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Morphological Parameter and Response of Salt Stressed Maize Genotype to Foliar Treatment of Iron Sulphate

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Abstract

Maize is a cross-pollinated, polymorphic plant in nature. It is commonly a moderately salt-sensitive crop. Salinity stress is the main abiotic factor that arrests the physiological characteristics and plant growth of a maize plant. This current study was conducted to assess the response of salt-stressed maize genotype to the foliar treatment of iron sulfate under salt stress in the wirehouse of the Old Botanical Garden in UAF. Different levels of NaCl (0 and 150mM) and iron sulfate (0 and 100ppm) were applied by adding 1ml tween 20 as a surfactant on two varieties of maize. Each treatment was applied thrice. After the seedling and growth, morphological parameterswere calculated. Exposure of maize genotype under salinity and control situations. Salt stress concealed the growth parameters of plants. Completely Randomized Design (CRD) was used. Maize (*Zea mays*) belongs to the grass family Poaceae and is foremost beneficial to the cereal crop in the global as nutriment. Sodium chloride also known

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as salt or halite is an ionic compound. The effect of NaCl on plants firstly, as increase NaCl in the soil solution reducing growth rate and chlorophyll contents.

Keywords: Abiotic factor, Physiological character, Salt- stress, Maize, UAF

Introduction:

Soils with an excessive number of soluble salts or interchangeable sodium in the root zone are referred to as salt-affected soils. Owing to limited rainfall and high evapotranspiration demand, coupled with poor soil and water management practices, salt stress has become a serious threat to crop production in arid and semi-arid regions of the world [1]. Large numbers of crops are grown in Pakistan, maize is one of the most important cash crops [2]. Maize (Zea maize) is a very significant cereal crop that is grown worldwide after wheat and rice. Its demand increased 5% per annum [3]. The investigation of FAO that world production in 2012 was over 875millionthe global cereal crops Committee has forecasted production increasing towering 990 million in 2014-2015 grown almost 200 million hectares. Per capita, almost 98 g/person/day corn is utilized [4]. The Poaceae family contains cereals of the monocotyledonous family. The most important crop in the world is maize and originated in central Mexico almost utilizing instantly, maize is introduced to European countries. This crop cultivation also reflects on maintenance of land management as well as considered major nutrients like rice, wheat, and potato [5]. Maize (*Zea mays* L.) is considered a good source of food many people spending life, utilizing it indirectly as a feed crop, so it is known as a vital element of universal foodstuff safety measures [6]. Maize is a significantcornflakebecause it was greatly used by people as well as animals. It contains high energy of protein, carbohydrates, and oil. The level of growth is reduced extremely by negative effects of salinity as well as reactive oxygen species increased in plants [7,8]. Property of seed and texture of sowing area play important role in yield production of all crops. [9]. Maize grain contains a high energy ratio fit for human consumption, on the other hand, the green fodder of maize is rich in protein. Though, this plant is gentlysusceptible to salinityshows poor germination under different stress [10,11]. Maize is used as a staple food all over the world [12]. Sodium is the primarycontaminated ion for maize. As maize is a C4 plant that's why NADP-malic enzyme-type photosynthesis is used by it [13]. For a large number of food supplements for the current and future generation of the increasing population, it is very necessary to enhance the rate of crop production and yield Many biotic and abiotic factors are involved in degraded soil fertility but salinity is one of the main factors which badly affect irrigated land 19.5% and dry-land of agriculture 2.1% present on the world. Poor yield and rate of growthdue tosalinity and ionic effects so in resulting the plants adopt different mechanisms for dealing the un-favorable condition [14,15]. Salinity has been acknowledged as the major germ area of soil aspect which badly affects the crop production in arid and pasture domains [16]. Plants can face salinity and drought by developing a mechanism i.e osmatic adjustment hence, in salinity the osmotic potential of plants reduce a result of inorganic ion (Na^+, Cl^-) and companionable organic solutes deposition[17,18]. The current study aims to assess the harmful effect of salt stress (NaCl) on the

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physiological mechanism and other activities in plants and assess the role of foliar spray of iron sulfate ($FeSO_4$) for the reduction of salt stress.

Material And Methods

This study was conducted in an old botanical garden in UAF, Pakistan. For purpose of study two varieties of maize (Desi makai and Neelum) were cultivated in plastic pots. Twenty-four pots are arranged in rows so, twelve, pots are used for one type of maize. Seeds of both varieties were collected from Sargodha. To observe the salinity effect on maize growth NaCl (0 and 150m*M*) and iron sulfate (0 and 100ppm) were useful. All the parameters are analyzed by using three replicates.

Data collection:

For measuring the analysis of all parameters i.e morphological, Physiological, Gas exchange, mineral nutrient's purpose, enzyme oxidation. So, for this purpose, healthy plants were chosen from each pot. Leaves were conserved for further biochemical analysis.

MORPHOLOGICAL PARAMETERS:

Once all treatments were achieved according to the attributes data was recorded it includes height, root, shoot length, root, shoot fresh weight, several leaves, and leaf area was recorded. After taking fresh weight roots and shoots were dried in an oven at 65oc for 72 hours for measuring the dry weight. There are different morphological parameters ie, Root length measured by using a measuring tape, shoot length measured by using measuring tape. For measuring the height of the plant following formula was usedPlant Height= root length+ shoot length, Count the number of leaves of each plant, after harvest the from each pot measure the fresh weight of the shoot by using electrical balance, After harvest, the from each pot measure the fresh weight of root individually by using electrical balance, Dried the plants in sunlight for one week after drying keep them in the oven at 65°C. The dry weight of the shot measure by using electrical balance, Dried the plants in sunlight for one week after drying keep them in the oven at 65°C. The dry weight of the root measure by using electrical balance, For the calculation of leaf area the top leaf plant was chosen, and measure the total length and width of the selected leave following formula was usedLeaf area=total leaf L×total leaf W × correction factor (0.68) and the analysis of net transpiration rate (*E*), CO2 assimilation rate (*A*), water use efficiency (*A*/*E*), Stomatal conductance (g_s) and substomatal CO_2 concentration (*Ci*) used youngest fully developed leaf mostly 3rd leaf of the top of each sample using a manageable scheme infra-red gas analyzer (IRGA) (ACD LCA-4 Analytical Development, Hoddesdon, UK).

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Biochemical Analysis:

Chlorophyll Contents

According to Arnon (1949) for the determination of all chlorophyll contents and carotenoids homogenized the upper leaf 0.5 by using pestle and gun in 80% acetone and maintained the volume 5 mL and filtered. The absorbance of the filtrate was read at 645 and 663 nm for chlorophyll a and b and at 480 for carotenoids respectively with the help of a spectrophotometer (Hitachi-U-2001, Japan). All chlorophyll parameters were designed according to Yoshida *et al.* (1976), but for carotenoid calculation used the method of Davies (1976), as like

Chl. *a* (mg/g) = [12.7(OD663)-2.69(OD645)] x V/1000 x W

Chl. *b* (mg/g) = [22.9(OD645) - 4.68(OD663)] x V/1000 x W

Total Chl. (mg/g) = [20.2(OD645) + 8.02 (OD663)] x V/1000 x W

Car = [(OD 480) +0.114 (OD 663) – 0.638 (OD 645)] x V/1000 x W

Where

V = Volume of the acetone used in extract (ml)

W = Weight of fresh leaf tissue (g)

Determination of Minerals:

Digestion procedure

For the determination of minerals, nutrients take 2 ml of digestion mixture (H_2SO_4) dissolve in (0.1 g dried) ground material of each sample in the digestion flask. All the flask conserved for 24 hours at 25^0c. After it heated the flask at 150^0c with the addition of 0.5 ml sulphuric acid then the flask was kept in a digestion block and heated at 250^0c until the fumes appeared. This process repeated and again until all the samples display colorless. By using a volumetric flask maintain the (50 ml) volume of digested material. Finally, the extracted filter was used for the cation (Na^+, K^+, $Ca^{2+)}$ analysis. Potassium (K^+), Sodium (Na^+), and Calcium (Ca^{2+}) contents were analyzed by using a flame photometer. Follow the Bradford method (Bradford, 1976) for total soluble proteins the following Reagent is used in like Bradford reagent: Dissolve 100 mg Coomassie Brilliant Blue G-250 in 50 ml 95% ethanol; add 100 ml 85% (w/v) phosphoric acid. Dilute to 1 liter when the dye has completely dissolved, and filter through Whatman #1 paper just before use. For the extraction of a protein select fresh healthy leaf 0.5g and ground it by using a tissue grinder in 5 ml of 50 m*M* waiting for cooling phosphate buffer (PH 7.8) in the ice bath. Centrifuged the mixture at 15000 rpm for 15 min at 4^0C. For determining protein upper layer was separated which is called supernatant. Requiring sample 100 µl, separated in Eppendorf tube

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and 1.0 ml of Bradford reagent mix in it and incubated this solution at 37 ^{o}C for 10-15 minutes along with the blank and absorbance was noted at 595 nm.

Statical Analysis

Analysis of variance (ANOVA) for all parameters arranged in a completely randomized design (CRD) of three replicates. So, data was calculated by using statical COSTAT computer software.

Results:

Morphological parameters

Data regarding S.F.W in cv. Desi makai and cv. Neelumin two different situations control and salinity in which iron sulfate applied exogenously as given in (Table.No.1). Significant ($p \leq 0.05$) reduction occurred due to salt stress Although, in Neelum maximum reduction was observed at the high level of NaCl 150mM. However, iron sulfate application caused a significant improvement ($p \leq 0.05$) in control situation as given ingrown under control and sodium chloride stress with exogenously applied iron sulfate is accessible in (Table.No.1)

Table.No.1. ANOVA for shoot fresh weight of two maize genotypes under control and salinity along with a foliar spray of iron sulfate.

S.O.V	Df	MS	F-Value	P-Value
Stress	1	72.3491	11.1051	0.0042**
Spray	1	0.61723	0.0947	0.7621 ns
Varieties	1	209.152	32.1032	0.0000***
Stress x spray	1	214.740	32.961	0.0000***
Stress x varieties	1	330.701	50.761	0.0000***
Spray x varieties	1	46.6754	7.1642	0.0165*
Stress x Spray x varieties	1	6.05034	0.9288	0.3496 ns
Error	16	6.51445		

Df = degree of freedom. Non-significant=^{ns}. ***, **, * significant at 0.0004, 0.003, 0.02 separately

Significant ($p \leq 0.05$) interactionpresents in both varieties. in general, Desi makai performed better than Neelum. Data composed forR.F.W using different varieties of maize cv. Desi makai and cv. Neelum in control and sodium chloride stress with exogenously applied iron sulfate as given in (Table.No.2).

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TableNo.2. ANOVA for root fresh weight of two maize genotypes under control and salinity along with a foliar spray of iron sulfate.

S.O.V	df	MS	F-Value	P-Value
Stress	1	2.1664	11.350	0.0039 **
Spray	1	0.0043	0.0205	0.886 ns
Varieties	1	11.830	61.993	0.0000 ***
Stress x spray	1	1.7225	9.0277	0.0084 **
Stress x varieties	1	0.2791	1.4645	0.2438 ns
Spray x varieties	1	0.4086	2.1393	0.1630 ns
Stress x Spray x varieties	1	0.1858	0.9728	0.3388 ns
Error	16	0.1907		

Df = degree of freedom. Non-significant= ns. ***, **, * significant at 0.0004, 0.003, 0.02 separately.

Significant ($p \leq 0.05$) reduction was observed due to NaCl in R.F.W but comparatively, in Neelum mostly reduction observed when 150mM Sodium Chloride applied. However, iron sulfate application caused significant ($p \leq 0.05$) induction in R.F.W in the control condition as given inTable.No2. Significant ($p \leq 0.05$) interaction was observed in both cultivars. largely, Desi makai performed better than Neelum. Data collected for S.D.W of two maize varieties cv. Desi makai and cv. Neelum in two different situations control and salinity in which iron sulfate applied exogenously as given in Table.No.3. Significant ($p \leq 0.05$) reduction in S.D.W occurred due to salt stress Although, in Neelum maximum reduction was observed at the high level of NaCl 150mM. However, iron sulfate spray caused a significant enhancement ($p \leq 0.05$) in the control situation as given in (Table.No.3).

Table.No.3. ANOVA data for shoot dry weight of two maize genotypes under control and salinity along with a foliar spray of iron sulfate.

S.O.V	df	MS	F-Value	P-Value
Stress	1	10.088067	7.1969799	0.0163*
Spray	1	0.4428167	0.3159121	0.5819 ns
Varieties	1	1.0416667	0.7431408	0.4014 ns

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Stress x spray	1	0.4320167	0.3082072	0.586 5ns
Stress x varieties	1	0.2562667	0.1828245	0.06747 *
Spray x varieties	1	2.49615	1.7807913	0.2007 ns
Stress x Spray x varieties	1	0.3800167	0.2711097	0.6097 ns
Error	16	1.4017083		

Df = degree of freedom.Non significant= ns. ***, **, * significant at 0.0004, 0.003, 0.02 separately

Non-Significant interaction was observed in both varieties. Overall, Desi makai performed better than Neelum. Data composed for R.D.W in two varieties of maize cv. Desi makai and cv. Neelum in control and sodium chloride stress with exogenously applied iron sulfate as given in Figure.No.1.

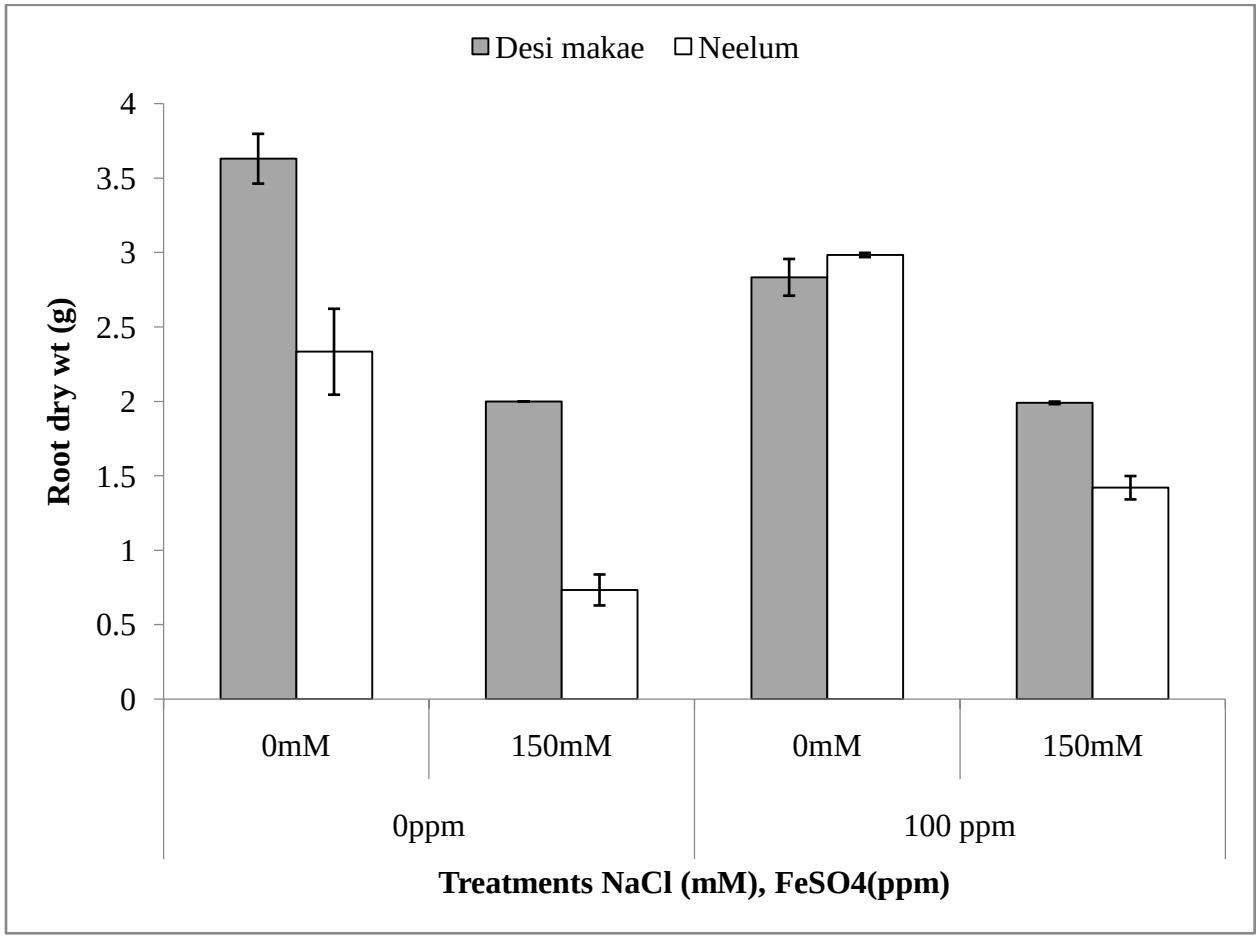

Figure.No.1: R.D.W (g) of different maize varieties under control and salinity along with a spray of iron sulfate.

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Significant ($p \leq 0.05$) reduction was observed due to NaCl in R.D.W but comparatively, in Neelum mostly reduction observed when 150mM Sodium Chloride applied. However, iron sulfate application caused significant ($p \leq 0.05$)induction in R.D.W in the control condition as given in Table.No.4.

Table.No.4. ANOVA for root dry weight of two maize genotypes under control and salinity along with a foliar spray of iron sulfate.

S.O.V	df	MS	F-Value	P-Value
Stress	1	8.449066	84.052891	0.0000 ***
Spray	1	1.008612	10.033741	0.0060**
Varieties	1	0.88935	8.8474197	0.0089**
Stress x spray	1	0.12041763	1.1979275	0.2899 ns
Stress x varieties	1	0.614423	6.112658	0.0250*
Spray x varieties	1	0.2016667	2.0062176	0.1758 ns
Stress x Spray x varieties	1	0.140167	0.1394404	0.7137 ns
Error	16	0.1005208		

Df = degree of freedom.Non significant= ns. ***, **, * significant at 0.0004, 0.003, 0.02 separately.

Significant ($p \leq 0.05$) result was observed in both varieties. Anyhow, Desi makai performed better than Neelum. Data regarded for plant height (cm) of different maize varieties cv. Desi makai and cv. Neelum in two different situations salinity and control in which iron sulfate applied exogenously as shown in Table.No5.

4.5. ANOVA table for plant height (cm) two maize genotype under control and salinity along with a foliar spray of iron sulfate.

S.O.V	df	MS	F-Value	P-Value
Stress	1	2816.6667	193.69628	0.0000 ***
Spray	1	1.53223	0.1031519	0.7522 ns
Varieties	1	560.66667	0.103151	0.0000 ***
Stress x spray	1	1261.533	86.750716	0.0000 ***

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Stress x varieties	1	24345	1.6504298	0.2172 ns
Spray x varieties	1	48.166667	3.3123209	0.0875 ns
Stress x Spray x varieties	1	60.166667	4.1375358	0.0589 ns
Error	16	14.541667		

Df = degree of freedom. Non significant= ^{ns}. ***, **, * significant at 0.0004, 0.003, 0.02 separately

A high concentration of salt caused a significant ($p \leq 0.05$) reduction in plant height, but the maximum reduction was observed in cv. Neelum after the application of 150Mm NaCl. However, the application of iron sulfate source of significant improvement ($p \leq 0.05$) under control is given in Table5. In both cultivars, the varietal difference was significant ($p \leq 0.05$). in general, Desi makai performed better than Neelum Figure.No.2.

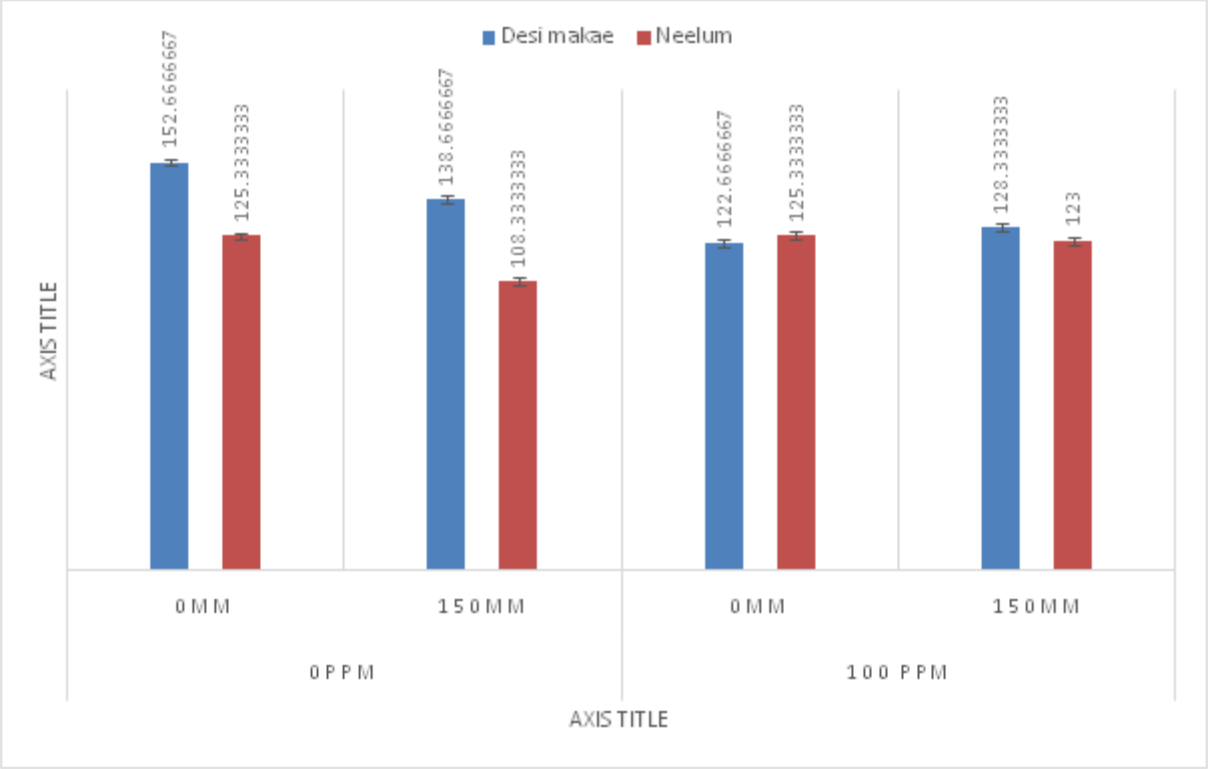

Figure.No.2: Height (cm) of different maize varieties under control and salinity along with a foliar spray of iron sulfate

Data was collected for Shoot length by using different maize varieties cv. Desi makai and cv. Neelum in non-saline and saline conditions with exogenously applied iron sulfate as given in Table.No.6.significanteffect of salinity on this parameter slightly reduction was observed in maize genotype when 150mM salt stress was applied. However, iron sulfate application caused a

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significant improvement ($p \leq 0.05$) shoot length in both maize genotypes of plants under saline conditions as given in Table.No.6.

Table.No.6. ANOVA for shoot length (cm) of two maize genotypes under control and salinity along with a foliar spray of iron sulfate.

S.O.V	df	MS	F-Value	P-Value
Stress	1	1855.0417	193.56957	0.0000 ***
Spray	1	0.0416667	0.0043478	0.9482 ns
Varieties	1	360.375	37.604348	0.0000 ***
Stress x spray	1	672.04167	70.126087	0.0000 ***
Stress x varieties	1	63.375	6.6130435	0.0205 *
Spray x varieties	1	18.375	0.0033476	0.1852 ns
Stress x Spray x varieties	1	92.041667	9.6043478	0.0069 **
Error	16	9.5833333		

Df = degree of freedom. On significant= ^{ns}. ***, **, * significant at 0.0004, 0.003, 0.02 separately.

A significant ($p \leq 0.05$) relation was present in both cultivars in this parameter. generally, Desi makai showed good performance than. Neelum. Data collected for root length in different varieties of corn cv. Desi makai and cv. Neelum in control and sodium chloride stress with exogenously applied iron sulfate as shown in Table.No.7. Significantly ($p \leq 0.05$) reduction in both cultivars due to salt stress but growth was highly reduced in Neelum when 150mM salt stress was applied. However, iron sulfate application caused a significant improvement ($p \leq 0.05$) root length in maize genotype of plants under non-saline conditions as given in Table.No.7.

4.7. ANOVA table for root length (cm) of two maize genotypes under control and salinity along with foliar spray iron sulfate.

S.O.V	df	MS	F-Value	P-Value
Stress	1	100.04167	24.252525	0.0002 ***
Spray	1	1.0416667	0.2525253 .	0.6221 ns
Varieties	1	22.041667	5.34343	0.0345 *

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Stress x spray	1	92.041667	22.313131	0.0002 ***
Stress x varieties	1	9.375	2.2727273	0.1512 ns
Spray x varieties	1	7.0416667	1.7070707	0.2098 ns
Stress x Spray x varieties	1	3.375	0.8181818	0.3791 ns
Error	16	4.125		

Df = degree of freedom. On significant= ns. ***, **, * significant at 0.0004, 0.003, 0.02 separately.

A significant ($p \leq 0.05$) relation was observed regarding this parameter. generally, Desi makai performed better than Neelum. ANOVA constructed for the number of leaves of maize varieties cv. Desi makai and cv. Neelum in different environment salinity and control in which iron sulfate applied exogenously isgiven in Table.No.8. A high level of salt caused significant ($p \leq 0.05$)reducing leavesalthough the greatest reduction was observed in cv. Neelum when 150mM Sodium Chloride is applied with foliar spray of iron sulfate. However, application of iron sulfate significant enhancement ($p \leq 0.05$) per plant leaves numbering in control situation Table.No.8.

4.8. ANOVA table for a plant number of leaves of two maize genotypes under control and salinity along with a foliar spray of iron sulfate.

S.O.V	df	MS	F-Value	P-Value
Stress	1	0.1666667	0.1333333	0.7198 ns
Spray	1	0.1666667	0.1333333	0.7198 ns
Varieties	1	8.1666667 .	6.5333333	0.0211 *
Stress x spray	1	1.53241	1.25562	0.2895 ns
Stress x varieties	1	0.1666667	0.1333333	0.7198 ns
Spray x varieties	1	8.1666667	6.1333333	0.0211 *
Stress x Spray x varieties	1	1.53345	1.24361	0.2895 ns
Error	16	1.25563		

Df = degree of freedom. On significant= ns. ***, **, * significant at 0.0004, 0.003, 0.02 separately.

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Both varieties showed a significant ($p \leq 0.05$) relation. overall, Desi makai performed betterthan Neelum. ANOVA constructed for L.An of two maize varieties cv. Desi makai and cv. Neelum in two different situations control and salinity in which iron sulfate applied exogenously is given in (Table.No.9. Salinity caused a significant ($p \leq 0.05$) reduction in L. An Although, in Neelum maximum reduction, was observed at a high level of NaCl 150mM. However, iron sulfate foliar spray showed significant progress ($p \leq 0.05$) in the control situation as given in Table.No.9.

Table.No.9. ANOVA for leaf area (cm^2) of two maize genotypes under control and salinity along with a foliar spray of iron sulfate.

S.O.V	df	MS	F-Value	P-Value
Stress	1	14891.198	40.68253	0.0000 ***
Spray	1	1324.0261	3.6172196	0.0753 ns
Varieties	1	6962.5454	19.02157	0.0005 ***
Stress x spray	1	72.03735	0.196805	0.6633 ns
Stress x varieties	1	155.55042	0.4249614	.5237 ns
Spray x varieties	1	906.01882	2.4752298	.1352 ns
Stress x Spray x varieties	1	6306.9868	17.230594	.0008 ***
Error	16	366.03422		

Df = degree of freedom. Non-significant= ^{ns}. ***, **, * significant at 0.0004, 0.003, 0.02 separately.

A significant ($p \leq 0.05$) improvement was observed according to this parameter. However, Neelum performed better than Desi makai.

Discussions

Many biotic and abiotic factors affecting the agricultural land due to which the loss of yield occurred, but salinity is one of the major threats to crop production. Large numbers of crops growing in Pakistan in which Maize is very important cereal crop after wheat and rice belong to family Poaceae and it is also known as queen crop[19]This crop has greater importance for both human being and animals because it contains high value of oil, protein, and energy. Sodium is considered a toxic ion that absorbs the water and loss water in plant activity and different physiological mechanisms are performed in the presence of water to reduce the rate of growth [20].in our current study Increasing population day by day so demand for food also increased but due to these factors (drought, salinity, waterlogging) requirement of people is not fulfilled. Under salinity, the plant does not perform its function normally and delayed the rate of germination,

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and sometimes stunt growth or different symptoms appear on the plant [21]. In our study, the morphological parameters like root and shoot length were decreased by the application of different levels of salt. But the application of iron sulfate mitigates the damaging property of salinity, and these results were following the previous results in which root and shoot length decreased significantly in salinity because roots relate to soil and high concentration of salt present in soil surface due to which water cannot uptake by plants easily and it is an important clue for reduction of these parameters [22]. In our study biochemical parameters like chlorophyll contents (a, b) were decreased by application different levels of salt. But the application of iron sulfate mitigates the damagingproperty of salinity and these results were supported by in which the chlorophyll contents (a, b), grain yield reduced significantly in salinity because a high level of salt in leaf lower rate of photosynthesis, stomata closed leading to reduce light absorbance so the chlorophyll contents also significantly decreased[23,24]. In our different factor study, the morphological parameters like height, leaf area, the number of leaves per plant were decreased by application different levels of salt. But the application of iron sulfate mitigates the damaging ropery of salinity and these results were assimilated with the previous observation of in which leaf area and number of leaves per plant highly decreased due to high level of salt in the leaf tissue [25,26,27]

Conclusion:

Many factors are responsible at large scale of food safety but now a day drought and salinity are the main factors involved in the greater loss to agriculture. Salinity is major abiotic stress of yield loss and agriculture production. It contains a great number of humans and animals. Corn is categorized as the third rank in all the crops. The main producing areas of maize in the world are America, India, and Africa. Maize belongs to the family Poaceae and very important cash crop because in hilly areas many people spend their lives eating corn directly or indirectly. Many food products are made by using maize, so it is also called queen crop. The effect of salinity in maize varied from species to species. The experiment was conducted for exploring the Response of salt-stressed maize genotype to the exogenous application of iron sulfate. For this purpose, two varieties of corn were used i.e Desi make and Neelum. This study was conducted in the old botanical garden in the Agriculture University of Faisalabad. Observe the effects of salinity on physiological and morphological parameters. Two-level of salt were applied (0, 150mM) with exogenous application of iron sulfate (0 and 100ppm). All the parameters were calculated by using three replicates.

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