ASSESSMENT OF MORPHOLOGICAL AND BIOCHEMICAL RESPONSE OF DIFFERENT ONION CULTIVARS UNDER SALT STRESS CONDITIONS

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ABSTRACT: Salinity is among the major abiotic challenges that limit soil's ability to supply nutrients to the plants which is detrimental to crop optimum productivity. Onion is globally an important vegetable crop that is affected by salt stress. Present study is focused on the impact of salinity on growth, production and quality of different onion cultivars. Ten onion cultivars were tested against three salinity levels i.e. 40, 80 and 120 mM NaCl during the year 2017-18. A completely randomized design (CRD) having four replications was used for analyzing the results statistically. The results pertaining to the days of germination indicated that the cv. Red Nasik took the minimum days (5.46) to attain 50% germination as compared to cv. Robinia (14.0). High salt concentration increased the time for germination and took maximum days (11.27) for 50% germination in120 mM NaCl salt level with highest plant mortality (14.06%) in cv. Faisal Red. Similarly, maximum germination (95.62%) was recorded in onion cv. Red Nasik. In the control, germination was 84.63% while the higher salt concentrations (120 mM NaCl) resulted in the minimum seed germination (61%). Plant height, the number of leaves and chlorophyll contents decreased with rising salinity levels. Similarly, chlorophyll contents decreased with each additional increased in salt concentration and the maximum value for this trait was recorded in control as compared to the minimum in 120 mM NaCl i.e. 58.42 SPAD. However, maximum proline contents (30.87 µ mol g-1) were recorded in 120 mM NaCl as compared to the minimum (9.86 µ mol g-1) at control. Similarly, TSS improved with increasing salinity and the minimum TTS (13.00 °Brix) were recorded in control as compared to the maximum in 120 mM NaCl) i.e. 16.98 °Brix.

Key words: Salt stress, Onion, Growth, Proline, Chlorophyll, Yield.

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INTRODUCTION

Onion (*Allium cepa* L.) is a member of *Alliaceae*, belongs to the genus *Allium* used as vegetable around the globe. The onion plant have biennial nature however it is grown annually to dig the reasonable size of bulb from the soil. Seeds/Seedlings and cut pieces are used to grow the green onions infrequent season. The green onion separately used as a vegetable that is hollow, fleshy and cylindrical with one side flattened. Bulbs are rich in calcium, phosphorus and carbohydrates. The Onions the significant source of ascorbic acid, potassium with ample amount of dietary fibers, folic acid, iron, calcium and protein contents. Onions have less amount of sodium with low-fat content as well; it did not cause hypertension and obesity in the human body (National Onion Association, 2001). In the Sub-continent onion is the part of local culture and fresh food.

They are included amongst the most abundantly produced vegetables around the world; with 93.22 million tons in 2019 (FAO, 2019). Pakistan is the 8th abundantly-onion producing country around the globe (FAO, 2019). In Pakistan, onions are cultivated on 148.7 thousand hectares with a production of 2.75 M. tons. (Anonymous, 2020). Sindh is the most abundantly onion producing province with 45.6 thousand hectares under cultivation with 0.745 M. tons of fresh onions (Anonymous, 2020). Pakistan's 6.3 million hectares are affected by salinity that is of 14% of cultivated irrigated land. The estimated yield loses due to salinity is 64% (Enciso *et al*., 2015). Globally 45 million hacteres of land that about 20% of total aerated area is saline. Each year more than 1.5

million ha of land become further unfit for cultivation due to various factors of abiotic nature such as low water availability, rise in sea level and low precipitation (Munns and Tester, 2008). It is estimated that increasing intensity of salts in aerated soils at present speed will barren more than 50% cultivated land until middle of 21st Century (Shock, 2013). Increasing population will be needed more food hence high productivity from present or slightly altered high productive varieties in day by day deteriorating land resources. It is estimated that if the conditions sustained as today there will be 70% more food requirement for about 2.3 billion additional population by 2050. In present situations it is difficult to coupe with poverty in up-coming future. It is necessary to use available resources efficiently to fight against poverty and threats of climate change to save the human race with expected famine and hunger (FAO, 2019). The expected saline land since 21st century is 9000×10^6 hectare that is a vibrant threat to global agriculture. Salinity is the consequence of high surface evaporation, poor management of irrigation water, heavy irrigation with saline water and seawater basin such as Thatta district in Pakistan.

Highly sensitive plant species with soil salinity are categorized as glycophytes while salt tolerant species are halophytes. Salt-sensitive plant species did not absorb salt and maintain osmo-equilibrium by synthesizing compatible substances such as sugars, glycine-betaine (GB) and proline in that conditions (Moore *et al.,* 2010). The abiotic stress complex is the most important factor for decreasing yield in plants (Dar *et al.,* 2018). Tolerance in response to abiotic stresses in plants is controlled by numerous genes that exchange signal transduction pathways. Although onion is categorized as salt-sensitive crop however limited literature is available upon the plant agronomic, morphological and biochemical behavior such as development of optimum pungency and level of various nutrients in pulp. Increased levels of salinity with NaCl not only disturb the fresh weight or size of bulbs but also change the odor and flavor at various plant maturity stages. Leaf and bulb moisture level have parallel response to various NaCl levels. The higher levels of Na^+ and Cl^- uni-molecular radicle that accumulate in leaves and other portions. This accumulation consequently leads to the burning and necrosis of young and older leaves (Mahmood *et al.,* 2016). The present study is designed to evaluate various onion cultivators against different level of salinity in irrigation water for recommendation of high yielding onion cultivars in saline water areas of Punjab and other provinces.

MATERIAL AND METHODS

The Experiment was conducted at vegetable research area of Barani Agriculture Research Institute, Chakwal during year 2017-2018. Plants were raised in pots during their normal growing period in winter. Salinity of levels control (T_0) ,40 mM NaCl (T_1), 80 mM NaCl (T_2), 120 mM NaCl (T_3) was developed. Salinity was developed in tap water and saline water was applied for irrigation in plants. Cultivars selected for study were; V_1: cv. Red Almost, V_2: cv. Desi Red, V_3: cv. Red Nasik, V_4: cv. Pusa Red, V_5: cv. PK-10321, V_6: cv. Robinia, V_7: cv. Faisal Red, V_8: cv. Early Red, V_9: cv. Mirpurkhas, V_{10}: cv. Desi Large.

Seeds of onion cultivars were sown during last week of September, 2017 in seedbeds that was made by softening of soils. Seedlings of 12 cm height (around 60 days old) were transplanted in pots of 12 cm diameter singly as five pots/plant were placed in each treatment. The experiment was carried out in a completely randomized design with factorial arrangement with four replications (Steel *et al*., 1997). The Least Significant Difference (LSD) test at 5% probability level was applied to compare the treatment's means (Gomez and Gomez, 1984). These plants were, initially, irrigated with tap water with the help of hand shower, followed by the irrigation with saline water. Urea and DAP fertilizers were applied as a source of nitrogen and phosphorus to transplants. Whole amount of phosphorus and 1/3 of nitrogen was applied after transplantation and 2/3 of nitrogen was applied half after one month of transplantation and other half after 2nd month of transplantation. Plant protection measures were adopted to keep crop free from weeds, insect pests and diseases through hoeing and chemical sprays. After 25 days of transplantation, salinity treatment i.e. prepared saline water irrigation was applied in each pot. Agronomic practices, apart from saline irrigation, were kept uniform till harvesting. Crop was harvested at reaching maturity in May, 2018. To evaluate the response of cultivars grown in saline conditions the attributes of Days to complete 50% germination, germination percentage (%) and chlorophyll contents (Barr and Weathery, 1962), bulb growth: bulb fresh weight (gm), moisture (%), proline content (Bates *at al*., 1973) and TSS (°Brix) was recorded.

RESULTS

Plant agronomic, morphological and biochemical response of various onion cultivars in saline water application: The results ($P\leq0.05$) showed significant difference in plant agronomic, morphological and biochemical response of various onion cultivars with saline water application. Onion cultivars showed variable response in growth and development for various growth attributes with cv. Red Nasik showed earliest 50% germination (5.46 days) and highest seed germination (95.63%). The 50% germination was observed after more days (7.82) and least number of seed germinated (59.69)

in cv. Faisal Red. The results of plant response in agronomic and morphological characters in plant height (32.33) and number of leaves were better in cv. Early Red. Less plant height (26.13), number of leaves (3.74) while higher leaves water contents (70.80%) was observed while less water contents (63.45) were observed in cv. Desi Large. Attributes related to bulbs of various onion cultivars showed variable response in mean effects of salinity applied through varying saline water applications. The bulbs water contents showed the health and growth of bulbs because better growth of bulbs is the prime need for the cultivation of onion. The better and highest moisture contents were observed i.e. 86.67 % in cv. Faisal Red while highest fresh weight (89.35 gm) was observed in cv. Early Red. The highest Dry weight (12.11 gm) of bulbs were observed in cv. Desi Red while lowest dry weight (8.02) was observed in cv. Mirpurkhas. The highest bulbs diameter (29.62) and lowest bulb mortality (4.25) was observed in cv. Early Red, while the lowest bulb diameter (23.45) in cv. Mirpurkhas and highest bulbs mortality (14.25) was observed in Faisal Red. More bulbs neck (3.03) was observed in cv. PK10321 while shortest bulbs neck (2.40) was observed in cv. Pusa Red. The biochemical attributes of onion plant indicate somewhat similar response of SPAD value. Significantly higher SPAD value (65.36) and Proline (16.64) was observed in cv. Early Red while least SPAD value (62.44) and Proline contents (12.45) were observed in cv. Desi Large.

Agronomic, morphological and biochemical response of various onion cultivars to varying saline water levels: The results ($P \leq 0.05$) showed the effect of various salinity levels applied through irrigation. The results revealed that normal water without salts allowed better plant growth, development for agronomic, morphological, bulbous and biotechnological attributes of onion, while proline contents were more at higher level of salinity of i.e. 120 mM. The gradual increasing of salinity levels decreased plant growth (Table 2). More days to (11.27) 50% germination were recorded in bulbs @ 120 mM saline water, germination at this level might be due to short initial tolerance of seed due to hard core. Highest rate of germination (84.63), plant height (37.65cm) and number of leaves (6.05) in water without salts. Lowest leaves water contents (58.21%) and lesser water contents in bulbs (85.23 %) were observed in control treatment while highest fresh weight (132.10), bulbs dry weight (19.61), bulbs diameter (37.20) and highest neck diameter (6.14) were observed when no salts treatments were applied to the plants through watering. More chlorophyll (66.41) was recorded in control where no salts stress was applied. Similarly, more proline contents were observed in 120mM of salinity level as well. Proline contents help plants to fight abiotic stresses for survival. Tolerant varieties of onion reshape their epigenetic level to produce more proline contents in response to high level of salts.

Overall response of various onion cultivars to different levels of saline water application: The results revealed that best performing variety @ 40 (mM) was Early Red (43.70), while Robin (36.99) on 80 (mM) and Red Nasik (35.58) at 120 (mM). Table 3 shows that increasing level of salinity decrease the growth response of the onion varieties while cv. Red Nashik, Robin and Early red performed better @120, 80 and 40 (mM) levels of salinity respectively.

Table 1: Mean plant agronomic, morphological and biochemical response of various onion cultivars in saline water application.

Attributes	Plant Agronomy and Morphology					Plant Bulbs Morphology						Plant Biochemistry	
Varieties	**Days for 50% germination**	**Seed germination percentage**	**Plant height (cm)**	**Number of leaves**	**Leaves water contents (%)**	**Bulbs water contents**	**Bulbs fresh weight**	**Bulbs dry weight**	**Bulbs diameter**	**Bulb neck diameter**	**Bulbs mortality %**	**SPAD value**	**Proline contents**
Red Almost	9.94± 2.33^{bc}	71.88± 4.32^{bc}	29.21± 5.45^{bcd}	4.09± 1.23^{a}	62.62± 14.32^{a}	84.84± 16.78^{b}	67.82± 12.45^{de}	10.40± 2.34^{bcd}	25.4± 5.34^{cd}	2.88± 0.34^{a}	4.69± 0.92^{bcd}	56.59± 12.34^{c}	13.29± 2.54^{cd}
Desi Red	11.24± 2.13^{ab}	73.44± 10.56^{bc}	30.94± 6.55^{ab}	4.16± 0.91^{a}	70.80± 13.65^{a}	85.76± 12.45^{ab}	83.56± 13.25^{abc}	12.11± 1.78^{ab}	28.96± 5.43^{ab}	2.95± 0.14^{a}	6.25± 0.87^{cb}	62.14± 11.23^{ab}	13.23± 0.62^{bcd}
Red Nasik	5.46± 2.43^{d}	95.63± 5.45^{a}	26.45± 5.67^{de}	4.10± 0.89^{a}	65.98± 14.35^{a}	86.05± 11.56^{ab}	70.16± 13.78^{cde}	9.94± 3.21^{bcde}	25.54± 4.91^{cd}	2.81± 0.79^{a}	9.38± 1.42^{bcd}	59.75± 10.98^{bc}	10.39± 4.34^{cd}
Pusa Red	10.63± 3.12^{bc}	76.88± 11.89^{bc}	26.13± 6.89^{e}	3.74± 1.13^{a}	65.38± 11.23^{a}	86.01± 10.98^{ab}	65.74± 14.87^{de}	9.29± 2.45^{de}	24.71± 3.87^{d}	2.40± 0.92^{a}	12.5± 2.45^{ab}	61.81± 09.87^{abc}	13.72± 3.23^{cd}
PK10321	10.90± 4.32^{b}	74.38± 13.23^{bc}	28.81± 4.56^{bcde}	4.26± 1.22^{a}	65.62± 12.34^{a}	86.14± 09.76^{ab}	76.28± 11.56^{bcd}	10.29± 3.56^{bcde}	26.63± 7.65^{abcd}	3.03± 0.98^{a}	9.38± 2.98^{bc}	60.36± 13.23^{abc}	15.45± 5.45^{bc}
Robinia	14.00± 3.54^{a}	66.56± 7.89^{cd}	29.65± 7.16^{abc}	3.96± 0.97^{a}	64.48± 13.45^{a}	85.77± 11.45^{ab}	92.30± 11.25^{a}	12.95± 3.21^{a}	29.59± 3.98^{a}	2.71± 0.89^{a}	9.38± 3.21^{bcd}	60.93± 11.28^{abc}	12.82± 5.65^{bc}
Faisal Red	11.99± 2.34^{ab}	59.69± 9.78^{d}	27.35± 8.77^{cde}	3.90± 1.12^{a}	66.52± 14.98^{a}	86.67± 13.45^{a}	71.51± 17.87^{cde}	9.68± 3.21^{cde}	25.60± 5.78^{bcd}	2.93± 0.51^{a}	14.06± 4.32^{a}	60.36± 12.87^{abc}	14.91± 3.98^{bcd}
Early Red	7.82± 1.34^{cd}	77.50± 12.76^{bc}	32.33± 4.67^{a}	4.39± 0.94^{a}	67.26± 10.34^{a}	85.62± 11.89^{ab}	89.35± 12.34^{ab}	11.82± 3.21^{abc}	29.62± 5.12^{a}	2.93± 0.82^{a}	4.25± 0.76^{cd}	65.36± 13.98^{a}	16.64± 5.43^{a}
Mirpurkhas	9.53± 3.54^{bc}	76.25± 14.56^{bc}	27.60± 4.98^{cde}	4.04± 0.87^{a}	67.62± 15.67^{a}	86.35± 15.67^{a}	60.38± 10.9^{8e}	8.02± 3.23^{e}	23.45± 7.65^{d}	2.88± 0.71^{a}	9.38± 3.23^{bc}	62.64± 11.54^{ab}	16.25± 2.45^{ab}
Desi Large	9.93± 2.56^{bc}	77.81± 16.75^{b}	30.84± 3.45^{ab}	4.11± 1.45^{a}	63.45± 17.65^{a}	85.89± 14.78^{ab}	77.40± 12.55^{bcd}	10.87±3.31 ^{abcd}	28.60± 3.45^{abc}	2.64± 0.56^{a}	4.69± 1.23^{cd}	62.44± 12.78^{ab}	12.45± 3.23^{d}

Table 2: Mean plant agronomic, morphological and biochemical response of various onion cultivars in saline water application.

Attributes	Plant Agronomy and Morphology					Plant Bulbs Morphology						Plant Biochemistry	
	Days for 50% germination	Seed germination percentage	Plant height (cm)	Number of leaves	leaves water contents	Bulbs water contents	Bulbs fresh weight	Bulbs dry weight	Bulbs diameter	Neck diameter	Mortality %	Total chlorophyll contents	Proline contents
0 mM	8.13± 1.23^{b}	84.63± 11.23^{a}	37.65± 6.54^{a}	6.05± 0.98^{a}	58.21± 6.7^{8b}	85.23± 6.71^{b}	132.10± 18.72^{a}	19.61± 3.45^{a}	37.20± 6.55^{a}	6.14± 1.23^{a}	8.42± 1.21^{c}	66.14± 7.67^{a}	14.71± 3.22^{ab}
40 mM	10.26± 2.12^{a}	80.88± 12.34^{a}	33.65± 5.12^{b}	4.56± 0.78^{b}	69.48± 9.87^{a}	87.50± 5.45^{a}	100.96± 16.78^{b}	12.45± 4.23^{b}	31.70± 5.44^{b}	2.27± 0.9^{8b}	6.88± 1.34^{b}	61.22± 10.23^{b}	13.92± 3.65^{ab}
80 mM	10.91± 2.34^{a}	73.75± 9.87^{b}	24.42± 5.23^{c}	3.24± 0.81^{c}	67.71± 7.23^{a}	85.48± 4.13^{b}	41.75± 6.78^{c}	6.16± 1.54^{c}	21.48± 4.67^{c}	0.99± 0.12^{c}	9.38± 2.34^{b}	59.17± 6.7^{8b}	19.72± 5.43^{b}
120 mM	11.27± 3.12^{a}	60.75± 8.76^{c}	20.01± 4.97^{d}	2.46± 4.67^{d}	68.50± 8.76^{a}	85.43± 8.43^{b}	26.99± 7.67^{d}	3.93± 1.23^{d}	16.88± 2.34^{d}	1.85± 0.91^{b}	18.13± 4.34^{a}	58.42± 9.87^{b}	40.86± 2.45^{a}

Table 3: Mean response of various onion cultivars in saline water application of salinity level of 0, 40, 80 and 120 (mM).

Varieties	Salinity levels				
	0 (mM)	**40 (mM)**	**80 (mM)**	**120 (mM)**	Mean
Red Almost	39.51±5.41^{e}	42.17±5.65^{e}	32.22±3.44^{e}	31.34±4.56^{e}	36.31±5.45^{D}
Desi Red	43.94±5.44^{ce}	43.38±6.54^{de}	35.75±5.43^{b}	33.74±6.45^{bcd}	39.20±6.65^{AB}
Red Nasik	42.75±6.98^{d}	42.98±7.32^{c}	34.62±6.77^{bcd}	35.58±5.87^{a}	38.98±8.81^{AB}
Pusa Red	44.37±7.23^{cd}	40.84±6.55^{cd}	33.89±6.98^{bc}	33.08±4.51^{cd}	38.05±6.56^{AB}
PK10321	47.05±4.98^{b}	41.80±7.65^{de}	33.80±5.65^{bc}	33.20±6.23^{cd}	38.96±5.12^{AB}
Robinia	44.79±8.89^{bc}	44.27±4.66^{a}	36.99±9.23^{a}	33.23±6.78^{bcd}	39.82±5.67^{AB}
Faisal Red	44.01±7.23^{c}	40.38±6.78^{ab}	33.19±8.34^{c}	32.37±5.12^{d}	37.49±8.12^{AB}
Early Red	48.88±6.98^{a}	43.70±9.55^{b}	36.40±5.98^{a}	33.65±7.81^{bcd}	40.65±5.43^{A}
Mirpurkhas	39.39±7.65	40.94±7.67^{ab}	36.36±8.98^{ab}	33.76±6.98^{bc}	37.61±3.45^{C}
Desi Large	44.56±8.98^{cd}	42.41±5.67^{de}	35.03±6.87^{bc}	34.70±4.45^{ab}	39.18±6.19^{BC}
Mean	43.92±5.34^{A}	42.29±6.56^{B}	34.83±8.98^{C}	33.46±4.88^{D}	

DISCUSSION

The interactions of various salinity levels and onion cultivars were found non-significant. Similarly, Nagaz *et al.,* (2012) observed that the germination % and root length (cm) were significantly reduced due to excessive salt concentration among various cultivars of onion. Different cultivars respond differently to varying NaCl levels. The attributes such as seed germination (%) and germination intensity decrease by increasing levels of salinity, as salinity and germination have antagonistic response (Baba *et al.,* 2010 and Patel *et al.,* 2020). These results confirmed with findings of Yohannes *et al*. (2020), who reported that increasing levels of salinity stops the plant growth hence decreasing the plant height.

The increasing level of salinity reduces fresh and dry mass of leaves and plants. Furthermore, lesser number of leaves were also observed with increasing levels of salinity (Yohannes *et al*., 2020). This is might be due to the absorbance of less water due to plant tolerance mechanism activity. Due to regularization of optimum level of salt in plant body, plants generally close the plasmodesma cell for checking excessive salt absorbance. The plasmodesma closure also reduces the water level in plant body. The results are confirmed by the international reports of increasing levels of NaCl salt stress decreased growth and total chlorophyll contents in various onion cultivars (Hansi *et al*., 2016). Fresh and dry weight of onion bulbs reduce due to the effect of higher salt levels of both soil and water (Yohannes *et al*., 2020). Higher level of salts affects the plant growth that ultimately resists the transfusion of water and nutrient from the soil. Limited supply of nutrients and minerals with effect of salinity stop the plant growth in response where significant decrease in bulbs weight was observed (Yohannes *et al*., 2020). Moreover, interaction between cultivars and salinity levels was significant and all the varieties showed same response at higher level of salinity. The data showed that higher the level of salts, lower the agronomic, morphological and biochemical response of plants (Sheikhani *et al.,* 2018). Previous literature reported significant lowering of bulbs weight by increasing level of salinity (Sheikhani *et al*., 2018). The saline water application to onion leads to shortening of onion bulbs neck. Similar, shortening of the bulbs neck has been observed by Baba *et al.,* (2010) and Hanci and Cebeci (2015). The increasing levels of salinity decrease germination capacity in onions. Our results are confirmed by previous findings that increasing levels of salinity decrease the germination percentage (Jafarzadeh and Aliasgharzad, 2007). Similarly, increasing salt level lowers the growth and development of plant, which starts from the germination. However, plant defense mechanisms activates to remediate the salinity effects, ultimately salinity increase the emergence of leaves which consume more energy and lower the size of bulbs (Jafarzadeh and Aliasgharzad, 2007; Patel *et al.,* 2020). Increased levels of salinity increase proline contents in plants leaves. The production and increasing of proline contents is the sign of activation of plant survival mechanism. Higher proline contents are connected with productive survival of plant in saline environment as described by Hanci and Cebeci (2015). It was also observed that proline contents in onion (*Allium cepa* L.*)* increase five parts in response to higher level of NaCl concentration.

Conclusion: The results presented here indicate the effect of salinity treatments on onion growth, yield and physiological traits. Constraints imposed by salt stress limited the onion productivity, although some physiological responses such as proline contents are increased as plants tend to acclimatize to mild salt stress at 40 mM of NaCl. Similarly, plants ability to produce chlorophyll is reduced hence, yield of bulbs is less. Despite having some difference in growth, yield and physiological responses at lower stress level i.e. 40 mM, higher stress levels are very detrimental for onion crop. Hence, varieties can be selected to perform better under less saline conditions. Furthermore, better germination and lesser mortality in cv. Robina make it better suited under moderate saline conditions.

Ethical Issues: There are no ethical issues associated with this publication.

Authors Contribution: Naveeda Anjum and Muhamamd Aqeel Feroze and and Muhammad Rizwan Saeed conducted the experiments and complied the data, Monis Hussain Shah and Rizwan Rafique and Tanzila Rafique analyzed the data, interpreted results and assisted in writing manuscript, Tehseen Ashraf, Muhammad Asim and Bushra Zulifqar supported in write up process reviewed the manuscript. Whereas, Dr. Rehan Rafique and Dr. Shaukat Ali reviewed the manuscript. All authors have significant contributions to this study.

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