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Evaluation of mitigating effects of salicylic acid against various levels of salinity in onion (*Allium cepa*)

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Abstract

Background: Onion (*Allium cepa*) is a vital vegetable as well as medicinal crop grown all over the world. A considerable area in Pakistan is under salinity stress which markedly reduce the average yield of many crops. Salicylic acid is an important growth regulator and has mitigating effects against saline adversities.

Methods: A study was conducted in complete randomized design (CRD) using two onion varieties, Phulkaara and Desi Red, under Faisalabad, Punjab conditions. The level of salinity was kept 50 mM NaCl and 100 mM NaCl while 400 ppm of salicylic acid was applied weekly. The data was analyzed through Statix 8.1 to compute analysis of variance (ANOVA).

Results: All the growth and physiological parameter of onion seedlings were decreased significantly with the increasing level of salinity while increased with the application of salicylic acid except total soluble protein. Salicylic acid (@400ppm) mitigated the adverse effects of salinity by enhancing photosynthetic activities. The total soluble protein was increased in leaves with increasing salinity stress whereas the application of SA has non-significant effects. The performance of both varieties remained significantly same for the parameters dry shoot weight, plant height, chlorophyll a, b and total chlorophyll contents. The variety Desi Red performed better for shoot length while Phulkaara was better in fresh shoot/root weight, length of root, dry root weight and total soluble protein.

Conclusion: The application of foliar spray of salicylic acid is potential growth regulator to increase the growth parameters of onion under saline stress conditions.

Introduction

Onion (A*llium cepa* L.) is key vegetable crop grown all around the world with its additional flavor, nutrition and medicinal values. During 2016 the world observed 40.69 million productions of onion utilizing an area of 4.95 million hectare. Pakistan occupies a position of 6th, 5th and 23rd by production, area and yield of onion in comparison to other countries in world [1]. Production of onion stood 2.099 million tons occupying 0.154 million hectare area during fiscal year 2020-21 facing a reduction of 1.1% in comparison to last year [2].

There exists a considerable yield gap in comparison to other countries producing onion in world. Salinity stress is major factor that affects the growth of crops like onion to reduce the overall yield especially in semi-arid /arid areas of country.

It is dire need of the time to increase per unit yield onion to meet the requirement of country. So, per unit production of onion needs to be enhanced in country utilizing best suited techniques and available germplasm combating with saline conditions.

Onion *(Allium cepa* L.) being a popular vegetable have pungent bulbs and flavorful leaves that are liked by consumers. It is member of genus *Allium* and family Alliaceae. More than 500 species have been reported under the genus, onion being the most popular among them in most parts of world. Onion contains phytochemical, vitamin C, flavonoids and sulphuric compounds. It also has anti-cancerous and antimicrobial properties that are beneficial for human health. Normally onion consists of following constitutes, water 89% then carbohydrates 9% and 1% protein along with negligible amount of fat. An amount energy of 40 kilocalories from 100g of onion is obtained [3]. Salicylic acid (2-hydroxybenzoic acid) is considered vital growth regulator which is found in plant kingdom. It is molecule belonging to diverse set of phenolic group that is synthesized by plants under various conditions of climate [4]. It has been revealed that foliar application of SA has played significant role in enhancing the yield of brinjal [5]. However, little amount of literature has been reported for its role in onion crop. It has also been studied that salicylic acid has positive effects on biometric parameters of many crops[6] . Salicylic acid can be used in plants to enhance tolerance against abiotic and biotic stresses[7,8].

When the concentration of salts exceeds to higher level than natural occurrence then then it is stated as salinity. It has negative and adverse effects on the growth of plants as it disturbs the uptake of ions from soil leading stunted growth. It has been threatening problem in world for agriculture production. By the increasing level of salinity the accumulation level of Na+ and Cl- increases that have deleterious effects in plant growth. Among these two the concentration of Cl- is more deleterious in plant [9]. According to some approximations about 20% cultivated area in world is affected by salinity. It cause osmotic and ionic stress in plant that disturbs the plant growth leading to lower economic yield [10].

In present study two onion varieties, Phulkaara and Desi Red, were assessed with the objective to examine varietal response against salinity, the effects of foliar application of salicylic acid and its mitigating effects on morpho-physiological parameters under salt stress conditions.

Methods

A pot experiment was conducted during 2020-21 at Government College Women University Faisalabad deploying completely randomized design (CRD). The experiment consisted of two onion varieties, Phulkaara and Desi Red. The coordinates of field were 31.4181° N and 73.1199° E of latitude and longitude respectively.

Sr #	Parameters	Description (Value)
1	Saturation	35%,
2	pH	7.5
3	EC	1.10 dS m^{-1}
4	Organic matter	1.91%,
5	Total N	8.0 mg Kg^{-1}
6	Available P	8.0 mg Kg^{-1}
7	Available K	1.52 mg Kg^{-1}

Table 1: The soil analysis of experimental soil.

It indicated that the soil texture is loamy with saturation. The data on weather was taken from weather observatory of plant physiology section, Agronomic Research Institute-AARI, Faisalabad (Table 2).

Month	Mean temperature (°C)	Mean relative humidity (%)	Total rainfall (mm)
September	30.3	63	20
October	25.9	60.6	1
November	18.7	66	1.2
December	15.4	63	3.9
January	13.8	66	0
February	18.1	61	7.3
March	24.2	55.6	12.1
April	29.8	58.6	11.2

Table 2: Weather data for whole crop season (2020-21) of Onion cultivation.

The seed of both varieties was grown on bed to get germinated seedling which were shifted to earthen pots after 30 days. The seedlings were grown under six treatments:

- T1 : 0 mM NaCl
- T2 : 50 mM NaCl
- T3 : 100 mM NaCl
- T4 : 400ppM SA
- T5 : 50 mM NaCl + 400 ppm SA

- T6 : 100 mM NaCl +400 ppm SA

The concentration of salinity was made according to each treatment using molar mass. The treatment 50 mM NaCl was prepared using molar mass 58.44g (NaCl). Hence 2.9 g of NaCl was used to prepare 50 mM NaCl solution that was used in soil media preparation. Foliar spray of Salicylic acid was used after an interval of seven days. Growth parameters (Fresh Shoot and Root Weight (g), Shoot and Root Length (cm), Dry Root/Shoot Weight (g) and Plant Height (cm)) and physiological (Chlorophyll Contents (mg/g), Total Soluble Protein (mg/L)) parameters were recorded after three months. Fresh leaves of seedlings were taken for the measurement of photosynthetic pigments (Chlorophyll a+ and total chlorophyll as mg/100g of fresh leaves weight) according to method proposed by [11]. Total soluble protein was also determined by using the methodology proposed by [12].

Statistical Analysis

The data was subjected to Statistix 8.1 for analysis. The CRD trial data was analyzed using two way interaction at 5% value of least significant difference (LSD) [13]. The analysis of variance (ANOVA) was applied to data [14]. The mean values were tabulated, and results were inferred.

Results

Two onion genotypes Desi Red and Phulkaara tested under various concentrations of NaCl along with different combinations of salicylic acid to check its mitigating effects against salinity stress.

Fresh Shoot/ Root Weight (g)

Fresh root weight observed declining trend with increase in salinity level. However, maximum mean value for this parameter was observed in 400ppm SA treatment level showing significantly higher value in comparison with all other treatments. The mitigating effects were observed while using the 400ppm SA along with 50 mM NaCl and 100 mM NaCl. Similarly, fresh root weight was reduced with increase in salinity level. The foliar application of salicylic acid impacted non-significantly. However, maximum fresh root weight was observed in 400ppm SA (Table 3).

Shoot/ Root Length (cm)

The maximum value for root and shoot length was observed in 400ppm SA level of treatment. A decreasing trend with increasing salinity level in soil was observed for both parameters. The foliar application of salicylic acid resulted in mitigating the toxicity of salinity in significant way. However, in case of shoot length its effects were non-significant for the treatment level of 100 mM NaCl +400 ppm SA (table 3).

Dry Root/Shoot Weight (g)

Dry shoot and root weight showed that the increasing salinity level affects adversely these parameters. A significant difference was observed with the increasing level of salinity. Maximum value for both characters was observed in case of 400ppm SA showing the better growth while applying salicylic acid in the absence of salinity. A significant mitigating effects were revealed up to 50 mM NaCl while applying salicylic acid but its mitigating effects were non-significant at 100 mM NaCl +400 ppm SA level of treatment. It shows that higher level of salinity has adverse effects on plant growth leading to less mitigating effects even by application of foliar application of salicylic acid (table 3).

Plant Height (cm)

The increasing salinity level also affected the plant height significantly. Maximum height of seedlings of onion was found in case of 400ppm SA showing its positive effects on plant height. The mitigating effects of application of 400ppm SA in combinations with 50 mM NaCl and 100 mM NaCl showed significant difference. It showed the salicylic acid has positive effects to reduce the salinity adversities and to enable plants to cope with adverse effects of salinity on growth (table 3).

Chlorophyll Contents (mg/g)

The chlorophyll contents showed significant reduction with the increasing level of salinity. It may be due to reduction in the photosynthesis activity of onion seedlings. The application of foliar spray effected positively, and maximum value of chlorophyll a, b and total chlorophyll was observed at 400ppm SA level of treatment. The mitigating effects of salicylic acid were non-significant in case of 50 mM NaCl + 400 ppm SA while significant effects were observed in 100 mM NaCl +400 ppm SA level of treatment.

Total Soluble Protein (mg/L)

The protein contents in the leaves of onion seedlings were increased with the increasing level of salinity in soil. The maximum amount of total soluble protein was found in 100 mM NaCl level of treatment. The amount of protein showed increasing trend with the increase of salinity level in soil. The lowest mean value was observed in control treatment and 400ppm SA level of treatment.

Performance of both Genotypes

The comparison of both varieties showed that the onion variety Desi Red showed significant better performance

	Treatments	Fresh Shoot Weight (g)	Fresh Root Weight (g)	Shoot Length (cm)	Root Length (cm)	Dry Shoot Weight (g)	Dry Root Weight (g)	Plant Height (cm)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total Chlorophyll (mg/g)	Total Soluble Protein (mg/L)
T1	0 mM NaCl (Control)	5.1	0.208	43.6	15.8	0.415	0.031	81.5	0.251	0.193	0.483	3.182
T2	50 mM NaCl	3.9	0.173	40.7	15.2	0.284	0.026	55.9	0.269	0.169	0.437	4.099
T3	100 mM NaCl	3.1	0.175	37.7	13.1	0.338	0.021	50.7	0.180	0.178	0.359	5.165
T4	400ppm SA	6.2	0.257	54.1	22.3	0.565	0.036	76.3	0.469	0.196	0.664	3.015
T5	50 mM NaCl + 400 ppm SA	5.0	0.198	46.4	18.5	0.350	0.031	64.8	0.302	0.180	0.482	3.749

in case of shoot length. On the other hand the variety Phulkaara showed significantly superior performance over Desi Red for fresh shoot/root weight, length of root, dry root weight and total soluble protein. The performance of both verities remained significantly similar for the traits dry shoot weight, plant height, chlorophyll a, b and total chlorophyll contents (table 4). The graphical representation of performance of both varieties showed that only minor difference occur in both varieties for showing their response under salt stress conditions and foliar application of salicylic acids. However, the variety Phulkaara showed little edge over Desi Red as its performance remained superior for more traits in comparison to Desi Red (Figure 1).

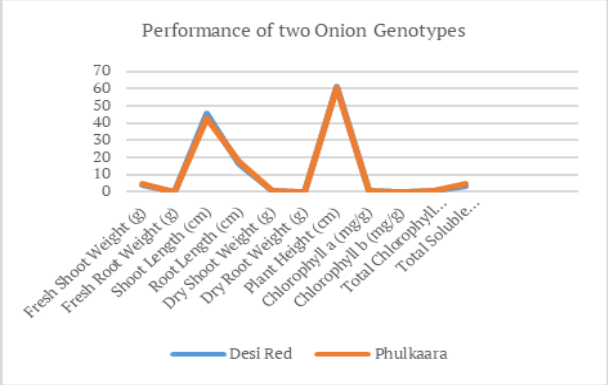

Figure 1: The Performance of Genotypes of Onion for various Biometric and Physiological Traits

The percentage as high as 12%. 7.5 % of East African haplogroup (N9a1), 5% South Asian haplogroup (M31a1, M3), 2.5% East Asian haplogroup (A+152+16362), 2.5% of African haplogroup, 2.5% of Southeastern haplogroup (B4), 2.5% of Central Asian haplogroup (C4a1), 2.5% of Southeast Asian haplogroup (R2). The genetic diversity of Wakhi population was high (0.998) with lower random match probability is (0.026) and in turn high power of discrimination (0.974) indicating rich gene pool (Table 3).

Discussion

Researchers have elaborated the increasing trend of fresh shoot and root weight with the increasing of salinity level on onion crop [15]. It was found that root weight and shoot weight in onion decreases when the salinity level increases in soil [16]. It looks that with the increasing level of salinity concentration in soil the reduction in water uptake capacity of seedlings occurs. This leads to poor growth of onion crop. The toxicity of NaCl may also make the nutrients uptake imbalance leading to stunted growth in onion seedlings. Other scientists investigated that that fresh biomass of wheat plants increases significantly by applying foliar spray of salicylic acid [17]. Similar trend of fresh shoot and root weight as also observed in the research conducted on wheat cultivars under different levels of salicylic acid [18,19].

Many researchers reported that root and shoot length decreases of these parameters in their research investigation under stress conditions [15,16]. The results of current research experimentation also show agreement with results obtained by various research

trials [20,21]. Root and shoot length in wheat increases with the application of foliar application of salicylic acid. It also been found that dry and fresh weight of seedlings of wheat increased positively and significantly by foliar application of SA [17]. The application of salicylic acid also affected root length in positive and significant manner [18]. A trend of reducing dry root weight has also been studied on onion research experiments by increasing stress level in soil [15]. It found that dry and fresh weight of seedlings of wheat increased positively and significantly by foliar application of SA [22]. Dry biomass in onion also increases while applying the SA in foliar spray [23]. Increasing trend of dry biomass was also observed in wheat cultivars under various doze application of salicylic acid [22][18]. Dry matter was also enhanced by applying SA under salinity and drought stress in onion [24]. Plant height increases with the foliar application of salicylic acid [22]. It may be due to increasing photosynthesis of onion plant that enhances the growth of seedlings. It also increased positively and significantly by the application of different dozes of salicylic acid in *Dieffenbachia picta* [23,25]. In pea and other plants the plant height was also increased by application of SA [22,26,27]. The application of foliar salicylic acid caused the reduction of adverse effects of salinity level in case chlorophyll contents (mg/g). The results are in line with the research made by various researchers [22][17]. It was also observed that the chlorophyll contents enhanced by using SA in foliar application mitigating the stress conditions in various varieties of wheat. Elevation of chlorophyll contents as also found by applying salicylic acid [27].

The protein contents were increased with the increase of salinity level in soil [28]. The amount of protein, with application of SA or without its application, remained same [19]. It was investigated that the application of salicylic acid did not affect the total soluble protein, previously similar results were reported [29]. However, some researchers indicated positive and significant effects of SA foliar application for total soluble proteins [30]. It was also discovered that increasing level of NaCl increased the level of soluble proteins [31]. It may be due to plant action as defense mechanism to produce more enzymes for its survival. The increasing no of enzymes leads to more protein contents. Increasing trend of soluble protein was noticed with increasing water stress level [32]. The protein level enhanced in seeds of all varieties under study with the higher level of NaCl. There are some contradictions in literature about the mechanism of mitigating effects of salicylic acid in case of total soluble protein. However, in current studies application of salicylic acid affected non-significantly and values remained at par with control treatment (table 3). It indicated that application of SA normalized the plant physiological mechanism by reducing the extra enzymes produced in stress conditions.

Yield attributing characters also influenced the total yield of the onion crop by better efficacy of photosynthesis resulting in increased dry matter which ultimately affected the total onion yield of the plants. It was also observed that foliar spray also takes part in uptake of NPK and increase the assimilation of photosynthates resulting in high vegetative production [22,27].

Salicylic acid being an important plant growth regulator has positive and significant effects to enhance the growth parameters of onion seedlings which may lead to healthy plant growth ultimately higher output of crop. The physiological parameters also showed better performance under application of salicylic acid (@400ppm) as compared to control treatments where no salicylic acid used. The salinity has severe adversities on plants that was potentially mitigated by applying salicylic acid in foliar application. This may future hope and strategy in country that may lead to improve our national yield that will lead to increase our national economy.

Competing interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Authors' Contribution

Dr. Rashda Naheed: designed the research experiment and supervised/ wrote manuscript. Noreen Akhtar: conceptualized and performed the research experiment. Muhammad Shahzad Afzal: wrote the manuscript and analyzed the data. Fozia Farhat: provided technical guidelines for research experimentation. Dr. Muhammad Umer Farooq: reviewing and editing.

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