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BIOCHEMICAL AND METABOLIC STUDIES OF WHEAT (TRITICUM AESTIVUM L.) IN RESPONSE TO SALINITY UNDER LABORATORY AND FIELD CONDITION

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ABSTRACT:

The research work titled "Biochemical and Metabolic Studies of Wheat (Triticum aestivum L.) in Response to Salinity Under Laboratory and Field Conditions" aimed to screen 10 wheat genotypes Kharchia-65, KRL-3-4, FLW-16, PBW-757, DBW-252, FLW-2, FLW-1, DBW-11, HD-2851, and FLW-5 for salinity stress tolerance. Salinity stress treatments were administered at 25mM, 50mM, 75mM, and 100mM, along with a control at the Instructional Farm of Acharya Narendra Deva University of Agriculture & Technology, Kumarganj, Ayodhya. Genetic variation was observed in germination percentage, with a drastic reduction in genotypes FLW-5 and FLW-16 under stress, while Kharchia-65 and KRL 3-4 showed better performance. Seedling shoot length significantly decreased in FLW-1 and FLW-2, and root length in DBW-11 under stress, whereas Kharchia-65 and DBW-252 performed better. Plant height saw a drastic reduction in HD-2851 and FLW-5, while Kharchia-65 and KRL 3-4 maintained better heights. Tiller number significantly reduced in FLW-16, FLW-2, and FLW-1, with Kharchia-65 and KRL 3-4 less affected. Spikelet length drastically reduced in DBW-252, FLW-5, and FLW-2, with Kharchia-65 and KRL 3-4 performing better. Salinity stress at the reproductive stage severely reduced yield, especially in FLW-1, while HD-2851, Kharchia-65, and KRL 3-4 were less affected. Catalase, peroxidase activities and SDS-PAGE increased under stress, with Kharchia showing the highest increments.Proline levels increased significantly, especially in Kharchia-65 and KRL 3-4, while protein levels decreased, with HD-2851 and FLW-5 showing the highest reductions. These findings show the differential response of wheat genotypes to salinity stress, with Kharchia-65 and KRL 3-4 consistently showing better performance across various parameters, providing insights for selecting and breeding wheat varieties with enhanced salinity tolerance.

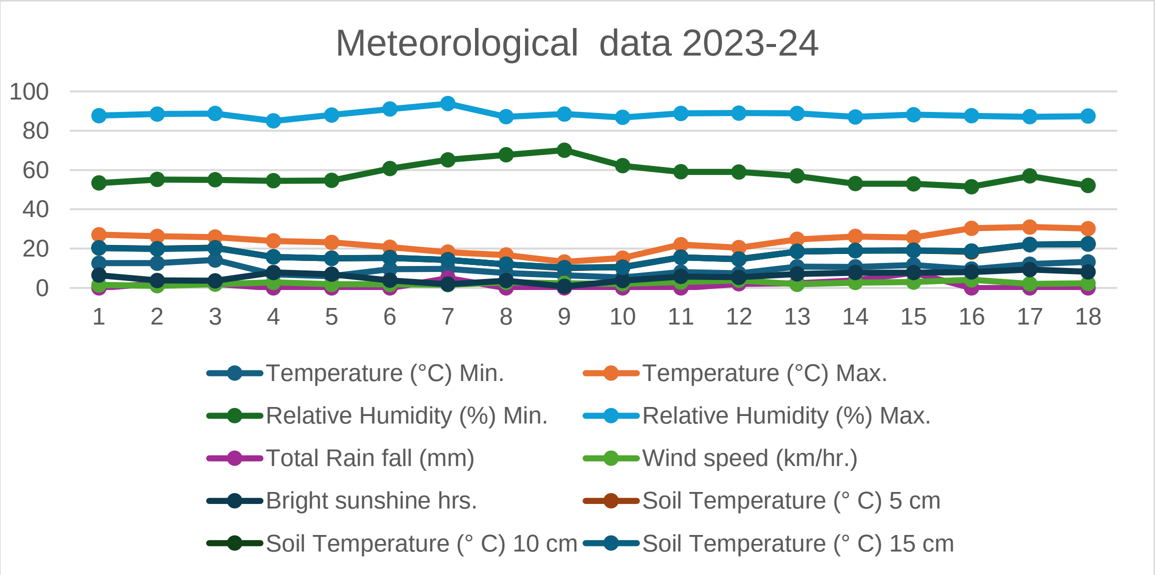
Keywords: salinity, genotypes, stress tolerance

1. Introduction

The basic food, wheat (*Triticum aestivum* L.), is a member of the Poaceae family. It is a hexaploid annual species that has chromosome number 42. About 2.5 billion people rely on wheat as a staple meal, making it one of the most widely grown cereal crops in the world. More than half of the calories consumed in the West and Central Asian and North African regions come from wheat, the main staple grain. Following rice in terms of calories and nutritional content, wheat is one of the primary protein sources in middle-income and developing nations. Wheat is grown in two seasons: winter and spring, all across the world. The most frequent abiotic stressor that negatively impacts plant development and production is salinity. Currently, salt affects more than 6% of the world's geographical surface, primarily in arid and semiarid regions. (Bui *et al.* 2013) Worldwide, 2.1% of dry land agriculture and 19.5% of irrigated land are affected by salinity. Seven million hectares of land in India are affected by salinity alone. (Giri*et al.*, 2003). The osmotic potential is lowered when salt-rich water builds up, which reduces the amount of water that is available to the plants. Reactive oxygen species (ROS) are produced by plants under salt stress, which leads to oxidative damage that negatively impacts plant growth. (Baisak*et al.*, 1994). Salt stress significantly inhibits crop growth and development, especially wheat, resulting in decreased grain production and quality. (Guo *et al.*, 2012; Turki *et al.*, 2012; Desoky and Merwad, 2015). Wheat plant development, reproduction, and yield are all impacted by soil salinity in different ways. Plants undergo morphological, physiological, and biochemical changes in response to salt stress due to modifications in hormone balances, photosynthesis, ion transport systems, anatomy, and water transport systems. (Ashraf and Harris, 2013). The growth phases, physiological and genetic characteristics, the amount of salt in the soil that is poisonous, and the length of time that the plant is exposed to salt stress all affect how sensitive a plant is to it. (Bacu*et al.*, 2020). The morphological, biochemical, structural, and molecular reactions to salt stress in wheat are covered in this section. Salinity stress causes osmotic stress and ion toxicity by increasing the uptake of Na+ ions and decreasing the Na+/K+ ratio because of decreased osmotic potential inside the plant roots. Additionally, by influencing the absorption and transport of other important required ions in target cells, these ionic imbalances disrupt important plant processes and activities. Salinity has a detrimental effect on wheat crop productivity. Salinity levels between 6 and 8dSm1 cause a decline in wheat crop yield. (Kunika et al., (2019).

Materials and methods

The experimental materials of the study comprised of 10 Saline Wheat genotypes 1. Kharchia-65, 2. KRL-3-4, 3. FLW-16, 4. PBW-757, 5. DBW-252, 6. FLW-2, 7. FLW-1, 8. DBW-11, 9. HD-2851, 10. FLW-5from check Kharchia-65 & KRL 3-4. These genotypes were produced from collection of genetic stock available in Wheat section, Department of Molecular Biology & Biotechnology, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya. This experiment is totally biased on saline conditions of wheat genotype.The geographical situation lies between range from 240.47 north to 260.56 north a longitude 810.12 east and 830.98 on an altitude of 113 meters above the mean sea level.



In this research paper, observation of seedling shoot and root length, plant height, Number of tillers plant^{-1}, catalase (CAT) activity (Unit min/mg/protein), peroxidase (POX) activity (Unit min/mg/protein) and spikelet length(cm) are recorded over the control.

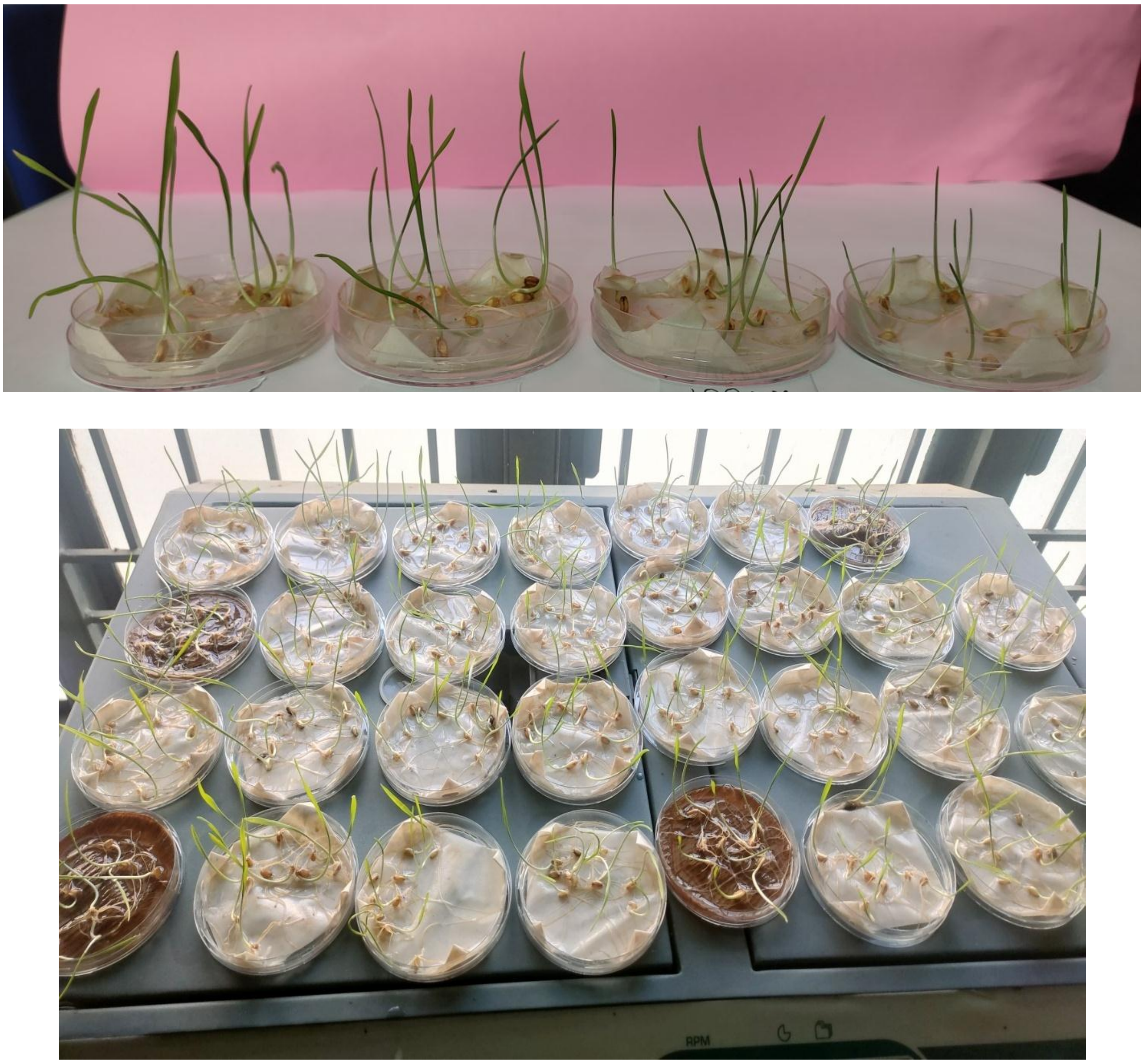


Fig4.4 Wheat genotypes at seedling stage showing symptoms of salt stress

Field Trail View Wheat Crop

2. RESULTS AND DISCUSSION

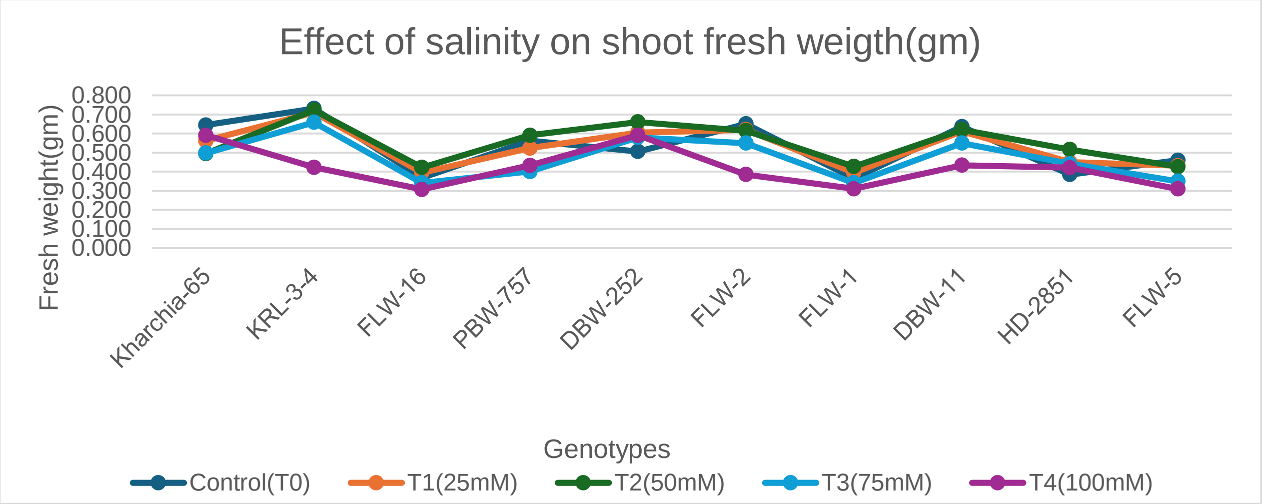
Growth parameters

Seedling shoot length (cm): seedling shoot lengths are documented and presented in a table 4.2, revealing the effects of salinity on early plant development. Genotypes that maintain longer seedling shoot lengths under higher salinity conditions, such as KRL 3-4 and Kharchia-65, are identified as resistant. For example, KRL 3-4 continues to show robust shoot growth at 100 mM NaCl, indicating strong salinity tolerance. Conversely, genotypes like FLW-1, FLW-2 & DBW-11 exhibit significantly reduced shoot lengths as salinity levels increase, indicating higher susceptibility. Similar results also reported by (Ghosh *et al*., 2013)

Table 4.1 Effect of salinity on seedling shoot length after 14 days of germination.

S.No.	**Genotypes**	**Control% (T0)**	**Treatment%**				
			T1 (25mM)	**T2 (50mM)**	**T3 (75mM)**	**T4 (100mM)**	**Mean**
1.	Kharchia-65	14.50	13.50	12.00	11.50	10.90	11.58
2.	KRL-3-4	17.5	15.00	16.00	14.00	11.50	14.80
3.	FLW-16	8.30	9.00	6.30	5.50	5.30	6.88
4.	PBW-757	13.50	11.00	9.13	7.55	6.00	9.44
5.	DBW-252	10.00	8.50	6.50	4.23	2.50	6.35
6.	FLW-2	8.20	9.00	9.20	6.30	2.25	6.99
7.	FLW-1	10.50	10.50	8.26	6.50	2.15	7.58
8.	DBW-11	8.50	7.34	6.14	5.50	3.10	6.12
9.	HD-2851	10.00	12.00	8.50	7.88	5.50	8.78
10.	FLW-5	10.00	9.11	7.89	6.14	4.20	7.47

Root length (cm)

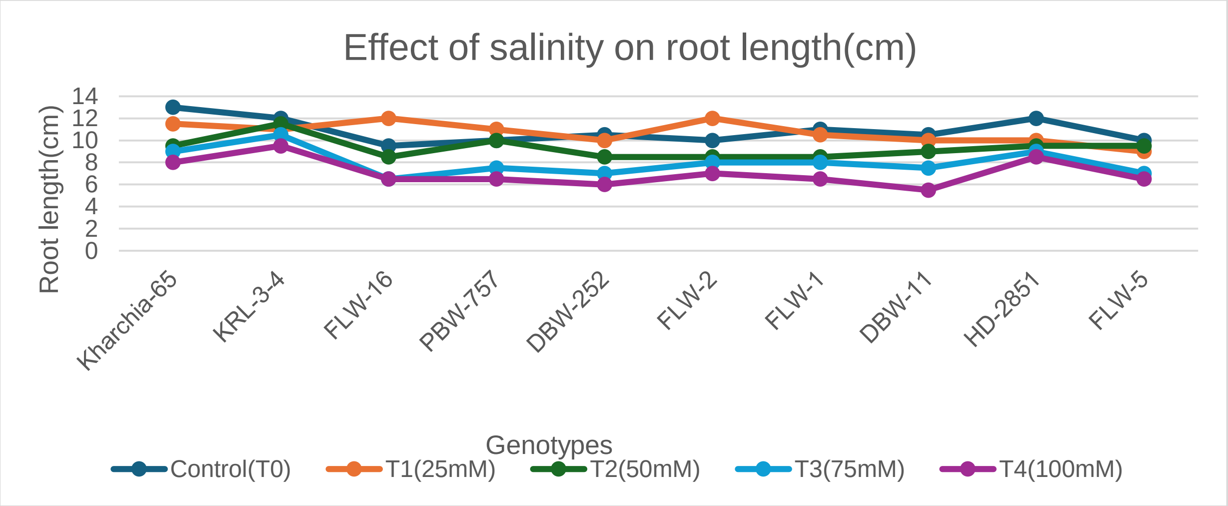


The root lengths are measured and presented in the table 4.2, illustrating how salinity affects root development across different genotypes. Genotypes that maintain longer root lengths under higher salinity conditions are considered more resilient, as they can sustain root growth despite the stress. For instance, Kharchia-65 and KRL-3-4, with mean root lengths of 10.20

and 10.90 respectively, demonstrate strong salinity tolerance. Conversely, genotypes like DBW-252 and FLW-5 exhibit significantly shorter root lengths as salinity increases, with means of 8.40 each, indicating higher susceptibility to salinity stress. Similar results also reported by (Ghosh *et al*., 2013)

Table 4.3: Effect of salinity on root length after 14 days of germination.

S.No.	**Genotypes**	**Control% (T0)**	**Treatment%**				
			T1 (25mM)	**T2 (50mM)**	**T3 (75mM)**	**T4 (100mM)**	**Mean**
1.	Kharchia-65	13.00	11.5	9.50	9.00	8.00	10.20
2.	KRL-3-4	12.00	11.00	11.50	10.50	9.50	10.90
3.	FLW-16	9.50	12.00	8.50	6.50	6.50	8.60
4.	PBW-757	10.00	11.00	10.00	7.50	6.50	8.80
5.	DBW-252	10.50	10.00	8.50	7.00	6.00	8.40
6.	FLW-2	10.00	12.00	8.50	8.00	7.00	9.10
7.	FLW-1	11.00	10.5	8.50	8.00	6.50	8.90
8.	DBW-11	10.50	10.00	9.00	7.50	5.50	8.50
9.	HD-2851	12.00	10.00	9.50	9.00	8.50	9.80
10.	FLW-5	10.00	9.00	9.50	7.00	6.50	8.40



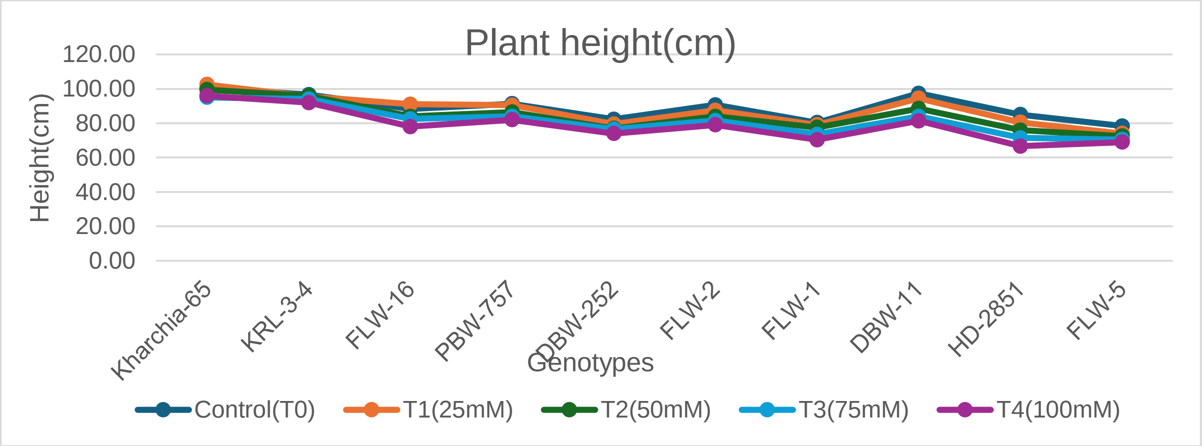
Plant height (cm)

The mean height for each genotype across all treatments is also provided for comparison presented in table 4.3. differences in genotype response to salinity stress are apparent from the data. Some genotypes, like Kharchia-65 and KRL-3-4, exhibit relatively minor reductions in height across the salinity treatments, indicating a degree of tolerance to salt stress. Conversely, genotypes such as FLW-1 and HD-2851 display more pronounced decreases in height, suggesting higher susceptibility to salinity-induced growth inhibition. Similar results also reported by (Hernandez *et al*., 2002).

Table 4.3: Effect of salinity on plant heights (cm)

S. No.			**Treatment**

	Genotypes	**Control**	**T1**	**T2**	**T3**	**T4**	**Mean**
1	Kharchia-65	100.33	102.45	99.50	95.00	96.00	98.24
2	KRL-3-4	96.66	95.50	96.00	94.00	92.00	94.38
3	FLW-16	88.33	91.00	84.00	82.50	78.00	83.88
4	PBW-757	91.33	90.50	86.50	84.00	82.00	85.75
5	DBW-252	82.33	79.50	77.00	76.50	74.00	76.75
6	FLW-2	90.66	87.50	84.00	81.50	79.00	83.00
7	FLW-1	80.33	79.00	77.50	73.50	70.33	75.08
8	DBW-11	97.33	94.50	88.50	84.00	81.33	87.08
9	HD-2851	85.00	80.50	76.00	71.50	66.67	73.67
10	FLW-5	78.33	74.00	72.50	70.50	69.00	71.50



Number of tillers plant^{-1}

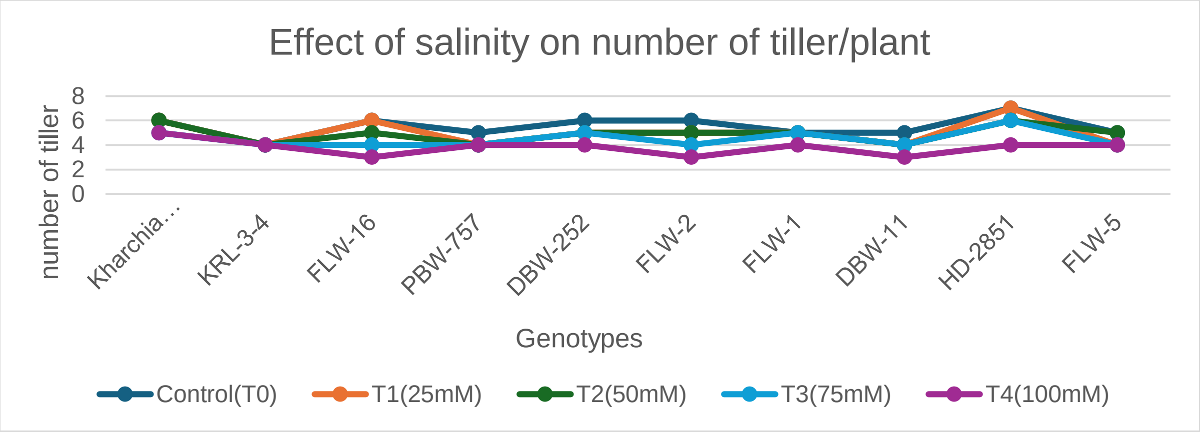
The data presented in table 4.4 illustrated that the control column represents the number of tillers per plant under normal conditions, while the subsequent columns labeled T1, T2, T3, and T4 signify increasing levels of salinity treatment at 25mM, 50mM, 75mM, and 100mM respectively. The mean number of tillers per plant across all treatments is also provided for each genotype. Some genotypes exhibit more resilience to salinity-induced tiller suppression than others. For example, Kharchia-65 and KRL-3-4 maintain a relatively stable number of tillers even under higher salinity treatments, indicating a degree of tolerance to salt stress in terms of tiller development. Conversely, genotypes like FLW-16 and HD-2851 show more pronounced decreases in tiller numbers, suggesting higher sensitivity to salinity stress. Similar results also reported by (Hernandez *et al*., 2002).

Table 4.4: Effect of salinity on number of tiller/plant

S. No.	**Genotypes**	**Control**	**Treatment**				
			T1	**T2**	**T3**	**T4**	**Mean**
1	Kharchia-65	6.00	6.00	6	5	5	5.50
2	KRL-3-4	4.00	4.00	4	4	4	4.00
3	FLW-16	6.00	6.00	5	4	3	4.50
4	PBW-757	5.00	4.00	4	4	4	4.00
5	DBW-252	6.00	5.00	5	5	4	4.75
6	FLW-2	6.00	5.00	5	4	3	4.25
7	FLW-1	5.00	5.00	5	5	4	4.75
8	DBW-11	5.00	4.00	4	4	3	3.75
9	HD-2851	7.00	7.00	6	6	4	5.75

10	FLW-5	5.00	4.00	5	4	4	4.25

Biochemical studies



Catalase (CAT) and peroxidase (POX)

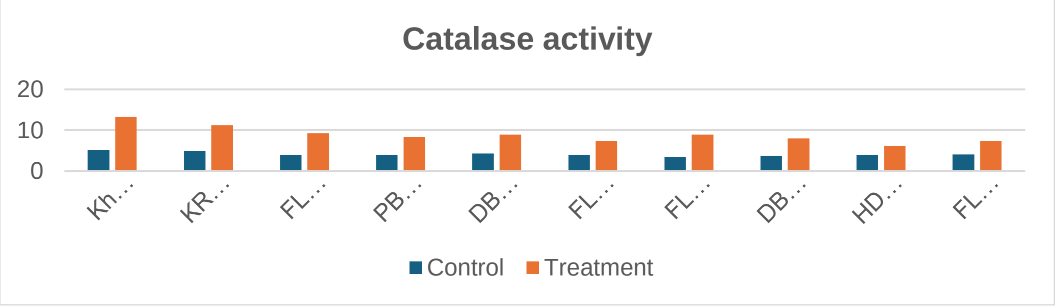
Catalase and peroxidase activities were studied in leaf at vegetative stage and results are presented in table 1 and table 2 respectively. Activity of both enzymes were significantly increased in Kharchia-65 and KRL 3-4 while same was reduced in other varieties and maximum reduction was in HD 2851.

Effect of salt stress on catalase (CAT) activity (min/mg/protein) in wheat genotypes.

The data presented in table 4.5 illustrated that effect of salt stress on catalase (CAT) activity in different wheat genotypes. Catalase is an important antioxidant enzyme that helps in detoxifying hydrogen peroxide (H_2O_2) into water and oxygen, thereby protecting cells from oxidative damage, particularly under stress conditions such as salinity.Similar results also reported by (Ahmed *et al*., 2008).

Table 4.5: Effect of salt stress on catalase (CAT) activity (Unit min/mg/protein) in wheat genotypes.

S. No.	Genotypes	Control	Treatment	Mean
1	Kharchia-65	5.12	13.25	9.19
2	KRL-3-4	4.91	11.23	8.07
3	FLW-16	3.91	9.25	6.58
4	PBW-757	4.00	8.27	6.14
5	DBW-252	4.26	8.92	6.59
6	FLW-2	3.91	7.38	5.65
7	FLW-1	3.45	8.91	6.18
8	DBW-11	3.72	8.01	5.87
9	HD-2851	3.98	6.18	5.08
10	FLW-5	4.09	7.37	5.73

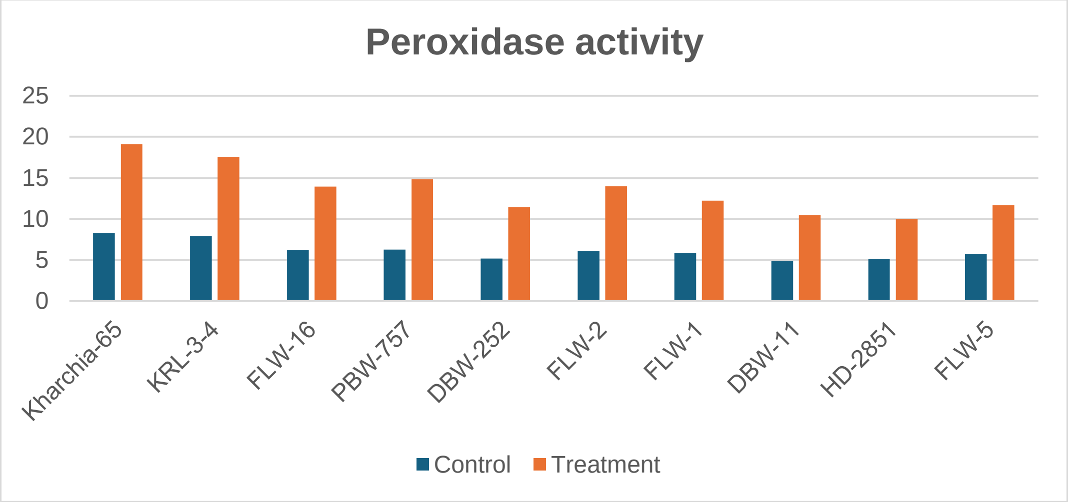


Peroxidase (POX) activity (min/mg/protein) in wheat genotypes.

The data presented in table 4.4 illustrated that data on peroxidase (POX) activity in wheat genotypes under control and salt stress conditions reveals a significant increase in enzyme activity in response to salinity. Similar results also reported by (Ahmed *et al*., 2008).

Table 4.6: Effect of salt stress on peroxidase (POX) activity in wheat genotypes.

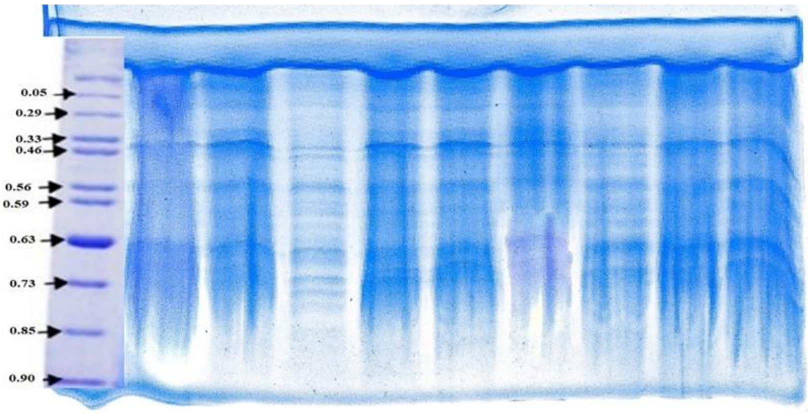
S. No.	Genotypes	Control	Treatment	Mean
1	Kharchia-65	8.31	19.12	13.72
2	KRL-3-4	7.89	17.56	12.73
3	FLW-16	6.23	13.95	10.09
4	PBW-757	6.26	14.81	10.54
5	DBW-252	5.19	11.46	8.33
6	FLW-2	6.07	13.98	10.03
7	FLW-1	5.88	12.24	9.06
8	DBW-11	4.92	10.46	7.69
9	HD-2851	5.13	10.01	7.57
10	FLW-5	5.72	11.68	8.70



SDS-PAGE 10% analysis of leave sample

The banding pattern all 10 wheat genotypes is give below:

kDa 1 2 3 4 5 6 7 8 9 10



The RM value of wheat genotypes are given in the table. The leaves protein of wheat showed variability in banding pattern of polypeptide are 12% acrylamide gel during SDS-PAGE. Relative mobility value of different genotypes of wheat was observed by protein profiling. The SDS-PAGE cluster analysis divided into two major clusters and similarity coefficient ranged from 0.5333 to 1. Paired with the highest similarity coefficient, followed by the check varieties Kharchia-65 to DBW-11 (0.7333), PBW-757 (0.6667), HD-2851(0.6667) & lowest similarity coefficient followed by the check Kharchia-65 to FLW-16(5.333), FLW-2, FLW-1. Similar results also reported by (Grigorova *et al.*, 2011)

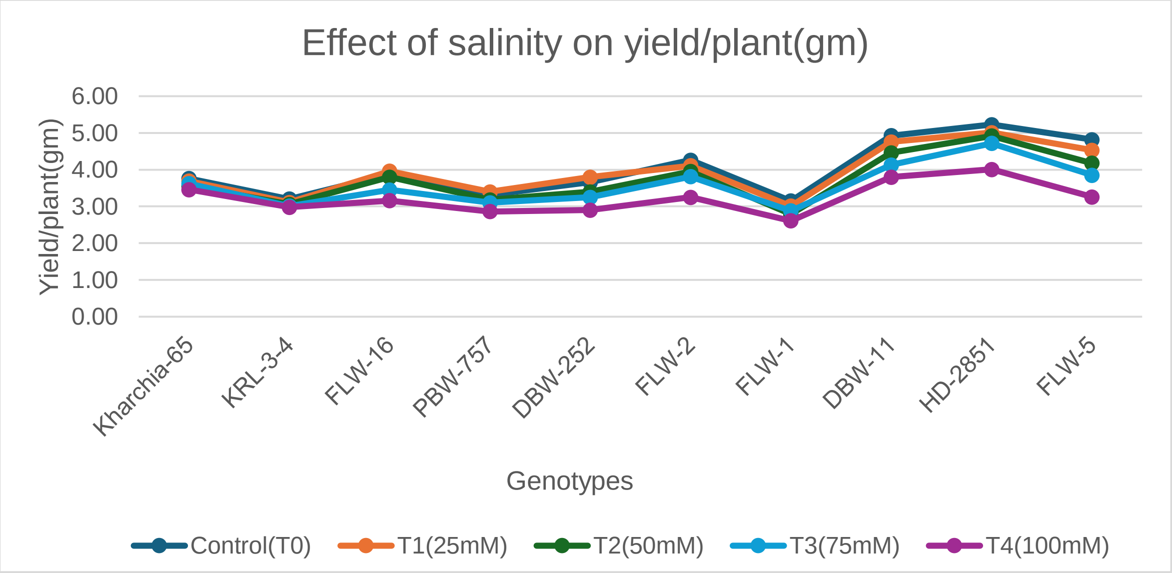
Yield parameters

Effect of salinity on spikelet length (cm)

The effect of salinity on spikelet length (measured in centimeters) across ten different wheat genotypes under experimental conditions. The genotypes investigated include Kharchia-65, KRL-3-4, FLW-16, PBW-757, DBW-252, FLW-2, FLW-1, DBW-11, HD-2851, and FLW-5. As in previous tables, the control column represents spikelet length under normal conditions, while the subsequent columns labeled T1, T2, T3, and T4 indicate increasing levels of salinity treatment at 25mM, 50mM, 75mM, and 100mM respectively. The mean spikelet length across all treatments is also provided for each genotype. Similar results also reported by (Grigorova *et al.*, 2011)

Table 4.7: Effect of salinity on Spikelet length (cm) of wheat genotypes.

S. No.	**Genotypes**	**Control**	**Treatment**				
			T1(25mM)	T2(50mM)	T3(75mM)	T4(100mM)	Mean
1	Kharchia-65	12.00	11.66	10.50	10.55	09.95	10.93
2	KRL-3-4	10.33	9.66	8.23	8.00	7.90	8.82
3	FLW-16	9.33	8.98	8.50	8.45	8.00	8.65
4	PBW-757	10.66	10.33	9.80	9.50	9.10	9.88
5	DBW-252	8.33	7.66	7.40	7.10	7.15	7.53
6	FLW-2	9.33	8.33	8.11	7.98	7.50	8.25
7	FLW-1	9.33	8.75	8.60	8.51	8.10	8.66
8	DBW-11	11.33	10.33	9.26	9.89	9.10	9.98
9	HD-2851	10.33	10.07	9.80	9.65	8.95	9.76
10	FLW-5	9.00	8.89	8.26	8.15	7.80	8.42



3. CONCLUSION

1. There was a genetic variation in **seedling shoot and root length(cm)** under control and salinity stress condition in wheat genotypes. Shoot length of wheat genotypes drastically reduced under FLW-1 & FLW-2 in stress condition. Root length of wheat genotypes drastically reduced under DBW-11 in stress condition. While less in Kharchia-65 & KRL 3-4. Means Kharchia-65 & DBW-252 better perform in stress.
2. There was a genetic variation in **plant height(cm)** under control and salinity stress condition in wheat genotypes. Plant height of wheat genotypes drastically reduced under HD-2851 & FLW-5 in stress condition. High reduction was recorded in HD-2851 while less in Kharchia-65 & KRL 3-4. Means Kharchia-65 & KRL 3-4 better perform in stress.
3. There was a genetic variation in tiller per plant under control and salinity stress condition in wheat genotypes. **Number of tillers plant^{-1}** of chickpea germplasm drastically reduced under salinity stress condition. High reduction was recorded in FLW-16, FLW-2 & FLW-1 while less in Kharchia-65 & KRL 3-4.
4. There was a genetic variation in spikelet length under control and salinity stress condition in wheat genotypes. **Spikelet length(cm)** of wheat genotypes drastically reduced under DBW-252, FLW-5 & FLW-2 in stress condition. High reduction was recorded in DBW-252 while less in Kharchia-65 & KRL 3-4. Means Kharchia-65 & KRL 3-4 better perform in stress.
5. **Catalase activity (min/mg/protein)** abruptly increased in all wheat genotypes after salinity stress. Kharchia showed high percent increment and less increment was noted in HD-2851 & FLW-5.
6. **Peroxidase activity (min/mg/protein)** abruptly increased in all wheat genotypes after salinity stress. Kharchia showed high percent increment and less increment was noted in HD-2851, FLW-5 & DBW-252.
7. **Proline** abruptly increased in all wheat genotypes after salinity stress. Kharchia-65 & KRL 3-4 showed high percent increment and less increment was noted in HD-2851.
8. In **SDS-PAGE** similarity matrix Kharchia-65 found high similarity with DBW-11.

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