors in tea (*Camellia* L. SPP.) cultivars under water stress. J. Plant. Crops 18: 83-83.

Ramegowda, V. and Senthil-Kumar, M. 2015. The interactive effects of simultaneous biotic and abiotic stresses on plants: mechanistic understanding from drought and pathogen combination. J. Plant Physiol. 176: 47-54.

Rawat, J. M., Rawat, B., Tewari, A., Joshi, S. C., Nandi, S. K., Palni, L. M. S. and Prakash, A. 2017. Alterations in growth, photosynthetic activity and tissue-water relations of tea clones in response to different soil moisture content. Trees 31 (3): 941-952.

Sabry, S. R. S. 1995. Osmoregulation in spring wheat under drought and salinity stress. J. Genet. Breed. 49: 55-60.

Saradhi, P. P., Alia Arora, S. and Prasad, K. V. S. K. 1995. Proline accumulates in plants exposed to UV radiation and protects them against UV-induced peroxidation. Biochem. Biophys. Res. Commun. 209 (1): 1-5.

Saraswathy, V. M., Venkataramanan, D. and Ramaiah, P. K. 1992. Drought tolerance in exotic robustas. J. Plant. Crops. 20: 123-123.

Sato, F., Yoshioka, H., Fujiwara, T., Higashio, H., Uragami, A. and Tokuda, S. 2004. Physiological responses of cabbage plug seedlings to water stress during low-temperature storage in darkness. Sci. Hortic. 101(4): 349-357.

Satyanarayana, N. and Cox, S. 1994. Factors influencing productivity of tea in drought. J. Plant. Crops. 22: 87-87.

Schuppler, U., He, P. H., John, P. C. and Munns, R. 1998. Effect of water stress on cell division and Cdc2-like cell cycle kinase activity in wheat leaves. Plant Physiol. 117 (2): 667-678.

Sharma, P. and Kumar, S. 2005. Differential

display-mediated identification of three drought-responsive expressed sequence tags in tea [*Camellia sinensis* (L.) O. Kuntze]. J. Biosci. 30 (2): 231-235.

Singh, H. P., Ravindranath, S. D. and Singh, C. 1999. Analysis of tea shoot catechins: spectrophotometric quantitation and selective visualization on two-dimensional paper chromatograms using diazotized sulfanilamide. J. Agric. Food. Chem. 47 (3): 1041-1045.

Singh, I. D. and Handique, A. C. 1993. Breeding for resistance in water stress in tea. Two and a Bud. 40 (1): 41-49.

Slama, I., Messedi, D., Ghnaya, T., Savoure, A. and Abdelly, C. 2006. Effects of water deficit on growth and proline metabolism in *Sesuviumportulacastrum*. Environ. Exp. Bot. 56 (3): 231-238.

Stewart, A. J., Chapman, W., Jenkins, G. I., Graham, I., Martin, T. and Crozier, A. 2001. The effect of nitrogen and phosphorus deficiency on flavonol accumulation in plant tissues. Plant Cell Environ. 24 (11): 1189-1197.

Sudoi, V., Cheramgoi, E., Langat, J. K., Kamunya, S. M. and Wachira, F. N. 2011. Screening of Kenyan tea clones for susceptibility to mite attack at different ecological zones. Int. J. Curr. Res. 3: 328-332.

Szabados, L. and Savoure, A. 2010. Proline: a multifunctional amino acid. Trends Plant Sci. 15 (2): 89-97.

Thomas, J., Kumar, R. R. and Pius, P. K. 2004. Screening of tea germplasm under soil moisture stress for productivity. J. Plant. Crops 32: 50-53.

Upadhyaya, H., Datta, B. K. and Panda, S. K. 2016. Drought induced physiological and biochemical changes in leaves of developing seedlings of tea [*Camellia sinensis* (L.) O. Kuntze] cultivars. J. Tea Sci. Res. 6 (4): 1-11.

Upadhyaya, H. and Panda, S. K.2004. Responses of *Camellia sinensis* to drought and rehydration. Biol. Plant. 48 (4): 597-600.

Upadhyaya, H., Panda, S. K. and Dutta, B. K. 2008. Variation of physiological and anti-oxidative responses in tea cultivars subjected to elevated water stress followed by rehydration recovery. Acta Physiol. Plant. 30 (4): 457-468.

Wijeratne, M. A. and Fordham, R. 1996. Effects of environmental factors on growth and yield of tea (*Camellia sinensis* L.) in the low-country wet zone of Sri Lanka. Sri Lanka J. Tea Sci. 64: 21-34.

Xu, Z. Z. and Zhou, G. S. 2007. Photosynthetic recovery of a perennial grass *Leymuschinensis* after different periods of soil drought. Plant Prod. Sci. 10 (3): 277-285.

Yoshida, S., Forno, D. A. and Cock, J. H. 1971. Laboratory manual for physiological studies of rice. Laboratory manual for physiological studies of rice. 61 pp.