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Growth, Physiology and Yield of Onion (*Allium cepa* L.) Under Salt Stress

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ABSTRACT

Onion production and productivity is constrained by biotic and abiotic factors. Soil salinity is one of the factors mentioned by producers in Ethiopia. Hence, the study was conducted to determine the relative tolerance of onion cultivars to salt stress levels under field condition on soil media filled in box at Melkasa Agricultural Research Center. Factorial combined treatments (4×5) were arranged in Randomized Complete Block Design with three replications. Data collected were subjected to analysis of variance using SAS version 9.3. The analysis revealed variation in growth, yield and yield related parameters among cultivars ($p \leq 0.05$) and salt stress levels ($p \leq 0.001$). Bombey Red, Nafis and Nasic Red cultivars showed the highest performance in number of leaves per plant, pseudo stem diameter, plant height, leaf length and width, fresh and dry above ground weight. The cultivars also showed the highest bulb length, width, fresh and dry weight. Chlorophyll and stomatal conductance was highly significant for main factors and interactions ($p \leq 0.001$). Photosystem-II also showed significant difference at 40th and 68th (DAT) days after transplanting among cultivars ($p \leq 0.05$) and at 40 and 54 DAT stages within salt levels ($p \leq 0.001$). With 1.2 dSm-1 salt level, the highest growth and yield performances were observed. The highest photosystem-II was recorded for Bombey Red, Nafis and Nasic Red, whereas Adama Red showed the least. Generally, during early growth stages, growth variables were not affected up to 4 dSm-1. Thus, Bombey Red, Nafis, and Nasic Red can be used for salt levels less than 4 dSm-1. Indeed, the experiment should be repeated under controlled environment with multi-cultivars and salt levels not more than 4 dSm-1.

INTRODUCTION

In Ethiopia onion covers an estimated annual total production area of 22,767 ha both under rain-fed and irrigated condition (CSA, 2015). However, onion production and productivity is constrained by biotic and abiotic factors among which is soil salinity (Etissa et al., 2014). According to Abebe et al. (2015), out of 15,256.2 ha, Amibara irrigation scheme alone, 34% (5239.8 ha) of the command area has been mapped as saline soil electrical conductivity (EC) > 4 dSm^{-1} and sodium absorption ratio (SAR) < 13). Expansion of the highly saline Basaka lake may aggravate salinity towards middle and lower Awash basin, the East and Northeast direction, due to the topography of the area (Olumana et al., 2009), where downstream irrigated area production is highly constrained.

Salt accumulation can limit the germination and development of various food crop species (Barroso et al., 2010). This leads to morphological, cellular, biochemical and molecular alterations that hinder the agricultural yield in response to the decrease in the water potential of the soil solution, induced by the high osmolarity (Lima and Bull, 2008). In addition, ionic toxicity promotes an imbalance in the absorption of essential nutrients, causing metabolic disorders, which inhibit growth (Maia et al*.,* 2012). Salt stress can also lead to an excess intracellular production of reactive oxygen species (ROS) such as the superoxide radical (O^{-2}), the hydroxyl radical (OH^-), hydrogen peroxide (H_2O_2), and singlet oxygen (O_2) (Stanisavljevic et al., 2011).

Salt stress may cause nutrient deficiencies or imbalances, due to the competition of sodium (Na^+) and chlorine (Cl^-) with nutrients such as potassium (K^+), calcium (Ca^{2+}) and nitrate (NO_3^-) (Hu and Schmidhalter, 2005). It was observed that increasing nitrogen (N) rates and salinity levels interacted to reduce chilli pod yield (Villa-Castorena et al*.,* 2003), while salt-stressed chilli performs well when adequately fertilized, over-fertilization during early crop development may contribute to salinity and decreased pod yield. It was also observed that foliar application of mono potassium phosphate on onion crop was seriously affected at higher salinity of irrigation water (4000 ppm), compared to moderate salinity level (2000 ppm) (El-Dewiny et al., 2013). In addition to increasing the salinity of irrigation water, it caused a reduction in the contents of N, P, K, Ca and magnesium (Mg) nutrients, as a result of competition between Na^+, Cl^- under high saline water and these nutrients. Thus, a better understanding of the role of mineral nutrients in plant resistance to drought and salinity will contribute to an improved nutrient management in arid and semi-arid area such as Awash Melkasa and in regions suffering from temporary drought.

Tolerance to salt stress is complex physiological traits, metabolic pathway, and molecular or gene networks (Gupta and Haung, 2014). However, adaptive response to salt stress being identified varies within species and cultivars. Research work in Turkey indicated that the effects of drought and salt tolerance of four onion cultivars showed morphological and physiological variables differences for both drought and salinity (Hanci and Cebeci, 2015). According to Beinsan et al. (2015), great genetic diversity in terms of free proline synthesis enables to identify cultivars that have a good tolerance to salt of collected local land-races observed. In Ethiopia, there is scanty of information regarding salt stress tolerance of onion cultivars. In view of this the research was done with objectives of evaluating onion cultivars for yield and yield components performance under salt stress levels on soil media.

MATERIALS AND METHODS

Description of the study site

The study was conducted at Melkasa Agricultural Research Center (MARC) under open field from November 2017 to May 2018. MARC is located 8°24'N latitude and 39°19'E with an altitude of 1550. The area receives an average annual rainfall of 786 mm. the soil of the area is loam and clay loam textural class. It has a dry climate with an average maximum and minimum temperature of 35.4 and 20.63°C (MARC, 2017).

Experimental materials, treatments and designs

Four released onion cultivars *viz.*, Nafis, Nasic Red, Bombey Red and Adama Red and five levels of root zone salinity (Awash water (0.3), 1.2, 4, 8 and 12 dSm^{-1}) were factorially combined; thus, 20 treatments (4 × 5) each replicated three times and the total of 60 experimental units were laid down using factorial arrangements in Randomized Complete Block Design.

Growing media preparation and field management

Top soil from MARC field station and black gravel were collected, sieved by mesh wire sized 2.36 mm and sterilized by solar, separately. A composite made of 3 ratio top soil and 1 ratio sieved gravel was mixed and one sample taken to MARC soil laboratory for soil physical and chemical properties determination (Table 1). A total of 64 kg soil media was filled in the box raised 40 cm from the and having 40 cm × 60 cm × 40 cm volume with 2:1:3 layers, coarse gravels, sieved gravel alone and mix of sieved gravel and top soil (3:1) from the bottom layers to upper layer, respectively to facilitate easiness of drainage problems.

Uniform and vigor seedlings were transplanted on January 24, 2018 by maintaining 5 and 10 cm spacing between plants and rows, respectively having a total of 36 plants per experimental unit sized 0.24 m^2. Salinized irrigation water application was started on February 12, 2018 or 19 days after transplanting (DAT). It was applied using watering cane according to onion crop water requirement calculated from climate data of MARC with the aid of CROPWAT 8.0 software and recently predetermined crop (Kc) of onion at the center. The irrigation water was fixed to 0.8, 2.6, 5.2 and 7.6 dSm^{-1} by considering root zone salinity to be at 1.2, 4, 8 and 12 dSm^{-1} according to FAO (1985)'s (Equation 1) .

$$ECe = 1.5\ ECw \quad (1)$$

Where, ECw= electrical conductivity of irrigation water, ECe = Electrical conductivity at root zone. Leaching requirement calculation given by FAO and SAFR (2002) (Equation 2) was used to maintain root zone soil salinity to the designed levels. Thus, additionally 17% of Awash water (0.3 dSm^{-1}) was added to each experimental unit to maintain designed salt level considering 90% leaching efficiency.

$$LR = \frac{ECw}{5ECe - ECw} \times \frac{1}{LE} \quad (2)$$

Where, LR= leaching requirement, ECw= electrical conductivity of irrigation water, ECe= electrical conductivity at root zone and LE= leaching efficiency 90% used.

Table 1. Physical and chemical properties of experimental soil.

Physical properties		**Chemical properties**			
Particle distributions (%)		pH		8.07	
Sand	40	Electrical conductivity (dSm^{-1})		0.54	
Silt	30	Total N (%)		0.14	
Clay	30	Total organic carbon (C) (%)		0.82	
		Available phosphorus (P) (ppm)		18.97	
		(Cmol/kg) soil			
		K^+	Ca^{++}	Mg^{++}	Na^+
		1.24	26.31	9.13	11.2

Fertilizer was applied using banding method according to the required rate. Chemical spray was done four times following fungal diseases and trips occurrence. During early stage at 36 and 56 days after sowing Ridomil Gold (2.6 g/L), four days after transplanting Tracer and Corragen with rate of 0.75 and 2 g/L, respectively and after 31 transplanting days Ridomil Gold (2.6 g/L) and Dursban (2.5 ml/L) in water solutions were applied. Other cultural management weeding and hoeing practices were applied based on the requirement of the crop.

Data collection

Growth parameters like number of leaves per plant, pseudo-stem diameter (neck diameter) and plant heights were recorded three times at different growth stages from five randomly selected plants within two weeks' intervals after two weeks of salt application. Whereas, leaf length, leaf width, plant height at full development, shoot fresh and dry weights and yield as well as related data like bulb length, bulb width, total soluble solids**,** fresh and dry bulb weight were taken from five plants per experimental unit according to International Plant Genetic Resources Institute IPGRI (2002) descriptor for *Allium* spp. Physiological data were taken three times from a recently developed leaf portion of each three plants per experimental unit after one month of saline water irrigation within two weeks interval. Accordingly, leaf chlorophyll contents were measured by Konica Minolta *SPAD*-502 expressed as SPAD unit, stomatal conductance was expressed by leaf porometer expressed as Steady State Diffusion Porometer *Model Decagon SC-1* and quantum yield of photosystem II) expressed as Fluorophen FP 100 at 9:00-11:00 sunny hours at different growth stages.

Data analysis

All variables collected were tested for assumptions of ANOVA prior to analysis. Parameters with no normal distribution such as bulb and fresh above ground biomass weight and bulb and dry above ground biomass weight were transformed.

RESULTS AND DISCUSSION

Number of leaves per plant

Analysis of variance revealed non-significant differences among cultivars (P= 0.70) and their interaction (P= 0.10) in leaf numbers per plant after two weeks of irrigating salinized water 30 days after transplanting (DAT) stages; but among salt levels showed significant differences p= 0.03 (Table 2). The highest average number of leaves was recorded with Awash water and 4 dSm^{-1} (3.73 per plant) salt levels which were statistically similar with 1.2 and 8 dSm^{-1} treated experimental units; the lowest number of leaves was recorded with 12 dSm^{-1} (3.05). Similar work was reported by Chang (2003); onion plant growth was visibly affected by increasing sodium chloride (NaCl) concentrations in the nutrient solutions within one week. Salinity stunted growth through reduced leaf initiation and expansion (Munns et al., 2000).

At 44 DAT and 58 DAT, after irrigating salinized water for a month and two weeks, there were significant differences in number of leaves per plant recorded for cultivars (P= 0.02) and salt level (P= 0.0001); whereas, their interaction did not show any significant difference (P= 0.58).

The highest number of leaves was recorded for Bombey Red (4.24) and Nasic Red, which was statistically at par with Nafis; whereas the lowest number of leaves was observed on Adama Red cultivar across the second and third stages. The variations among cultivars might be due to the genetic make-up of cultivars by which they maintain their physiological and metabolic activity. Salt stress levels up to 4 dSm^{-1} showed statistically similar value at 44 DAT. At 58 DAT the highest average number of leaves per plant was observed on experimental units receiving Awash water (4.27) which was at par with 1.2 dSm^{-1}; whereas number of leaves was reduced to 2.34 at the highest salt concentration of 12 dSm^{-1}. Similar work was reported by Sta-Baba et al. (2010) on onion. The average number of leaves and leaf diameter were severely affected than control. Leaves were changed from rich green to dull blue-green with salt stress; and leaf tips showed burning symptoms, typically associated with salinity stress. At higher salt stress levels, gradual decline in growth rate was observed. Reductions in the number and sizes of leaves induced by increasing salinity indicated that growth was affected at both the meristematic level and subsequent leaf expansion stages (Munns et al., 2000). There are fewer numbers of average healthy leaves per plants at high salt stress levels because leaves are scorched prior to fully leaf growth and expansion. It was also reported by Rahneshan et al. (2018) that at high NaCl concentrations, necrosis occurs and chlorophyll is lost severely in leaves. Specifically, inhibition of leaf expansion observed in the salt-treated plants was partly related to low photosynthetic rates. Also, lower water potentials of plants in the high-salt treatment might have affected cellular expansion through effects on cell turgor, resulting in reduced leaf expansion (Cosgrove, 1986).

Table 2. Mean number of leaves per plant, plant height and pseudo-stem diameter of four onion cultivars as influenced by levels of salt stress at three different growth stages of irrigating salinized water at MARC in 2018

Treatments	Number of leaves per plant, Pseudo-stem diameter and Plant height (cm)								
	30 DAT	44 DAT	58 DAT	30 DAT	44 DAT	58 DAT	30 DAT	44 DAT	58 DAT
Cultivars									
Adama Red	3.45	3.64^{b}	2.82^{b}	16.01	18.41^{b}	16.77^{c}	3.58	3.58	4.20^{c}
Bombey Red	3.55	4.24^{a}	3.84^{a}	17.21	24.95^{a}	26.12^{a}	3.72	3.72	5.36^{a}
Nafis	3.29	4.08^{ab}	3.52^{a}	15.01	20.91^{ab}	21.71^{b}	3.31	3.31	4.59^{bc}
Nasic Red	3.47	4.12^{a}	3.83^{a}	15.59	22.08^{ab}	24.07^{ab}	3.70	3.70	4.96^{ab}
CR (5%)	ns	0.45	0.37	ns	4.35	3.84	ns	ns	0.73
Salt levels (dSm^{-1})									
Awash water (0.3)	3.70^{a}	4.75^{a}	4.27^{a}	17.47^{a}	26.86^{a}	29.98^{a}	3.84	5.61^{a}	6.30^{a}
1.2	3.47^{ab}	4.37^{a}	3.95^{ab}	16.38^{abc}	24.83^{a}	26.53^{ab}	3.71	4.90^{a}	5.49^{b}
4	3.73^{a}	4.42^{a}	3.75^{b}	17.23^{ab}	24.79^{a}	24.57^{b}	3.73	4.81^{a}	4.93^{b}
8	3.25^{ab}	3.50^{b}	3.18^{c}	14.68^{bc}	17.40^{b}	17.52^{c}	3.27	3.41^{b}	3.93^{c}
12	3.05^{b}	3.07^{b}	2.38^{d}	14.03^{c}	14.05^{b}	12.24^{d}	3.35	3.32^{b}	3.23^{c}
CR (5%)	0.50	0.50	0.42	2.46	4.86	4.29	ns	0.10	0.81
CV%	17.43	15.08	14.35	20.9	27.23	23.42	19.28	27.34	20.55

Means in the column followed by the same letter(s) are not significantly different at 5% level of significance. CR (0.05) = Critical Range at the 5 % level; and CV (%) = coefficient of variation in percent. ns= non-significant.

Plant height at different growth stages

Plant height was significantly influenced by salt stress levels (P=0.04) at 30 DAT, but non-significant differences was observed among cultivars (P= 0.33) and their interactions (P= 0.43). The tallest plant height (17.47 cm) was observed with Awash water (0.3 dSm^{-1}) in which it was statistically similar up to 4 dSm^{-1}; whereas the shortest was recorded with 12 dSm^{-1} salt stress level (14.03 cm) and was also statistically at par with 8 dSm^{-1}. Second and third stages of plant height records showed highly significant differences among salt stress levels (P= 0.0001). The highest plant height was observed with Awash water (1.2 and 4 dSm^{-1}); whereas the shortest was recorded on 8 and 12 dSm^{-1} salt stress levels at 44 DAT. At 58 DAT plant height was extremely affected with 12 dSm^{-1} where the least value recorded was 12.24 cm. The tallest plant height was observed with Awash water (29.98 cm), which was statistically similar with 1.2 dSm^{-1}. Across the three stages, it was observed that plant height was gradually increased from 17.47 to 29.98 cm for Awash water irrigated units, while decreasing trend was seen at the highest salt level (12 dSm^{-1}) from 14.03 to 12.24 cm. Increase in salinity stress was accompanied by significant reduction in plant growth. Number of leaves per plant and plant height followed similar pattern with significant maximum value in control and reduction as salt level increased. This could be because sodium affects growth by increasing soil pH and directly creating nutrient deficiencies or imbalances and toxicity (Machado and Serralheiro, 2017). Salt stress was reported to affect different metabolic processes such as CO_2 assimilation, protein synthesis, respiration or phytohormone turnover (Hepaksoy, 2004), which visibly reduce plant growth and development.

For the main factor, the cultivars were significant (P=0.03) on the second stage and highly significant (P=0.0001) on the third stage; but their interaction did not show significant differences (P=0.07 and 0.21 respectively) at both stages above (Table 2). Among the cultivars, the tallest plant height was recorded for Bombey Red (24.95 cm) which was at par with Nasic Red and Nafis at second stage and with Nasic Red at third stage; whereas Adama Red had the shortest height. This indicated that Bombey Red, Nasic Red and Nafis cultivars might have their own physiology and metabolic process to maintain their photosynthetic capacity and growth than Adama Red cultivar.

Table 3. Mean comparison of leaf length, leaf width, plant height, shoot fresh and dry weights of onion cultivars under salt stress levels at MARC in 2018.

Treatments	Vegetative parameter				
	Leaf length (cm)	Leaf width (mm)	Plant height (cm)	Shoot fresh weight (g)	Shoot dry shoot weight (g)
Cultivars					
Adama Red	19.13^{b}	1.51^{b}	24.28^{b}	26.25^{c} (1.07)	4.76^{b} (1.41)
Bombey Red	23.91^{a}	1.72^{a}	29.62^{a}	38.12^{bc} (1.06)	7.67^{a} (1.46)
Nafis	23.48^{a}	1.78^{a}	27.84^{ab}	44.05^{ab} (1.07)	7.09^{a} (1.46)
Nasic Red	23.53^{a}	1.80^{a}	28.19^{a}	51.33^{a} (1.09)	7.97^{a} (1.45)
CR (5%)	3.09	0.21	3.65	0.024	0.026
Salt levels (dSm^{-1})					
0.3	29.15^{a}	2.16^{a}	34.94^{a}	70.01^{a} (1.06)	11.67^{a} (1.52)
1.2	26.87^{a}	1.87^{b}	32.51^{a}	64.18^{a} (1.10)	10.91^{a} (1.51)
4	22.11^{b}	1.71^{b}	28.02^{b}	34.35^{b} (1.08)	5.98^{b} (1.44)
8	17.08^{c}	1.35^{c}	20.48^{c}	11.40^{c} (1.06)	2.43^{c} (1.38)
12	15.50^{c}	1.32^{c}	19.11^{c}	10.65^{c} (1.03)	2.05^{c} (1.37)
CR	3.47	0.23	4.10	0.03	0.03
CV%	17.25	15.31	16.69	3.01	2.43

Means in the column followed by the same letter(s) are not significantly different at 5% level of significance. CR (0.05) = Critical Range at the 5 % level; and CV (%) = coefficient of variation in percent. Numbers in brackets are transformed by log 10 for fresh shoot weight, while log (x+C) for dry shoot weight data.

Pseudo-stem diameter

Non-significant differences were observed for pseudo-stem diameter at 30 DAT for cultivars (P= 0.33), salt levels (P= 0.19) as well as their interactions (P= 0.17). After two weeks of first stage (at 44 DAT), significant differences were observed among salt stress levels (P=0.0001), while cultivars (P= 0.21) and their interaction (P= 0.33) did not show significant differences. At 58 DAT, pseudo-stem diameter was highly significantly influenced by salt stress levels (P=0.0001), whereas significance differences were observed among cultivars (P=0.01). However, their interaction did not show any significance (P= 0.36).

At the growth stage of 44 DAT, the highest diameter was recorded with Awash water (0.3 dSm^{-1}), 1.2 and 4 dSm^{-1} salt levels, and the thinnest value for 8 and 12 dSm^{-1}. As aging increased to 58 DAT, the highest stem diameter was also recorded with Awash water (6.30 mm), where the lowest value was recorded with 8 and 12 dSm^{-1} salt stress level (Table 2). This result indicated that increased salt stress decreased pseudo-stem diameter by bringing physiological drought. This corroborates with the findings of Ghodke et al. (2018) who reported that, increased drought stress in onion reduced pseudo-stem diameter of the crop. Among the cultivars, Bombey Red showed the thickest pseudo-stem diameter (5.36 mm) compared to Adama Red which had the thinnest diameter. Our findings indicated that Bombey Red cultivar showed mild growth performance at different stages of development in comparison to other cultivars. This variation in performance was also observed under field condition during the experiment compared to Adama Red which was dead at early growth stage with the highest salt stress levels.

Leaf length

Leaf length was highly and significantly affected by cultivars (P= 0.01) and salt levels (P=0.0001), respectively. However, leaf length has not been affected by their interaction (P=0.12). The highest leaf length was recorded for Bombey Red, Nasic Red and Nafis while the shortest plant height (19.13 cm) was recorded for Adama Red (Table 3). This result confirms the field performance observed during the experiment where Adama Red cultivar was very short and poorly performed. The main differences seen among cultivars in plant height may be due to the intrinsic effects existing among themselves.

Increase in salt stress level decreased plant leaf height, where the highest leaf height 29.15 cm was recorded with Awash water (0.3) and 1.2 dSm^{-1} irrigated units and the shortest plant height was observed at 8 and 12 dSm^{-1} salt stress levels. Similar work was reported on onion leaf number and leaf length that were negatively affected at high NaCl concentrated irrigation water (Stab-Baba et al., 2010; Hanci and Cebeci, 2015). Salt stress was reported to inhibit plant height; increasing levels of NaCl in the soil showed a diminished net photosynthetic rate, which automatically limited the photosynthetic CO_2 assimilation (Saleem et al., 2011).

Leaf width

ANOVA revealed significant differences among cultivars (P=0.04) for leaf width and highly significant differences among salt stress levels (P=0.001). However, their interaction did not show significant differences (P= 0.24). The highest leaf width value was recorded for Nasic Red, Bombey Red and Nafis while the lowest value (1.51 mm) was recorded for Adama Red (Table 3). Our results indicated that Adama Red cultivar responded differently from the rest cultivars via reducing its leaf size to the salt stress level. In line with the report of Hernandez et al. (2003), salt stress inhibited cell division and cell expansion; this consequently led to leaf expansion and leaf width.

Of all the salt stress levels, the widest leaf width (2.16 mm) was recorded with Awash water (0.3), whereas 1.2 and 4 dSm^{-1} showed intermediate leaf width. The lowest value was recorded at 8 and 12 dSm^{-1} salt levels. Our results indicated that linear increase in concentration of NaCl stress levels significantly reduced leaf width of the onion cultivars. Similar finding was also supported by the work of Munns (2002) who reported that plants change their normal morphological structure in order to defend themselves from stress. It was also reported that salinity reduced final leaf width and emergency of number of lateral shoots in soybean. Dolatabadian et al. (2011) reported that salt reduced the leaf area of beet root at the whole-plant level (leaf area ratio) and at the individual leaf level (specific leaf area) (Rozema et al., 2015).

Plant height at full development

Analysis of variance showed that there were significant differences among cultivars (P=0.01) and there were highly significant differences observed among salt levels (P=0.001) in plant height. Their interaction did not show any significance (P= 0.36). The tallest plant height was recorded for Bombey Red and Nasic Red cultivars which were statistically at par with Nafis, where the lowest plant height was recorded for Adama Red (24.28 cm) cultivar.

Experimental units irrigated with Awash water (0.3 dSm^{-1} and 1.2 dSm^{-1}) showed the highest plant height, whereas the lowest plant height was observed at 8 and 12 dSm^{-1} salt levels (Table 3). At 4 dSm^{-1}, intermediate plant height was observed. Similar work was reported by Hanci and Cebeci (2015) in onion, Girma et al. (2015) in rice; salinity concentration affected plant height negatively. It was observed that onion plant height at highest salt levels (more than 4 dSm^{-1}) gradually decreased and final plants height was severely stunted. The plant height was stunted because enough hormone was released to trigger leaf abscission (Dodd, 2005), and also due to the earliest response of glycophytes to salt stress (Munns and Termaat, 1986). The decrease in the availability of cytokinins may also cause growth in inhibition of salt-stressed crops (Raghavendra, 1991). The observed reduction in the plant height may be considered as an avoidance mechanism, which minimizes water loss by transpiration when the stomata are closed (Acosta-Motos et al., 2017). Furthermore, a decrease in leaf and pseudo-stem creates a reduction in all aerial part sizes and in the plant height.

Shoot fresh and dry weight

Shoot fresh and dry weights were significantly and highly significantly affected by cultivars (P=0.04 and P= 0.0007); highly significant differences were observed among salt levels (P= 0.0001), respectively. Their interaction did not show any significant difference (P= 0.22).

Nasic Red cultivar showed the highest fresh weight (51.33 g) was statistically at par with Nafis, whereas the lowest fresh weight was recorded for Adama Red (26.25 g). The highest dry above ground biomass weight was also recorded for Nasic Red, Bombey Red and Nafis cultivars, while the least dry weight value was recorded for Adama Red (4.76 g) cultivar. The result showed that Adama Red cultivar was relatively less performing not only in dry and fresh biomass weight, but also in quantum yield, leaf number, leaf length, and plant height parameters. This could be due to the variability in internal factors through which the crops maintain their physiology and morphological characters from the induced stress.

The highest fresh and dry biomass weight was recorded with Awash water at 1.2 dSm^{-1} salt stress levels and the least were recorded at 8 and 12 dSm^{-1} salt stress levels. In this study, salt stress significantly reduced plant fresh and dry weight of onion cultivars as shown above (Table 3). These results are similar to those of tomato (Sholi, 2012), sunflower (Akram and Ashraf, 2011), mustard (Hayat et al., 2011) and okra (Saleem et al., 2011; Azeem et al., 2017). This reduction in biomass either in fresh or dry weight might be due to salt stress that significantly reduced growth parameters and photosynthetic attributes which finally reduced photo-assimilate production and translocation (Azeem et al., 2017).

Chlorophyll content

Statistical analysis revealed that chlorophyll content measured by SPAD- meter showed highly significant (P= 0.001) differences for the main factors as well as their interaction at 49 DAT, 63 DAT and 77 DAT stages.

Table 4. Interaction effects of onion cultivars and levels of salt stress on chlorophyll (in SPAD mmolm^{-2}s^{-1}) and stomatal conductance (in mmolm^{-2}s^{-1} determined with a porometer) at three growth stages under MARC in 2018.

Cultivars	Salt levels	Physiological parameter					
		SPAD (*mmolm^{-2}s^{-1}*)			Porometer (*mmolm^{-2}s^{-1}*)		
		49 DAT	**63 DAT**	**77 DAT**	**51 DAT**	**65 DAT**	**79 DAT**
Adama Red	0.3	17.76^{ab}	4.6^{gh}	9.58^{bc}	135.35^{cd}	105.32^{cd}	146.06^{a}
	1.2	8.19^{efg}	7.38cdefg	5.45^{ef}	178.41^{a}	100.94^{cd}	83.81^{gh}
	4	20.36^{a}	7.65cdefg	7.06^{cdef}	107.55^{defgh}	109.19^{c}	87.55^{fgh}
	8	17.90^{ab}	9.27^{bcd}	4.88^{ef}	76.19^{hij}	90.4^{de}	96.36^{defg}
	12	11.89^{cde}	8.15^{cdef}	4.24^{f}	115.8^{cdef}	76.92^{ef}	125.37^{b}
Bombey Red	0.3	8.62^{efg}	6.03^{efg}	9.56^{bc}	62.87^{j}	99.54^{cd}	108.28^{cd}
	1.2	7.64^{fg}	9.82^{bc}	6.6cdef	106.07^{defgh}	89.01^{de}	89.78^{efgh}
	4	18.103^{ab}	9.27^{bcd}	14.5^{a}	96.98^{efghi}	52.27^{g}	52.72^{j}
	8	8.98^{defg}	2.65^{h}	9.28^{bcd}	82.93^{fghij}	96.17^{cd}	70.16^{i}
	12	8.6^{efg}	8.79^{cde}	9.53^{bc}	74.49^{hij}	60.79^{fg}	99.22^{def}
Nafis	0.3	7.07^{g}	12.11^{b}	14.71^{a}	129.44^{cde}	66.01^{fg}	84.47^{gh}
	1.2	9.62^{defg}	29.88^{a}	6.5cdef	107.43^{defgh}	62.65^{fg}	48.98^{j}
	4	12.39^{cd}	6.54^{defg}	15.8^{a}	193.38^{a}	144.10^{b}	102.11^{de}
	8	10.94^{def}	5.79^{efg}	6.97^{cdef}	113.28^{cdefg}	105.13^{cd}	71.61^{i}
	12	6.257^{g}	7.25^{defg}	5.96^{def}	69.62^{ij}	98.07^{cd}	79.07^{hi}
Nasic Red	0.3	14.823^{bc}	9.53^{bcd}	11.2^{b}	168.11^{ab}	133.16^{b}	144.38^{a}
	1.2	7.81fg	9.93^{bc}	8.3bcde	81.02^{ghij}	187.5^{a}	114.81^{bc}
	4	9.87^{defg}	4.91^{gh}	6.79^{cdef}	143.06^{bc}	147.50^{b}	96.04^{defg}
	8	8.97^{defg}	5.36^{fgh}	5.54^{ef}	139.58^{bcd}	71.27^{f}	89.22^{fgh}
	12	17.79^{ab}	10.46^{bc}	5.917^{def}	129.31^{cde}	95.43^{cd}	84.64^{gh}
	CR (5%)	3.219	2.77	2.99	29.57	15.77	11.42
	CV%	16.56	19.32	22.03	15.64	9.68	7.52

Means in the column followed by the same letter(s) are not significantly different at 5% level of significance. CR (0.05) = Critical Range at the 5 % level; and CV (%) = coefficient of variation in percent.

The interaction effects indicated that the highest SPAD value was recorded for Adama Red at 4 dSm^{-1} (20.36 mmolm^{-2}s^{-1}), it was statistically at par with Adama Red with Awash water and 8 dSm^{-1} and also Nasic Red at 8 dSm^{-1}. Nafis with 1.2 dSm^{-1} (29.88 mmolm^{-2}s^{-1}) at second stage and Nafis with Awash water and 4 dSm^{-1}, Bombey Red with 4 dSm^{-1} showed the highest SPAD values at third stage (Table 4). The least SPAD value interacted with Nafis at 12 dSm^{-1} (6.25 mmolm^{-2}s^{-1}), Adama Red with Awash water (4.6 mmolm^{-2}s^{-1}) and Adama Red at 12 dSm^{-1} salt levels (4.24 mmolm^{-2}s^{-1}), at 49 DAT, 63 DAT and 77 DAT, respectively. Thus, the result implied that each cultivar had independent response to maintain water content of leaves and leaf chlorophyll after prolonged stress duration may indicate a potential mechanism of osmotic adjustment in low to moderately high salinity (Stavridou et al., 2017). However, it was reported that SPAD value of chlorophyll decreased significantly in the stressed leaves, because of salinity which either inhibits synthesis and/or accelerates the degradation of the existing chlorophyll molecules (Wani et al., 2013).

Stomatal conductance

ANOVA for the main factors and their interaction effects on stomatal conductance (gs) determined by porometer across the three stages (51 DAT, 65 DAT, and 79 DAT) showed highly significant variances (P= 0.001). The highest gs was recorded for Nafis at 4 dSm^{-1} and Adama Red at 1.2 dSm^{-1} which was statistically at par with Nasic Red with Awash water at first stage. Nasic Red with 1.2 dSm^{-1} (187.5 mmolm^{-2}s^{-1}) at second stage and Awash water with Adama Red and Nasic Red showed the highest gs at third stage (Table 4). The least gs was observed in Bombey Red with Awash water (62.87 mmolm^{-2}s^{-1}) at 51 DAT, which was not statistically different from Adama Red with 8, Bombey Red with 8 and 12, Nafis with 12 and Nasic Red with 1.2 dSm^{-1}. At 65 DAT, Bombey Red with 4 dSm^{-1} (52.27 mmolm^{-2}s^{-1}) which was par with Bombey Red with 12, Nafis with Awash water and 1.2 dSm^{-1} and at third stage Bombey Red with 4 dSm^{-1} and Nafis with 12 dSm^{-1} showed the least gs. Likely Azeem et al. (2017) reported that increased salt levels decreased gs of okra cultivars. A significant decrease in the gs of plants exposed to increased levels of salt stress diminished net photosynthetic rate, by limiting internal CO_2 concentration and transpiration rate (Saleem et al., 2011). Although the current study did not show linear increase or decrease of gs on salt stress levels because cultivars responded independently, salt stress levels above 8 dSm^{-1} were highly affected leaf gs.

Table 5. Mean quantum yield of onion cultivars as influenced by levels of salt stress at three different growth stages at MARC in 2018.

Treatments	**Quantum yield**		
	40 DAT	**54 DAT**	**68 DAT**
Cultivars Adama Red	0.531 [b]	0.462 [b]	0.413 [b]
Bombey Red	0.599[a]	0.511[ab]	0.538[a]
Nafis	0.614[a]	0.536[a]	0.532[a]
Nasic Red	0.612[a]	0.543[a]	0.476[ab]
CR (5%)	0.05	0.057	0.09
Salt levels (dSm^{-1}) Awash water (0.3)	0.635 [a]	0.58 [a]	0.537
1.2	0.611[a]	0.55 [a]	0.491
4	0.602[a]	0.55 [a]	0.49
8	0.585[a]	0.45[b]	0.479
12	0.522[b]	0.43[b]	0.451
CR (5%)	0.05	0.06	ns
CV%	11.19	15.03	24.81

Means in the column followed by the same letter(s) are not significantly different at 5% level of significance. CR (0.05) = Critical Range at the 5 % level; and CV (%) = coefficient of variation in

Quantum yield

Two way analysis of variances showed highly significant differences for quantum yield of photosystem II (PS II) among cultivars at 40 DAT and 54 DAT (P= 0.004), and 68 DAT (P=0.02), but their interaction did not show any significant difference for the cultivars (P=0.06, 0.55 and 0.90) respectively. Salt stress levels significantly influenced quantum yields at 40 DAT and 54 DAT (P=0.004), while the third stage (68 DAT) did not show any significant difference (P=0.55).

The highest quantum yield was recorded for Nafis, Bombey Red and Nasic Red, whereas Adama Red showed the lowest at the first stage (Table 5). At 54 DAT Nafis and Nasic Red showed the highest quantum yield which was at par with Bombey Red. At third stage Bombey red and Nafis showed the highest quantum yield, which was also statistically similar with Nasic Red. Across the three stages, the least quantum yield value was recorded for Adama Red cultivar (0.531, 0.462, and 0.413, respectively). This result indicated that Adama Red cultivar was more salt sensitive than other cultivars. Decrease in quantum yield was reported to be an indicative parameter for salt sensitivity in rape genotypes (Pak et al., 2009) and tomato (Moniruzzaman et al., 2013). This also ensures the field performance of Adama Red cultivar, in which there was early dead and scorched leaves, especially at 12 dSm^{-1} salt level.

The highest quantum yield value was recorded from Awash water (0.3 dSm^{-1}), up to 8 and 4 dSm^{-1} salt levels at 40 DAT and 54 DAT, respectively. The least values were observed with 12 dSm^{-1} (0.522) at first stage, whereas the second stages of records showed the least quantum yield with 8 and 12 dSm^{-1}. The result showed that as salt stress level increased quantum yield response showed a decreasing trend. Similar work was reported by Pak et al. (2009) in salt sensitive genotypes of rape genotypes and Moniruzzaman et al. (2013) in tomato genotypes. Satoh et al. (1983) in red algae noticed that as salt level increased quantum yield of the genotypes decreased. Photosystem II (PSII) is a multisubunit chlorophyll protein complex that drives electron transfer from water to plastoquinone using energy derived from light (Minagawa and Takahashi, 2004). According to

Murata et al. (2007), salt stress suppressed not only synthesis of the D1 protein, but also the synthesis of almost all other proteins. They found that salt stress, due to 0.5 M NaCl, inhibited the repair of photo damaged PSII, but did not directly accelerate photo damage to PSII. It was also reported that, high concentrations of NaCl inactivate the translational machinery (or ribosomes), inactivate RuBisCO and inhibit CO_2 fixation; salt stress induces the generation of reactive oxygen species (ROS), which, in turn, inhibit protein synthesis, inactivate ATP synthase and decrease the intra-cellular level of ATP, which is essential for protein synthesis (Nishiyama et al., 2011). Thus, increase in the electrical conductivity of the irrigation water reduces the quantum efficiency of PSII and this effect was reported to be the attribution of low capacity of synthesis of proteins present in the thylakoid membranes (Sousa et al., 2016) (Table 5).

Table 6. Mean comparison of bulb length, width, fresh and dry weight and total soluble solid of onion cultivars evaluated under five salt stress levels at MARC in 2018.

Treatments	Bulb length (mm)	Bulb width (mm)	Bulb fresh weight (g)	Bulb dry weight (g)	TSS (°Birx)
Cultivars Adama Red	26.39^{b}	15.18^{b}	28.15^{b} (1.08)	4.55^{b} (1.55)	11.73^{b}
Bombey Red	30.73^{a}	20.41^{a}	76.87^{a} (1.57)	8.85^{a} (1.59)	11.52^{b}
Nafis	28.84^{ab}	22.07^{a}	49.43^{ab} (1.56)	7.37^{ab} (1.58)	11.70^{b}
Nasic Red	30.50^{a}	18.90^{a}	64.05^{a} (1.48)	8.15^{a} (1.59)	12.47^{a}
CR (5%)	2.8	3.55	0.2	0.04	0.68
Salt levels (dSm^{-1})					
Awash water (0.3)	35.28^{a}	26.54^{a}	106.75^{a} (1.93)	11.01^{a} (1.62)	11.71^{bc}
1.2	34.04^{a}	25.49^{a}	90.73^{a} (1.91)	11.32^{a} (1.63)	12.78^{a}
4	29.55^{b}	19.16^{b}	43.50^{b} (1.41)	7.42^{ab} (1.58)	12.21^{ab}
8	22.43^{c}	10.55^{c}	10.33^{c} (0.98)	4.73^{bc} (1.54)	11.41^{bc}
12	22.22^{c}	11.64^{c}	9.90^{c} (0.78)	1.08^{c} (1.51)	10.94^{c}
CR (5%)	3.14	4.02	0.22	0.04	0.77
CV%	12.36	24.08	17.75	3.11	7.47

Means in the column followed by the same letter(s) are not significantly different at 5% level of significance. CR (0.05) = Critical Range at the 5 % level; and CV (%) = coefficient of variation in percent. Numbers in brackets are transformed by log 10 for fresh bulb weight, while log (x+C) for dry bulb weight data.

Bulb length and width

Two ways of analysis showed highly significant variations for bulb length and bulb width among the cultivars (P=0.004). The analysis also depicted highly significant differences of bulb length and width among the salt levels (P= 0.0001). Their interaction did not show any significant differences for bulb length and width (P= 0.19).

The highest bulb length was recorded for Bombey Red and Nasic Red, whereas the highest bulb width was recorded for Nafis, Bombey Red and Nasic Red. The least bulb length (26.39 mm) and width (15.18 mm) were recorded for Adama Red cultivar. Although, it is difficult to definitely determine performance based on only bulb length and width, due to its dependence on nature of crop bulbs shape. This result indicated that Adama Red cultivar was reduced in bulb length and width than others. Table 6 indicated that for Nafis cultivar, bulb length showed medium length; while bulb width was the highest due to its nature of bulb shapes. Generally, the result indicated that Bombey Red, Nasic Red and Nafis were the most performing cultivars in bulb width.

The highest bulb length and width were recorded on Awash water (0.30 dSm^{-1}) and 1.2 dSm^{-1} salt levels; whereas the least bulb length and width were observed at 8 and 12 dSm^{-1} salt levels. This result showed that an increased salt concentration levels more than threshold (1.2 dSm^{-1}) radically reduced bulb length and width. Supportive work was reported by Kahouli et al. (2014) in carrot; increase in salt concentration decreased root diameter and length. The reduction in bulb length and width under high NaCl salt concentration could be because salts induced internal water deficit which caused partial or complete closure of stomata. Azeem et al. *(*2017) finally inhibited leaf expansion, reduced net photosynthetic capacity of the plants, leading to reduction in biomass production (Saleem et al., 2011).

Bulb fresh and dry weight

Fresh and dry bulb biomass weights were significantly influenced by cultivars (P=0.02 and P=0.03), respectively. There were also highly significant differences in fresh and dry bulb biomass weights observed among salt levels (P= 0.0001). However, their interaction did not show any significance (P=0.46) for the two parameters.

The highest fresh and dry bulb biomass weights were recorded for Bombey Red and Nasic Red which were statistically similar with Nafis; whereas the least fresh and dry bulb biomass weight was recorded for Adama Red (28.15 g) (Table 6). The result pinpointed Bombey Red, Nasic Red and Nafis cultivars to perform better than Adama Red cultivar, which has relatively low performance. The differences among cultivars might be due to internal factor by which they maintain their morphological and physiological parameters. This finally influences their dry matter accumulation and yield.

The highest fresh bulb biomass weight was recorded on Awash water (0.3 dSm^{-1}) and 1.2 dSm^{-1}, whereas the least was recorded on 8 and 12 dSm^{-1} salt stress levels. However, the least dry bulb biomass weight was recorded on 12 dSm^{-1} which was statistically similar with 8 dSm^{-1}; whereas the highest dry biomass was recorded on Awash water and 1.2 dSm^{-1} which were statistically at par at 4 dSm^{-1} salt level. The result indicated that increase in salt concentration reduces fresh and dry biomass of onion cultivars. It was also reported by Backhausen et al. (2005) that fresh and dry weight decreased by 30% due to the increase in NaCl more than 5 dSm^{-1}. There was an increase in dry weight at 1.2 dSm^{-1} irrigated units than the control. This might be because salt concentration to certain limit may increase total soluble solid. Hepksoy (2004) reported that salinity increases total sugar contents and all sugar fractions of fruits *Sastuma madrin c.* Owari. Up to 4 dSm^{-1} salt levels of irrigation water mild dry weight and fresh biomass weights were recorded.

Total soluble solid (TSS)

TSS was significantly and highly affected by cultivar (P=0.04) and salt levels (P= 0.0002), respectively. Statistical analysis did not reveal significant differences among their interaction p= 0.30.

The highest TSS was recorded for Nasic Red (12.47 ^{o}brix), while the lowest Brix was observed in Bombey Red, Adama Red and Nafis cultivars. Under natural condition Nasic Red and Nafis are known to have the highest 10- 18 ^{o}Brix of TSS while Bombey Red was the least of all (Zelleke and Derso, 2015). Under current study similar result was found in respective of their proportion. This variation in TSS among the cultivars might be due to their genetic constituents.

The highest TSS in 12.78 ^{o}Brix was observed on 1.2 dSm^{-1} which was statistically similar with 4 dSm^{-1} salt stress level; whereas the least was recorded on 12 dSm^{-1} (10.94 ^{o}Brix) which was statistically at par with 0.3 dSm^{-1} and 8 dSm^{-1} treated units (Table 6). This indicated that salt stress to certain level might increase TSS as compared to the lowest salt concentration. Similar work was reported by Ghodke et al. (2018) that under forced drought stress TSS was slightly elevated in comparison to routinely irrigated plot. Hepaksoy (2004) also reported that *Sastuma madrin c.* Owari orange orchard grown near sea compared to that farthest from the sea showed the highest TSS. The increase in these TSS was reported to regulate its osmosis, and improve its metabolic processes during stress conditions (Ripoll et al., 2014).

CONCLUSIONS

Salt stress is limiting onion production and productivity due to crop sensitivity to salt stresses. Our study results indicated that growing onion cultivars with salt stressed water significantly affected growth variables, physiology, yield and yield components of onion across the growing stages. Onions leaf length, leaf width, fresh and dry above ground biomass weight, fresh and dry bulb biomass weights, TSS, bulb length and width were affected significantly ($p \leq 0.05$) by cultivars and highly significantly ($p \leq 0.001$) by salt levels. However, during early growth stage of up to 4 dSm^{-1} did not affect the number of leaves per plant, pseudo stem diameter, plant height, but gradually decreased after 58 DAT. The highest number of leaves per plant, plant height and pseudo stem diameters were observed in Bombey Red and Nasic Red, which were statistically similar with Nafis; whereas Adama Red cultivar showed the lowest number of leaves, shortest and thinnest across the stages. Physiological parameters like chlorophyll and stomatal conductance taken across the stages showed highly significant variations among the main factors and their interactions; whereas quantum yield of PSII showed significance variation at 40th DAT and 68th DATs among cultivars and salt levels at 40 and 54 DATs stages. The highest quantum yield was recorded for Nasic Red, Nafis and Bombey Red, where Adama Red showed the least. Quantum yield was reduced as salt concentration level increased. Bombey Red, Nafis and Nasic Red cultivars showed the highest performance in leaf length and width, plant height, bulb length and width, fresh and dry bulb weight, dry above ground biomass. The highest leaf length, plant height, leaf width, TSS (12.78 ^{o}Brix), dry bulb weight (11.32 g), fresh and dry above ground biomass weight (70.01 g and 11.67g), bulb length and width were recorded at 1.2 dSm^{-1} salt stress level. Generally, most of the variables during early growth stages were not affected up to 4 dSm^{-1}; whereas the highest growth and yield performances were recorded at 1.2 dSm^{-1} salt stress levels. It is concluded that our cultivars cannot resist salt stress beyond 4 dSm^{-1} and Bombey Red, Nafis and Nasic Red can be used for salt levels less than 4 dS/m. However, the experiment should be repeated under controlled environment adding more cultivars and less than 4 dSm^{-1} salt levels in the future.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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