

Effects of drought stress on growth, physiological and biochemical parameters of two Ethiopian red pepper (*Capsicum annum* L.) cultivars

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Abstract

Drought is the most predominant abiotic factor that affects plant growth and development. The present research examined how drought stress affected the growth, physiological, and biochemical responses of Local and Markofana red pepper (*Capsicum annum* L.) cultivars. Five-week-old cultivars were exposed to well-watered, low, moderate, and severe drought conditions and kept at 100, 80, 60 and 40 % of field capacity, respectively. Nine-week-old cultivars were sampled to analyze the effects of the stress on different parameters of both cultivars. Compared with the control group, drought stress caused a reduction in growth, physiological and biochemical parameters; nevertheless, negative effects of the stress were more noticeable in the Local cultivar. Severe drought stress significantly reduced shoot length in Markofana (53.71 %) compared to the control group. Significant variation was observed in relative water content in the Local cultivar (20.26 %) exposed to drought. In the Local cultivar, the total chlorophyll content and chlorophyll fluorescence declined significantly by 77.28 % and 3.33 %, respectively. Therefore, the cultivar Markofana was relatively less affected by drought stress. In general, these differences in cultivar responses to drought stress may aid in developing drought tolerance genotypes that can withstand drought stress conditions with minimal yield losses.

Key words: Abiotic stress, drought, biochemical content, red pepper, root length, photosynthesis rate, proline content, chlorophyll content

Introduction

Drought is a long-lasting natural phenomenon in which the water condition is less sustainable than the local normal situation. Global warming and the El Nino weather phenomenon have led to frequent drought disasters worldwide in recent years, including in East Africa (Shukla *et al.*, 2019) and North America (Cook *et al.*, 2007). In agriculture, different abiotic stresses cause considerable yield losses and are becoming more widespread due to global warming change effects (Stefanelli *et al.*, 2010). Drought is the most predominant abiotic factor that affects plant growth and development (Ferrara *et al.*, 2011). It remarkably impacts crop plants' growth, physiology, and yield worldwide (Jalil and Ansari, 2020).

Due to drought and conflict, 1.3 million people are displaced in Ethiopia (UNICEF, 2017). Globally, agriculture is the biggest consumer of water, accounting for almost 70 % of withdrawals of water resources in developed countries and up to 95 % in developing countries (Wada *et al.*, 2011). A total of 86 % of cultivated land in the world comes under rain-fed agriculture. Because of global warming, a water deficiency is expected to cause extreme drought in the inland area from 1 to 30 % by 2100 (Xu *et al.*, 2010). Thus, increasing crop productivity while alleviating drought will be among the most significant challenges in the coming years. Cell growth is the most affected physiological process by water stress (Anjum *et al.*, 2011). The stress greatly reduces the growth of plants, root biomass, and shoot biomass (Boutraa *et al.*, 2010).

Drought can reduce tissue concentrations of chlorophylls and carotenoids primarily because of the production of reactive oxygen species (ROS) in thylakoids that cause permanent damage to the photosynthetic apparatus (Anjum *et al.*, 2011). It significantly reduces the content of chlorophyll a, chlorophyll b, and total chlorophyll in different crops such as marigolds and sunflowers (Pratap and Sharma, 2010). Photosynthesis and many other physiological processes are negatively affected by drought stress (Erdal *et al.*, 2021). For example, water deficit damages the basic organizational structure of photosynthetic apparatus and inhibits carbon assimilation (Ali and Ashraf, 2011). This may be due to the inhibition of the photosystem II complex and a loss of chlorophyll pigments (Wang *et al.*, 2018). These alterations are reflected by chlorophyll fluorescence (Fv/Fm), which is conveniently used for detecting and quantifying plant tolerance to stressful conditions (Wang *et al.*, 2018). Drought in Ethiopia has shown a spatial and temporal distribution over the last fifty years. Certain regions in the country are affected more frequently: the eastern, south eastern and Rift valley regions (Getachew, 2018). It may become more severe in the following years due to deforestation and other anthropogenic activities.

Red pepper (*Capsicum annum* L.) is the world's 3rd essential vegetable crop, following potato and tomato in terms of production quantity. Today, farmers produce the greatest percentage of hot peppers in Ethiopia (Getahun and Habtie, 2017) and worldwide. Despite extensive pepper production practised in Ethiopia, the production system has several constraints. The

absence of improved varieties is among the major challenges of production. Farmers usually use local varieties of low-quality seeds with poor growth performance and low productivity. The susceptibility of local varieties to drought is also among the major challenges of the production practice. However, the effect of drought stress on the growth, physiological and biochemical features of red pepper cultivars was not conducted broadly in the studied area to get the cultivars that are tolerant to drought stress. Therefore, the present study examines the effects of drought on the growth, physiological, and biochemical parameters of red pepper cultivars. The projection detailed in this study will help to have hybrid cultivars with resistance to drought stress.

Materials and methods

Experimental setup: The experiments were conducted at the University of Gondar, Botany Laboratory in the Central Gondar Administrative Zone of Ethiopia. The study area is located at 12°35'11.7" N latitude and 37°26'27" E longitude. The altitude of the site is about 2148 m above sea level. The minimum and maximum temperatures are 13.3 and 28.3°C, respectively. Mean relative humidity and yearly precipitation are ~56 % and 1161 mm, respectively. During the experimental period (March-April), relative humidity was 50 %, and no rainfall was observed during experimentation.

The seeds of Local and Markofana cultivars (*Capsicum annum* L.) obtained from Bahir Dar Agricultural Research Centre were sterilized with ethanol (80%) for around 15 min, bathed with distilled water, and then sown in plastic pots containing farmyard manure (FYM 25 %) and soil (75 %). After two weeks of germination, uniform-sized seedlings of the cultivar were shifted in plastic pots (25 cm width × 26 cm height) filled with 6 kg of sandy loam soil with 2 kg manure in a ratio of 3:1 (75 % soil and 25 % FYM) as recommended by (Husen *et al.*, 2018) and seeded at a depth of ~ 2 cm on the soil. Each plastic pot contained three seedlings. The potted seedlings were watered with tap water daily at a field capacity of 100 % (FC) for up to 3 weeks, which was the accommodation period. After five weeks, a completely randomized design was adopted with three replications per treatment and three plants per replication for both cultivars.

Drought treatments: The red pepper plants were allowed to grow under normal conditions for up to five weeks. After five weeks, three different levels of drought stress concerning FC, *i.e.* lower drought stress (80 % FC or T₁), moderate drought stress (60 % FC or T₂), and severe drought stress (40 % FC or T₃) were imposed. In contrast, control or well-watered with 100 % FC (T₀) was kept for comparison. A moisture meter TRIME-EZ/-IT (IMKO Micromoduletechnik GmbH, Germany) was used to monitor stress treatments regularly. The specified drought treatments were applied until the 40th day of drought exposure. The treatments were arranged in a completely randomized design (CRD) under a factorial arrangement.

Plant growth parameters: Some plant growth parameters of both cultivars were recorded for each treatment, *i.e.* control to severe drought stress. The size of the root and stem was measured in cm, and opened leaves were also counted. The stem's ground-line basal diameter (mm) was measured with an electronic digital calliper. In addition, the length, width (each in mm), and leaf area (mm²) were measured using a Leaf Area Meter (AM 300, ADC Bio Scientific Limited, UK).

Relative water content: Leaf relative water content (LRWC) was determined from the leaves collected at the midsections of crop plants to minimize age effects. Sample leaves of the plants were taken and immediately weighed using a digital electronic balance to get fresh weight. Then, the LRWC was calculated using the formula given by (Aguayoh *et al.*, 2013)

Chlorophyll fluorescence: Chlorophyll fluorescence (CF) was measured using a portable Multi-Mode OS5P Chlorophyll Fluorometer (Opti-Sciences, Inc., USA) from 10:00 to 11:00 AM using the methods of (Husen *et al.*, 2018; Almeselmani *et al.*, 2011) in the calculation.

Moreover, net photosynthetic rate (A), transpiration rate (E), and stomatal conductance (gs) were recorded from fully expanded attached leaves with the help of a portable leaf gas exchange system (ADC Bio Scientific Limited, UK). All these measurements were taken on whole plants from each treatment.

Chlorophyll content: One plant per replica was used for chlorophyll determination. Fresh samples and homogenization were done. In the end, the concentration of chlorophyll "a" and "b" total chlorophyll content was determined using the following formula;

$$\text{Chl. } a \text{ (mg g}^{-1}\text{FW)} = 12.7x \text{ (A663)} - 2.69x \text{ (A645)}$$

$$\text{Chl. } b \text{ (mg g}^{-1}\text{FW)} = 2.9x \text{ (A663)} - 4.68x \text{ (A645)}$$

$$\text{Total chlorophyll concentration (mg/g FW)} = 20.2x\text{A663} + 8.02\text{A645}$$

Where (A663) and (A645) represent absorbance values read at 663 and 645 nm wavelengths. This was collected at the end of the drought stress period to compare the chlorophyll contents of stressed and non-stressed red pepper plants.

Determination of internal proline content: Proline content was determined based on the reaction of proline with ninhydrin. Then, the absorbance at 520 nm was determined using a Microprocessor UV-Vis double-beam spectrophotometer.

Determination of total phenolic compounds: The extract's total phenolic was determined using the following formula at the end of the experiment.

$$\text{Total phenolic content} = \text{Gallic acid equivalent (mg/L)} \times \text{total volume of methanol extract} \times \text{sample weight (kg/g)} / \text{Dilution factor (L/mL)}$$

Biomass estimation: At the end of the experiment, plants were harvested carefully. Then, the shoot and root fresh weight of each replica of the treatments for two cultivars were measured using a digital electronic balance (CY510, Citizen Scale, Poland) and the mean values were taken as the shoot and root fresh weight of the red pepper cultivars.

To get the root-to-shoot ratio of biomass, the whole plants were uprooted, rinsed, separated into shoot and root, and oven-dried for 24 hours at 72 °C at the end of the experiment. Then, the root-to-shoot ratio was computed using the formula given by (Luvaha *et al.*, 2009).

Data Analysis: All the collected data were subjected to analysis of variance (ANOVA), means comparison was done using LSD, and graphical comparison was presented using the software SPSS version 20. The significance level of data was accepted at $P \leq 0.05$ and rejected when $P \geq 0.05$ confidence interval level.

One-way ANOVA was used to determine statistically significant differences between the means of the parameters of the two red pepper cultivars under drought levels. Each of the cultivars' parameters was then measured on the same independent variable, having undergone the same condition. On the other hand, two-way ANOVA was used to analyze the interaction effect of both CT and watering regime. A correlation was also made to determine the relationship's direction and measure the strength of association between two continuous variables.

Results and discussion

The data regarding the effect of drought on Local and Markofana red pepper cultivars on their various growth parameters are given in Table 1. Drought stress markedly inhibited plant growth for both the cultivars at $P \leq 0.05$ level. Severe drought stress (40 % FC) showed a comparable effect in Local and Markofana as it reduced the shoot length by 54.40 and 53.71 % and root length by 20.99 and 46.89 %, respectively. This finding was in line with the study conducted by (Chutipaijit *et al.*, 2012; Emam *et al.*, 2010; Riaz *et al.*, 2010; Maleki *et al.*, 2013). The reduction in plant height is due to the stress effects on growth-promoting hormones that reduce cell turgor (Pandey *et al.*, 2014).

The present study showed significant differences between treatments (T_0 and T_3) with stem thickness in Markofana and Local cultivars. This result agrees with the Luvaha *et al.* (2009) report on mango seedlings. This study's result revealed a reduction in leaf development under severe drought stress compared to the control group. This finding is similar to the previous reports (Boutraa *et al.*, 2010; Anjum *et al.*, 2011; Riaz *et al.*, 2013; Hayatu *et al.*, 2014). Reduced production of new leaves and increased senescence of older leaves causes a reduction in leaf area under water stress (Hayatu *et al.*, 2014). The number of leaves in this study reduced significantly in the Markofana cultivar ($P < 0.05$). This was consistent with the previous reports (Riaz *et al.*, 2013). Mosenda *et al.* (2020) also found similar findings for spider plants under water stress conditions. The leaf area also declined significantly in the local and Markofana cultivars under severe drought stress conditions. Similar results have been reported by Mosenda *et al.* (2020).

The result of the study revealed that the root length was reduced significantly in the Markofana cultivar ($P < 0.05$). The reductions in root length for both cultivars are displayed in Table 1. This confirmed the previous reports (Riaz *et al.*, 2013; Afzal *et al.*, 2014; Salazar *et al.*, 2015).

Number of buds and flowers: The sum of flower buds recorded

Table 1. Effects of drought stress on the shoot and root growth of the cultivars

Cultivar	Treatment	Shoot length (mm)	Root length (mm)	Stem thickness	Number of leaves (Plant ⁻¹)	Leaf area (mm ²)	Leaf width (mm)
Local	Control	46.89 ^a	17.58 ^a	5.27 ^a	43.55 ^a	9688.67 ^a	56.2 ^a
	80%FC	36.55 ^b (22.34)	18.00 ^a (2.39)	4.89 ^a (7.21)	33.89 ^a (22.18)	9425.33 ^a (2.72)	55.13 ^a (1.90)
	60%FC	25.33 ^c (45.85)	15.34 ^a (12.74)	3.96 ^b (24.86)	23.78 ^a (45.39)	7011.67 ^b (27.63)	46.97 ^a (16.42)
	40%FC	21.33 ^d (54.40)	13.89 ^a (20.99)	3.64 ^c (30.93)	20.78 ^a (52.28)	4235.67 ^c (56.28)	36.33 ^a (35.36)
Markofana	Control	42.00 ^a	18.83 ^a	4.91 ^a	46.67 ^a	10645.67 ^a	102.10 ^a
	80%FC	32.89 ^b (21.69)	12.89 ^b (10.88)	4.73 ^a (3.67)	45.56 ^a (2.38)	7601.33 ^b (28.59)	51.06 ^a (49.99)
	60%FC	26.11 ^c (37.83)	12.89 ^b (31.55)	3.70 ^b (21.77)	29.89 ^b (35.95)	5319.00 ^c (50.04)	40.20 ^a (60.63)
	40%FC	19.44 ^d (53.71)	10.00 ^c (46.89)	3.47 ^c (29.33)	22.33 ^c (52.15)	4151.00 ^d (61.01)	33.53 ^a (67.16)

F.C. = Field capacity, the data represent the Mean±SE of the three replicates in the experiment. Means followed by the different letters in a column are significantly different at $P < 0.05$ level according to the L.S.D. test values within parenthesis are percent variation as obtained from the control plants of respective cultivars. (CV- Cultivar, Trt.- treatments)

in the treatments of all the cultivars is presented in Fig. 1. As a result, the number of buds was reduced in the Local and Markofana cultivars under severe drought stress. The reduction in number of buds may be due to the great effect of the stress on the number of branches. Severe drought stress also affected the flowers produced in the two cultivars (Fig. 2). The number of flowers was significantly reduced in both cultivars under drought-stress conditions. This is in agreement with the report of Andersson (2011) on *Impatiens walleriana* plants.

Effects of drought on the physiological traits of the cultivars:

Drought stress greatly reduced the physiological efficiency of leaves in the two cultivars compared to the controls. The degree of reduction of LRWC was high in Local (20.26 %), and a lower decrease of LRWC was for the Markofana (17.33 %) cultivar (Table 2). The reduction in LRWC was statistically significant in the local cultivar. The results of our study were consistent with the findings obtained by Hegazi *et al.* (2014) on soybean leaves under drought stress. However, the LRWC between the control and the respective treatments in the Markofana was statistically insignificant. This may be due to the variation in the ability of red pepper cultivars to avoid stress by maintaining tissue turgor osmotically.

In the present study, drought stress imposed for 40 days significantly affects the PS II photochemical efficiency (Fv/Fm)

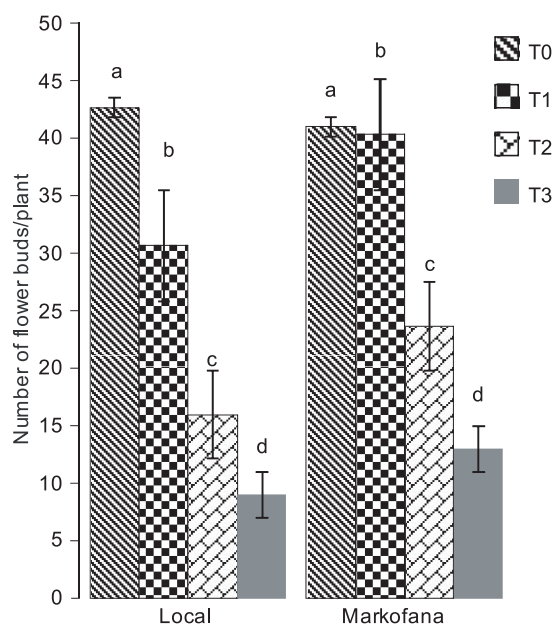


Fig. 1. Effect of drought stress on the number of buds in the cultivars. Bars with different letters represent significant differences at $P \leq 0.05$.

Table 2. Effect of drought stress on physiological traits of the cultivars

Cultivars	Treatments	LRWC	CF	A ($\mu\text{ mol CO}_2\text{ m}^{-2}\text{ s}^{-1}$)	E ($\text{m mol m}^{-2}\text{ s}^{-1}$)	Gs ($\text{mol m}^{-2}\text{ s}^{-1}$)
Local	Control	83.86 ^a	0.7533 ^a	16.87 ^a	5.11 ^a	0.199 ^a
	80%FC	82.26 ^a (1.92)	0.6823 ^a (1.59)	15.71 ^b (20.70)	5.27 ^b (3.33)	0.188 ^b (1.11)
	60%FC	77.77 ^a (7.26)	0.6690 ^a (3.50)	14.27 ^{bc} (23.56)	4.67 ^c (4.35)	0.182 ^c (2.55)
	40%FC	66.87 ^b (20.26)	0.6702 ^b (3.33)	12.78 ^d (26.78)	3.23 ^d (5.45)	0.111 ^d (11.45)
Markofana	Control	86.44 ^a	0.7630 ^a	15.79 ^a	5.81 ^a	0.185 ^a
	80%FC	85.14 ^a (1.50)	0.6533 ^a (4.35)	15.32 ^b (21.45)	5.23 ^a (3.35)	0.188 ^b (1.08)
	60%FC	78.08 ^a (9.67)	0.6423 ^a (5.96)	14.35 ^c (22.21)	4.97 ^a (4.11)	0.167 ^c (3.83)
	40%FC	71.46 ^a (17.33)	0.6577 ^a (3.70)	13.56 ^d (24.35)	4.11 ^a (5.00)	0.134 ^d (10.97)

F.C. = Field capacity, the data represent the Mean \pm SE of the three replicates in the experiment. Means followed by the different letters in a column are significantly different at $P\leq 0.05$ level according to the L.S.D. test values within parentheses are percent variation as obtained from the control plants of respective cultivars.

of red pepper cultivars. The values of the reduction percentage of Fv/Fm for each cultivar in different treatments are given in Table 2. As discussed by Wang *et al.* (2018), photochemical efficiency of PS II was decreased after 33 days of drought treatment on young apple tree leaves. As discussed elsewhere (Liu *et al.*, 2011; Zlatev and Lidon, 2012), the photochemical efficiency of photosystem II was decreased significantly during drought stress in crop plants due to the photoinactivation of PS II centres. The changes in PS II activity under water deficit stress are linked to photoinhibition and to the development of slowly relaxing quenching processes.

The photosynthetic rate of both the Local and Markofana cultivars was also reduced significantly at $P\leq 0.05$ under severe drought conditions (Table 2). This finding was consistent with the report on sorghum (Mafakheri *et al.*, 2010), rice (Yang *et al.*, 2016), and chickpea cultivars (Zhang *et al.*, 2019) under drought stress. According to Mafakheri *et al.* (2010), the decrease in photosynthesis can be due to stomatal and non-stomatal factors. The stomatal conductance also decreased significantly ($P<0.05$) with increasing drought stress levels in the study. This determines plant tolerance to drought (Lauteri *et al.* 2014). This restricts gas exchange between the atmosphere and the inside of the leaf, which is one of the first responses of plants to drought. Allen *et al.* (2011) found that stomatal movement is critical in observing water transpiration and CO₂ absorption under drought stress. The transpiration rate declined significantly ($P<0.05$) in the local cultivar under severe drought stress in this study. A significant reduction in transpiration rate was also observed under drought stress conditions in crops such as wheat, rice, and maize.

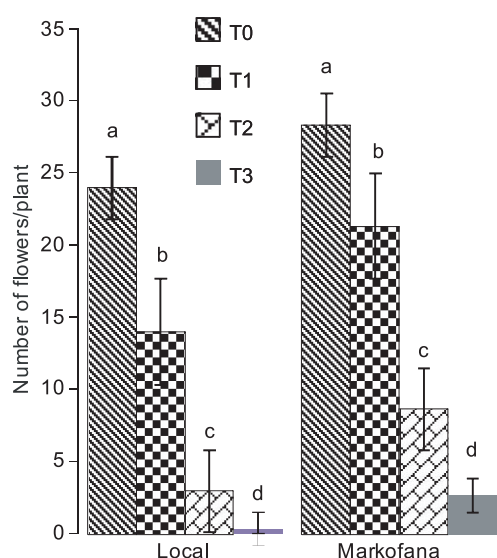


Fig. 2. Effect of drought stress on the number of flowers in the cultivars. Bars with different letters represent significant differences at $P\leq 0.05$.

Effects of drought on biochemical traits of the cultivars: The number of leaf pigments (chlorophyll a, chlorophyll b, and total chlorophyll) dropped with an increase in the stress level (Table 3). The total chlorophyll, chlorophyll a, and chlorophyll b contents declined significantly in the Local cultivar under severe drought stress levels (40 % FC). This result is in close agreement with the previous reports (Sikuku *et al.*, 2010). The decline in chlorophyll 'a' may result from a reduced synthesis of the main chlorophyll pigment complexes encoded by the cab gene family. On the other hand, there were insignificant differences in the treatments of the Markofana cultivar compared to the control. This may be due to the production of osmolytes during stress in the cultivar. This was consistent with the previous report on chili pepper by Ichwan *et al.* (2017) against drought stress.

In the present study, drought stress affected proline content and the total phenolic content in both the Local and Markofana cultivars (Table 3). However, the result revealed a significant increase in proline and total phenolic content observed in the Markofana cultivar under severe drought stress. This was consistent with the previous reports (Liu *et al.*, 2011; George *et al.*, 2015). Plants counteract drought stress by accumulating osmoprotectants in response to stress (Anjum *et al.*, 2017; Tanveer *et al.*, 2019). Proline is the most important osmolytes produced against drought stress in plants. Proline supports the plant in reducing oxidative damage, and this is a vital strategy for plants to resist drought stress (Chegah *et al.*, 2013).

Shoot and root fresh weight: All plants were harvested for biomass analysis after the experiment. The result showed that shoot fresh weight declined significantly in the Local and Markofana cultivars under severe drought stress compared to the control group (Fig. 3). Similarly, the root fresh weight of the Local and Markofana cultivars was reduced significantly under severe drought stress conditions (Fig. 4). This confirmed the previous study conducted by Kumar *et al.* (2012), and Rostampour *et al.* (2012). This may be because water deficit stress reduced the leaf area index, resulting in reduced photosynthesis. It also might be related to leaf openings being closed by signals of roots (Saradadevi *et al.*, 2014).

Shoot and root dry weight: Drought stress significantly affected the cultivars' shoot and root dry weight (Table 4). The Root-to-shoot ratio was increased significantly in the Markofana due to drought stress compared to the control group. This finding confirmed the work of Liu *et al.* (2011). This may be due to the more extensive growth of adventitious and tap roots in plants exposed to severe water deficit than the control one (Luvaha *et al.*

Table 3. Effects of drought stress on biochemical parameters

Cultivars	Treatments	Chl. A (mg g ⁻¹ FW)	Chl. b (mg g ⁻¹ FW)	Total Chl. (mg g ⁻¹ FW)	Proline Content (μmol/g)	Total phenolic (mg/100g)
Local	Control	23.80 ^a	1.79 ^a	49.61 ^a	3.80 ^a	19.79 ^a
	80%FC	16.59 ^a (52.66)	4.85 ^b (1.71)	30.78 ^a (37.96)	4.59 ^a (12.66)	27.85 ^a (14.89)
	60%FC	10.78 ^a (54.71)	1.61 ^c (10.06)	19.74 ^a (60.21)	5.78 ^a (14.71)	36.51 ^a (19.56)
	40%FC	6.63 ^b (72.14)	1.28 ^d (28.49)	11.27 ^b (77.28)	9.63 ^a (22.14)	45.28 ^a (22.57)
Markofana	Control	16.21 ^a	1.80 ^a	33.92 ^a	4.21 ^a	21.61 ^a
	80%FC	7.60 ^a (48.89)	1.85 ^a (2.78)	20.49 ^b (39.59)	5.60 ^b (18.89)	29.85 ^b (13.59)
	60%FC	7.71 ^a (52.44)	2.16 ^a (20.00)	19.18 ^a (43.45)	6.71 ^c (19.44)	31.26 ^c (28.00)
	40%FC	6.97 ^a (57.00)	2.22 ^a (23.33)	18.59 ^a (45.19)	10.97 ^d (37.00)	36.32 ^d (44.12)

F.C. = Field capacity, the data represent the Mean±SE of the three replicates in the experiment. Means followed by the different letters in a column are significantly different at $P \leq 0.05$ level according to the L.S.D. test values within parenthesis are percent variation as obtained from the control

Table 4. Effects of drought stress on biomass of red pepper cultivars

Cultivars	Treatments	Root DW (g)	Shoot DW (g)	Root:shoot ratio
Local	Control	3.26 ^a	26.72 ^a	12.07 ^a
	80%FC	2.31 ^a (29.14)	15.27 ^b (11.45)	15.25 ^a (26.35)
	60%FC	1.14 ^b (65.03)	6.59 ^c (75.34)	17.64 ^a (46.15)
	40%FC	0.91 ^c (72.09)	4.48 ^d (83.23)	19.97 ^a (65.45)
Markofana	Control	2.08 ^a	16.55 ^a	12.61 ^a
	80%FC	1.98 ^a (4.81)	12.99 ^b (21.51)	15.74 ^b (24.82)
	60%FC	0.89 ^b (57.21)	6.16 ^c (62.78)	14.65 ^c (16.18)
	40%FC	0.66 ^c (68.27)	4.04 ^d (75.29)	16.13 ^d (27.91)

FC = Field Capacity, the data represent the Mean±SE of the three replicates in the experiment. Means followed by the different letters in a column are significantly different at $P \leq 0.05$ level according to the L.S.D. test values within parenthesis are percent variation as obtained from the control plants of respective cultivars.

al., 2009). It may also be due to the increasing phytohormones under stress conditions over the normal period.

Analysis of interaction effect on some parameters of the cultivars: The interaction effect of watering level and CT on the shoot length, leaf area, shoot fresh weight, root dry weight, and total chlorophyll are presented in Table 5. There was a statistically significant interaction between the effects of watering level and CT on the root fresh weight of the cultivars ($P=0.003$). However, statistically insignificant interaction effects of the CT and watering level were observed on shoot length, leaf area, shoot fresh weight, total chlorophyll, and root dry weight among the two cultivars. The result also showed a statistically insignificant difference between the cultivars under severe stress conditions in

Table 5. Interaction effect of watering level and CT on the cultivars

Parameters	Source of MS variation	F	Sig.	
Shoot length	WL	1083.796	120.958	0.000
	CT	70.919	7.914	0.002
	WL * CT	8.373	0.934	0.489
Leaf area	WL	56266014.028	63.013	0.000
	CT	18690886.861	20.932	0.000
	WL * CT	1867180.194	2.091	0.092
Root fresh weight	WL	360.722	63.664	0.000
	CT	95.109	16.786	0.000
	WL * CT	25.969	4.583	0.003
Root dry weight	WL	7.188	49.720	0.000
	CT	0.936	6.474	0.006
	WL * CT	0.167	1.158	0.361
Total chlorophyll	WL	879.703	5.397	0.006
	CT	148.565	0.911	0.415
	WL * CT	152.930	0.938	0.486

WL= Watering level, CT= Cultivar Type

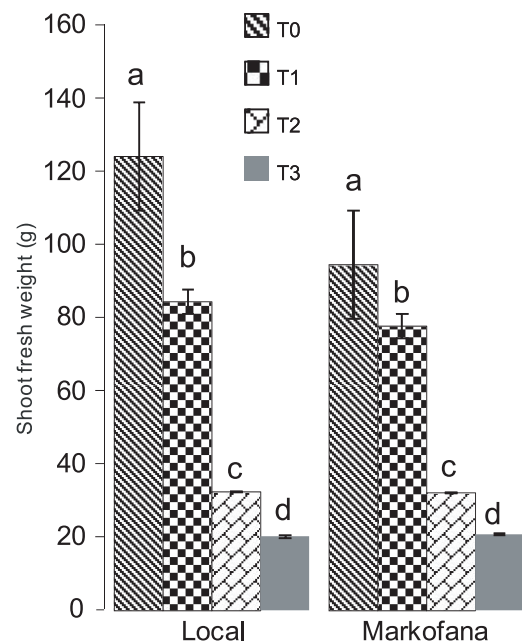


Fig. 3. Effect of drought stress on shoot fresh weight of the cultivars. Bars with different letters represent significant differences at $P \leq 0.05$.

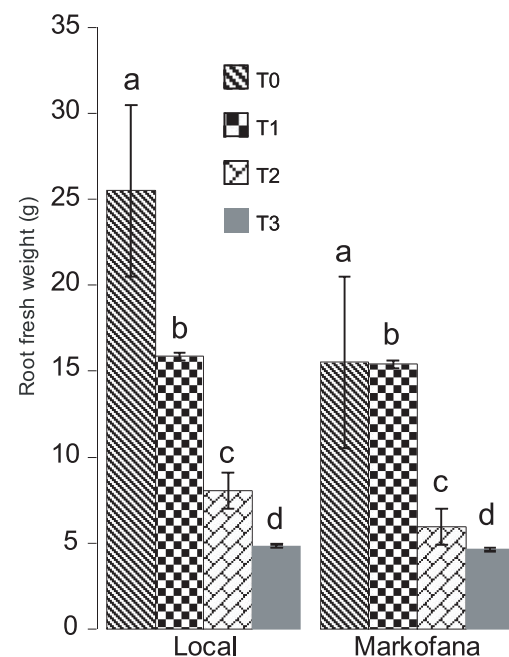


Fig. 4. Effect of drought stress on root fresh weight of the cultivars. Bars with different letters represent significant differences at $P \leq 0.05$.

shoot fresh weight ($P=0.055$) and total chlorophyll ($P=0.415$). On the other hand, there were statistically significant differences between shoot length, leaf area, and shoot fresh weight ($P\leq 0.05$).

Correlation results in the two cultivars: Correlations between various morphological and physiological parameters were made and the summaries are presented in Tables 6 and 7 for Local and Markofana cultivars ($P\leq 0.01$, or $P\leq 0.05$ level). The results revealed that the most correlated parameters were shoot fresh weight and shoot dry weight in Local ($r=0.985$, $P\leq 0.01$) and in Markofana ($r=0.997$, $P\leq 0.01$). This indicates that an increase in shoot fresh weight increases the shoot dry weight in the cultivars. On the other hand, the least correlated parameters were root length with shoot fresh weight ($r=0.754$) at 0.01 level in the Local cultivar. Root fresh weight with shoot length is the least correlated parameter in Markofana ($r=0.873$) at a 0.01 level.

Table 6. Correlation between different morphological and physiological parameters in Local cultivar

Parameters	RL	SFW	RFW	SDW	RDW	Total Chl.
SL	.677*	.925**	.942**	.926**	.886**	.722**
RL	1	.754**	.651*	.690*	.598*	.359
SFW		1	.977**	.985**	.955**	.615*
RFW			1	.978**	.962**	.670*
SDW				1	.943**	.655*
RDW					1	.547

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed). SL- Shoot length, RL-Root length, SFW- Shoot fresh weight, RFW- Root fresh weight, SDW- Shoot dry weight, RDW-Root dry weight, Total Chl. - Total chlorophyll

Table 7. Correlation between different morphological and physiological parameters in Markofana cultivar

Parameters	RL	SFW	RFW	SDW	RDW	Total Chl.
SL	0.889**	0.922**	0.873**	0.932**	0.895**	0.895**
RL	1	0.913**	0.896**	0.915**	0.922**	0.313
SFW		1	0.952**	0.997**	0.957**	0.280
RFW			1	0.935**	0.990**	0.199
SDW				1	0.942**	0.329
RDW					1	0.247

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed). SL- Shoot length, RL-Root length, SFW- Shoot fresh weight, RFW- Root fresh weight, SDW- Shoot dry weight, RDW-Root dry weight, Total Chl. - Total chlorophyll

The strength of the linear relationship between shoot length and root length in the Local cultivar is presented in Table 8. Shoot length and root length have a positive linear relationship ($r=0.884$; $R^2=0.781$). Similarly, shoot length and fresh weight have a positive linear relationship ($r=0.998$ $R^2=0.996$) in the same cultivar (Table 8). Markofana had a strong positive linear relationship between shoot length and root length ($r=0.977$; $R^2=0.954$). Similarly, a strong linear positive relationship existed between shoot length and shoot fresh weight ($r=0.963$; $R^2=0.927$), as presented in Table 9.

The study revealed that the stress affects different parameters of the cultivars. However, the two cultivars showed adaptive changes to drought exposure in the study period. Growth parameters such as shoot length, root length, stem thickness, number of leaves, and leaf area were significantly affected by drought stress in the Markofana cultivar. At the physiological level, the Local cultivar responds to drought stress by reducing leaf-relative water content, chlorophyll fluorescence, photosynthetic rate, stomatal conductance, and transpiration rate. The stress also affected

Table 8. Correlation analysis for the local cultivar

Parameter	Shoot length	Root length	Shoot fresh weight
Shoot length	1	0.844	0.998
Root length	0.844	1	0.855
Shoot fresh weight	0.998	0.855	1

Table 9. Correlation analysis for the Markofana cultivar

Parameter	Shoot length	Root length	Shoot fresh weight
Shoot length	1	0.9774	0.9634
Root length	0.9774	1	0.9731
Shoot fresh weight	0.9634	0.9731	1

the total biomass and significantly increased in the Markofana cultivar and insignificantly in the Local cultivar. Both watering level and CT showed insignificant interaction effects on shoot length, leaf area, shoot fresh weight, root dry weight, and total chlorophyll on the cultivars. However, root fresh weight was affected significantly by the interaction.

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