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Full Length Article

Effects of Salinity and Drought Stresses on the Physio-Morphological Attributes of Onion Cultivars at Bulbification Stage

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Abstract

Onion (*Allium cepa* L.) has huge importance due to its health benefits. Salinity and drought stress appear to be the major threats towards the productivity of crops and vegetables across the globe. Although in literature several studies summarize responses of agricultural crops to abiotic stresses but data for onion appears to be limited. In this greenhouse study, seven onion cultivars were compared for their morphological and physiological responses to salinity stress (SS) and drought stress (DS). Salinity stress was applied to SS group through irrigating with water containing increasing doses of NaCl (100, 125, 150, 175 and 200 m*M*) in 3-day intervals, whereas water was suspended for 20 days to DS group for drought stress application. Salinity and drought stress decreased photosynthetic rate, lower leaf number, leaf length and bulb yield. SS interfered with root length and diameter, whereas thicker and elongated root length was noticed in response to DS. The cultivars 'Elit', 'Hazar' and 'Sampiyon' exhibited the reduction in photosynthetic rate. Maximum damage to chlorophyll contents was observed in cultivars 'Hazar' and 'Sampiyon' under SS and DS conditions. The cultivar 'Perama' manifested the highest bulb weight under SS and DS condition. The cultivars 'Perama' and 'Seyhan' were referred to as tolerant based on their overall performance under SS and DS, supported by the results of principal component analysis (PCA), however, the cultivars 'Elit', 'Hazar', and 'Sampiyon' performed poorly, so grouped as sensitive cultivars. Results of this study can be helpful in screening of tolerant and susceptible onion cultivars which will be useful for future breeding programs.

Keywords: Abiotic stress; Onion cultivars; Photosynthesis; Root morphology; Yield

Introduction

Onion (*Allium cepa* L.) is a monocot vegetable that belongs to the Alliaceae family. It is ranked second after tomato (*Solanum lycopersicum* L.) depending upon its consumption (Brice *et al.* 1997). Onion is the prime bulbous vegetable crop cultivated commercially in most parts of the world (Hanci and Gökçe 2016; Havey and Ghavami 2018). China is the leading onion producer with 17.8 million tons while Turkey holds 4^{th} position in the world with a production of 2.0 million tons and covering an area of 105,000 ha (FAOSTAT 2020). Onion is adapted to a wide range of climatic conditions, but its growth is observed best at mild climatic conditions without extreme climate (Mubarak and Hamdan 2018). It also requires sufficient moisture content in soil with a pH of 6.0–7.0 for good production (Ibrahim 2017). The crop is consumed as green and mature forms (Astley 1990). Onions vary in taste from sweet to mild flavoured and pungent for making an onion aroma as a condiment in almost every cuisine (Crowther *et al.* 2005).

Stress is considered as an external environmental factor that affects the growth and productivity of plants and causes an ultimate reduction in plant's yield (Ozturk *et al.* 2002). Abiotic stress covers a diverse class ranging from unwanted fluctuations in temperature to drought caused by limited water availability yearly, monthly, and even on daily basis. Drought rises due to inadequate rainfall results in decreased water content in the soil, and it is predicted to be increased by 2090 (Kogan and Guo 2016). Water scarcity is a versatile problem imposing a negative influence on plant physiological functions resulting in retarded morphological growth due to the closure of stomata that disrupts photosynthetic machinery causing reduced photosynthesis and transpiration (Farooq *et al.* 2009). Salinity, on the other hand, is more common in arid regions due to excessive evaporation which causes accumulation of inorganic salts disrupting the plant metabolism (Shahzad *et al.* 2017; Murtaza *et al.* 2017). Although saline soils have existed long before agriculture, poor quality irrigation water had worsened this problem (Zhu 2001). High salt concentration

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interferes with the absorption of water by plant roots; therefore, it also aggravates drought stress effects on crops (Gökçe and Chaudhry 2020).

Researchers have been struggling to circumvent abiotic stress issue and unravelling defending mechanisms for crop tolerance for a long time (Gokce *et al*. 2020; Maggio *et al*. 2006), especially by focusing on drought and salinity negatively influencing the production and yield of staple food crops up to 70% (Mittler 2006). In addition, it is predicted that abiotic stress conditions will become even worse in the coming future due to global climate change as reported by the Intergovernmental Panel on Climate Change (IPCC 2014). Due to a shallow root system penetrating only up to 60 cm, salinity and drought stress negatively affects normal onion growth which ultimately reduces its bulb yield (Hanci and Cebeci 2015). Therefore, onion production suffers from yield losses due to inadequate availability of water resulting in moderate to severe drought stress conditions and salinity problems across Turkey (Ardahanlioglu *et al*. 2003; Sönmez *et al*. 2005; Kendirli *et al*. 2005; Cemek *et al*. 2007; Bilgili *et al*. 2011). Although this is the case, contrary to many reports on the investigation of responses of agricultural crops to these two most important abiotic stresses, literature for onion is extremely limited. Thus, this study aimed to observe the effects of salinity and drought stresses on onion growth, root functioning and fluctuations in physiological changes of selected short-day Turkish onion cultivars. To the best of our knowledge, this is the first study comparing morphological and physiological variables in several onion cultivars in response to salinity and drought stress treatments. It was hypothesized that; different onion cultivars would be screened as tolerant or susceptible to salinity and drought stresses, which can be used for future breeding programs.

Materials and Methods

Experimental site

This study was conducted in semi-controlled greenhouse conditions (no artificial lightening, temperature control with 20°C day/10°C night until bulbing, 25°C day/15°C night during stress treatment, ambient humidity with 40 to 70% during the growing season) at Niğde Ömer Halisdemir University, Niğde, Turkey.

Plant material and growth conditions

Seven short-day onion cultivars 'Elit' (round bulb shape with yellow-colored scale), 'Hazar' (top bulb shape with brownish-colored scale), 'Inci' (top bulb shape with white-colored scale), 'Naz' (round bulb shape with yellow-colored scale), 'Perama' (round bulb shape with pinkish-colored scale), 'Seyhan' (globe bulb shape with straw yellow-colored scale) and 'Sampiyon' (round bulb shape with yellowish-brown colored scale) were used. Pots (10 L) were filled with torf and perlite in a 3:1 ratio. Twelve onion seeds were sown in each pot and necessary management practices were taken to ensure proper growth. After germination, ten plants per pot were maintained, and onion plants were divided into three groups [*i.e*., control (C), salinity stress (SS) and drought stress (DS)]. The first group consisted of control plants (C) without stress application, while the second group of plants was under salinity stress (SS) and the third group was subjected to drought stress (DS). The treatments were arranged according to a completely randomized design under factorial arrangements with three replications. Stress application was started at the onion bulbification stage or leaf bases begin to thicken or expand stage (BBCH 41) (BBCH Monograph 2001). Plants were daily watered in the control group, whereas water was suspended for 20 days for drought-stressed onion plants. For salinity stress treatments, irrigation was performed in 3-day intervals with water containing increasing amounts of NaCl, starting with 100 m*M* followed by 125 m*M*, 150 m*M*, 175 m*M* and 200 m*M* (Hanci and Cebeci 2015). In total, onion cultivars were exposed to 750 m*M* of salinity stress in 20 days. The plants were subjected to salinity and drought stresses after which drought stressed plants were well–watered, and salinity stressed plants were washed with normal irrigation water to drain all the salts from the pot to get rid of excess salt. Afterward, the rescued plants were allowed to grow for a further two weeks until harvesting. When onion plants showed bending of neck and started falling, they were harvested through uprooting for measuring bulb yield traits.

Physiological measurements

Physiological parameters were measured from all three groups (C, SS and DS) from three different plants in three replicates from each group. All the physiological measurements were taken during 09:00–13:00.

Gaseous exchange traits

Gaseous exchange traits were measured at 0, 10th and 20th day after stress application. Photosynthetic rate, transpiration rate, and stomatal conductance measurements from control (C) and stress application groups (SS and DS) were conducted with the help of constant light intensity of photosynthesis device (1500 μmol m^{-2} sec^{-1}), CO_2 amount (400 μmol) and airflow (500 μmol s^{-1}) and when photosynthesis gained steady state, the measurement was recorded using a portable gas exchange system LiCor 6400 XT (Li-Cor Biosciences, USA).

Relative water content (RWC)

Onion leaf segments of about 5 cm long (3rd or 4th leaf) in three replications were collected from C, SS and DS groups.

The fresh weight of onion leaves was measured and the turgid weight of the leaves was determined after keeping them in distilled water overnight. The leaves were dried in a microwave at 500 W for 10 min followed by oven drying at 95°C for 2–3 h to ensure complete drying of the leaves before determining dry weight. Relative water content (RWC) values of plants were calculated according to the following equation:

$$\text{RWC}(\%) = [(\text{Fresh weight} - \text{Dry weight})]/[(\text{Turgor weight} - \text{Dry weight}) \times 100]$$

Chlorophyll index

Leaf chlorophyll index was measured from control as well as from salt and drought-stressed plants with SPAD 502 Chlorophyll-Meter (Soil Plant Analysis Development; Minolta, Japan). Onion leaf (3^{rd} or 4^{th} fresh leaf) was selected from each pot and measurements were carried out as the average of three replications.

Leaf temperature

Leaf temperature was measured using Infrared Thermometer (IRT) device (MASTECH BM380). The values were represented as the average of three measurements from each pot.

Chlorophyll and carotenoids contents

Leaf Chl *a* and *b* content and carotenoids were determined by 0.5 g of fresh onion leaves (3^{rd} or 4^{th} fresh leaf) homogenized in 80% of 10 mL acetone. The homogenized solution was kept at 4°C overnight for complete extraction. The mixture was centrifuged at 10,000 rpm for 5 min at 4°C. The absorption of the supernatant was measured by using a UV–Vis spectrophotometer (UV–1800, Shimadzu) at 470 nm, 646 nm, and 663 nm. Calculation of chl *a*, chl *b*, and carotenoids contents were determined by formula defined by Arnon (1949).

$$\text{Chl } a\ (\mu\text{g/mL}) = 12.7(\text{A663}) - 2.69(A645)$$

$$\text{Chl } b\ (\mu\text{g/mL}) = 22.9(\text{A645}) - 4.68(A663)$$

$$\text{Total chlorophyll}\ (\mu\text{g/mL}) = 20.2(\text{A645}) + 8.02(A663)$$

Morphological measurements

All the morphological measurements were taken from three plants from each pot. Leaf length (cm) of the onion cultivars were measured with a measuring tape. The number of leaves was averaged by counting three plants from each pot. The onion bulbs were harvested from every pot at harvest time and the weight of bulb per plant was determined. The bulb length (cm) and bulb diameter (cm) per plant were determined from each pot in three replicates.

Root morphology

The roots were collected from each pot and in three replicates for each group. The root samples were washed with distilled water to remove dirt. The roots were placed in 20 cm wide and 30 cm long acrylic tank with one-inch distilled water and placed on EPSON scanner. The scanned images of roots were analyzed by WinRHIZO$_{TM}$ 2013 (Régent Instruments Inc., 2013) software for root morphological traits including total root length (cm), the total surface area of the root (cm^2), average root diameter (mm), and root volume (cm^3).

Statistical analysis

The data collected was analysed statistically using Fisher's Analysis of Variance (ANOVA) technique. The least significant difference (LSD) test at $P \leq 0.05$ was applied to compare the treatment mean values (Steel *et al*. 1997) using Statistical Package Statistix 8.1 (Tallahassee Florida, USA). Principal component analysis (PCA) was performed for visualizing differences between the treatments among cultivars by using XLSTAT-2014.

Results

Gaseous exchange parameters

Stomatal conductance was found to be consistently reduced compared to control in response to salinity and drought stress irrespective of onion cultivar. The cultivars acclimatized to stress conditions showed a significant reduction ($P \leq 0.05$) in stomatal conductance activity when compared with their respective controls. The cultivar 'Perama' excelled the other cultivars with the least decrease in stomatal conductance after 10 days of salinity and drought stress. In contrast, cultivar 'Hazar' showed a decrease of 70 and 84% in stomatal conductance after 10 days of SS and DS, respectively. The response of onion cultivars was also noted after 20 days of stress imposition. The cultivars 'Hazar' and 'Sampiyon' showed a significant decrease up to 90 and 93% in stomatal conductance after 20 days of salinity and drought stress (Table 1). The photosynthetic rate was significantly ($P \leq 0.05$) suppressed in all cultivars (Table 1). The maximum photosynthetic rate was observed in cultivar 'Perama', whereas the minimum photosynthetic rate was measured from cultivar 'Hazar' with a reduction of 42 and 43%, after 10 days of SS and DS, respectively. The cultivars 'Perama' and 'Seyhan' showed the least decline in the photosynthetic rate at 20^{th} day of stress treatments while all other cultivars showed a lower photosynthetic rate under both stress conditions with a reduction of 39–50%. Results revealed that SS and DS resulted in a reduction in the transpiration rate of all the cultivars. The cultivar 'Perama' showed the least decline in transpiration rate by 56 and 52% with the exposure of 20

Table 1: Effect of salinity and drought stress on gaseous exchange traits of onion cultivars

Cultivars	Photosynthesis rate (μmol m^{-2} s^{-1})			Stomata conductance (mol m^{-2} s^{-1})			Transpiration rate (mmol m^{-2} s^{-1})		
	0 day	10th day	20th day	0 day	10th day	20th day	0 day	10th day	20th day
Elit–C	21.35 ± 2.70	22.65 ± 1.5*	19.33 ± 0.65*	0.16 ± 0.06	0.25 ± 0.07*	0.12 ± 0.01*	2.79 ± 0.26	2.60 ± 0.43*	2.12 ± 0.32*
Elit–SS	22.31 ± 1.31	14.77 ± 1.92	11.20 ± 0.98	0.17 ± 0.12	0.09 ± 0.03**	0.02 ± 0.01	2.88 ± 0.48	1.95 ± 0.30*	0.43 ± 0.33**
Elit–DS	22.50 ± 1.99	15.58 ± 2.07	11.31 ± 0.67	0.16 ± 0.11	0.04 ± 0.01	0.01 ± 0.01	2.91 ± 0.40	1.76 ± 0.05	0.24 ± 0.32
Hazar–C	20.24 ± 1.20	21.31 ± 1.82*	21.02 ± 1.51*	0.19 ± 0.05	0.25 ± 0.10*	0.10 ± 0.02*	2.69 ± 0.60*	3.51 ± 0.37*	2.65 ± 0.52*
Hazar–SS	20.58 ± 1.90	16.40 ± 1.86	12.16 ± 0.56	0.22 ± 0.07*	0.07 ± 0.07**	0.01 ± 0.01	2.50 ± 0.09	1.97 ± 0.41**	0.62 ± 0.50
Hazar–DS	20.51 ± 0.80	16.67 ± 0.65	11.96 ± 0.55	0.20 ± 0.08	0.04 ± 0.01	0.01 ± 0.01	2.31 ± 0.63	1.75 ± 0.35	0.56 ± 0.32
Inci–C	22.67 ± 1.29	24.52 ± 2.96*	24.29 ± 3.90*	0.18 ± 0.03	0.24 ± 0.09*	0.15 ± 0.01*	3.20 ± 0.27*	3.60 ± 0.73*	2.80 ± 0.61*
Inci–SS	24.07 ± 3.51	15.30 ± 0.78	12.91 ± 0.45	0.21 ± 0.15	0.07 ± 0.01	0.03 ± 0.01	2.86 ± 0.44	1.48 ± 0.24	1.29 ± 0.69**
Inci–DS	24.61 ± 1.76	14.65 ± 2.81	12.07 ± 1.49	0.17 ± 0.05	0.05 ± 0.01	0.01 ± 0.01	3.22 ± 0.59*	2.05 ± 0.50**	0.62 ± 0.21
Naz–C	23.31 ± 1.90	21.48 ± 2.22*	19.76 ± 2.91*	0.20 ± 0.07*	0.21 ± 0.04*	0.13 ± 0.01*	3.20 ± 0.79*	2.91 ± 0.15*	1.99 ± 0.35*
Naz–SS	22.39 ± 2.88	12.31 ± 1.93	13.74 ± 0.99**	0.18 ± 0.04	0.05 ± 0.01	0.02 ± 0.01	2.72 ± 0.24	1.45 ± 0.14	0.87 ± 0.22**
Naz–DS	22.60 ± 1.48	17.22 ± 2.26**	11.05 ± 0.22	0.21 ± 0.04	0.04 ± 0.01	0.01 ± 0.01	3.27 ± 0.44**	1.66 ± 0.43**	0.44 ± 0.27
Perama–C	21.04 ± 0.82	24.18 ± 2.02*	20.12 ± 2.30*	0.22 ± 0.08	0.18 ± 0.07*	0.15 ± 0.01*	3.03 ± 0.74	3.03 ± 0.74*	3.13 ± 0.33*
Perama–SS	25.02 ± 0.64*	19.06 ± 2.84	14.20 ± 0.44	0.20 ± 0.08	0.12 ± 0.05**	0.02 ± 0.01	3.05 ± 0.29**	2.60 ± 0.10	1.39 ± 0.47
Perama–DS	22.44 ± 1.51	18.53 ± 1.23	13.18 ± 1.69	0.19 ± 0.08	0.08 ± 0.01	0.02 ± 0.01	2.81 ± 0.29	2.46 ± 0.43	1.49 ± 0.37
Seyhan–C	21.93 ± 1.22	24.17 ± 1.72*	19.76 ± 0.66*	0.20 ± 0.01	0.21 ± 0.08*	0.13 ± 0.02*	3.11 ± 0.39*	3.66 ± 0.33*	3.03 ± 0.38*
Seyhan–SS	23.31 ± 2.14*	17.20 ± 2.48	14.82 ± 0.34**	0.22 ± 0.09	0.05 ± 0.01	0.03 ± 0.01	2.97 ± 0.24	1.56 ± 0.27	1.29 ± 0.46**
Seyhan–DS	20.87 ± 0.40	15.72 ± 2.36	12.64 ± 0.33	0.19 ± 0.07	0.05 ± 0.01	0.01 ± 0.01	3.01 ± 0.51	1.64 ± 0.05	0.82 ± 0.17
Sampiyon–C	21.78 ± 1.53	25.74 ± 2.92*	23.35 ± 2.40*	0.18 ± 0.05	0.26 ± 0.05*	0.15 ± 0.01*	3.44 ± 0.03	3.43 ± 0.96*	3.53 ± 0.34*
Sampiyon–SS	22.55 ± 2.91	17.57 ± 1.89	14.53 ± 2.80**	0.21 ± 0.04	0.07 ± 0.01	0.01 ± 0.01	3.59 ± 0.29*	2.15 ± 0.19**	0.71 ± 0.27**
Sampiyon–DS	23.55 ± 1.99*	17.95 ± 0.11	12.18 ± 1.89	0.22 ± 0.05*	0.05 ± 0.01	0.01 ± 0.01	3.21 ± 0.40	1.84 ± 0.35	0.26 ± 0.18

Values represent mean value ± standard deviation. C control plants, SS salt stressed plants and DS drought stressed plants. (*) shows significant difference with stressed group plants ($P \leq 0.05$). (**) shows significant difference among stressed counterparts (drought or salinity stress) ($P \leq 0.05$)

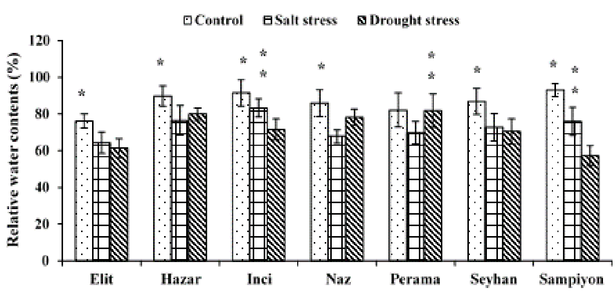

Fig. 1: Effect on relative water contents of different onion cultivars subjected to salinity and drought stress conditions. Asterisk (*) represents significant difference ($P \leq 0.05$). Two vertical asterisks (**) shows significant difference among stressed counterpart (salt or drought stress) ($P \leq 0.05$)

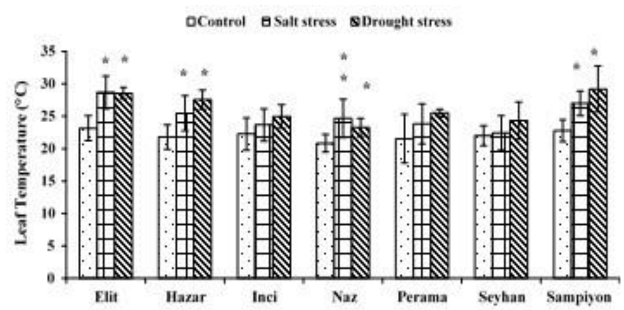

Fig. 2: Leaf temperature of different onion cultivars subjected to salinity and drought stress conditions. Asterisk (*) represents significant difference ($P \leq 0.05$). Two vertical asterisks (**) shows significant difference among stressed counterpart (salt or drought stress) ($P \leq 0.05$)

days of SS and DS, respectively. The cultivar 'Elit' showed a reduction by 80% under SS, a 93% decrease was observed in cultivar 'Sampiyon' under DS (Table 1).

Physiological parameters

Relative water contents of onion cultivars significantly reduced in all onion cultivars exposed to salinity and drought stress compared to their respective controls (Fig. 1). Control onion cultivars did not show any significant change. The RWC contents were measured on 20th day and the cultivar 'Inci' had the highest RWC (83%) in the case of SS while cultivar 'Perama' had the highest RWC (81%) in case of DS even with no significant ($P \leq 0.05$) difference from the control. The lowest RWC was assessed from cultivar 'Naz' (68%) in the case of SS, whereas cultivar 'Sampiyon' had the lowest RWC (57%) under DS. A change in leaf temperature was observed before terminating stress treatment (Fig. 2). The leaf temperature of the cultivar 'Inci' was lowest under SS (23.6°C) and DS (24.9°C) on 20th day, respectively, with no significant ($P \leq 0.05$) difference from control plants. However, the cultivar 'Elit' had the highest rise in leaf temperature under SS (28.6°C) and the cultivar 'Sampiyon' under DS (29.1°C). Chlorophyll index was also quantified which indicated deterioration in all onion cultivars with prolonged stress. The cultivar 'Hazar' showed a decrease of 17% in response to SS and a 23% reduction in cultivar 'Sampiyon' was recorded under DS when compared with their respective control plants (Fig. 3). The chlorophyll (*a*, *b*, and total) contents indicated that stress impositions damaged the photosynthetic pigments (Fig. 4). Salt in irrigation water damaged chlorophyll *a* and *b* content in cultivar 'Hazar' with a rate of 28 and 34%, respectively.

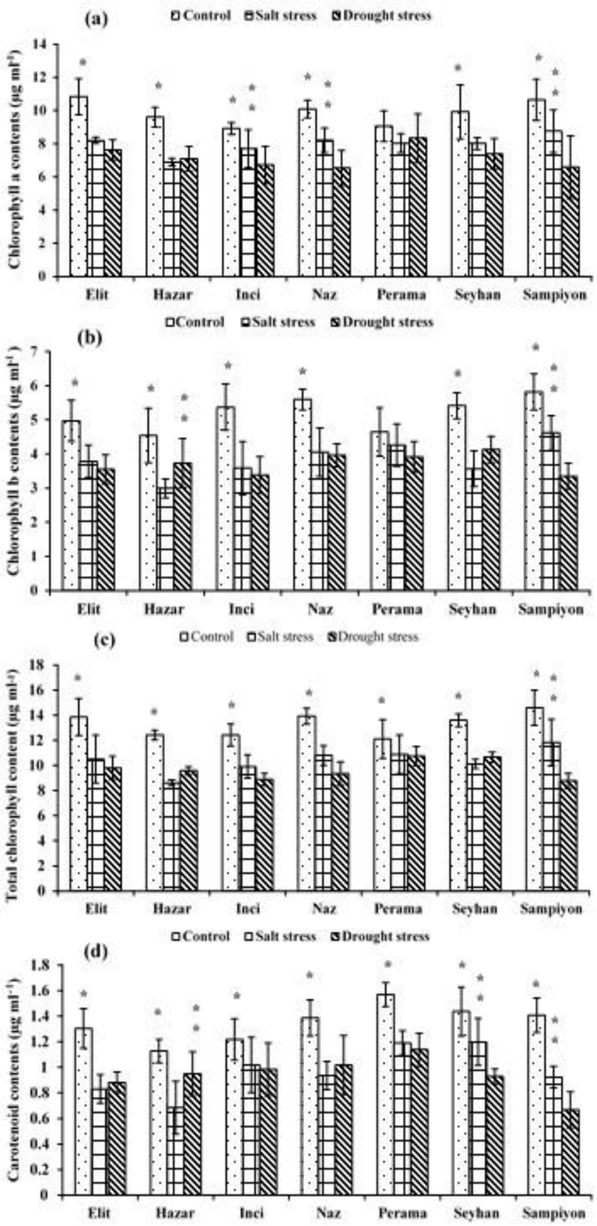

Fig. 4: Effect on photosynthetic pigments of different onion cultivars subjected to salinity and drought stress conditions. (**a**) Chlorophyll *a* content (μg mL^{-1}) (**b**) Chlorophyll *b* content (μg mL^{-1}) (**c**) Total chlorophyll content (μg mL^{-1}) (**d**) Carotenoid content (μg mL^{-1}). Asterisk (*) represents significant difference ($P \leq 0.05$). Two vertical asterisks (**) shows significant difference among stressed counterpart (salt or drought stress) ($P \leq 0.05$)

However, cultivar ‘Sampiyon’ had maximum damage to chlorophyll *a* and *b* content under DS with a reduction of 38 and 42%, respectively. Carotenoid contents were also decreased with both stress conditions, whilst the lowest value was observed in the cultivar ‘Hazar’ with a decrease of 40% under SS and 51% in ‘Sampiyon’ under DS (Fig. 4).

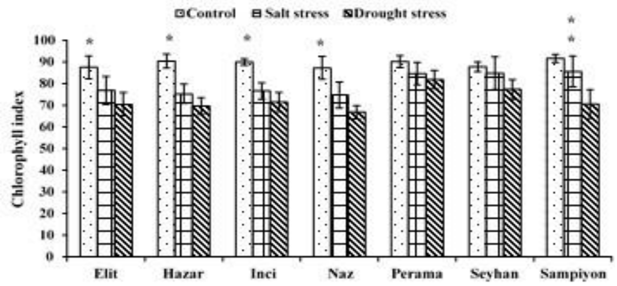

Fig. 3: Chlorophyll index of different onion cultivars subjected to salinity and drought stress conditions. Asterisk (*) represents significant difference ($P \leq 0.05$). Two vertical asterisks (**) shows significant difference among stressed counterpart (salt or drought stress) ($P \leq 0.05$)

Morphological parameters

All the morphological changes showed a reduction in vegetative growth at prevailing stress conditions (Fig. 5). It was noticed that there were fewer number of leaves per plant in response to both stresses. The maximum number of leaves per plant was noted in cultivar ‘Perama’ and ‘Seyhan’ under SS and DS, respectively. The minimum number of leaves was counted from cultivar ‘Naz’ with a decrease of 40 and 53% under SS and DS when compared to its control. The decrease in leaf diameter was also observed in stressed plants. Under DS, cultivars ‘Naz’ and ‘Sampiyon’ showed a 40% decrease and under SS, cultivars ‘Inci’ and ‘Naz’ showed a 30% decrease; while a higher leaf diameter was observed in cultivar ‘Perama’ followed by cultivar ‘Seyhan’. The cultivars ‘Hazar’ and ‘Elit’ exhibited stunted leaf length under SS and DS conditions with a decrease of 32 and 28%, respectively (Fig. 5a).

Yield related parameters

Bulb characteristics are important economic traits to evaluate bulb yield (Fig. 6). Onion bulb length and bulb diameter were affected significantly ($P \leq 0.05$) under stress conditions. The reduction in bulb diameter was more compared to the decrease in bulb length. The cultivar ‘Perama’ showed the highest bulb weight under SS (50.6 g) and DS (44.2 g) conditions, respectively. The lowest bulb weight was observed from cultivar ‘Hazar’ with a reduction of 51% under SS and 53% in cultivar ‘Sampiyon’ under DS (Fig. 6d).

Root morphological parameters

Roots response towards SS resulted in retarded growth as compared to DS group, which showed an increase in root characteristics. Salinity stress suppressed the total root length by 14% in cultivar ‘Sampiyon’ contrarily least reduction by 4% in ‘Inci’, while under DS the cultivar ‘Sampiyon’ exhibited 17% increase in root length followed by 13% in cultivar ‘Inci’ (Fig. 7a). The root surface area

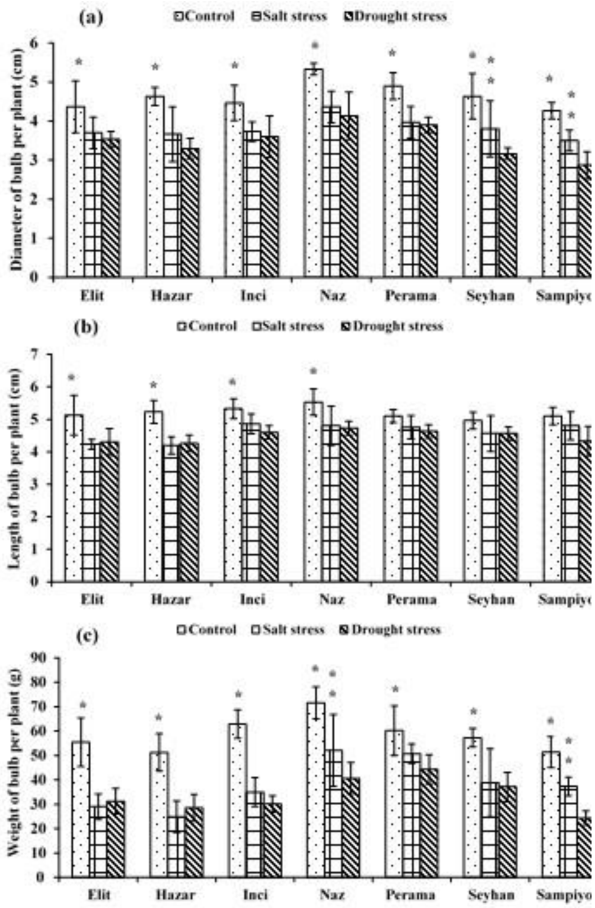

Fig. 6: Yield parameters of different onion cultivars subjected to salinity and drought stress conditions. (**a**) Diameter of bulb per plant (**b**) length of bulb per plant (**c**) total weight of bulb per plant. Asterisk (*) represents significant difference ($P \leq 0.05$). Two vertical asterisks (**) shows significant difference among stressed counterpart (salt or drought stress) ($P \leq 0.05$)

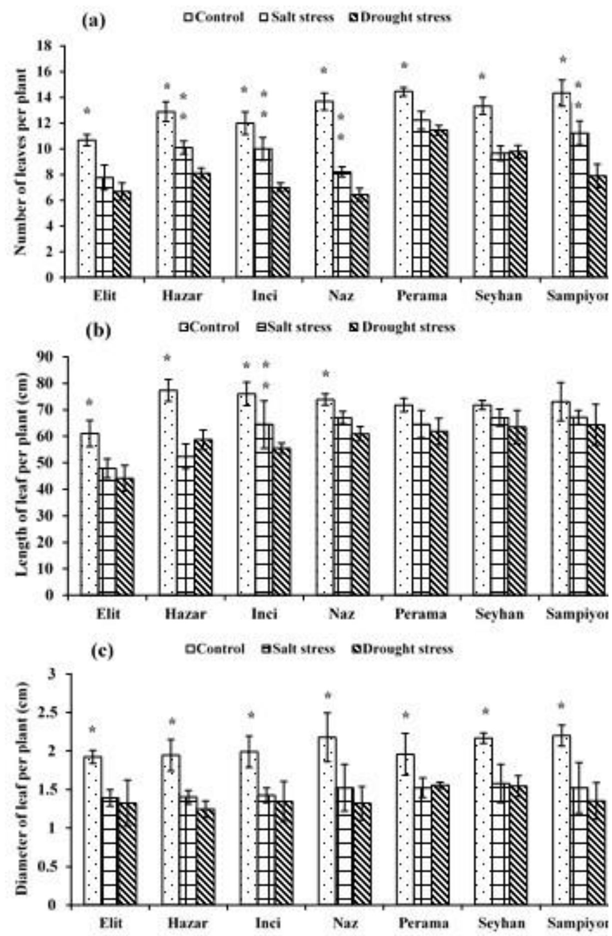

Fig. 5: Vegetative growth of different onion cultivars subjected to salinity and drought stress conditions (**a**) Number of leaves per plant (**b**) Length of leaves per plant (**c**) Diameter of leaf per plant. Asterisk (*) represents significant difference ($P \leq 0.05$). Two vertical asterisks (**) shows significant difference among stressed counterpart (salt or drought stress) ($P \leq 0.05$)

also decreased under SS, whereas the same variable depicted an increasing trend under DS. The cultivars 'Sampiyon' and 'Elit' showed a decline of 12% in root surface area in response to SS, while the cultivars 'Hazar' and 'Sampiyon' resulted an increase of 16 and 13%, respectively in root surface area under DS (Fig. 7b). The average root diameter showed a 15%, reduction with the imposition of SS in the cultivars 'Elit' and 'Naz'. On the other side, the cultivar 'Seyhan' showed 3% increase in root diameter. The cultivar 'Perama' showed 16% increase in root diameter under DS (Fig. 7c). The root volume was decreased by 31% in cultivar 'Inci' followed by 23% in cultivars 'Elit' and 'Hazar' under SS. A reverse trend was observed with an increase in root volume by 35% in the cultivar 'Inci' under DS (Fig. 7d).

Principal component analysis

The interrelationship among selected onion cultivars along with the tested variables under SS and DS conditions were analysed by biplot principal component analysis (PCA) as shown in Fig 8. It revealed that the first two components explained 60.78% variance (contributed by PC1 38.78%, and PC2 22.00%) under SS conditions. DS conditions showed a total variation of 71.36% (contributed by PC1 53.12%, and PC2 18.24%) among the onion cultivars for the measured traits. PCA biplot grouped the onion cultivars based on their response to the tested morphological and physiological variables/traits. In SS, the cultivars 'Perama', 'Seyhan' and 'Inci' depicted positive PC1 values. The cultivar 'Perama' showed best performance for chlorophyll index, total chlorophyll contents, photosynthesis, length of leaf and length of bulb. The cultivar 'Seyhan', was best performing for the traits such as root surface area, average root diameter, diameter of bulb, carotenoid content, stomatal conductance, and transpiration rate. The cultivar 'Inci', was best in total root length and RWC under SS. The cultivars

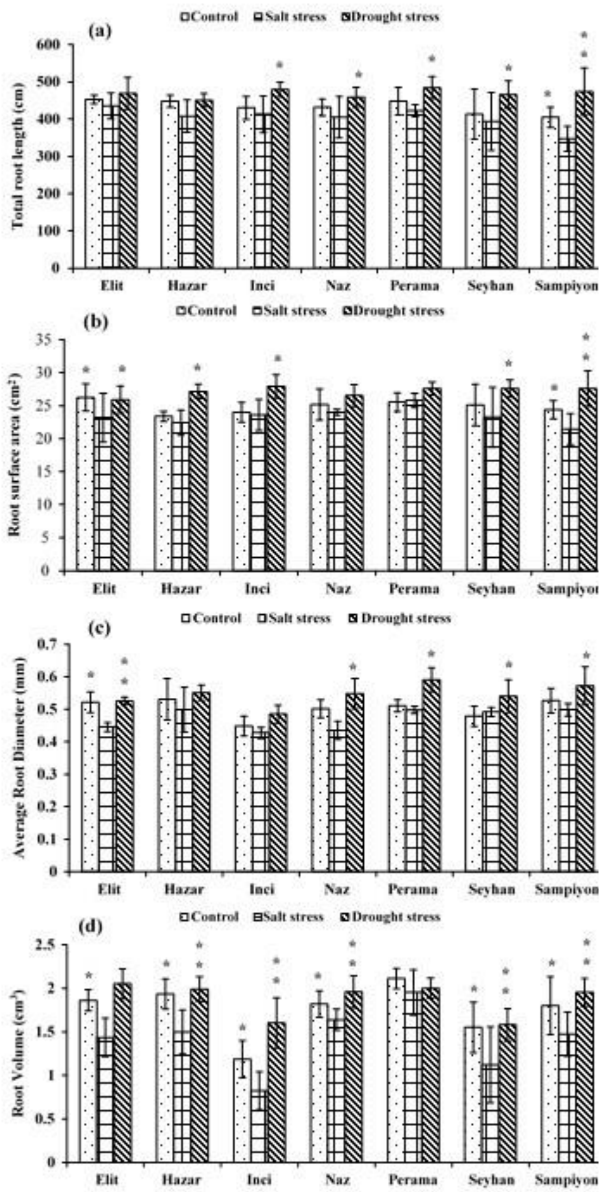

Fig. 7: Effect on root morphology of different onion cultivars subjected to salinity and drought stress conditions. (**a**) Total root length (cm), (**b**) root surface area (cm^2), (**c**) root diameter (mm), (**d**) root volume (cm^3). Asterisk (*) represents significant difference ($P \leq 0.05$). Two vertical asterisks (**) shows significant difference among stressed counterpart (salt or drought stress) ($P \leq 0.05$)

'Perama' and 'Seyhan', were referred to as tolerant based on their response to the tested variables, whereas the cultivars 'Hazar', 'Elit' and 'Sampiyon' were salt sensitive. The cultivar 'Inci' showed average response (Fig. 8). In DS condition, the cultivar 'Seyhan' showed maximum chlorophyll index, root diameter, and length of bulb, while the cultivar 'Perama' indicated the maximum diameter of leaf, number of leaves, RWC, photosynthesis, transpiration rate and weight of bulb. The cultivars 'Elit' 'Hazar' and 'Sampiyon', were drought sensitive according to the observed traits (Fig. 8).

Discussion

The results of current study highlighted the tolerance potential of short-day onion cultivars to SS and DS. Both stresses adversely affected the gaseous exchange characteristics (photosynthesis, stomatal conductance and transpiration rate) of the all the cultivars, however, some cultivars performed better in comparison to others (Table 1). All the cultivars showed least disruption in gaseous exchange traits during the initial 10 days out of the 20 days of SS and DS, which indicated that onion is less prone to short exposure of stress period. Contrarily, after exposure to 20 days of stress conditions, the cultivars 'Elit', 'Hazar' and 'Sampiyon' showed maximum decline in photosynthetic rate due to decreased stomatal activity. The reduction in transpiration rate in these cultivars was also more as compared to the remaining cultivars. Decreased gaseous exchange in these cultivars might have resulted due to the decline in leaf internal CO_2 concentration which is a consequence of stomatal closure and is evident by the decreased transpiration rate (Vesala *et al.* 2017). Sensitivity of these cultivars might also be attributed to the damaged photosynthetic apparatus caused by the generation of reactive oxygen species (ROS), which are accumulated under the circumstances of reduced CO_2 influx and excess/continued light exposure (Farooq *et al.* 2014). The reduction of cellular water caused by the contact of roots with the stressful environment subsequently reduces the transport of assimilates, that eventually affects the photosynthetic rate (Chaves *et al.* 2009). PCA analysis also endorsed the sensitivity of the cultivars 'Elit', 'Hazar', and 'Sampiyon' under both SS and DS conditions (Fig. 8). The least influence on gaseous exchange traits in cultivars 'Perama' and 'Seyhan' was due to their high-water contents. It includes changes in the cellular osmotic behaviour evident by high relative water contents in the cell under stress conditions (Hussain *et al.* 2018). The synthesis of osmo-protectants combined with high chlorophyll pigments and enhanced photosynthesis could be a major decisive factor for the stress tolerance response of these cultivars (Farooq *et al.* 2015). Our study grouped the cultivars 'Perama' and 'Seyhan' as 'tolerant' based on their better adaptive response to both the stresses. The PCA analysis revealed that these cultivars were the best performing for the gaseous exchange traits to both SS and DS. Demirel *et al.* (2020) investigated the effect of drought stress on gaseous exchange characteristics of potato cultivars and suggested that higher photosynthetic rate was a key attribute exhibited by tolerant cultivars. A similar trend was observed in our study.

Relative water content is an important physiological attribute to estimate the internal water status under stress conditions. The cultivars 'Elit', 'Hazar' and 'Sampiyon'

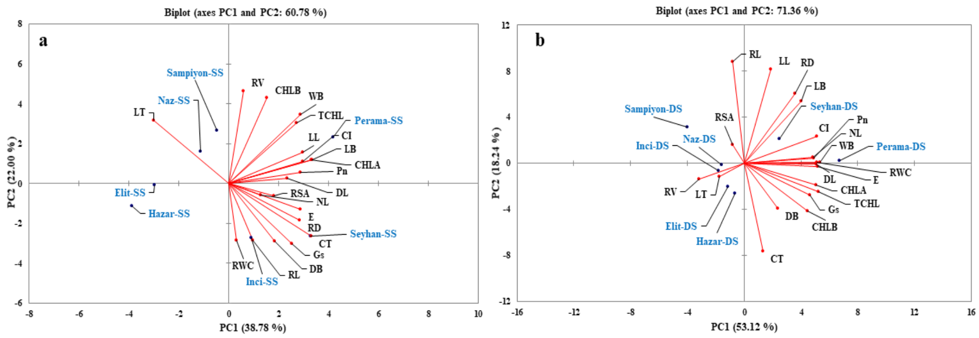

Fig. 8: PCA biplot for morpho-physiological variables of seven onion cultivars grown under salinity stress (**a**) and drought stress (**b**) conditions. PCA biplot is a combination of score plot of onion cultivars (represented in blue text) and loading plot of variables (represented by red vectors; black text). SS: salinity stress, DS: drought stress, NL: number of leaves, DL: diameter of leaf, LL: length of leaf, DB: diameter of bulb, LB: length of bulb, WB: weight of bulb, TRL: total root length, ARD: average root diameter, RV: root volume, RSA: root surface area, RWC: relative water content, LT: leaf temperature, CI: chlorophyll index, CHLA: chlorophyll a, CHLB: chlorophyll b, TCHL: total chlorophyll, CT: carotenoid content, Pn: Photosynthesis, Gs: stomatal conductance, E: transpiration rate

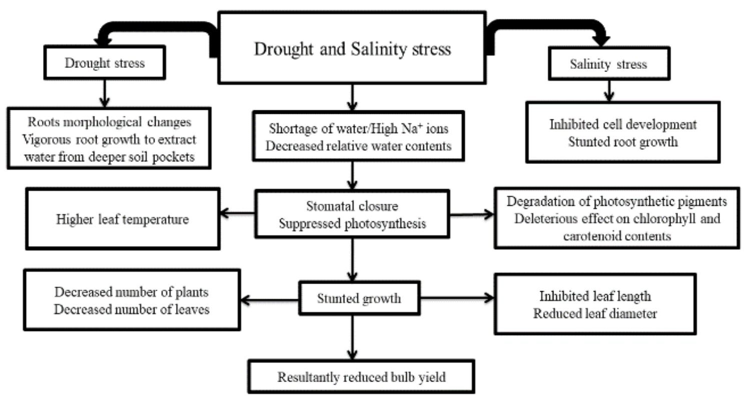

Fig. 9: Schematic diagram showing general effect of drought and salinity stress on onion. Drought and salinity stress negativley impacted relative water contents of onion dirupting photosynthesis. Salinity stress hindered uptake of water by onion roots along with weaker root development. Both stresses resulted in decreased vegetative growth and resultantly lower bulb yield

showed decreased RWC under given stresses (Fig. 1). As, RWC is considered as an indicator of stress tolerance (Dien *et al.* 2019), our results suggested that the cultivars 'Elit', 'Hazar' and 'Sampiyon' were susceptible to applied stress. It is evident from the PCA results that these cultivars showed poor performance and existed in the negative quadrate (Fig. 8). Our results and previous studies indicated that SS and DS affects negatively RWC which also leads to oxidative stress in plants (Egert and Tevini 2002; Astaneh *et al.* 2018). The cultivar 'Inci', showed more RWC only under SS condition, whereas 'Perama' and 'Seyhan' were the best performers under DS. Interestingly, these cultivars could have exhibited higher osmotic regulation through accumulation of osmolytes to alleviate SS and DS (Moharramnejad *et al.* 2019).

Leaf temperature was elevated under both the stressed conditions with a higher increase under DS (Fig. 2). The higher leaf temperature was noticed in the cultivars 'Elit', 'Hazar' 'Naz' and 'Sampiyon' under both the stresses, which is attributed to lower RWC and disruption in gaseous exchange traits. Isoda (2010) reported that water-stressed plants showed lower transpiration rates resulting in higher leaf temperature. The reason is that there is a close relationship between stomatal closure and increased leaf temperature (Liu *et al.* 2011). The cultivar 'Inci' exhibited comparatively lower leaf temperature under SS, due to its capability to preserve higher levels of RWC. The cultivar 'Seyhan' prevented the tissue damage by regulating metabolic processes such as increased stomatal opening, transpiration rate, and enhanced photosynthesis that resulted in lowering of leaf temperature. Other factors that might have contributed to better adaptation response of this cultivar under both stresses is linked to the manipulation of antioxidant system to scavenge oxidative stress caused by SS and DS (Farooq *et al.* 2019).

In this study, exposition of onion plants to SS and DS damaged the photosynthetic pigments of all the cultivars. The maximum impairment in chlorophyll contents was depicted by the cultivars 'Elit', and 'Hazar' in response to SS and in 'Sampiyon' under DS (Fig. 4). This might be due to the damage caused by ROS in these cultivars. It resultantly destroyed the chloroplast structure of the cultivars. Moreover, decrease in chlorophyll of stressed plants is a general symptom of oxidative stress which is attributed to inhibition in synthesis of chlorophyll (Santos 2004). The findings of damaged chlorophyll contents are also supported by earlier study of Romdhane *et al*. (2020). Total chlorophyll content was higher in the cultivar 'Perama' that was credited to the minimal effect on the light harvesting complexes present on the thylakoid membrane, as evident from enhanced photosynthesis compared to others. Carotenoid is an antioxidant with the potential for detoxifying the harmful effects of ROS in plants. Current study reported the negative impact of SS and DS with damaged carotenoid contents causing photoinhibition. In cultivars 'Elit' and 'Sampiyon', a significant reduction of carotenoid contents indicated susceptibility to SS and DS, whereas the cultivar 'Hazar' performed poorly under SS. These cultivars showed decline in RWC and total chlorophyll content. Contrarily, the cultivars 'Inci' and 'Seyhan' were the richest in carotenoid content and this was possible by retaining higher RWC under SS. This finding was also supported by previous reports in onion (Hanci and Cebeci 2014,; Hanci and Cebeci 2015; Hanci *et al*. 2015; Semida 2016).

Salinity and drought stresses negatively influenced the above-ground biomass of onion (Fig. 5). The cultivar 'Naz' showed minimum number of leaves under both the stresses whereas the cultivars 'Elit', 'Inci' and 'Sampiyon' demonstrated decreased number of leaves under DS. The stressed condition inhibited the growth of the cultivars with alteration in cell size division and resulted in decreased production of leaf and promoted senescence (Ghodke *et al*. 2018). Leaf length was decreased in the cultivars 'Hazar' and 'Elit' whereas the cultivars 'Perama' and 'Seyhan' showed the least reduction in leaf length. The differences in vegetative growth among the cultivars found in this study can be attributed to disruption in physiological characteristics. The sensitive cultivars might have experienced a decrease in turgor pressure limiting the expansion of leaf (Fahad *et al*. 2017). Metwally (2011) also demonstrated the negative effects of stress on growth of onion.

Stress at bulbification stage significantly reduced the yield traits in all cultivars tested in response to the SS and DS (Fig. 6), as expected based on the previous reports (Pelter *et al*. 2004; Zayton 2007; Metwally 2011). The highest bulb weight of the cultivar 'Perama' is attributed to the tolerance in response to stress conditions. Bulb characteristics are known to show a reduction in response to stress among the cultivars due to the difference in soil water intake and evapotranspiration flux (Pelter *et al.* 2004; Lipiec *et al.* 2013). In the present study, cultivars 'Elit' 'Hazar' and 'Sampiyon', having lower photosynthetic activity, higher leaf temperature and lower chlorophyll contents compared to other cultivars, showed the lowest bulb weight in response to SS and DS, which can be explained as the reduction in morphological and physiological characteristics of susceptible cultivars resulting into smaller cell size (Tisne *et al.* 2010).

Salinity and drought stress altered the root morphological characteristics of the cultivars under study. To the best of our knowledge, this is the first study that focuses on the effects of SS and DS on the root morphology of onion. Under DS, all the onion cultivars used in this study showed elongated root development. However, in case of the SS, opposite results were obtained (Fig. 7). The decreased root length in the cultivar 'Sampiyon' under SS is due to higher osmotic pressure in the vicinity of roots which prevented uptake of water and resulted in shorter roots (Sadat-Noori *et al*. 2008). The results obtained regarding inhibited root length with exposure to SS are in accordance with Basu *et al*. (2017). The increase in root length of the cultivar 'Inci' indicated the plasticity of root. It might be due to better cell division and expansion of root apical meristem. Thus, it suggests that salinity stress altered root growth with the enhanced and reduced cell division and cell expansion (West *et al.* 2004). The growth increment in root could be due to its ability to alleviate osmotic stress by maintaining osmotic potential. The absorbed ions by the root might be quickly separated into vacuoles without its higher accumulation, therefore increasing turgor of the cell and stimulated cell elongation (Mukami *et al*. 2020). Increased root length of cultivar 'Sampiyon' under the DS is probably due to its sensitivity to moisture deficiency. It is known to force plant roots to extract water from deeper soil pockets (Fang *et al*. 2017). The decreased root diameter was observed with the application of SS in all cultivars, whereas DS resulted in increased root diameter. The reduction in root diameter of the cultivars 'Elit' and 'Naz', is due to ionic toxicity and osmotic pressure (Fricke *et al.* 2006). The root surface area and root volume decreased in response to SS while it increased under DS. The cultivars 'Elit' 'Sampiyon', and 'Hazar' showed a decline in root surface area and root volume, respectively. The reduction under SS was due to inhibited root growth due to osmotic stress and hampers root meristem size (Jiang *et al*. 2016). In contrast, a reverse trend was noticed in the cultivars 'Hazar', 'Sampiyon' and 'Elit' resulted in increase in root surface area and root volume under DS. It might be due to moisture deficiency that triggers synthesis of abscisic acid for the closure of stomata (Hussain *et al*. 2016). These cultivars also showed poor performance *i.e*., lower RWC, damage to photosynthesis and photosynthetic pigments regarding which triggers oxidative stress. These disruptions in physiological processes exert pressure with enlarged root surface area and root volume to extract water. Our findings are consistent with previous studies that reported a similar influence of stress on root architecture of garlic, potato, tomato, eggplant, and pea (Akinci *et al.* 2004; Al-Safadi and Faoury 2004; Karni *et al*. 2010; Khenifi *et al*. 2011; Pereira *et al*. 2020). In view of the examined parameters during the stress duration and yield traits, an illustrative diagram is described in Fig. 9.

Conclusion

This study was conducted to evaluate the performance of seven onion cultivars under salinity and drought stresses. Our results revealed a differential response of onion cultivars. It was concluded that cultivars 'Seyhan' and 'Perama' showed higher tolerance compared to other cultivars under both stresses with minimal decline in morphological and physiological traits evaluated. Cultivars 'Elit', 'Hazar' and 'Sampiyon', on the other hand, exhibited susceptibility compared to other cultivars studied. The resilient cultivars 'Seyhan' and 'Perama' can be used in future breeding studies to increase abiotic stress tolerance of onion against salinity and drought stresses. Furthermore, these will be the most attractive onion cultivars for stress related studies at the molecular level.

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Author Contributions

Usman Khalid Chaudhry, Zahide Neslihan Öztürk Gökçe and Ali Fuat Gökçe conceptualized the idea and designed the study. Usman Khalid Chaudhry performed the experiment, collected the data, analyzed the data and wrote the initial draft. Zahide Neslihan Öztürk Gökçe and Ali Fuat Gökçe edited and reviewed the draft of paper. All the Authors, read and approved the final draft.

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