Original Research

EVALUATION OF SALT AND HEAVY METAL STRESS FOR SEEDLING TRAITS IN WHEAT

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Abstract: *Wheat (Triticum aestivum L.) is an important cereal crop of the world. It is one of the staple foods for major portion of world population. There are various biotic and abiotic factors responsible for low production of wheat in our country. Among these factors, soil salinity is major problem playing an important role in soil degradation, thus consequently reducing wheat production and quality. This study was conducted to evaluate the effect of various salinity and heavy metal levels against three wheat cultivars fir salinity resistance. Three different varieties of wheat were screened against the salinity under controlled conditions in the laboratory of tissue culture, University of Lahore. Seeds of three wheat varieties (Anaj-2006, Faisalabad-2008 and Inqalab-91) were sown in seedling trays. NaCl and $CuSO_4$ were applied as salinity and heavy metal treatment upon wheat cultivars. In order to evaluate, hazardous effects of salinity and heavy metal on wheat certain growth parameters were observed i.e. leaf length and width, leaf area, stem and root length, fresh and dry weight of leaf, stem and root, root shoot length ratio and photometry of leaf, stem and root was measured. Results depicts salinity and heavy metal application has negative correlation with growth parameters of wheat particularly combine application of NaCl and $CuSo_4$ have led to impose major detrimental effects on wheat cultivars. Regarding varietal comparison, "Anaj-2006" proved to be comparatively better in context of less salt's residual accumulation in leaf, stem and roots along with lower root to shoot length ratio thus exhibiting a strong genetic potential to keep surviving and maintain healthy growth. However, it was concluded that salinity and heavy metal have adversely affected growth and yield potential of "Faisalabad-2008". So conclusively, there is dare need to screen out indigenous and exotic wheat germplasm available throughout the country for finding some suitable genetic resources having moderate to high resistance levels against salinity and heavy metal which can be further used for breeding purpose in varietal improvement program.*

Keywords: *Triticum aestivum*, salinity, heavy metals, germplasm, root length, shoot length

Introduction

Wheat main crop of Pakistan because is used as staple food and attains supreme position during the country's agricultural policy construction. The specimen has been cultivated in the field of eighty lac hectares exhibiting a decline in production as last year it was cultivated on nine million hectares. However, an estimated 24.2 million tons of bumper wheat crop has increased by 3.9 percent over 23.3 million tons last year's crop (Mazher et al., 2007). Pakistan's domestic average remains very small relative to other wheat-producing nations. Considering the impacted part of the salt is situated within the control channel region, and may enhance the salt tolerance of wheat may lead significantly to improving output per hectare, as well as Pakistan's success in wheat manufacturing with other nations. Reduced growth and productivity is the eventual result of decrease of plant nutrients or excess of hazardous or harmful material. These inhibiting growth conditions has a higher impact on life cycle of plant. Distinguishing the environmental conditions is very hard as the other stress element is also involved during the stress event. Plants are exposed to various kinds of abiotic pressures: excess of salt, excess or shortage of water and inadequacies in nutrients. Salt stress is very prevalent amongst the abiotic stresses that affect plant health (Pessarakli *et al.*, 1991). Across globe, it is estimated that more than eight Mha of salinity are influenced mainly by sodium (434 Mha) or others (397 Mha), which is more than six half of the world's ground region (FAO, 2005). However, due to irrigation and land clearing, both sodium and most natural salinity has become a big percentage of grown property lately salty. UNEP and FAO report that approximately 0.45 billion farms of 230 million farms of irrigated soil are salinized (FAO, 2005). 15 percent irrigated soil out of total area under cultivation is yielding much higher about one fifth of the world's complete requirement (Munns 2005).

A greater number of living and non-living elements that narrow and eventually affect crop development and output. Dry spell, weather, salinity and timber extraction are prominent biotic factors and abiotic factors. Excess of water and salt are among the most significant issues factor affecting plant output in today's world's irrigated soil. These issues are the primary barriers to poor crop efficiency in different regions of Pakistan.

Salinization is among the main soil degeneration variables that noxiously reduce plant growth and efficiency throughout the world. Approximately 7% of the complete region of the globe is influenced by water (Szabolcs, 1989). The scenario is further worsened worldwide, with salinization increasing by 10% worldwide, especially in nations where unnatural irrigation is vital agricultural support (Flowers and Hajibagheri, 2004). In North Indian Plain, the British governor improved issues linked to salt with the introduction and spread of the irrigation scheme. Pakistan is one of the countries in this area with the world's biggest adjacent ground irrigation scheme. This is the area where the per capita magnitude of soil assets is restricted, vulnerable to natural and anthropogenic disturbances, and susceptible to degradation due to predicted climate change and increased demographic stress.

In the water, salts prevent crop development for two purposes. Firstly, the capacity of the plant to handle water is decreased, thus reducing crop development. This is a salinity deficiency or osmotic consequence. Secondly, in the documents, salts can reach the transpiration column of crops that develop and harm cells, as well as decrease plant growth leaching. This is a salinity-specific ion surplus impact on development speed (Munns, 2005). It contributes to a salinity reaction of the crop in two phases. In the first stage, crops under drought and physiological reaction impacted by outside salinity and compelled crops are similar to the reaction to drought stress. Sodium chloride accumulates in surplus quantities that hinder development instead of accommodating this development of increasing tissue spaces when they come to xylem. Which are mostly supplied in phloem, in the event of meristematic tissue, acids were effectually avoided (Munns, 2002). Subsequently next phase, affected to a greater degree by the salinity factory inside the plant. In the previous documents, salt is deposited in the excess by crops: a very elevated amount of NaCl outcomes in a continuous transition of salt to the flow of transpiration over a lengthy time of time and is left to decay. Maybe the vacuolar ability to weigh the toxic salt species is triggered by congestion. For plant survival mortality rate is very critical. On other side reason of dehydration is might they construct in cell wall (Munns *et al*., 2005). If mortality is greater than average of emerging new leaves it will difficult for plant to survive. It is seen there is rise in manufacturing of "ROS i.e. O^-, O^{-2}, H_2O_2 and OH" in cell. manufacturing of these radicals mainly occuring in areas located in peroxisome, chloroplast and mitochondrial electron transport chain. Plants also modified themselves against injurious effects of ROS. Main defending system of plants are mostly antioxidant enzymes such as CAT, POD and SOD.

Superoxide anion (O-2) radicals resulting from environmental stress variables such as plant salinity will be catalyzed by SOD to H_2O_2 and oxygen (O_2). As a result of this dismutation response, CAT converts H2O2 into air (H_2O) and O2 which demonstrates very toxic impacts in living systems. Not like CAT, POD by oxidation of co-substrate i.e. decaying H_2O_2 by flavonoids or tannins. In plants, CAT occur in microbody (peroxisomes and glyoxisomes) and its main purpose is to erase H_2O_2 produce during β-oxidation and photorespiration of fatty acids. Adding in it, higher plants have many different PODs involved in various processes, such as salt stress, and these are found in the cell wall, cytosol and vacuoles. Many physical (engineering), Biological and chemical method are initiated for production on such soils. Due to limitation in environment and economic reasons cohesive use of these methods is imperative. Foliar or basal application of fertilizers has obtained much focus for minimizing hazardous impact of salt content (Raza et al., 2006). An exogenous implementation of potassium in corn (Akram et al., 2007), Ca in fruit (Awada et al., 1995), along with action of nitrogen on beans (Save et al., 1994) improved the antagonistic impact of salt content.

Materials and methods

Seeds of three varieties (Inqalab-91, Faisalabad-2008, Anaj-2006) of wheat were collected from market. Pots, peat moss soil, seeds, falcons, water, spatula, permanent marker, scotch tape, weighing balance and UV spectrophotometer were used as equipment. NaCl, CuSO4 and ethanol were used as reagents. Forty-five seeds from each variety were sown in seedling trays filled with soil and silt media. Experiment was done under controlled environmental conditions in Lab, Department of biotechnology University of Lahore, where average temperature was kept at 20±5°C during day and 12±3°C at night during the experimental period. While relative humidity was kept in a range of 50-85%. Ideal moisture levels for germination along with seedling development was kept with regular irrigation. T_o, T_1, T_2, T_3, T_4, T_5, T_6, T_7 and T_8 were denoted as control, 1M NACL, 0.5 M NACL, 1M $CuSO_4$, 0.5M $CuSO_4$, T1+T_3, T_1+T_4, T_2+T_3 and T_2+T_4, respectively.

Procedure

- Sow the seeds in the pots filled with peat moss soil.
- Once at the 4th leaf stage the data of the plants was collected, then the treatment was initiated, once the plant reached the 4th leave stage.

- After the treatment data was initiated again
- Again, apply the treatments after 7 days of first spray
- Take the results after three days
- Spectrophotometric analysis of leaf, stem and root

Parameters to be evaluated

Plant were selected from each pot for absolute growth studies, for this purpose, following method will be adopted.

Root, stem and leaf length (cm)

Length of root, stem and leaf was measured on centimeter scale by using meter rod.

Root, stem and leaf fresh weight (g)

Root, stem and leaf fresh weight was measured in "grams" by using electric balance.

Root, stem and leaf dry weight (g)

After weighing the fresh weight, samples were kept for 24h at biotechnology lab of UOL. After it, dry weight of root, stem and leaf was measured by using electric balance.

Spectrophotometric Analysis

Spectrophotometry is scientific method used for estimating solutes level in a certain mixture by amounting light percentage absorbed in such solutes. It's a very precise and trustful method because various liquids have variant ability to absorb different wavelengths at different intensities. Results are amounted by evaluating light that passes through the mixtures, we can ascertain specific mixed ingredients in solution and can also measure their level in these mixtures. Because of such abilities it is used for analyzing different mixtures in laboratory.

Using spectrophotometer

Generally, these machines are need to warm up for better working yielding precise results. Therefore, turn on machine and stable it for a period of fifteen minutes earlier then using treatment.

Clean the cuvettes

Two types of cuvettes can be used for running samples, glass cuvettes or disposable ones so if one is using spectrophotometer in lab one can use disposable cuvettes so it is not necessary to clean. While using reusable cuvettes, these should be properly cleaned before any use by using distilled water.

Cuvettes should be handled with very care because these are very costly specially when they are makeup of glass or quartz. Particularly quartz cuvettes are mainly produced for using UV-visible spectrophotometer.

Some other point should also be taken care mainly handling cuvette, take care before touching sides of glass tube because light have to pass through these. If these are touched, please clean these sides with tissues.

Load the proper volume of the sample into the cuvette

Glass tubes or cuvettes used in spectrophotometer are mostly of 1 ml in size while test tubes are mostly of 5 ml. For much the time, wavelength is passing through the solution, precise results were obtained.

In case work is done with help of micropipette sample should be taken with new tip every time.

Use control Solution

Control solution termed as blank, it only contains solution made of chemicals without plant sample or test sample. If one uses salt in water, blank should only contain water. One other thing is also kept in mind that blank solution is also of same volume as test sample and reading is taken in same size cuvette as that of test sample.

Clean the outside surface of cuvette

Before keeping the cuvette in spectrophotometer for analysis one should clean it as much possible as it can to prevent intervention resulting from dirt or dust particles. For this purpose, use a soft tissue paper and clean all water drops or any dust on cuvette.

Running the experiment

Select and confirm wavelength needed for running experiment and getting results from experiment, it is recommended to use one wavelength for getting more precise results. Some other care to be taken is that color of light should be absorbed by test chemical and its solute whose concentration has to be measured.

- Wavelength will be selected and confirmed by experimenter
- Sample color and wavelength color should be different for getting more accurate results because same color wavelength will be reflected totally

Calibrate the machine with the blank

Initially put the blank sample in cuvette and run the spectrophotometer after closing lid. On spectrophotometer screen, different varying values will start to appear depending upon intensity of light received and detected. After a stable value has been obtained record value and auto zero by pressing that button

- Digital spectrophotometers are also calibrated by using similar method. After reading set blank to autozero by using that specific button
- This will help as after removing blank sample the calibrated value will be constant and still in that place so ultimately when other samples will be used and run for examination this absorbance value will automatically ahead of that blank value and that will be minus from calibrated auto zero or blank value

Place the test samples in machine

After successful measurement of blank samples now place the test samples in cuvette.

- After proper calibration of machine by using blank, reading will be zero
- In case it is not zero run the blank again
- In case of still dealing with issues make call to assistance or machine dealer to check out it

Measuring test sample absorbance

After removing blank sample, keep the test sample in spectrophotometer holder in a way that it stands upright. Wait for ten seconds until reading become constant then note the values. This absorbance value is also termed as optical density (OD).

- More the light pass through sample less will be received and absorbed by solution
- If results are out of range or not appropriate please dilute the sample and measure again
- Repeat the whole process for at least three times for every sample and make a mean of all these

Statistical Analysis

Results were statistically evaluated by variance method (Steel et at, 1997). The other proposed techniques were Regression, correlation and skewness.

Results

Root length

The average root length under all treatments was recorded as 3.2907±0.1903cm. The coefficient of variation was 10.01%. The results indicated that the highest root length was found under the treatment of control and 1M NACL (4cm) followed by T_1+T_3 (3.6833cm), T_2+T_3 (3.3cm), 0.5M $CuSO_4$ (3.2cm), T_1+T_4 (3.1cm), T_2 +T_4 (3cm). 0.5M NACL (2.75cm) and 1M $CuSO_4$ (2.58cm). Among varietal comparison it was assessed that wheat cultivar "Inqlab-91" performed better followed by "Faisalabad-2008" while "Anaj-2006" concluded as least performing cultivar in regard of leaf width.

Stem length

maximum avg. stem length (6.6 cm) was recorded by "Anaj-2006" trailed by "Inqlab-91" and "Faisalabad-2008" with avg. value of 6.05 cm and 5.30 cm respectively. Whereas interaction of salinity treatment with wheat cultivars depicts most severe impact of 1mM $CuSO_4$ application in "Inqlab-91" recording 2 cm stem length followed by 0.5mM NaCl + 1mM $CuSO_4$ treatment in both "Inqlab-91" and "Anaj-2006" yielding out 3 cm stem length. results showed that 1mM NaCl + 1mM $CuSO_4$ treatment have shown most severe results as compared to other salt and heavy metals applied.

Leaf length

The average leaf length of wheat was recorded as -16.452 ± 0.211cm under all treatments of wheat. The results were consistent and reliable because of low coefficient of variation (3.86%). The highest leaf length (25.667cm) was found under control the treatment of control while followed by treatment of 1M NACL (21.167cm) while T_1+T_4 (21.367cm) 0.5M CuSO4 and T_2+T_4 (19.800cm), 0.5M NACL (18.800cm), T_1+T_3 (8.167cm), T_2+T_3 (7.33cm), and the lowest leaf length (5.667cm) was found under the treatment of 1M $CuSO_4$. Whereas among varietal comparison it was assessed that wheat cultivar "Anaj-2006" performed better followed by "Faisalabad-2008" while "Inqlab-91" concluded as least performing cultivar in regard of leaf length.

Leaf width

The average leaf width was recorded as 0.4±0.0277cm. The results were less consistent because the coefficient of variation was high (12.01%). The highest leaf width was found under the treatment of control and T_1+T_4 (0.4833cm) followed by 1M NACL (0.4500cm), 0.5M CuSO4 (0.4500cm), T_2+T_4 (0.4500), 0.5M NACL (0.4167cm), T1+T_3 (0.3167cm) 1M $CuSO_4$ (0.2667cm). while the lowest leaf width was found under the treatment of T_2+T_3 (0.1833cm). Moreover, results regarding interaction of genotypes and treatment have exhibited that application of 0.5 mM NaCl + 1mM $CuSO_4$ have caused damage by reducing leaf width in all three wheat cultivars by recording 0.2 cm in "Anaj-2006" and "Faisalabad-2008" while 0.3 cm width in "Inqalab-91" respectively.

Leaf area

With respect to leaf area, varietal performance was significant and "Inqlab-91" performed quite better whereas "Anaj-2006" and "Faisalabad-2008" have homogenous performance. regarding impact of salinity treatment on leaf area concludes 1mM $CuSo_4$ as most severe levels as it recorded minimum leaf area in all wheat cultivars. minimum leaf area were recorded in 1mM $CuSO_4$, 1mM Nacl+ 1mM $CuSO_4$ application in "Inqlab-91", "Anaj-2006" and "Faisalabad-2008"

Fresh leaf weight

The varietal evaluation assessment showed that wheat cultivar "Inqlab-91" performed better followed by "Faisalabad-2008" while "Anaj-2006" concluded as least performing cultivar in regard of fresh leaf weight. While regarding impact of salinity treatment by 1mM NaCl + 1mM $CuSO_4$ was damaging as it recorded minimum fresh leaf width (0.101 g) followed by 0.5mM NaCl + 1mM $CuSO_4$ recording 0.1006 g of fresh leaf width. Whereas the interaction of salinity treatment with wheat cultivars depicts most severe impact by 0.5mM NaCl + 1mM $CuSO_4$

application in "Inqlab-91", "Anaj-2006" and "Faisalabad-2008"

Fresh root weight

1mM NaCl + 1mM $CuSO_4$ was most devastating as it recorded minimum fresh root weight (0.1003 g) followed by 1mM $CuSo_4$ recording (0.1007 g) of root fresh weight (Table 4.20). Moreover the salt processing and wheat cultivars are inter-linked which clearly depicts substantial outcome as minimum fresh root weight (0.1001, 0.1002 and 0.1003 cm) was assessed in case of "Anaj-2006" and "Faisalabad-2008" by application of 1mM NaCl + 1mM CuSO4, 0.5mM NaCl + 1mM $CuSO_4$, in "Faisalabad-2008" respectively. wheat cultivar "Faisalabad-2008" performed better followed by "Inqlab-91" while "Anaj-2006" concluded as least performing cultivar in regard of fresh root weight.

Fresh stem weight

The average stem weight under all the treatment was 0.1026g. The coefficient of variation was 0.89%. Results showed that wheat cultivar "Anaj-2006" performed better followed by "Faisalabad-2008" while "Inqlab-91" concluded as least performing cultivar in regard of fresh stem weight. While regarding impact of salinity treatment by 1mM $CuSo_4$ was damaging as it recorded minimum fresh shoot width (0.1009 g) followed by 0.5mM NaCl (0.101 g) of fresh shoot weight.

Dry root weight

The average dry root weight was recorded as 0.0187g. The coefficient of variation was 4.37%. results show that1mM NaCl + 1mM $CuSo_4$ as most severe because it yields minimum dry root weight (0.014 g) followed by 1mM $CuSo_4$ recording (0.019 g) of root dry weight (Table 4.29). Moreover the salt processing and wheat cultivars are inter-linked which clearly depicts substantial outcome as lowest RDW (0.050 g) was compromised in 1mM NaCl + 0.5mM $CuSo_4$ treated plants

Dry stem weight

The coefficient of variation was 15.64%. The maximum stem dry weight (0.009 g) was attained after 1mM $CuSo_4$ application in both wheat cvs. "Anaj-2006" and "Inqlab-91". Whereas the interaction of salinity treatment with wheat cultivars has concluded that in general salinity application have resulted in reduced stem growth and assimilate accumulation thus lowering stem dry weight. In general, 0.5mM NaCl + 1mM $CuSO_4$ treatment have resulted in minimum stem dry weight in all three wheat cultivars

Dry leaf weight

Overall wheat cultivar "Inqlab-91" performed quite better whereas "Anaj-2006" and "Faisalabad-2008" have homogenous performance. 1mM NaCl + 1mM $CuSo_4$ was most devastating as it recorded minimum fresh root weight followed by 0.5mM NaCl + 1mM $CuSo_4$. Moreover, the salt processing and wheat cultivars are inter-linked which clearly depicts substantial outcome as higher leaf dry weight (0.055 g) compromised in control treatments. While among treated plants, results expressed that higher leaf dry weight was found in case of application of lower doses of salinity application as 1mM NaCl application has recorded good leaf dry weight (0.043-0.047g) in "Inqlab-91" and "Anaj-2006"

Leaf spectrophotometry

The results indicated that the average carotenoids in leaves were recorded as 0.3098 The coefficient of variation was 17.67%. it was assessed that wheat cultivar "Inqlab-91" performed better followed by "Faisalabad-2008" while "Anaj-2006" concluded as least performing cultivar in regard of photometry of leaf. While regarding impact of salinity treatment on photometry of leaf depicts was most devastating as it recorded minimum leaf phoyometric residual contents in 1mM $CuSo_4$ of leaf in "Anaj-2006". Moreover the salt processing and wheat cultivars are inter-linked which clearly depicts substantial outcome which were assessed in case of "Inqalab-91" and "Faisalabad-2008" by application of 1mM NaCl and 1mM $CuSO_4$ respectively

Stem spectrophotometry

It was found that the average carotenoids in stem were 0.608mg/g fresh leaf weight in wheat seedlings under treatments of water. It was observed that there was low coefficient of variation (1.39%). Anaj-2006 showed minimum salt accumulation. While regarding impact of salinity treatment on stem photometry analysis reveals that most devastating treatment was 1mM $CuSo_4$ as maximum salts were observed under this treatment. Moreover, relation among salt processing and wheat cultivars clearly depicts substantial outcomes which were assessed in case of "Anaj-2006" and "Faisalabad-2008" by application of 1mM NaCl respectively

Root spectrophotometry

It was found that there was very low coefficient of variation (3.88%). among all wheat cultivar "Anaj-2006", "Faisalabad-2008" and "Inqlab-91" followed respectively regarding their performance for salt accumulation in roots. Among these, best results were obtained in "Anaj-2006" as its root have depicted lowest values of salts storage in their roots. While regarding impact of salinity treatment on root photometry analysis reveals that most devastating treatment was 1mM NaCl + 1mM $CuSo_4$ as maximum salts accumulates were observed under this treatment. Moreover, relation among salt processing and wheat cultivars clearly depicts substantial outcomes which were assessed in case of "Anaj-2006" and "Faisalabad-2008" by application of 1mM NaCl respectively.

Root shoot length ratio

The maximum root shoot length ratio was found under 1M NaCl + 1mM $CuSo_4$ treatment in all wheat cvs. "Anaj-2006", "Inqlab-91" and "Faisalabad-2008". Whereas the results regarding salinity treatment have depicted combine application of 1M NaCl + 1mM $CuSo_4$ have resulted in higher root shoot length ratio thus affects plant growth and productivity. Among varietal comparison, "Faisalabad-2008" recorded highest values for root shoot ratio after treated with salt and heavy metals.

Discussions

Salinity, a severe environmental threat which is reducing growth, yield and quality of produce. Salinity among one of main abiotic factors is worsening condition due to excessive use of ground water pumping as irrigation water. The detrimental consequence of salinity can differ depending on environmental circumstances (Acosta-Motos et al., 2014; Bajracharya et al., 2014). Plants are classified into various kinds i.e. glycophytes or euhalophytes and this classification is done on the basis of their response to saline conditions via ion intake or uptake, osmotic balance, CO_2 storage, electron transport, levels of chlorophyll, intensity of ROS generation and antioxidant capacity. Salinization, major issue that is affecting and causing excessive soil degradation, is indirectly or directly affecting plant expansion as well as fertility globe-wide. Till now the global area of about seven percent is salt affected (Szabolcs, 1989; Munns and Tester 2008; Cassaniti et al., 2012). This condition is harsening throughout the world at the rate of ten percent increment per anum mainly in countries where artificial irrigation is done to grow crops (Flowers and Hajibagheri, 2004). With the advent of irrigation system in Indo-Gangetic plains by British lead to increment of salinity issues uphere. Pakistan, important country of Asia with the biggest surface irrigation system of the world. It is estimated that per anum 40,000 ha cultivated land is degraded because of salinity (Li et al., 2006). salt stress causes reduction of avg. leaf area. While, shortened leaf size is the main and initial reaction of glycophytes reacted with salt stress (Munns and Termaat, 1986). Whereas, decrease of canopy size may also be reflected as resistance strategy to reduce loss of water and solutes happening through transpiration even under closed stomatal conditions (Save et al., 1994). This can also enhance preservation of hazardous atoms in roots, and avoiding their entry in aerial potions. Under salinity circumstances, properties of cell wall become altered also the leaf turgor pressure and reduction of photosynthesis thus limiting leaf area (Ruiz-Sánchez et al., 2000). Stem growth is severely affected by excess salt levels. While reduction of leaf and stem size results in overall reduction of all aerial part sizes and general plant height (Rodríguez et al., 2005). alteration in cell turgor pressure of roots have been observed when grown under salinized media thus will ultimately affects stomatal closure leading to reduction of photosynthesis. While this reduction can also be due to loss of chlorophyll contents under saline conditions. Shoot and leaf weight have been witnessed much loss under saline conditions in comparison to control. Munns, (1992) revealed that old leaves senescence is encouraged by excessive salt accumulation which reduces carbohydrates supply along with PGR's to new growing regions thus affecting overall growth and minimizing growth. Presently available wheat germplasm in Pakistan should be exploited for breeding purposes in order to yield suitable cultivars for diverse areas of the country. Presently evaluated wheat cultivars exhibit morphological diversity in different aspects i.e. grain weight and yield, plant height and taste.

Among tested varieties, Inqlab-91 and Anaj-2006 performed better under salt stress as compared to Faisalabad-2008 and these two could be utilized under saline situations in Pakistan. There is a dare need to start wide-ranging varietal improvement program for the upgradation of wheat cultivars in Pakistan. Morphological, biochemical and molecular characterization has provided the basic information thus facilitating researchers to achieve clear objective. Therefore, a research trial was conducted to evaluate wheat germplasm against salinity stress. Three wheat cultivars; Inqalab-91, Anaj-2006 and Faisalabad-2008 was grown for evaluation against salinity stress under lab conditions. Different vegetative and physiological parameters were recorded to determine hazardous effects of salinity upon wheat cultivars

Conclusion

Results conclude that treatment (salt and heavy metal dose), germplasm and their interaction have significant effect. Among parameters evaluated it was assessed that upon single and combine application of NaCl and $CuSO_4$, $CuSO_4$ application have recorded in reducing growth parameters. While the combine application of NaCl + $CuSO_4$ also led to impose detrimental effects on plant growth and development behavior.

Conflict of Interest

There is no conflict of interest between authors.

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Table 1: Analysis of variance for various traits of wheat under salt and heavy metal stress conditions

Source	Leaf length	Leaf width	Stem length	Root length	Fresh leaf weight	Fresh stem weight	Fresh root weight	Leaf dry weight	Stem dry weight	Root dry weight	Leaf photo metry	Stem photome try	Root photome try	Leaf area	Root shoot length ratio
Replication	2.16	3.009E-36	3.5780	0.8313	1.746E-06	4.091E-07	7.407E-10	8.167E-08	3.130E-06	5.934E-06	0.00271	1.25E-05	6.69E-06	0.441	0.00001
Genotype	35.325*	0.02667	7.7269*	15.0902	1.180E-04*	1.824E-06*	2.175E-05*	1.015E-05	1.027E-05*	9.522E-05*	0.02387	2.005E-04*	7.17E-05	22.3919*	0.50789*
Treatments	320.50*	0.0450	69.7046*	1.5617	0.00252*	7.292E-06*	1.478E-05*	1.212E-05	1.701E-05*	0.00246*	0.00536	1.304E-04*	3.712E-05*	95.2948*	0.44429*
Genotype× Treatments	5.467*	0.00292	11.4156*	3.7023	1.684E-04*	5.645E-06*	2.492E-06*	6.296E-06	5.410E-06*	1.710E-04*	0.00373	1.335E-04*	3.614E-05*	1.2931	0.12849*
Error	0.401	0.00231	0.1372	0.1086	7.838E-07	8.410E-07	8.818E-09	7.974E-08	2.073E-07	1.569E-06	0.003	7.11E-07	4.88E-06	0.8432	0.00824
Grand mean	16.419	0.4000	5.9907	3.2907	0.1285	0.1026	0.1025	2.68E-03	2.91E-03	0.0287	0.3098	0.068	0.0569	7.1578	0.6730
CV	3.86	12.01*	6.18*	10.01*	0.69*	0.89*	0.09*	10.52*	1.564*	4.37*	17.67*	1.39*	3.88*	12.83*	13.49*
Standard error	0.3657	0.0277	0.2138	0.1903	5.111E-04	5.295E-04	5.421E-05	1.630E-04	2.629E-04	7.233E	0.0316	4.868E-04	7.362E-04	0.3061	0.0303

Table 2. Pair-wise mean comparisons for various traits of wheat under salt and heavy metal stress conditions

Treatments	Leaf length	Leaf width	Stem length	Root length	Fresh leaf weight	Fresh stem weight	Fresh root weight	Leaf dry weight	Stem dry weight	Root dry weight	Leaf photo-metry	Stem photometry	Root photometry	Leaf area	Root shoot length ratio
T control	25.667 A	0.4833 A	14.000 A	4.0000 A	0.1485 A	0.1025 ABC	0.1044 A	4.43E-03 A	2.50E-03 BCD	0.0485 A	0.3233 C	0.0585 C	0.0562 BC	12.400 A	0.2977 D
T 1	21.167 B	0.4500 A	6.350 C	4.0000 A	0.1382 D	0.1020 BCD	0.1032 C	3.23E-03 BC	2.00E-03 D	0.0382 C	0.3525 A	0.0560 D	0.0560 BC	9.517 BC	0.6278 C
T 2	18.800 C	0.4167 A	8.000 B	2.7500 CD	0.1437 BC	0.1031 ABC	0.1033 C	3.28E-03 BC	2.62E-03 BCD	0.0437 B	0.3165 D	0.0638 B	0.0577 AB	7.870 C	0.4722 CD
T 3	5.667 E	0.2833 B	3.000 F	2.5833 D	0.1019 F	0.1041 A	0.1007 E	2.47E-03 D	6.83E-03 A	0.0019 E	0.3125 D	0.0697 A	0.0605 A	1.692 D	0.8893 B
T 4	19.800 C	0.4500 A	5.967 C	3.2000 BCD	0.1430 C	0.1028 ABC	0.1036 B	3.58E-03 B	2.33E-03 CD	0.0430 B	0.2765 E	0.0565 D	0.0530 C	8.947 BC	0.5220 C
T 5	8.167 D	0.3167 B	3.117 EF	3.6833 AB	0.1013 F	0.1015 CD	0.1003 F	2.50E-04 E	3.13E-03 BC	0.0014 E	0.2520 E	0.0640 B	0.0607 A	2.658 D	1.2070 A
T 6	21.367 B	0.4833 A	4.917 D	3.1000 BCD	0.1332 E	0.1033 ABC	0.1030 D	2.92E-03 CD	2.92E-03 BC	0.0332 D	0.3043 D	0.0563 D	0.0557 BC	10.367 B	0.5753 C
T 7	7.33 D	0.2667	3.817	3.3000	0.1023	0.1005	0.1005	4.67E-04	5.33E-04	0.0035	0.3177	0.0633 B	0.0572	2.000	0.8700

		B	E	BC	F	D	F	E	E	E	D		ABC	D	B
T 8	19.800C	0.450 A	4.750 D	3.0000 CD	0.1448 B	0.1033 AB	0.1035 B	3.52E-03 B	3.33E-03 B	0.0448 B	0.3330 B	0.0592 C	0.0550 BC	8.970 BC	0.5953 C
Standard Error	0.2111	0.016	0.1235	0.1099	2.951E-04	3.057E-04	3.130E-05	9.413E-05	1.518E-04	4.176E-04	0.0183	2.810E-04	7.362E-04	0.3061	0.0303

Table 3. Mean comparison of wheat genotypes for various traits under salt and heavy metal stress conditions

Genotype	Leaf length	Leaf width	Stem length	Root length	Fresh leaf weight	Fresh stem weight	Fresh root weight	Leaf dry weight	Stem dry weight	Root dry weight	Leaf photometry	Stem photometry	Root photometry	Leaf area	Root shoot length ratio
INQLAB-91	18.022 A	0.4444 A	6.6111 A	3.9944 A	0.1303 A	0.1029 A	0.1032 A	3.14E-03 A	3.78E-03 A	0.0303 A	0.3340 A	0.0641 A	0.0586 A	8.4150 A	0.7804 A
FAISALBAD – 2008	15.800 B	0.3778 B	6.0556 B	3.6222 B	0.1298 A	0.1025 A	0.1031 A	3.09E-03 A	2.48E-03 B	0.0298 A	0.3275 A	0.0610 B	0.0574 A	6.7711 B	0.7591 A
ANAJ - 2006	15.433 B	0.3778 B	5.3056 C	2.2556 C	0.1256 B	0.1023 A	0.1012 B	1.82E-03 B	2.47E-03 B	0.0261 B	0.2679 B	0.0574 C	0.0547 B	6.2872 B	0.4794 B