# GERMINATION, GROWTH, PHYSIOLOGY AND YIELD OF ONION (Allium cepa L.) UNDER SALT STRESS

M. Sc. THESIS

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# Germination, Growth, Physiology and Yield of Onion (Allium

# cepa L.) under Salt Stress

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M.Sc. Thesis

Submitted to the Department of Horticulture and Plant Sciences, Jimma University, College of Agriculture and Veterinary Medicine, in Partial Fulfillment of the Requirements for the Degree of Master of Science in Horticulture

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October, 2018 Jimma, Ethiopia

# DEDICATION

This thesis work is dedicated to my beloved father Daba Regessa and my mother Desta Gragn.

# **STATEMENT OF THE AUTHOR**

I declare that this thesis is my genuine work and I have duly acknowledged all sources of materials used for writing it. This thesis has been submitted in partial fulfillment of the requirements of M.Sc. degree, Jimma University, College of Agriculture and Veterinary Medicine and is deposited at the University Library to be made available to borrowers and readers under the rules and regulations of the library.

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### **BIOGRAPHICAL SKETCH**

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# ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
CR	Critical Range
CSA	Central Statistical Agency
DAT	days after transplanting
DMRT	Duncan Multiple Range Test
ECe	electric conductivity of soil at root zone
ECw	electrical conductivity of irrigation water
LE	leaching efficiency
LR	leaching requirement
MARC	Melkasa Agricultural Research Center
PSII	photosystem two
ROS	reactive oxygen species
SAR	Sodium Absorption Ratio
SVI	seedling vigor index
TSS	total soluble solid

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Germination, Growth, Physiology and Yield of Onion (Allium cepa L.) under Salt Stress

# ABSTRACT

Onion is one of irrigated crop produced by smallholder farmers and commercial growers for both local and export markets in Ethiopia. However, low productivity is reported by producers due to salinity. In view of this, study was initiated with the objective of determining the relative tolerability of onion cultivars to salt stress levels in laboratory and field condition on soil media filled in box, irrigated with salinized water at Melkasa Agricultural Research Center, Five onion cultivars (Adama Red, Bombey Red, Nasic Red and Nafis) and distilled water plus four salt levels (1.2, 4, 8 and 12  $dSm^{-1}$ ) were factorially (5x5) arranged in Complete Randomized Design with three replications for germination test. Except Agrifound, four of the above cultivars and Awash water (0.3  $dSm^{-1}$ ) instead of distilled water, were combined factorially (4 x5) arranged in Randomized Complete Blocked Design with three replications in the field. Data from laboratory and field were subjected to analysis of variance using SAS version 9.3. Laboratory result showed variations in seed germination percentage, seedlings root and shoot length, shoot to root length ratio, seedling vigor index, fresh and dry weight among salt levels ( $p \le 0.001$ ). Except, for germination rate and shoot to root length ratio non-significant differences were observed among cultivars (P>0.05). Germination percentages and seedling vigor index were higher with distilled water and gradually decreased as salt stress levels increased to 12  $dSm^{-1}$ . The fastest germination rate was recorded for Adama Red and Nafis. Adama Red showed maximum (5.52) seedling shoot to root ratio while Agrifound (4.20) was the least. Field data analysis revealed variation in leaf length, leaf width, plant height, fresh and dry above ground biomass weight, Total Soluble Solid (TSS), bulb length and width among cultivars ( $p \le 0.05$ ) and salt stress levels  $(p \le 0.001)$ . Chlorophyll in SPAD- meter and stomatal conductance in porometer across three stages showed highly significant ( $p \le 0.001$ ) variations for main factors and interactions, whereas quantum yield showed significant difference at 40<sup>th</sup> DAT and 68<sup>th</sup> day after transplanting (DAT) among cultivars ( $p \le 0.05$ ) and salt levels ( $p \le 0.001$ ) at 40 and 54 DAT stages. The highest quantum was recorded for Nasic Red, Nafis and Bombey Red, where Adama Red showed the least. Leaf length and width, plant height, fresh and dry above ground biomass weight, fresh and dry bulb weights, TSS, bulb length and width were affected significantly by cultivars ( $p \le 0.05$ ) and salt stress levels ( $p \le 0.001$ ). 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 <sup>o</sup>brix), dry bulb weight (11.32 g), fresh and dry above ground weight (70.01 g and 11.67g), bulb length and width were recorded with 1.2 dSm<sup>-1</sup> salt level. Generally most of germination variables and early growth stages were not affected up to 4  $dSm^{-1}$ , whereas the highest growth and yield performances were recorded with 1.2 dSm<sup>-1</sup> salt stress levels in the field. Bombey Red, Nafis, and Nasic Red can be used for salt levels less than 4 dS/m. Indeed, experiment should be repeated under controlled environment including other cultivars and reducing salt levels to 4 dSm<sup>-1</sup> in the future.

Key words: NaCl, quantum yield, leaf length, bulb, chlorophyll, porometer

## **1. INTRODUCTION**

The genus *Allium* consists of onion (*Allium cepa* L.) which is a subspecies and primary member of Alliaceae family. Onion is an ancient crop that is thought to originate in Central Asia and has been cultivated for over 5000 years but today distributed over worldwide (Platt, 2003; Khosa *et al.*, 2016).

Currently, in Ethiopia, it is a high-value bulb crop produced by smallholder farmers and commercial growers for both local and export markets (Nega *et al.*, 2015). Onion is important in the daily Ethiopian diet and all the crop parts are edible, bulbs are widely used as a seasoning in various dishes (Abdissa *et al.*, 2011; Reddy and Kanna, 2016). It is one of the most economically important horticultural crops in the country. It is an indispensable part of the daily meal of the Ethiopian dish as it improves the taste and scent of the food because of its distinct pungent flavor and is an essential ingredient for the cuisine in many regions (White and Zellner, 2008). The bulb used in soups, sauces, condiments, spice, in medicine, seasoning of many foods and for the preparation of value added edible products like powder, flakes, and salts (Opara, 2003).

In Ethiopia onion covers an estimated annual total production area of 22,767 ha both under rain-fed and irrigated condition (CSA, 2015). Increasing of population number, soil fertility degradation needs extra land under cultivation by irrigation in addition to rainy season production to feed growing population. Central rift valley of Oromia which is the major onion production area uses irrigation facility (Asres, 2016). However, onion production and productivity was constrained by biotic and abiotic factors among which soil salinity claimed by producers (Etissa *et al.*, 2014). In parallel to irrigation expansion there is also expansion of salinity problems.

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 (ECe > 4 dSm<sup>-1</sup> and 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

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(Olumana *et al*, 2009; Tadesse *et al.*, 2010), where downstream irrigated area production is constrained. The salinization of soils dedicated to agriculture, caused by the accumulation of salts in irrigation water, causes these soils to become increasingly unproductive (Lima and Bull, 2008). When irrigation waters have a high concentration of salts and there is no possibility of exporting these brackish waters to a sink, they can accumulate and cause damage (Dos-Santos *et al.*, 2009). Such accumulation can limit the germination and development of various food crop species (Barroso *et al.*, 2010), leading 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 osmolality (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<sup>-2</sup>), the hydroxyl radical (OH<sup>-</sup>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and singlet oxygen (O<sub>2</sub>) (Stanisavljevic *et al.*, 2011).

Nutrient competitions in both drought as well as salt stressed areas reduce crop growth by affecting the availability, transport, and partitioning of nutrients (Hu and Schmidhalter, 2005). However, drought and salt stress may differently affect the mineral nutrition of onions. Salt stress may cause nutrient deficiencies or imbalances, due to the competition of Na<sup>+</sup> and CT with nutrients such as  $K^+$ ,  $Ca^{2+}$ , and  $NO_3^-$  (Hu and Schmidhalter, 2005). Study on the growth and yield of chilli pepper (Capsicum annuum L.) revealed that application of Nitrogen (N) rates of 140 kg ha<sup>-1</sup> or more increased soil salinities, by 4 dS m<sup>-1</sup> in some cases. It was observed that increasing 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 of onion crop seriously affected at higher salinity of irrigation water (4000 ppm) as 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 Mg nutrients as a result of competition between Na<sup>+</sup>, Cl<sup>-</sup> 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 vary within species of 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 proposal was initiated with the following general and specific objectives.

### **General objective**

To identify the relative tolerability of onion cultivars to salt stress levels in laboratory and field on soil media at Melkasa Agricultural Research Center.

### **Specific objectives**

- To determine germination ability of onion cultivars under salt stress levels in laboratory condition.
- To evaluate onion cultivars for yield and yield components performance under salt stress levels on soil media.

## **2. LITERATURE REVIEW**

#### 2.1. Overview of Salinity and Irrigated Agriculture

Worldwide crop productivity is limited by abiotic stresses such as salt stress, drought, nutrient deficiency, or toxicity, and flooding. The global annual cost of salt-induced land degradation in irrigated fields could be \$ 27.3 billion (Qadir et al., 2014). Salt affected and degraded 20% of cultivated land in the world, and 33% of irrigated land (Machado and Serralheiro, 2017). It was reported by Shrivastava and Kumar (2015) an estimated area of 45 million ha (20%) of irrigated agricultural land producing one-third of the world's food is salt-affected. In developing countries, where salt stress causes food and nutritional insecurity for large populations and poverty, particularly in rural areas this situation becomes more problematic. For example, drought stress has affected more than 70 million hectares of rice-growing land worldwide whereas, more than 100 million hectares of agricultural land is uncultivable because of salt stress and substandard use stress resulting in low outputs which further brought human malnutrition and less access to education and employment opportunities (Athar and Ashraf, 2009). Salinity is one of the fiercest abiotic factors limiting the productivity of crops due to crop sensitivity to salinity caused by over stresses of salts in the soil. At present, the size of agricultural land affected by salt stress is increasing from time to time. For most food crops, average yields are in the ranges from 20% to 50% of observed yields; these losses are mainly due to drought and soil salinity conditions which might worsen in several regions because of climate change (Shrivastava and Kumar, 2015).

Soil salinization is a major factor contributing to low productivity of agricultural soil (Mahajan and Tuteja, 2005). Although difficult to estimate accurately, the size of salinized soil is increasing, and this phenomenon is especially intense in irrigated lands. In Ethiopia, irrigated agriculture is affected by severe water logging and salinity problems challenging crops production resulting in significantly lower yields than the potential (Gebrehiwot, 2017). Small holders irrigated agriculture in highland area featured with vertisol; salinity and salinization are limiting crop productivity due to the drainage problem, whereas in lowland areas the large and medium scale irrigation schemes using river basins are affected by salt

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stresses. The frequent occurrence of water-logging and salt build-up in irrigation fields are as result of non-functional drainage system and poor water management practices (Gebrehiwot, 2017).

#### 2.2. Salinity Associated Problems and Trends in Ethiopia

Accumulation of salts over long periods of time, through weathering of parent materials containing soluble salts, groundwater results in salt stress as primary salt formation. It is caused by natural processes. Weathering processes break down rocks and release soluble salts of various types, mainly chlorides of sodium, calcium, and magnesium, and to a lesser extent, sulfates, and carbonates. Sodium chloride (NaCl) is the most soluble salt (Munns, 2017).

According to study in the Middle Awash Basin, around 34 % hectarage of the command area has been mapped as saline soil (ECe > 4 dSm<sup>-1</sup> and SAR < 13) whereas, only 0.05% (9.1 ha) scheme was classed as saline-sodic (ECe > 4 dSm<sup>-1</sup>, and SAR >13) (Abebe *et al.*, 2015). From the thematic maps generated, it is concluded that proportion of the field affected by salt stress is rapidly increasing. More and more field is fully abandoned due to salt stress problem. This is mainly as result of non-functional drainage system and poor water management practices (Gebrehiwot, 2017). Abebe *et al.* (2015) asserted that the water table control by rehabilitating the subsurface drainage system seems to be the only feasible way to improve the sustainability of the scheme. It was also reported that misuse of irrigation water, soil salt stress, water-logging, sedimentation, soil erosion and degradation were challenging vegetable production in Central Rift Valley of Oromia, including Awash Melkasa areas (Etissa *et al.*, 2014).

Salinity problems are encountered in all agro climatic condition and are consequences of natural (primary) and human-induced (secondary) processes (FAO, 2015). In Ethiopia, an estimated area of about 11 million ha land is exposed to salinity and sodicity (Qureshi, 2016). Bellete (2016), reported that about 1.5 million ha of fertile valley bottom soils are affected by salinity. In the Awash river basin the situation is feared to aggravate salinity in the future due to climate change induced elements (Qureshi, 2016). Increased irrigation water volume and

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irrigation water mis-management practices might aggravate the problem (Bellete, 2016). Soil salinity and sodicity problems are more common where rainfall is insufficient to leach salts and excess sodium ions out of the rhizosphere. Salt-affected soils often occur on irrigated lands, especially in arid and semiarid regions, where annual rainfall is insufficient to meet the evapotranspiration needs of plants and to provide for leaching of salt (FAO, 2015). Salt-affected soils must be restored to productivity and effective steps taken to prevent salinization of new areas being brought under irrigation at huge cost (Bellete, 2016). Increasing salinity remains a challenge to the sustainability of irrigated agriculture in Ethiopia and South Sudan as it reduces natural biodiversity as well as farm and livestock productivity (Qureshi, 2016).

### 2.3. Irrigated Onion Production in Central Rift Valley Areas of Ethiopia

Currently, the area of land potentially irrigable is estimated at 2.7 million hectares in Ethiopia (FAO, 2016). From maximum 5.7 million ha potential of available water and land resources, 3.7 million ha is commonly quoted among which around 83,000 ha are located in the Middle Awash Valley (Bekele, 2010). In Ethiopia, small-scale irrigated onion production is characterized by low irrigation water productivity (Derib et al., 2011). However, compared to non-irrigation users farmers, irrigation users are more profitable in income earning per hectare of land (Makombe et al., 2007). According to case study at Meki by Haileslassie et al. (2016), on-farm smallholder irrigation performance showed the highest irrigation productivity for onion and tomato with the magnitude of 14.55 and 10.29 tons/ha respectively. According to survey work conducted on small-scale irrigation users of 500 agro-pastorals households at Amibara and Fentale woreda's of the Awash basin, onion cultivar Bombay Red yielded on average 19.3 tons per ha at increasing rate of returns to production where the household generated to income in profitability rate (Nigussie et al., 2015). With the average landholding size per household of 4.7 ha, an average land allocation for onion productions are 2.2 and 1.1 ha per household in Amibara and Fentale woreda's respectively. This indicates how onion production is very important to generate income for the household in the area.

#### 2.4. Response and Variability of Onion Cultivars to Salinity Stress

Agricultural crops productivity hindered under excessive soil salt stress of which most vegetables are particularly sensitive throughout the developmental phases of the plant. The salinity threshold (ECt) of the majority of vegetable crops is low (ranging from 1.0 to 2.5  $dSm^{-1}$  in saturated soil extracts) and vegetable salt tolerance decreases when saline water is used for irrigation (Machado and Serralheiro, 2017). Its threshold level is reported to be 1.2  $dSm^{-1}$  (Maas and Hoffman, 1977), whereas salt stresses less than 2, 2-4, 4-8, 8-16  $dSm^{-1}$  are found to be non-saline, slightly saline, moderately saline, and highly saline respectively. Even though onion classified as salt-sensitive vegetable crop, its production found in saline soils of the World (Chang and Randle, 2004). The reaction to salinity is reported to be varying among different onion cultivars (Ansari and khaleghi, 2009).

Developmental phases of the plant, multiple biochemical pathways that facilitate retention and/or acquisition of water, protect chloroplast functions, and maintain ion homeostasis determines the ability of plants to tolerate salt (Parida and Das, 2005). Sta-baba et al. (2010) reported that gradual increase in saline irrigation water application reduces leaf number, plant height and final bulb size of onion cultivars. Growth is first reduced by a decrease in the soil water potential (osmotic phase) and later a specific effect appears as salt injury in leaves, which die because of a rapid increase in salt in the cell walls or cytoplasm when the vacuoles can no longer sequester incoming salts (ionic phase). Salt accumulation in the old leaves accelerates their death and thus decreases the supply of carbohydrates and/or growth hormones to the meristematic regions, thereby inhibiting growth (Munns, 1992). In onion salt stress reported to inhibit plant height, increasing levels of NaCl in the soil showed a diminished net photosynthetic rate, which will limit automatically the photosynthetic CO<sub>2</sub> assimilation (Saleem et al., 2011). The two main approaches employed to improve salt tolerance are the use of natural genetic variation by using direct selection in stressful environments or mapping quantitative trait loci and subsequent marker-assisted selection. For these screening cultivars under salt stress environment enables to identify tolerant cultivar/genotype. Hence, variability across stage may be different, where mainly germination and field performance of onion cultivars will be discussed.

#### 2.4.1. Germination

The survival and perpetuation of many crop species depend on the ability of seeds to germinate at high salt concentration in the soil (Bojovic *et al.*, 2010). However, high salinity reduces seedling viability and vigor, as well as activating the antioxidant defense system of germination and development of several plant species (Correa et al., 2013). Onion cultivars, viability and vigor decreased in parallel with increasing NaCl concentrations, whereas antioxidant enzyme activity increased. The study by (Correa et al., 2013) showed that one cultivar (Madrugada) less salt tolerance than Fepagro 27 and Petroline cultivars were found to be tolerant to salt stress. It was concluded that high NaCl concentrations have a negative effect on the physiological quality of onion seeds, resulting in lower seedling growth rates and increased antioxidant enzyme activity. This implies that there is genetic variability in onion cultivars to tolerate salt stress though the crop considered as salt sensitive one. Mostafavi (2012) reported that, decrease in germination percentage and germination rate under salt stress is related to reduction in water absorption into seeds at seed imbibition and turgescence stage; whereas, in salt stress tolerant cultivar the ability to overcome such problem related to intrinsic factor. In onion it was reported by Sta-baba et al. (2010) salinity levels up to 9.51 dSm<sup>-1</sup> have on effects on germination during the first eight days; however it is not to mean tolerant through the whole growing stage in which Shannon and Grieve (1999) have explained salt tolerance of onions to be high at germination and three to five leaf stage.

#### 2.4.2. Field performance

Salinity tolerance of onions reported to be high at germination and at about the three to five leaf stages, but very low during seedling growth (Shannon and Grieve, 1999). Sta-Baba *et al.* (2010), also found that different salinity levels up to 9.51 dSm<sup>-1</sup> have no effects on germination of onion during the first eight days, not related to later responses of the whole plant to salt. Soil salinity affects various physiological and biochemical processes which result in reduced biomass production. This adverse effect of salt stress appears on whole plant level at almost all growth stages including germination, seedling, vegetative and reproductive stages. During vegetative growth, salinity decreased bulb diameter, bulb weight, root growth,

plant height, and number of leaves per plant and mature earlier (Shannon and Grieve, 1999). This is due to salt stress limiting carboxylation photosynthesis rate and photo-assimilate translocation of the crop. In numerous studies salt-induced inhibition in photosynthesis is accompanied by stomata closure under short-term salt exposure and non-stomatal limitations under long-term salt exposure (Shahbaz et al., 2011). Salt stress decrease crops stomatal aperture which in turn reduces internal  $CO_2$  and enzymes activity (Negrao *et al.*, 2017). Shahbaz et al. (2011) reported that salt stress markedly reduced different gas exchange characteristics such as photosynthetic rate, water use efficiency (photosynthetic rate to transpiration rate ratio), transpiration rate and stomatal conductance in all examined sunflower cultivars. Usually, salt stress increased the chlorophyll a/b ratio because, during the process of chlorophyll degradation, chlorophyll b may be converted into chlorophyll a, consequently resulting in enhanced chlorophyll a content (Eckardt, 2009; Fang et al., 1998). Since salinity affected biological yield more than plant Na uptake, in such cases the percentage of nitrogen in leaves increased and leaves become darker than in the non-saline conditions; therefore, Chlorophyll Content Index (CCI) might be higher than in normal conditions. Pirasteh-Anosheh et al. (2014b) observed that there was no significant difference among the salinity treatments in terms of CCI until 14 days after sowing. Nevertheless, this increase was greatest at the highest salinity regime. On the other hand, the effect of salinity on CCI changes in plants might be different. Jaleel et al. (2008) observed that at lower salt stress levels, chlorophyll a and b and total chlorophyll content would be decreased slightly and under higher salt stress media a significant reduction in the content of these pigments could be observed. It is concluded that destruction of chlorophyll pigments and instability of the pigment-protein complex is the main result of the reduction of chlorophyll content in salinity media. It also could be due to the interference of salts with the *de novo* synthesis of proteins, the structural component of chlorophyll, rather than the breakdown of chlorophyll (Jaleel et al., 2007, 2008). On the other hand, it has been reported that in salt-tolerant species, chlorophyll content is increased while salinity decreases it in salt-sensitive species (Khan et al., 2009). Therefore, chlorophyll content could not be considered as an overall index for salt stress tolerance and must be integrated with other indices. Ashraf and Harris (2013) recommended the use of carotenoids as a reliable criterion for salt tolerance. They also

indicated that growth improvement in plants under salinity has been widely reported to be due to the significant role of zeaxanthin in alleviating oxidative damage of membranes.

### 2.5. Mechanism of Salinity Tolerance

Salt tolerance is the ability of a plant to grow and develop its life cycle in a medium that contains high percentage of soluble salts. Salt tolerance is usually measured as the relative yield production in saline compared to non-saline conditions during the growing season (Munns, 2002). Salt tolerance could be evaluated as plant survival, but for annual species, the amount of biological yield is more useful, as this is usually related to grain yield. The variation in salinity tolerance in dicotyledonous species is even greater than in monocotyledonous species (Lauchli, 1984; Munns and Tester, 2008). Results have shown that at a given salinity level, a salt-tolerant species such as sugar beet might have a reduction of only 20% in dry weight, a moderately tolerant species such as cotton might have a 60% reduction, and a sensitive species such as soybean might be dead (Greenway and Munns 1980). Plants use extra biochemical and molecular mechanisms to overcome salinity. Mechanisms of salt tolerance would be either low- or high-complexity processes. The former appears to involve an alteration in many biochemical pathways while the later involves changes that protect major mechanisms such as photosynthesis and respiration (Botella et al., 1994; Parida and Das, 2005). Some plants have adapted to cope with salt stress; however, the majority of crops are salt sensitive and will not survive under conditions of high salt ions in the root zone or will survive but with decreased biomass production (Hale and Orcutt, 1989). However, there are mechanisms by which plants survive under salt stress condition as mentioned below.

#### **2.5.1.** Role of the vacuole

There are two mechanisms used by the plant to exclude salt reaching the leaf from the cytoplasm. Salt ions can accumulate in the apoplast or move to the vacuole. A build-up of salt ions in the apoplast leads to an increase in the osmotic gradient between the inside and outside of the cell. To adjust a thermodynamic equilibrium, water inside the cell diffuses to the

intercellular spaces, leading to progressive cellular dehydration and, eventually, cell death. Thus, the vacuoles are responsible for potassium homeostasis in the cytosol, maintenance of a high ratio of  $K^+$  to  $Na^+$  cytosolic concentrations by removing  $Na^+$  ions from this compartment. This process is necessary, because Na<sup>+</sup> ions, which enter plant cytosol through low-selective ion channels in the plasmalemma (Andreev, 2001). Therefore, salt-tolerant traits are more associated with the amount of salt ions that accumulate in the cell vacuole (Volkmar et al., 1998). Salt ions pass across the cell membrane and the cytoplasm to enter the vacuole. The quantity of salt ions that pass across the cell membrane must not be more than the amount deposited into the vacuole to minimize the risk of salt hazard (Volkmar et al., 1998). The amount of salt flow is controlled by the storage capacity of the root and the salt concentration Therefore, salt-tolerant require in the soil solution. plants an active vacuolar compartmentation capacity to store the high amount of salt ions delivered from the xylem to the leaf (Lauchli and Epstien, 1990).

#### 2.5.2. Osmotic adjustment

The compartmentalization of salt ions between the cytoplasm and vacuole creates a strong osmotic gradient across the vacuolar membrane. This flow is balanced by an increase in the synthesis of chemical and biochemical molecules in the cytoplasm, a process known as osmotic adjustment. Osmotic adjustment is used by plants as an important mechanism to overcome salt stress (Pessarakli, 2014). Compatible solutes such as proline, glycine-betaine, proline betaine, B-alaninebetaine, D-sorbitol, D-mannitol, sucrose, glucose, fructose, D-pinitol, L-quebrachitol, Myoinositol, b-dimethylsulphone, and propionate are used by plants in osmotic adjustment mechanisms (Lauchli and Epstein, 1990). Among the organic osmolytes, proline (Pro) and glycine-betaine (GB) are the most important and efficient compatible solutes (Tang *et al.*, 2015). Under salt stress proline and glycine-betaine reported to increases cell membrane protection and salt tolerance in onion (Mansour and Ali, 1998; Beinsan *et al.*, 2015). Generally, compatible solutes are often used to describe these organic osmolytes because of their presumed compatibility with cytoplasmic entities and processes (Munns and Tester, 2008). For example, proline synthesis in tobacco plants increased up to 80 times under saline conditions. Genetic evidence of the importance of glycine-betaine in

improving salt tolerance has been shown in barley and maize (Volkmar *et al.*, 1998). Similar evidence has been demonstrated for mannitol, an important osmoprotectant in celery (Tarcynski *et al.*, 1993). Plants consume significant quantities of carbon to produce sufficient osmotic substances and this process potentially limits the normal growth and development of the plant (Munns and Tester, 2008). Plants also use high concentrations of inorganic ions for osmotic adjustment (Greenway and Munns, 1980). The energetic cost of this approach is much lower than the synthesis of organic components in the cell (Munns and Tester, 2008). In leaf cells, to accumulate one mole of NaCl as an osmoticum, about seven moles of ATP are needed. In comparison, the amount of Adenosine triphosphate (ATP) required to synthesize one mole of an organic compatible solute is markedly higher. The ATP requirement for the synthesis or accumulation of solutes has been estimated as 3.5 for Na+, 34 for mannitol, 41 for proline, 50 for glycinebetaine, and approximately 52 for sucrose (Munns and Tester, 2008). Overall, production of osmoticum might be an adaption for plants surviving in saline conditions but this mechanism affected growth of the plant due to ion toxicity and deficiency (Munns and Tester, 2008; Volkmar *et al.*, 1998).

#### 2.5.3. Salt inclusion versus exclusion

Since cell membranes have selection processes for ion absorption, the entrance of sodium becomes limited. Therefore, salt ion levels in the roots and stems of plants are sometimes higher than in the leaves. Due to variations in the selectivity of the membranes among plant species, they may be divided into salt excluders and salt non-excluders (Hale and Orcutt, 1989). Onions are relatively excluders of both Na and Cl and are sensitive to sulphates (Rao, 2016). Physiologically, excessive soil salt stress imposes initial water deficit that results from the relatively high solute concentrations in the soil, causes ion-specific pores resulting from altered K<sup>+</sup>/Na<sup>+</sup> ratio and leads to a build-up in Na<sup>+</sup> and Cl<sup>-</sup>concentrations that are detrimental to plants (Rao, 2016). Sodium exclusion by roots occurs to prevent toxic concentrations of Na<sup>+</sup> in leaves. Accumulation of Na<sup>+</sup> manifests its toxic effects after days or weeks, depending on the species, and causes premature death of older leaves (Munns and Tester, 2008). Salt-tolerant plants showed some evidence of exclusion of Na<sup>+</sup> from the leaf. This is especially true for many glycophytic species, including crop plants such as wheat and

barley, corn, chickpea and beans, as well as some halophytes (Volkmar et al., 1998). Since in most species,  $Na^+$  appears to reach a toxic concentration before CF does, many studies have focused on Na<sup>+</sup> exclusion mechanisms within the plant. However, for some species such as soybean, CF is considered to be the more toxic ion. Generally, plants tolerated high amounts of Na<sup>+</sup> and Cl<sup>-</sup> arriving in their leaves by use of some anatomical alterations and intracellular partitioning mechanisms (Munns and Tester, 2008). There are some differences between amounts of  $Na^+$  and  $C\Gamma$  in root and leaf cells. Roots had the lowest Cl concentration compared to leaves, which increased with increasing salinity, while Na<sup>+</sup> in leaves was much lower than CT (Chartzoulakis and Klapaki, 2000). In some dicotyledonous halophytes, there is a salt induced increase in cell size due to increases in vacuole volume (succulence), and in others, the excretion of  $Na^+$  and  $C\Gamma$  creates salt glands or bladders at the leaf or stem surfaces. Some evidence has shown that salt glands are the only anatomical adaptations that occur in some monocotyledonous halophytes (Munns and Tester, 2008). Barley crops that thrive in saline conditions showed, contrary to  $K^+$  a greater accumulation of  $CI^-$  in epidermal compared with mesophyll cells (Munns and Tester, 2008). Most halophytes use salt ions as an osmoticum to control the concentration of external ions. In many glycophytes, there is no obvious relationship between salt exclusion and salt tolerance. While Na<sup>+</sup> exclusion is a general characteristic reported in some salt-tolerant wheat lines, a salt-sensitive line had much lower shoot Na<sup>+</sup> levels than the more tolerant lines. In a similar experiment, tolerant maize cultivars transported more Na<sup>+</sup> to the shoot than intolerant cultivars. Therefore it seems that, at least in some glycophytes, salt exclusion is not necessarily associated with salt-tolerant characteristics (Volkmar et al., 1998).

### 2.5.4. Na+/K+ favoritism

It is indicated that the selection of ions by plants is a clear way to tolerate salt conditions. For example, in the Na<sup>+</sup>/K<sup>+</sup> discrimination concept, Na<sup>+</sup> uptake can be substituted by K<sup>+</sup> to allow the plant to tolerate salt conditions. Therefore Na<sup>+</sup>/K<sup>+</sup> discrimination could be considered as an important criterion in selecting commercial crops (Volkmar *et al.*, 1998). However, the Na<sup>+</sup>/K<sup>+</sup> discrimination trait is not necessarily a salt tolerance criterion in glycophytes. For example, some salt-tolerant cultivated barley strains and their wild relatives do not show the

enhanced  $Na^+/K^+$  discrimination trait. Similarly, while some wild relatives of wheat tend to be better at discriminating against  $Na^+$  than cultivated wheat, it is believed that this is not due to enhanced discrimination but rather, to greater control of salt accumulation (Munns and Tester, 2008). Halophytes prefer to include  $Na^+$  rather than  $K^+$ , as a tolerance tool for osmotic adjustment. There is a positive relationship between  $Na^+$  inclusion and salt tolerance in these plants (Volkmar *et al.*, 1998).

#### 2.5.5. Role of anti-oxidants

Salt stress induces an accumulation of ROS that are detrimental to cells at high concentrations because they cause oxidative damage to membrane lipids, proteins, and nucleic acids (Gomez *et al.*, 1999; Mittler 2002). Despite the potential of ROS for causing harmful oxidations, it is now well established that they are also implicated in the control of plant growth and development as well as priming acclamatory responses to stress stimuli (Foyer and Noctor, 2009). To cope with ROS, living organisms evolved antioxidant defense systems, comprised of enzymatic and non-enzymatic components. Several enzymes are involved in the detoxification of the activated oxygen species like superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR), and glutathione peroxidase (GPX). In onion antioxidants like superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) is responsible to reduce  $H_2O_2$  under salt stress (Correa *et al.*, 2013).

# **3. MATERIALS AND METHODS**

#### 3.1. Description of the Study Site

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

#### 3.2. Experimental Materials, Treatments and Designs

#### **Experiment I: Laboratory germination test**

The seed germination experiment was conducted in a laboratory at room temperature of MARC seed laboratory as described by Taffouo *et al.* (2009). Five onion genotypes, four released onion cultivars and one pipeline cultivar *viz.*, Nafis, Nasic Red, Bombey Red, Adama Red and Agrifound respectively were used (Appendix Table 1). The seeds were obtained from Melkasa Agricultural Research Center. Factorial experiment consists five onion cultivars and five levels of water having EC of (1.2, 4, 8, 12 dSm<sup>-1</sup>) and distilled water as control were combined with all possible combinations (Appendix 2). Thus, a total of 25 treatments (5 x 5) and each replicated three times and total of 75 experimental units were factorially arranged in Complete Randomized Design (CRD).

#### Experiment II: Field test on soil media

Four released onion cultivars *viz.*, Nafis, Nasic Red, Bombey Red and Adama Red and five levels of root zone salinity (Awash water, 1.2, 4, 8, 12 dSm<sup>-1</sup>) were factorially combined with all possible combinations (Appendix Table 3); thus, 20 treatments (4 x 5) each replicated three times and the total of 60 experimental units were laid down using factorial arrangements in Randomized Complete Block Design (RCBD).

#### **3.3. Experimental Procedure**

#### Experiment I: Laboratory germination test

#### Salinization of irrigation water

In the laboratory study five levels namely distilled water (control), 1.2, 4, 8, 12 dSm<sup>-1</sup> conductivity solution were used by dissolving 0.17 g, 2.35 g, 4.64 g and 6.96 g of oven dried NaCl in a liter of distilled water. The solutions were checked by portable *ECscan 30 C*onductivity Tester to adjust the proposed level.

#### Sterilization and germination management

Laboratory experiment was started by washing and sterilizing of petridishes thoroughly by soaking in hot water boiled at 70 °C for 5 to 10 minutes. Later petridishes were surface sterilized by wiping them with 95- 97% alcohol for 10–15 minutes. After sterilization, petridishes were lined with qualitative filter paper model 102 and arranged in a complete randomized design (CRD).

Seeds were soaked for five to ten minutes in 50% solution of alcohol (Rao *et al.*, 2006). Each petridishes were treated with 3 pippete of distilled water, 1.2, 4, 8 and 12 dSm<sup>-1</sup> of NaCl. About 150 seeds of each proposed cultivar were taken and 50 uniform seeds of each onion cultivars were placed on each petridish diameter size (9 cm) in a uniform distance following Kubisza *et al.* (2012). The petridishes were treated with 3- 5 pippetes of the respective concentrations of NaCl in alternate days.

#### Experiment II: field test on soil media

#### Soil collection and growing media preparation

Top soil from MARC field station and black gravel were collected and sieved separately by mesh wire sized 2.36 mm. Later, it was exposed to solar radiation for a month for sterilization. Finally composite was made by mixing 3:1 of top soil and sieved gravel respectively (Ketema, personal communication). A total of 64 kg of soil was added to the box in the ratio of 2:1:3 layers, of coarser gravels, sieved gravel alone and mix of sieved gravel and top soil from the bottom layers to upper layer respectively. From the composite one sample was taken to MARC soil laboratory for soil physical and chemical properties determination (Table 1).

Table	1. Physico-	chemical	propertie	es of	experimental	soil
	2					

Physical properties	Chemical properties	
Particle distributions	рН	8.07
Sand 40	Electrical conductivity dS	$m^{-1}$ 0.54
Silt 30	Total N (%)	0.14
Clay 30	Total organic C (%)	0.82
	Available P (ppm)	18.97
	(Cmol/kg) soil	
	K <sup>+</sup> Ca <sup>++</sup>	Mg <sup>++</sup> Na <sup>+</sup>
	1.24 26.31	9.13 11.2

### Field experiment management

Field experiment was conducted on soil media filled in box constructed from wood in the form of pot and framed by mats to hold the soil and facilitate easiness of drainage problems. The box had total height 80 cm, raised 40 cm from the ground in which designed to have 40 cm X 60 cm X 40 cm volume. A total of 64 kg of soil was added to the box in the ratio of

2:1:3 layers, of coarse gravels, sieved gravel alone and mix of sieved gravel and top soil (3:1) from the bottom layers to upper layer, respectively.

The seeds were sown by drilling on December 8, 2017 on the soil media filled in box, however to maintain uniformity and vigorosity it was up-rooted and transplanted on January 24, 2018 by maintaining 5 cm and 10 cm spacing between plants and rows, respectively having a total of 36 plants per experimental unit sized  $0.24 \text{ m}^2$ .

### Water salinization, irrigation and salt levels maintenance of field experiment

Table salt (NaCl) was sun dried to reduce the iodine concentration according to Diosady and Venkatesh (2000). Dry weight of NaCl 0.25 g, 1.52 g 3.04 g and 4.56 g were dissolved in one liter of Awash water ( $0.3 \text{ dSm}^{-1}$ ) to make 0.8, 2.6, 5.2 and 7.6 dSm<sup>-1</sup> of irrigation water, respectively. The solution were checked and re-checked by portable *ECscan 30* Conductivity Tester prior to each irrigation schedules. The irrigation water was fixed by considering root zone salinity to be at 1.2, 4, 8 and 12 dSm<sup>-1</sup> by the following formula according to FAO (1985).

ECe= 1.5 ECw ...... (Equ. 1). Where, ECw= electrical conductivity of irrigation water, ECe= conductivity at root zone

Salinized irrigation water application was started on February 12, 2018 or 19 day after transplanting. Irrigation 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 Kc of onion at the center. Additionally, Awash water (0.3 dSm<sup>-1</sup>) alone was being applied to maintain root zone soil salinity to the designed levels. The additional water required to maintain the designed levels of salinity concentration around root zone was calculated as (FAO SAFR, 2002) by the formula:

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$$LR = \frac{EC_{\Psi}}{5EC_{\theta} - EC_{\Psi}} \times \frac{1}{LE}$$
 (Equ. 2). Where, LR= leaching requirement

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

For this experiment 17% of leaching requirement was used to have 90% leaching efficiency. Fertilizer was applied using banding method according to the required rate. Chemical spray was done four times following fungal diseases and trips occurance. 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.

#### 3.4. Collected Data

#### 3.4.1. Lab experiment

*Germination Percentage (GP)*: From the third day after sowing, germinated seeds were counted daily once in 24 hours following germination of seeds. Those seeds considered as germinated were a radical length more than 2mm (Keshavarzi, 2012; Mostafavi, 2012). Counting was continued until germination stopped and final counting considered as final germination (Keshavarzi, 2012).

*Germination Rate (GR)*: was calculated using the following (Eq. 3) formula, where n1 is the number of seedlings germinated on the first day of germination,  $t_1$  is the days from beginning to the first germination, and  $X_n$  is the total number of seeds germinated

GR (number of germinated seeds in each day):- =  $(n_1t_1) + (n_2t_2) + \dots + (n_xt_x)/X_n \dots (Eq. 3)$ .

*Fresh Weight* (g): at the end of the experiment, five plants were selected from each treatment weighed for fresh weight measurement.

*Root and shoot length (cm)*: from five representative seedlings radicle and plumule were separated and root and shoot length was measured by ruler meter separately.

Dry weight (g): each repetition was dried on filter paper separately in the oven at 60 °C to constant weight and measurement was taken as described by Keshavarzi (2012).

Seedling vigor index (SVI): seedling vigor index was calculated by multiplication of seedling height (root length plus shoot length) with germination percentage as described by Mattew *et al.* (2002). Vigor index = seedling height x germination percentage.

*Shoot: root ratio:* was calculated by dividing the measured shoot height to root height and expressed in percent.

#### 3.4.2. Field experiment

*Leaf length* (*cm*): length of the longest leaf of five fully developed plants per experimental units was recorded at the maximum growth stage of the plants by ruler meter.

*Leaf width* (*cm*): width of the longest leaf of five fully developed plants per experimental units was taken by flatten it to measure easily according to IPGRI (2002) descriptor for *Allium spp*.

*Leaf number per plant:* at two weeks intervals number of healthy and fully developed leaves were counted three times at different growth stages from randomly selected five plants after two weeks salt application.

*Pseudo-stem diameter (neck diameter):* at two weeks intervals three times pseudo-stem diameter was measured by electronic digital caliper from five randomly selected plants after two weeks salt application.

*Plant height:* after two weeks salt application plant heights were measured three times by ruler meter at two weeks interval from five randomly selected plants and final plant height was recorded at full blooming stage.

*Fresh bulb weight (g):* at full bulbs maturity and 10 days of treatment application stopped five randomly selected plants from each experimental unit was weighed using sensitive balance.

**Bulb length** (*cm*):- five representative randomly selected bulbs were measured by electronic digital caliper from bottom tip to apex of bulb.

**Bulb width:** - same bulbs selected for bulb length data were considered for bulb width measurement using electronic digital caliper from middle portion of bulb length.

*Total soluble solids:* - three representative randomly selected bulbs were taken from each experimental unit and crushed by bulb crusher and poured into the Refractometer and reading was recorded.

*Dry bulb weight* (g):- five randomly selected plants from each experimental unit was chopped and put on paper bags separately and oven dried at 60 °C to constant weight and dry weight measurement was taken by sensitive balance as described by Keshavarzi (2012).

*Fresh above ground biomass weight* (g):- five randomly selected plants from each experimental unit were cut at tip of bulb and above ground part was measured using sensitive balance.

Dry above ground biomass weight (g):- five randomly selected plants from which above ground biomass fresh weight was taken were chopped and oven dried to constant weight and biomass was measured using sensitive balance.

*Leaf chlorophyll content (mmolm*<sup>-2</sup>*s*<sup>-1</sup>):- after one month of saline water irrigation, it was taken three times at two weeks interval from a recently developed leaf portion of each three plants per experimental unit using Konica Minolta SPAD-502 Plus chlorophyll meter expressed by SPAD unit.

*Stomatal conductance (mmolm*<sup>-2</sup>*s*<sup>-1</sup>):- was taken three times at two weeks interval from one green recently-matured leaf portion of each three plants per experimental unit starting from one month after treatment application using leaf porometer (Steady State Diffusion Porometer) *Model Decagon SC-1*.

*Quantum yield (photosystem II):-* after one month of treatment application it was taken three times at two weeks interval from one green recently-matured leaf portion of each three plants per experimental unit using Fluorophen FP 100 at 9:00-11:00 sunny hours.

### 3.5. Data Analysis

#### **3.5.1. Germination variables**

Germination variables were tested for their normality prior to analysis and those variables unfitted to normal distributions were subjected to data transformations. Likewise variables such as shoot length, shoot to root ratio were moderately positive skewed with zero values data transformed by log 10 (x + c), while root length, fresh and dry weights were negatively skewed data by sqrt (x - c), and germination percentage substantially positive skewed was transformed by log10 (x) formula.

Collected data were subjected to Analysis of Variance (ANOVA) using SAS 9.3. Regression analysis was done for significant variables. When the ANOVA shows significant differences between treatments, mean comparison, and separation was done by using Duncan Multiple Range Test (DMRT).

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## **3.5.2. Field experiment**

All variables collected were tested for assumptions of ANOVA prior to analysis transformed. Those parameters unfitted to normality like bulb and fresh above ground biomass weight substantially positive skewed by log 10, bulb and dry above ground biomass weight were moderately positive skewed with zero values data by log (x + c) were subjected to data transformation. Germination and field data variables correlation analysis was done by Pearson's correlation analysis method.

# **4. RESULTS AND DISCUSSION**

## 4.1. Influence of Salt Stress on Germination Variables

## 4.1.1. Germination percentage

Two-way analysis of variance exhibited highly significant variation with seed germination percentages among salt levels (P= 0.0001), but there were no significant differences observed for cultivars (P=0.79) and their interaction (P=0.17). The highest germination percentage (77.33%) was observed from distilled water while the lowest germination percentage (11.87%) was recorded on the highest level of salt concentration 12 dSm<sup>-1</sup> (Table 2). A linear regression (R<sup>2</sup>=0.88) between germination percentages and salt stress levels (Figure 1.) explained that, a unit increment in salt level reduced 5.53 percentages of onion germination. Similar results were reported in onion (Joshi and Sawant, 2012; Sudha and Riazunnisa, 2015), in cow pea (Gogile et al., 2013), in chick-pea. In addition to toxic effects of sodium ions, higher concentration of salt reduces the water potential in the medium which hinders water absorption by germinating seeds and thus reduces germination (Gulzar and Khan, 2001; Neamatollahi et al., 2009). A decrease in germination is related to salinity induced disturbance of metabolic process leading to increase in phenolic compounds (Hadas, 1977). Mostafavi (2012) reported that, decrease in germination percentage and germination rate under salt stress is related to reduction in water absorption into seeds at seed imbibition and turgescence stage.

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Figure 1. Relationships of salt stress levels on mean germination percentage of onion cultivars at MARC in 2018.

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Treatments	Germination		shoot leng	shoot length (cm)		root length (cm)	
	percentage	e (%)					
	original	transf	original	transf.	original	transf	
Cultivars							
Adama Red	47.20	(1.63)	1.62	(0.57)	0.30	(0.85)	
Agri found	51.87	(1.59)	1.63	(0.57)	0.38	(0.85)	
Bombey Red	50.27	(1.58)	1.77	(0.57)	0.36	(0.86)	
Nafis	50.93	(1.58)	1.85	(0.60)	0.36	(0.88)	
Nasic Red	51.73	(1.64)	1.46	(0.60)	0.30	(0.88)	
CR (5%)	ns		ns		ns		
Salt levels (dSm <sup>-1</sup> )							
Distilled water	77.33 <sup>a</sup>	(1.88)	$2.17^{a}$	(0.66)	$0.35^{a}$	(0.91)	
1.2	65.47 <sup>b</sup>	(1.81)	$1.76^{a}$	(0.64)	0.39 <sup>a</sup>	(0.94)	
4	60.40 <sup>b</sup>	(1.77)	1.53 <sup>a</sup>	(0.63)	$0.32^{ab}$	(0.91)	
8	35.00 <sup>c</sup>	(1.52)	$1.17^{b}$	(0.53)	$0.29^{b}$	(0.83)	
12	11.87 <sup>d</sup>	(1.06)	$0.70^{c}$	(0.46)	0.23 <sup>c</sup>	(0.75)	
CR (5%)	7.91		0.05		0.06		
CV%	9.83		12.5		9.13		

Table 2. Mean comparison among different salt stress levels on the germination percentage, shoot and root length of onion cultivars at seed Laboratory of MARC during 2018

Means in the column followed by the same letter(s) are not significantly differ at 5% level of significance. CR (0.05) = Critical Range at the 5 % level; and CV (%) = coefficient of variation in percent. ns= non-significant. Numbers in brackets are transformed by log 10 (x + c) for shoot length, root length by sqrt (x - c) and log 10(x) for germination percentage.

## 4.1.2. Seedling shoot length

The analysis of variance revealed highly significant variation on salt stress levels (P=0.0009) for mean seedling shoot length, but there were no significant differences observed among cultivars (P=0.63) and their interaction (P=0.08).

An increment in salinity level significantly reduced the mean seedling shoot length in all cultivars. The shortest seedling shoot length (0.7 cm) was recorded at 12 dSm<sup>-1</sup> salt level, while maximum shoot length (2.17 cm) was recorded on control (distilled water). The linear equation Y = -0.1113x + 2.0267 (Figure 2) indicates that an increase in 1 dSm<sup>-1</sup> salt stress reduces (0.1113 cm) of shoot length. However, it was observed that shoot length up to 4 dSm<sup>-1</sup> salt concentration were statistically non-significant in comparison to distilled water as above (Table 2). Similar finding was reported by Tolessa *et al.* (2013) on tomato shoot length was decreased with increased salt level from 0 % to 0.6 %. This reduction in shoot length development might be due to the toxic effects of NaCl and might be unbalanced nutrient uptake by seedlings (Hajibagheri *et al.*, 1989).



Figure 2. Relationships of salt stress levels on mean shoot length of onion cultivars at MARC in 2018.

## 4.1.3. Seedling root length

The analysis of variance revealed highly significant variation on salt levels (P=0.0001) for mean seedling root length, but there were no significant difference observed among cultivars (P=0.53) and their interaction (P=0.47).

An increase in salt stress levels had also significantly reduced the mean seedling root length in all of the cultivars. The shortest seedling root length (0.23 cm) was recorded with 12 dSm<sup>-1</sup> salt level, while the tallest root lengths were recorded with salt stress level of 1.2 dSm<sup>-1</sup> and distilled water which were statistically par with 4 dSm<sup>-1</sup> (Table 2). Similar finding was reported by Jafarzadeh and Aliasgharzad (2007), in sugar beet cultivars seedling root length were enhanced by low salinity level (2 dSm<sup>-1</sup>) in comparison with control (~0 dSm<sup>-1</sup>). This might be due to salt stress at low level may enhance initiation of growth hormones. This was reported by Janmohammadi *et al.* (2008), salt stresses increase the expression of aquaporins, enhancement of ATPase activity, RNA and acid phosphate, increase amylase, proteases or lipeases activity. High salinity may inhibit root and shoot elongation due to slowing down the water uptake (Werner and Finkelstein, 1995). It might be due to the ability of the root system to control entry of ions to the shoot is of crucial importance to plant survival in the presence of NaCl (Hajibagheri *et al.*, 1989).



Figure 3. Relationships of salt stress level on mean root length of onion cultivars at MARC in 2018.

#### 4.1.4. Germination rate

The analysis of variance revealed significant variations among onion cultivars (p=0.02) for mean germination rate, but there were no significant difference observed among salt stress levels (P=0.30) and their interaction (P=0.48).

As indicated in Figure 4 Nafis and Adama Red had shown the fastest germination rate (4.78 and 4.71 seedlings per day respectively) which were statistically similar with Agrifound and Bombey Red, while Nasic Red was the slowest one (4.35 seedlings per day). This may be due to the intrinsic effects of cultivars and/or their seeds physiological conditions. Differences in germination rates among cultivars were reported to happened as a result of the storage period effects on seed physiological potential (Rodo and Marcos-Filho, 2003; Sudha and Riazunnisa, 2015).



Figure 4. Mean comparison among different onion cultivars germination rates at MARC in 2018.

#### 4.1.5. Seedling shoot to root length ratio

Two way analysis of variances indicated that significant variance in mean seedling shoot to root length ratio was observed among cultivars (P=0.03) and salt levels (P=0.002). However, non-significant result was observed for their interaction (P=0.55) (Appendix Table 5).

The highest seedling shoot to root ratio was recorded for Adama Red, Bombey Red, Nasic Red and Nafis, whereas the least value (4.20) was recorded for Agrifound (Table 3). The highest shoot to root ratio was also recorded on distilled water up to 4 dSm<sup>-1</sup> salt level in which it was statistically par with 8 dSm<sup>-1</sup>. Increasing salt stress levels decreases shoot to root length ratio (Figure 5). Shoot length was highly affected by salt stress in comparison to root length, where a unit increase of 1 dSm<sup>-1</sup> reduced (0.0115 cm and 0.1113 cm) root and shoot lengths respectively (Figure 2 and 3). Singh *et al.* (2012) was also reported that the root growth of tomato appears to be less affected, while shoot length was affected drastically. This initial reduction in shoot growth than root was probably due to hormonal signals generated by the roots (Munns, 2002). Kevah (2011) reported that, above ground growth may decline due to the translocations of more assimilate to roots to improve its water uptake ability.



Figure 5. Associations of salt stress levels with the mean shoot to root ratio of onion.

### 4.1.6. Fresh and dry weights

The analysis of variance revealed highly significant variation among salt stress levels for mean dry and fresh weight (P=0.001), but there were no significant differences observed among cultivars (P= 0.59 and 0.36) and their interaction (P= 0.33 and 0.32).

Salt tolerance is usually assessed as the percent biomass production in saline versus control conditions over a prolonged period of time. An increment in salinity concentration level had significantly reduced the mean dry and fresh weight in all of the cultivars in the current study. The minimum fresh and dry weight recorded at 12 dSm<sup>-1</sup> salt level were (0.02 g and 0.01 g) respectively. The maximum fresh weight (0.49 g) was recorded on salt level of 1.2 dSm<sup>-1</sup> which was statistically par with 4 dSm<sup>-1</sup>; whereas the highest dry weight (0.07 g) was recorded from distilled water up to 4 dSm<sup>-1</sup> which was also statistically similar with 8 dSm<sup>-1</sup> salt levels as shown below (Table 3). From this it is possible to generalize that salts stress reduced fresh and dry biomass weight of onion cultivars. Similar works has been reported that salt stress reduces seedling fresh and dry weight biomass of tomato lines (Kaveh et al., 2011; Sholi, 2012; Tolessa et al., 2013). The reduction in biomass weight under salt stress was probably emanated from reduction in water uptake and/or salt toxicity (NaCl), in which cause physiological dryness, reduced cell expansions and multiplications (Zhu, 2001; Munns et al., 2000). It was reported by Shaheen et al. (2013), leaf water and osmotic potentials of eggplant plants were increased significantly (more negative), while leaf turgor potential was decreased due to addition of varying levels of salt to the growth medium.

Treatments	Shoot/ root		Fresh we	eight (g)	Dry weight (g)	
	original	transf	original	transf	original	transf
Cultivars						
Adama Red	5.52 <sup>a</sup>	(1.09)	0.33	(1.09)	0.04	(0.68)
Agrifound	4.20 <sup>b</sup>	(1.06)	0.47	(1.12)	0.12	(0.71)
Bombey Red	5.08 <sup>a</sup>	(1.07)	0.26	(1.07)	0.03	(0.67)
Nafis	5.10 <sup>a</sup>	(1.09)	0.43	(1.14)	0.06	(0.69)
Nasic Red	4.95 <sup>a</sup>	(1.12)	0.33	(1.11)	0.09	(0.71)
CR (5%)	1.31		ns		ns	
Salt levels (dSm <sup>-1</sup> )						
Distilled water	6.30 <sup>a</sup>	(1.15)	0.37 <sup>bc</sup>	(1.09)	$0.07^{a}$	(0.70)
1.2	4.64 <sup>a</sup>	(1.12)	0.49 <sup>a</sup>	(1.18)	0.07 <sup>a</sup>	(0.70)
4	4.99 <sup>a</sup>	(1.14)	$0.40^{ab}$	(1.16)	$0.07^{a}$	(0.71)
8	3.98 <sup>ab</sup>	(1.04)	0.23 <sup>c</sup>	(1.08)	$0.04^{ab}$	(0.69)
12	3.00 <sup>b</sup>	(0.97)	0.02 <sup>d</sup>	(1.00)	0.01 <sup>b</sup>	(0.66)
CR (5%)	1.31		0.09		0.03	
CV%	6.01		11.13		7.85	

Table 3. Mean comparison of four onion cultivars on shoot/root, fresh and dry weight evaluated under different salt levels at MARC in 2018

Means in the column followed by the same letter(s) are not significantly differ at 5% level of significance. CR (0.05) = Critical Range at the 5 % level; and CV (%) = coefficient of variation in percent. ns= non-significant. Numbers in brackets are transformed to by sqrt (x - c) for fresh and dry weight, while log 10 (x + c) for Shoot/ root.

## 4.1.7. Seedling vigor index

Analysis of variance showed that highly significant difference for the main factors cultivars, salt stress levels and their interaction (P=0.001) on mean seedling vigor index (Appendix Table 5).

The interaction showed that Nafis with distilled water resulted in the highest SVI (136.84), while the least SVI recorded with the interactions of Adama Red and Nasic Red cultivars with 12 dSm<sup>-1</sup> salt level (Figure 6). This highest SVI value was observed with control (distilled water), where it was gradually decreasing as salt stress levels increased. Similar works was reported by Sudha and Riazunnisa (2015), on onion higher SVI was recorded for the control relative to the higher salt stress levels. This might be as result of salt stress decreases water potential of growing medium in which it reduces germination percentage and seedling lengths. Among cultivars the highest seedling vigor index (SVI) was recorded for Nafis (136.84) while the lowest value recorded for Agrifound (72.01).



Figure 6. Interaction effects of salt stress levels and onion cultivars on seedling vigor index at MARC Seed Laboratory during 2018.

### 4.2. Growth Variables

#### 4.2.1. Leaf numbers per plant

ANOVA 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 DAT stages); but among salt levels showed significant differences p= 0.03 (Appendix Table 6). The highest average leaves numbers were recorded with Awash water and 4 dSm<sup>-1</sup> (3.73 per plant) salt levels which were statistically similar with 1.2 and 8 dSm<sup>-1</sup> treated experimental units while the lowest leaf numbers were recorded with 12 dSm<sup>-1</sup> (3.05). Similar work was reported by Chang (2003), onion plant growth was visibly affected by increasing NaCl concentrations in the nutrient solutions within one week. Salinity stunted growth through reduced leaf initiation and expansion (Munns *et al.*, 2000).

In the second at 44 DAT and third stages at 58 DAT, after irrigating salinized irrigation water for a month and one month and two weeks respectively, showed significant differences in leaf numbers per plant recorded for cultivars (P=0.02) and salt level (P=0.0001), whereas, their interaction did not show any significance difference (P=0.58).

The highest leaf number was recorded for Bombey Red (4.24) and Nasic Red which was statistically at par with Nafis, whereas the lowest leaf numbers were 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 maintains their physiological and metabolic activity. Salt stress levels up to 4 dSm<sup>-1</sup> showed statistically similar value at 44 DAT. At stage of 58 DAT the highest average leaf numbers per plant was observed on experimental units receiving Awash water (4.27) which was par with 1.2 dSm<sup>-1</sup>, whereas leaf numbers were reduced to (2.34) on the highest salt concentration 12 dSm<sup>-1</sup>. Similar work was reported by Stab-baba *et al.* (2010) in 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 declination in growth rate was observed. Reductions in the

number and sizes of leaves induced by increasing salinity indicated, development was affected at both the meristematic level and at subsequent leaf expansion stages (Munns *et al.*, 2000). It was observed that few numbers of average healthy leaves per plants at high salt stress levels due to scorching of leaves prior to fully leaf growth and expansion. It was also reported by Rahneshan *et al.* (2018) mainly necrosis and losing chlorophyll mostly severe in leaves at high NaCl concentrations. 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).

Treatments	Leaf numbers per plant			Plant height (cm)			
	30 DAT	44 DAT	58 DAT	30 DAT	44 DAT	58 DAT	
Cultivars							
Adama Red	3.45	3.64 <sup>b</sup>	2.82 <sup>b</sup>	16.01	18.41 <sup>b</sup>	16.77 <sup>c</sup>	
Bombey Red	3.55	4.24 <sup>a</sup>	3.84 <sup>a</sup>	17.21	24.95 <sup>a</sup>	26.12 <sup>a</sup>	
Nafis	3.29	4.08 <sup>ab</sup>	3.52 <sup>a</sup>	15.01	20.91 <sup>ab</sup>	21.71 <sup>b</sup>	
Nasic Red	3.47	4.12 <sup>a</sup>	3.83 <sup>a</sup>	15.59	22.08 <sup>ab</sup>	24.07 <sup>ab</sup>	
CR (5%)	ns	0.45	0.37	ns	4.35	3.84	
Salt levels (dSm <sup>-1</sup> )							
Awash water (0.3)	3.70 <sup>a</sup>	4.75 <sup>a</sup>	4.27 <sup>a</sup>	17.47 <sup>a</sup>	26.86 <sup>a</sup>	29.98 <sup>a</sup>	
1.2	3.47 <sup>ab</sup>	4.37 <sup>a</sup>	3.95 <sup>ab</sup>	16.38 <sup>abc</sup>	24.83 <sup>a</sup>	26.53 <sup>ab</sup>	
4	3.73 <sup>a</sup>	4.42 <sup>a</sup>	3.75 <sup>b</sup>	17.23 <sup>ab</sup>	24.79 <sup>a</sup>	24.57 <sup>b</sup>	
8	3.25 <sup>ab</sup>	3.50 <sup>b</sup>	3.18 <sup>c</sup>	14.68 <sup>bc</sup>	17.40 <sup>b</sup>	17.52 <sup>c</sup>	
12	3.05 <sup>b</sup>	3.07 <sup>b</sup>	2.38 <sup>d</sup>	14.03 <sup>c</sup>	14.05 <sup>b</sup>	12.24 <sup>d</sup>	
CR (5%)	0.50	0.50	0.42	2.46	4.86	4.29	
CV%	17.43	15.08	14.35	20.9	27.23	23.42	

Table 4. Mean leaf numbers and plant height of four onion cultivars as influenced by levels of salt stress at three different growth stages of irrigating salinized water at MARC in 2018

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

#### 4.2.2. Plant height

Plant height was significantly influenced by salt stress levels (P=0.04) at 30 DAT, but nonsignificant differences was observed among cultivars (P=0.33) and their interactions (P=0.43) according to the above (Appendix Table 6).

The tallest plant height (17.47 cm) was observed with Awash water (0.3 dSm<sup>-1</sup>) 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 statistically also par with 8 dSm<sup>-1</sup>. Second and third stage of plant height records were also statistically 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<sup>-</sup> <sup>1</sup>, whereas the shortest was recorded on 8 and 12 dSm<sup>-1</sup> salt stress levels at 44 DAT. At 58 DAT plant height was extremely affected with 12 dSm<sup>-1</sup> where the least value recorded was (12.24 cm) and the tallest plant height was observed with Awash water (29.98 cm) which was statistically similar with 1.2 dSm<sup>-1</sup>. Across the three stages, it was observed that plant height was gradually increased from 17.47 cm to 29.98 cm for Awash water irrigated units, while decreasing trend was seen at the highest salt level 12 dSm<sup>-1</sup> from 14.03 cm to 12.24 cm. Increasing 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 might be due to sodium affect growth through, increasing soil pH and directly creating nutrient deficiencies or imbalances and toxicity (Machado and Serralheiro, 2017). In another way salt stress was reported to affects different metabolic processes such as CO<sub>2</sub> assimilation, protein synthesis, respiration or photo hormone turn over (Hepaksoy, 2004), which visibly reduce plant growth and development.

For the main factor cultivars significant (P=0.03) on the second stage and highly significant (P=0.0001) on the third stage differences were observed, but their interaction did not show significant differences (P=0.07 and 0.21 respectively) at both stages as above (Table 4). Among cultivars the tallest plant height was recorded for Bombey Red (24.95 cm) which was par with Nasic Red and Nafis at second stage and with Nasic Red at third stage, whereas Adama Red showed 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.

#### 4.2.3. 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 growth stage of 44 DAT the highest diameter was recorded with Awash water  $(0.3 \text{ dSm}^{-1})$ , 1.2 and 4 dSm<sup>-1</sup> salt levels and the thinnest value for 8 and 12 dSm<sup>-1</sup>. As aging increased to 58 DAT the highest stem diameter also recorded with Awash water (6.30 mm) where the lowest value recorded with 8 and 12 dSm<sup>-1</sup> salt stress level. This result indicated that increased salt stress decreased pseudo-stem diameter by bringing physiological drought corroborating with the findings of Ghodke *et al.* (2018) who reported that, increased drought stress in onion reduced pseudo-stem diameter of the crop. Among cultivars Bombey Red showed the thickest pseudo-stem diameter (5.36 mm) compared to Adama Red was observed as 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.

Treatments	Pseudo-stem diameter (mm)			Quantum yield			
	30 DAT	44 DAT	58 DAT	40 DAT	54 DAT	68 DAT	
Cultivars							
Adama Red	3.58	3.58	4.20 <sup>c</sup>	0.531 <sup>b</sup>	0.462 <sup>b</sup>	0.413 <sup>b</sup>	
Bombey Red	3.72	3.72	5.36 <sup>a</sup>	0.599 <sup>a</sup>	0.511 <sup>ab</sup>	0.538 <sup>a</sup>	
Nafis	3.31	3.31	4.59 <sup>bc</sup>	0.614 <sup>a</sup>	0.536 <sup>a</sup>	0.532 <sup>a</sup>	
Nasic Red	3.70	3.70	4.96 <sup>ab</sup>	0.612 <sup>a</sup>	0.543 <sup>a</sup>	0.476 <sup>ab</sup>	
CR (5%)	ns	ns	0.73	0.05	0.057	0.09	
Salt levels (dSm <sup>-1</sup> )							
Awash water (0.3)	3.84	5.61 <sup>a</sup>	6.30 <sup>a</sup>	0.635 <sup>a</sup>	0.58 <sup>a</sup>	0.537	
1.2	3.71	4.90 <sup>a</sup>	5.49 <sup>b</sup>	0.611 <sup>a</sup>	0.55 <sup>a</sup>	0.491	
4	3.73	4.81 <sup>a</sup>	4.93 <sup>b</sup>	0.602 <sup>a</sup>	0.55 <sup>a</sup>	0.490	
8	3.27	3.41 <sup>b</sup>	3.93 <sup>c</sup>	0.585 <sup>a</sup>	0.45 <sup>b</sup>	0.479	
12	3.35	3.32 <sup>b</sup>	3.23 <sup>c</sup>	0.522 <sup>b</sup>	0.43 <sup>b</sup>	0.451	
CR (5%)	ns	0.10	0.81	0.05	0.06	ns	
CV%	19.28	27.34	20.55	11.19	15.03	24.81	

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

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

### 4.3. Physiological Variables

### 4.3.1. Quantum yield

Two way analysis of variances showed highly significant differences for quantum yield (photo-system 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 cultivar (P=0.06, 0.55 and 0.90) respectively. Salt stress levels significantly influences quantum yields at 40 DAT and 54 DAT highly significantly (P=0.004), while the third stage (68 DAT) did not show any significance 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. At 54 DAT Nafis and Nasic Red showed the highest quantum yield which was par with Bombey Red. At third stage Bombey red and Nafis showed the highest quantum yield in which 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) in respectively. This result indicated that Adama Red cultivar was more salt sensitive than other cultivars. Decreament in quantum yield was reported to be indicative parameter for salt sensitivity in rape genotypes (Pak *et al.*, 2009) and tomato (Moniruzzaman *et al*, 2013). This also assures the field performance of Adama Red cultivar in which was observed as early dead and scorching of leaves especially with 12 dSm<sup>-1</sup> salt level.

The highest quantum yield value was recorded from Awash water (0.3 dSm<sup>-1</sup>), up to 8 and 4 dSm<sup>-1</sup> salt levels at (40 DAT) and 54 DAT respectively. The least values were observed with 12 dSm<sup>-1</sup> (0.522) at first stage, whereas the second stages of records showed the least quantum yield with 8 and 12 dSm<sup>-1</sup>. The result showed that as salt stress level increases 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 as salt level increased quantum yield of the genotypes was decreased. Photosystem II (PSII) is a multisubunit chlorophyll protein complex that drives electron transfer from water to plastoquinone using energy derived from light (Minagawa1 and Takahashi, 2004). According to Murata *et al.* (2007), salt stress suppressed not only the

synthesis of the D1 protein de novo 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), inactivated rubisco and the inhibition of  $CO_2$  fixation by salt stress induces the generation of ROS, which, in turn, inhibit protein synthesis, inactivates ATP synthase and decreases 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, was reported to be the attribution of low capacity of synthesis of proteins present in the membranes of the thylakoids (Sousa *et al.*, 2016).

### 4.3.2. 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 (Appendix Table 8).

The interaction effects indicated that the highest SPAD value was recorded for Adama Red with 4 dSm<sup>-1</sup> (20.36  $mmolm^{-2}s^{-1}$ ) at first stage which was statistically par with Adama Red with Awash water and 8 dSm<sup>-1</sup> also Nasic Red with 8 dSm<sup>-1</sup>. Nafis with 1.2 dSm<sup>-1</sup> (29.88  $mmolm^{-2}s^{-1}$ ) at second stage and Nafis with Awash water and 4 dSm<sup>-1</sup>, Bombey Red with 4 dSm<sup>-1</sup> showed the highest SPAD values at third stage (Table 6). The least SPAD value at three stages interacted as Nafis with 12 dSm<sup>-1</sup> (6.25  $mmolm^{-2}s^{-1}$ ), Adama Red with Awash water (4.6  $mmolm^{-2}s^{-1}$ ) and Adama Red with 12 dSm<sup>-1</sup> salt levels (4.24  $mmolm^{-2}s^{-1}$ ) at stages of 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 either inhibits synthesis and/or accelerates the degradation of existing chlorophyll molecules (Wani *et al.*, 2013).

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

Table 6. Interaction effects of onion cultivars and levels of salt stress on chlorophyll in SPAD units and stomatal conductance in porometer at three growth stages under MARC in 2018

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

#### 4.3.3. Stomatal conductance

ANOVA for the main factors and their interaction effects on stomatal conductance by porometer across the three stages (51 DAT, 65 DAT, and 79 DAT) showed that highly significance variances (P=0.001).

The highest porometer was recorded for Nafis with 4 dSm<sup>-1</sup> and Adama Red with 1.2 dSm<sup>-1</sup> which was statistically par with Nasic Red with Awash water at first stage. Nasic Red with 1.2 dSm<sup>-1</sup> (187.5  $mmolm^{-2}s^{-1}$ ) at second stage and Awash water with Adama Red and Nasic Red showed the highest porometer at third stage (Table 6). The least stomatal conductance was observed in Bombey Red with Awash water (62.87  $mmolm^{-2}s^{-1}$ ) at 51 DAT in which it 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<sup>-1</sup>. At 65 DAT Bombey Red with 4 dSm<sup>-1</sup> (52.27 mmolm<sup>-2</sup>s<sup>-1</sup>) which was par with Bombey Red with 12, Nafis with Awash water and 1.2 dSm<sup>-1</sup> and at third stage Bombey Red with 4 dSm<sup>-1</sup> and Nafis with 12 dSm<sup>-1</sup> showed the least porometer. Likely Azeem et al. (2017) reported that increased salt levels decreased stomatal conductance of okra cultivars. A significant decrease in the stomatal conductance of plants exposed to the increasing levels of salt stress, reported to diminished net photosynthetic rate, by limiting internal CO<sub>2</sub> concentration and transpiration rate (Saleem et al., 2011). Although, the current study did not show linear increase or decrease of stomatal conductance on salt stress levels due to cultivars responded independently, salt stress levels above 8 dSm<sup>-1</sup> were highly affected leaf porometer.

#### 4.4. Yield and Yield Components

#### 4.4.1. Leaf length

Leaf length was highly significantly affected by cultivars (P=0.01) and salt levels (P=0.0001) respectively. However, leaf length did not affect by their interaction (P=0.12) (Appendix Table 9).

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 7). 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 exists among themselves.

Increment 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<sup>-1</sup> irrigated units and the shortest plant height was observed on 8 and 12 dSm<sup>-1</sup> salt stress levels. Similar work was reported on onion leaf number and leaf length was negatively affected at high NaCl concentrated irrigation water (Stab-baba *et al.*, 2010; Hanci and Cebeci, 2015). Salt stress reported to inhibit plant height, increasing levels of NaCl in the soil showed a diminished net photosynthetic rate, which will limit automatically the photosynthetic CO<sub>2</sub> assimilation (Saleem *et al.*, 2011).

## 4.4.2. Leaf width

ANOVA revealed that 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 (Appendix Table 9).

The highest leaf width value was recorded for Nasic Red, Bombey Red and Nafis while the lowest value (1.51 mm) recorded for Adama Red (Table 7). 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 the cell division and cell expansion, consequently leaf expansion and as a result leaf width is reduced.

Among salt stress levels the widest leaf width (2.16 mm) was recorded with Awash water (0.3), whereas 1.2 and 4 dSm<sup>-1</sup> showed intermediate leaf width, while the lowest value was recorded on 8 and 12 dSm<sup>-1</sup> the salt levels. Our results indicated that linearly increasing in concentration of NaCl stress levels significantly reduced leaf width of the onion cultivars. Our finding was also supported by the work of Munns (2002) who reported that plants changes their normal morphological structure in order to defend themselves from stress they faced. It was also reported that salinity reduced final leaf width and emergency of number of lateral shoots in soybean (Dolatabadian *et al.*, 2011), reduced leaf area at the whole-plant level (leaf area ratio) and at the individual leaf level (specific leaf area) in beet root (Rozema *et al.*, 2015).

## 4.4.3. Plant height

Analysis of variance showed that there were significant differences among cultivars (P=0.01) and 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 Nafis, where the lowest plant height was recorded for Adama Red (24.28 cm) cultivar.

Experimental units irrigated with Awash water  $(0.3 \text{ dSm}^{-1})$  and  $1.2 \text{ dSm}^{-1}$  showed the highest plant height, whereas the lowest plant height was observed on 8 and 12 dSm<sup>-1</sup> salt levels. On 4 dSm<sup>-1</sup> 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. From the observation during the experiment, onion plant height at highest salt levels (more than 4 dSm<sup>-1</sup>) gradually decreased and final plants height was severely stunted. The reason of stunting in plant height was assumed to be, releasing enough hormone

to trigger leaf abscission (Dodd, 2005), and the earliest response of glycophytes exposure 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.

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

Table 7. Mean comparison of leaf length, leaf width, and plant height, fresh shoot and dry weight of onion cultivars under salt stress levels at MARC in 2018

Means in the column followed by the same letter(s) are not significantly differ 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.

#### 4.4.4. Fresh and dry above ground biomass

Fresh and dry above ground biomass weights were significantly and highly significantly affected by cultivars (P=0.04 and P= 0.0007), and highly significant differences were observed among salt levels (P= 0.0001) respectively. Their interaction did not show any significant difference p=0.22 (Appendix Table 9).

Nasic Red cultivar (51.33 g) showed the highest fresh weight statistically 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 dry and fresh biomass weight, but also in quantum yield, leaf number, leaf length, and plant height parameters. The reason assumed to be the variability in internal factors through which the crops maintains their physiology and morphological characters from the induced stress.

Among salt stress levels the highest fresh and dry biomass weight was recorded with Awash water and 1.2 dSm<sup>-1</sup> salt stress levels and the least were recorded with 8 and 12 dSm<sup>-1</sup> salt stress levels. In this study, salt stress significantly reduced plant fresh and dry weight of onion cultivars as shown above (Tables 7). These results are in conformity with 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 significantly reduced growth parameters and photosynthetic attributes which finally reduces photo-assimilate production and translocation (Azeem *et al.*, 2017).

### 4.4.5. Bulb length and width

Two ways of analysis showed highly significant variations for bulb length and bulb width among 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 significance 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 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 determining 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. As (Table 8.) indicated 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  $(0.30 \text{ dSm}^{-1})$  and  $1.2 \text{ dSm}^{-1}$  salt levels, whereas the least bulb length and width was observed at 8 and 12 dSm<sup>-1</sup> salt levels. This result showed that an increased salt concentration levels more than threshold  $(1.2 \text{ dSm}^{-1})$  radically reduced bulb length and width. Supportive work was reported by Kahouli *et al.* (2014) in carrot, increasing in salt concentration decreased root diameter and length. The reduction in bulb length and width under high NaCl salt concentration might be as results of salts induced internal water deficit which cause partial or complete closure of stomata (Azeem *et al.*, 2017), and finally inhibit leaf expansion, reduces net photosynthetic capacity of the plants, leading to reduction in biomass production (Saleem *et al.*, 2011).

#### 4.4.6. Fresh and dry bulb biomass weight

Fresh and dry bulb biomass weights were significantly influenced by cultivars (P=0.02 and P=0.03), respectively. It was also highly significant differences in fresh and dry bulb biomass weights were 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 8). The result pinpointed as Bombey Red, Nasic Red and Nafis cultivars performing better than Adama Red cultivar in which it

was noticed as relatively low performing cultivar. The differences among cultivars might be due to internal factor by which they maintain their morphological and physiological parameters which finally influence their dry matter accumulation and yield.

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

Treatments	Bulb length	Bulb width	Fresh bulb	Dry bulb	TSS
	(mm)	(mm)	wgt. (g)	wgt.(g)	( <sup>o</sup> birx)
Cultivars					
Adama Red	26.39 <sup>b</sup>	15.18 <sup>b</sup>	28.15 <sup>b</sup> (1.08)	4.55 <sup>b</sup> (1.55)	11.73 <sup>b</sup>
Bombey Red	30.73 <sup>a</sup>	20.41 <sup>a</sup>	76.87 <sup>a</sup> (1.57)	8.85 <sup>a</sup> (1.59)	11.52 <sup>b</sup>
Nafis	28.84 <sup>ab</sup>	22.07 <sup>a</sup>	49.43 <sup>ab</sup> (1.56)	7.37 <sup>ab</sup> (1.58)	11.70 <sup>b</sup>
Nasic Red	30.50 <sup>a</sup>	18.90 <sup>a</sup>	64.05 <sup>a</sup> (1.48)	8.15 <sup>a</sup> (1.59)	12.47 <sup>a</sup>
CR (5%)	2.80	3.55	0.20	0.04	0.68
Salt levels (dSm <sup>-1</sup> )					
Awash water (0.3)	35.28 <sup>a</sup>	26.54 <sup>a</sup>	106.75 <sup>a</sup> (1.93)	11.01 <sup>a</sup> (1.62)	11.71 <sup>bc</sup>
1.2	34.04 <sup>a</sup>	25.49 <sup>a</sup>	90.73 <sup>a</sup> (1.91)	11.32 <sup>a</sup> (1.63)	12.78 <sup>a</sup>
4	29.55 <sup>b</sup>	19.16 <sup>b</sup>	43.50 <sup>b</sup> (1.41)	7.42 <sup>ab</sup> (1.58)	12.21 <sup>ab</sup>
8	22.43 <sup>c</sup>	10.55 <sup>c</sup>	10.33 <sup>c</sup> (0.98)	4.73 <sup>bc</sup> (1.54)	11.41 <sup>bc</sup>
12	22.22 <sup>c</sup>	11.64 <sup>c</sup>	9.90 <sup>c</sup> (0.78)	1.08 <sup>c</sup> (1.51)	10.94 <sup>c</sup>
CR (5%)	3.14	4.02	0.22	0.04	0.77
CV%	12.36	24.08	17.75	3.11	7.47

Table 8. Mean comparison of bulb length, width, fresh and dry weight and TSS of onion cultivars evaluated under five salt stress levels at MARC in 2018

Means in the column followed by the same letter(s) are not significantly differ 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.

#### **4.4.7.** Total soluble solid (TSS)

TSS was significantly and highly significantly 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 (Appendix Table 10).

The highest TSS was recorded for Nasic Red (12.47 <sup>o</sup>brix), while the lowest <sup>o</sup>brix was observed in Bombey Red, Adama Red and Nafis cultivars. Under natural condition report indicated that Nasic Red and Nafis known to have the highest 10- 18 <sup>o</sup>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 °brix) was observed on  $1.2 \text{ dSm}^{-1}$  which was statistically similar with 4 dSm<sup>-1</sup> salt stress level, whereas the least was recorded on 12 dSm<sup>-1</sup> (10.94 °brix) which was statistically par with 0.3 dSm<sup>-1</sup> and 8 dSm<sup>-1</sup> treated units (Table 8). This indicated that salt stress to certain level of salinity concentration might increase TSS as compared to the lowest salt concentration. Supportive work was reported by Abdallah *et al.* (2016) in rice, Ghodke *et al.* (2018) under forced drought stress TSS was slightly elevated in comparison to routinely irrigated plot. Hepksoy (2004) also reported that *Sastuma madrin c.* Owari orange orchard grown nearby sea with comparable with the farthest from the sea showed the highest TSS. The increment of these TSS reported to regulate its osmosis, improve metabolic processes during stress conditions (Ripoll *et al.*, 2014).

## 4.5. Pearson's Correlation

### 4.5.1. Correlation for germination variables

Pearson's correlation coefficients for most of germination parameters of onion cultivars under salinized water were showed weakly to strongly positive associations as shown in (Table 9). The result indicated germination rate was not significantly correlated with seedling vigor index, shoot length, germination percentage, shoot to root ratio, root length, fresh and dry shoot weight. Seedling vigor index was strongly and highly significantly associated with shoot length (r = 0.74, P = 0.0001), and highly significantly correlated with germination percentage (r= 0.71), shoot to root ratio (r= 0.58, P= 0.0001) and root length (r= 0.44, P= 0.0008), whereas dry weight (r= 0.17, P= 21) and fresh weight (r=0.25, P= 0.07) were not significantly correlated. This might be due to seedling vigor index is the multiplicative of germination percentage and seedling length (the sum of shoot and root length). As germination percentage and seedling length increased seedling vigor index also increased. Shoot length was very highly significantly (P= 0.0001) associated with, germination percentage (r= 0.74), shoot to root ratio (r= 0.93), root length (r= 0.81), dry shoot weight (r= (0.50) and fresh shoot weight (r= (0.47)). The associations of shoot length, germination percentage, shoot to root ratio, root length, fresh and dry shoot weight with germination percentage, shoot to root ratio, root length, fresh and dry shoot weight implied shoot length and root length were bases for seedlings considered as germinated. If shoot and root are not procurable the rest parameters may not exists. Germination percentage was highly significantly associated with, shoot to root ratio (r= 0.74, P= 0.0001), root length (r= 0.63, P=(0.0001), shoot fresh weight (r= 0.33, P= 0.004) and significance correlation with dry shoot weight (r= 0.27, P= 0.01). Shoot to root ratio, was very highly significantly associated with, root length (r= 0.74, P= 0.0001), fresh shoot weight (r= 0.42, P= 0.0002) and dry shoot weight (r= 0.38, P= 0.0007). Root length, was very highly significantly (P= 0.0001 associated with, fresh shoot weight (r=0.57) and dry shoot weight (r=0.42).

	GR	SVI	SHL	GP	S/R	RL	DW	FW
GR	1.00	0.00	$-0.02^{ns}$	-0.03 <sup>ns</sup>	0.00 <sup>ns</sup>	0.01 <sup>ns</sup>	0.02 <sup>ns</sup>	$-0.02^{ns}$
SVI		1.00	0.74**	0.71***	0.58***	0.44***	0.17 <sup>ns</sup>	0.25 <sup>ns</sup>
SHL			1.00	0.74 <sup>***</sup>	0.93***	0.81***	0.50***	0.47***
GP				1.00	0.74 <sup>***</sup>	0.63***	$0.27^{*}$	0.33***
S/R					1.00	0.74***	0.42***	0.38***
RL						1.00	0.42***	0.57***
DW							1.00	0.53***
FW								1.00

Table 9. Pearson's correlation coefficient of germination parameters of onion cultivars under salt stress levels in Seed Laboratory at MARC in 2018

\* indicates significance at p<0.05, \*\* at p<0.01, \*\*\* at p<0.001, ns- non significance, GRgermination rate, SVI- Seedling vigor Index, SHL- shoot length, GP- Germination percentage, S/R- shoot to root ratio, RL- root length, DW- dry shoot weight, FW, fresh shoot weight.

### 4.5.2. Correlation for yield and yield related parameters

The Pearson's correlation analysis of yield related and yield parameters of onion cultivars strongly and positively correlated to each other's as shown in (Table 10). The result indicated that leaf length was significantly (P= 0.0001) and positively correlated with leaf width (r= (0.81), plant height (r= 0.97), bulb length (r= 0.81), bulb width (r= 0.83), fresh bulb weight (r= 0.75), fresh shoot (r= 0.79), dry weight (r= 0.82) and TSS (r= 0.43, P= 0.001). Leaf width was highly significantly (P=0.0001) associated with plant height (r=0.83), bulb length (r=0.64), bulb width (r= 0.73), fresh bulb weight (r= 0.63), dry bulb weight (r= 0.67), fresh shoot (r= 0.72), dry shoot weight (r= 0.75) and TSS (r= 0.41, P= 0.002). Plant height was also highly significantly (P = 0.0001) associated with bulb length (r = 0.83), bulb width (r = 0.86), fresh bulb weight (r= 0.76), dry bulb weight (r= 0.77), fresh shoot (r= 0.80), dry weight (r= 0.84) and TSS (r = 0.45, P = 0.0008). Bulb length was highly significantly (r = 0.83, P = 0.0001) associated with, bulb width (r= 0.92), fresh bulb weight (r= 0.79), dry bulb weight (r= 0.72), fresh shoot weight (r= 0.86), dry weight (r= 0.86) and TSS (r= 0.40, P= 0.002). Bulb width was positively and highly significantly (P= 0.0001) correlated with, fresh bulb weight (r= (0.88), dry bulb weight (r= 0.84), fresh shoot weight (r= 0.85), dry weight (r= 0.91) and TSS (r= 0.46). Fresh bulb weight was also highly significantly (P= 0.0001) correlated with dry bulb weight (r= 0.84), fresh shoot weight (r= 0.81), dry shoot weight (r= 0.90) and TSS (r= 0.31). Dry bulb weight positively and highly significantly (P= 0.0001) correlated with fresh shoot weight (r = 0.69), dry shoot weight (r = 0.78) and TSS (r = 0.42, P = 0.001). Fresh shoot weight positively and highly significantly correlated with dry shoot weight (r= 0.95, P= (0.0001) and TSS (r= 0.35, P= 0.009).

Strongly and positively association between leaf length, leaf width, plant height with bulb length, width, fresh and dry weight and fresh and dry shoot weight implied that leaf length, width and plant heights were components of photo-assimilates synthesis which finally translocated to the rest of plant parts. Thus, increasing in leaf length, width, and plant heights also increases the photosynthetic production and further increase bulb length, width, fresh and dry weight, and shoot fresh and dry weight of onion cultivars.

	LL	LW	РН	BL	BW	FBW	DBW	FSW	DSW	TSS
LL	1.00	0.81***	0.97***	0.81***	0.83***	0.75***	0.75***	0.79***	0.82***	0.43***
LW		1.00	0.83***	0.64***	0.73***	0.63***	0.67***	0.72***	0.75***	0.41**
PH			1.00	0.83***	0.86***	$0.76^{***}$	0.77***	$0.80^{***}$	0.84***	0.45***
BL				1.00	0.92***	$0.79^{***}$	0.72***	0.86***	0.86***	$0.40^{***}$
BW					1.00	0.88***	0.84***	$0.85^{***}$	0.91***	0.46***
BFW						1.00	0.84***	0.81***	0.90***	0.31*
BDW							1.00	0.69***	$0.78^{***}$	0.42***
SFW								1.00	0.95***	0.35**
SDW									1.00	0.33**
TSS										1.00

Table 10. Pearson's correlation coefficients of growth, yield and yield related parameters of onion cultivars under salt stress levels at MARC under open field on soil growing media in 2018

\* indicates significance at p<0.05, \*\* at p< 0.01, \*\*\* at p< 0.001, ns- non significance, LL- leaf length, LW- leaf width PH- plant height, BL- bulb length, BW- bulb width, FBW- fresh bulb weight, DBW- dry bulb weight, FSW- fresh above ground shoot weight, TSS- total soluble solid.

# **5. SUMMARY AND CONCLUSIONS**

Salt stress is limiting onion production and productivity due to crop sensitivity to salt stress. In Ethiopia, onion is one of the commercial bulb crop grown under irrigation where production and productivity were affected by salt stress via irrigation water. Hence, study was conducted at MARC to determine onion cultivars to different salt stress levels in the laboratory and in the open field using saline irrigation water.

Laboratory result showed highly significant ( $p\leq0.001$ ) variations in seed germination percentage, seedling shoot length, seedling root length, shoot to root length ratio, seedling vigor index, fresh and dry weight within salt stress levels. Significant ( $p\leq0.05$ ) differences were also observed among cultivars in germination rate and shoot to root length ratio, but in seedling root length, shoot length, dry and fresh weight and main factors interaction did not show significant differences (p>0.05). The highest shoot length, root length, shoot to root ratio and seedling dry weights were observed up to 4 dSm<sup>-1</sup> salt stress level. Germination percentages and seedling vigor index were gradually decreasing from the higher values with distilled water to on 12 dSm<sup>-1</sup> salt stress level. The interaction effect showed Nasic Red with distilled water was the highest seedling vigor index (136.84), while the least was recorded in the interactions of Adama Red and Nasic Red cultivars with 12 dSm<sup>-1</sup> salt stress level. The fastest germination rate was recorded for Adama Red and Nafis in which statistically similar with Agrifound and Bombey Red, whereas Adama Red showed maximum (5.52) seedling shoot to root ratio while Agrifound (4.20) was the least.

Field experiment results indicated that growing onion cultivars with salt stressed levels significantly affected growth variables, physiology, yield, and yield components of onion. An average leaf numbers per plant, plant height and pseudo-stem diameter were highly affected with salt stress levels at 30, 44 and 58 days after transplanting. However, during the early growth stage up to 4 dSm<sup>-1</sup> did not affect leaf numbers per plants, pseudo stem diameter, plant height, but gradually decreased at third stage. Among cultivars the highest leaf numbers per plants were observed in Bombey Red and Nasic Red were statistically same with Nafis, while the highest plant height and pseudo stem diameter was recorded for Bombey Red statistically

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similar with and Nasic Red, whereas Adama Red showed the lowest leaf numbers, shortest and thinnest across the stages.

Physiological parameters like chlorophyll in SPAD- meter and stomatal conductance in porometer taken across the stages showed highly significant variations among main factors and their interactions, whereas quantum yield showed significance variation in at  $40^{\text{th}}$  DAT and  $68^{\text{th}}$  DATs among cultivars and salt levels at 40 and 54 DATs stages. The highest SPAD value was Adama Red with 4 dSm<sup>-1</sup> (20.36 *mmolm*<sup>-2</sup>*s*<sup>-1</sup>) salt level at first stage, Nafis with 1.2 dSm<sup>-1</sup> (29.88 *mmolm*<sup>-2</sup>*s*<sup>-1</sup>) at second stage and at third stage Nafis with 4 dSm<sup>-1</sup>. Nafis with 4 dSm<sup>-1</sup> (193.38 *mmolm*<sup>-2</sup>*s*<sup>-1</sup>) at first stage, Nasic Red with 1.2 dSm<sup>-1</sup> (187.5 *mmolm*<sup>-2</sup>*s*<sup>-1</sup>) at second stage and Adama Red with Awash water (146.06 *mmolm*<sup>-2</sup>*s*<sup>-1</sup>) showed the highest porometer. The highest quantum was recorded for Nasic Red, Nafis and Bombey Red, where Adama Red showed the least. Quantum yield was reduced as salt concentration level increased.

Onions leaf length, leaf width, plant height, fresh and dry above ground biomass weight, fresh and dry bulb biomass weights, TSS, bulb length and width were affected significantly ( $p \le 0.05$ ) by cultivars and highly significantly ( $p \le 0.001$ ) salt levels. 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 °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 on 1.2 dSm<sup>-1</sup> salt stress level.

Generally most of germination variables and early stage of growth were not affected up to 4  $dSm^{-1}$ , whereas the highest growth and yield performances were recorded with 1.2  $dSm^{-1}$  salt stress levels in the field. It is concluded that our cultivars cannot resist salt stress more than 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 other cultivars and no more than 4  $dSm^{-1}$  salt levels in the future.

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## **APPENDICES**

No.	Cultivars	Yield (ton/ha) on research field	Status released	Seed sources
1	Nafis	40	2010	MARC/EIAR
2	Nasic	35	2004	MARC/EIAR
3	Adama	35	1980	MARC/EIAR
4	Bombey Red	30	1980	MARC/EIAR
5	Agrifound		pipeline	MARC/EIAR

Appendix Table 1. Description of onion cultivars used for experiment

*Source(s):* (EARO, 2004; MoRDA, 2009, 2010)

Treatments	onion Cultivars	Salinity levels	Combinations
		$(dSm^{-1})$	Cultivar * dSm <sup>-1</sup>
Т 1.	Nafis	distilled water	Nafis*distilled water
T 2		1.2	Nafis*1.2
Т3		4	Nafis*4
T 4		8	Nafis*8
Т 5		12	Nafis*12
T 6	Nasic Red	distilled water	Nasic Red*distilled water
Т7		1.2	Nasic Red*1.2
T 8		4	Nasic Red*4
Т9		8	Nasic Red*8
T 10		12	Nasic Red*12
T 11	Adama Red	distilled water	Adama Red*distilled water
T 12		1.2	Adama Red*1.2
Т 13		4	Adama Red*4
T 14		8	Adama Red*8
T 15		12	Adama Red*12
T 16	Bombey Red	distilled water	Bombey Red*distilled water
Т 17		1.2	Bombey Red* 1.2
T 18		4	Bombey Red* 4
T 19		8	Bombey Red* 8
T 20		12	Bombey Red* 12
T 21	Agrifound (Roobaaf)	distilled water	Bombey Red*distilled water
T 22		1.2	Bombey Red* 1.2
T 23		4	Bombey Red* 4
Т 24		8	Bombey Red* 8
Т 25		12	Bombey Red* 12

Appendix Table 2. Treatment combinations for germination tests

Treatments	onion Cultivars	Salinity levels $(dSm^{-1})$	Combinations
			Cultivar * dSm <sup>-1</sup>
T 1.	Nafis	0.30 (Awash water)	Nafis*0.30
T 2		1.2	Nafis*1.2
T 3		4	Nafis*4
T 4		8	Nafis*8
Т 5		12	Nafis*12
Τ6	Nasic Red	0.30 (Awash water)	Nasic Red*0.30
Τ7		1.2	Nasic Red*1.2
T 8		4	Nasic Red*4
Т9		8	Nasic Red*8
T 10		12	Nasic Red*12
T 11	Adama Red	0.30 (Awash water)	Adama Red*0.30
T 12		1.2	Adama Red*1.2
T 13		4	Adama Red*4
T 14		8	Adama Red*8
T 15		12	Adama Red*12
T 16	Bombey Red	0.30 (Awash water)	Bombey Red*0.30
T 17		1.2	Bombey Red* 1.2
T 18		4	Bombey Red* 4
Т 19		8	Bombey Red* 8
Т 20		12	Bombey Red* 12

Appendix Table 3. Treatment combinations for field experiment

Source of	Df	Mean squares of parameters						
variation		Germination percentage (%)	Germination rate	Shoot length (cm)	root length (cm)			
cultivar	4	0.01 <sup>ns</sup>	0.49*	0.0033 <sup>ns</sup>	0.005 <sup>ns</sup>			
Salt level	4	1.67***	0.28 <sup>ns</sup>	0.1056***	$0.087^{***}$			
Cultivar*Level	16	0.03 <sup>ns</sup>	0.23 <sup>ns</sup>	0.0054 <sup>ns</sup>	0.006 <sup>ns</sup>			
Error	50	0.025	0.23	0.0054 <sup>ns</sup>	0.006			

Appendix Table 4. ANOVA for mean squares of germination percentage, rate, shoot and root length of onions under salt stress levels in Laboratory at MARC in 2018

\*, \*\*, \*\*\* indicates significance at p<0.05, p<0.01, p<0.001 respectively. Df- degree freedom, ns- non significance.

Appendix Table 5. ANOVA table for mean squares of shoot /root, seedling vigor index, fresh and dry weight of onions under salt stress levels in Laboratory at MARC in 2018

Source of	Df	Mean squares of parameters					
vanauon		Shoot /root	Seedling vigor index	Fresh weight(g)	Dry weight(g)		
cultivar	4	0.007*	9360.15***	0.01 <sup>ns</sup>	0.003 <sup>ns</sup>		
Level	4	0.092***	57362.29***	$0.08^{**}$	$0.006^{*}$		
Cultivar*Level	16	0.007 <sup>ns</sup>	5583.64***	0.02 <sup>ns</sup>	0.003 <sup>ns</sup>		
Error	50	0.004	58.47	0.02	0.003		

\*, \*\*, \*\*\* indicates significance at p<0.05, p<0.01, p<0.001 respectively. Df- degree freedom, ns- non significance.

Source of variation	Df	Mean squar Leaf numbe	res of er per plant		Plant height		
		30 DAT	44 DAT	58 DAT	30 DAT	44 DAT	58 DAT
Rep	2	1.18*	1.27*	0.44 <sup>ns</sup>	16.12 <sup>ns</sup>	92.94 <sup>ns</sup>	80.05 <sup>ns</sup>
cultivar	3	0.17 <sup>ns</sup>	1.03*	3.41***	13.08 <sup>ns</sup>	110.33*	243.07***
Level	4	1.03*	5.97***	6.68***	28.22*	368.80***	618.37***
Cultivar*Level	12	0.61 <sup>ns</sup>	0.72 <sup>ns</sup>	0.22 <sup>ns</sup>	11.60 <sup>ns</sup>	63.25 <sup>ns</sup>	37.53 <sup>ns</sup>
Error	38	0.36 <sup>ns</sup>	0.37	0.25	11.12 <sup>ns</sup>	34.56	26.94

Appendix Table 6. ANOVA for leaf number per plant and plant height of onions as influenced by levels of salt stress at three different growth stages in field at MARC in 2018

\*, \*\*, \*\*\* indicates significance at p<0.05, p<0.01, p<0.001 respectively. Df- degree freedom, ns- non significance.

Appendix Table 7. ANOVA for pseudo-stem diameter and quantum yield of onion cultivars as influenced by levels of salt stress at three different growth stages in field at MARC in 2018

Source of variation	Df	Mean squares of pseudo-stem diameter (mm)			Quantum yield			
	-	30 DAT	44 DAT	58 DAT	40 DAT	54 DAT	68 DAT	
Rep	2	1.35 <sup>ns</sup>	3.44 <sup>ns</sup>	2.58 <sup>ns</sup>	0.009 <sup>ns</sup>	0.0074 <sup>ns</sup>	0.0679*	
cultivar	3	0.55 <sup>ns</sup>	2.27 <sup>ns</sup>	3.70*	0.023**	0.0205	$0.0508^{*}$	
Level	4	0.77 <sup>ns</sup>	12.10*	17.92***	0.019**	0.0561***	0.01136	
Cultivar*Level	12	0.71 <sup>ns</sup>	1.70 <sup>ns</sup>	1.09 <sup>ns</sup>	0.008 <sup>ns</sup>	0.0059 <sup>ns</sup>	0.0074 <sup>ns</sup>	
Error	38	0.48	1.45	0.96	0.004 <sup>ns</sup>	0.0059	0.0148	

\*, \*\*, \*\*\* indicates significance at p<0.05, p< 0.01, p< 0.001 respectively. Df- degree freedom, ns- non significance. DAT- days after transplanting.

Source of variation	Df	Mean squa SPAD (mn	res of $nolm^{-2}s^{-1}$ )		Porometer	Porometer (mmolm <sup>-2</sup> s <sup>-1</sup> )			
		49 DAT	63 DAT	77 DAT	51 DAT	65 DAT	79 DAT		
Rep	2	4.99 <sup>ns</sup>	1.85 <sup>ns</sup>	0.46 <sup>ns</sup>	210.18 <sup>ns</sup>	61.12 <sup>ns</sup>	11.93 <sup>ns</sup>		
cultivar	3	100.49***	85.43***	50.66***	6661.51***	5687.57***	3553.84***		
Level	4	48.23***	142.00***	74.82***	2867.64***	1973.94***	3169.35***		
Cultivar* Level	12	52.72***	74.21***	16.49***	3835.19***	3187.49***	1144.82***		
Error	38	3.74	2.87	3.44	326.88	92.97	49.80		

Appendix Table 8. ANOVA for mean squares of SPAD ( $mmolm^{-2}s^{-1}$ ) and Porometer ( $mmolm^{-2}s^{-1}$ ) of onions under salt stress levels at three growth stages in field at MARC in 2018

\*, \*\*, \*\*\* indicates significance at p<0.05, p< 0.01, p< 0.001 respectively. Df- degree freedom, ns- non significance. DAT- days after transplanting.

Source of variation	Df	Mean squares parameters						
variation	-	leaf length (cm)	leaf width (m)	plant height (cm)	fresh shoot wgt.(g)	dry shoot wgt.(g)		
Rep	2	132.586***	0.22*	127.97***	0.0004 <sup>ns</sup>	0.0004 <sup>ns</sup>		
cultivar	3	86.51**	0.43**	92.14 <sup>*</sup>	0.003*	0.009***		
Level	4	356.34***	1.49***	491.97***	$0.008^{**}$	0.062***		
Cultivar *Level	12	25.37 <sup>ns</sup>	0.09 <sup>ns</sup>	24.23 <sup>ns</sup>	0.001 <sup>ns</sup>	0.0023 <sup>ns</sup>		
Error	38	15.38	0.07	21.39	0.001	0.0012		

Appendix Table 9. ANOVA for mean squares of leaf length, leaf width, plant height, fresh and dry shoot weight of onions under different salt stress levels in field at MARC in 2018

\*, \*\*, \*\*\* indicates significance at p<0.05, p<0.01, p<0.001 respectively. Df- degree freedom, wgt-weight, ns- non significance.

Appe	endix T	able 10. A	ANOVA	for mean	n square	s of b	ulb wid	th, bulb	length,	TSS,	Fresh	and	dry
bulb	weight	of onions	under sa	alt stress	levels ir	field	at MA	RC in	2018				

Source of	Df	Mean squares parameters						
variation		Bulb width (mm)	Bulb length (mm)	Fresh bulb wgt. (g)	Dry bulb wgt. (g)	TSS ( <sup>°</sup> brix)		
Rep	2	127.20 <sup>ns</sup>	50.65 <sup>*</sup>	0.24*	0.004 <sup>ns</sup>	0.37 <sup>ns</sup>		
cultivar	3	109.73**	68.47**	0.71***	0.006*	2.45*		
Level	4	600.77***	399.23***	2.99***	0.032***	5.55***		
Cultivar *Level	12	30.68 <sup>ns</sup>	10.08 <sup>ns</sup>	0.14 <sup>ns</sup>	0.0054 <sup>ns</sup>	0.96 <sup>ns</sup>		
Error	38	21.34	13.01	0.06	0.0024	0.78		

\*, \*\*, \*\*\* indicates significance at p<0.05, p< 0.01, p< 0.001 respectively. Df, degree freedom, ns- non significance, wgt-weight, DAT- days after transplanting, TSS- total soluble solid.