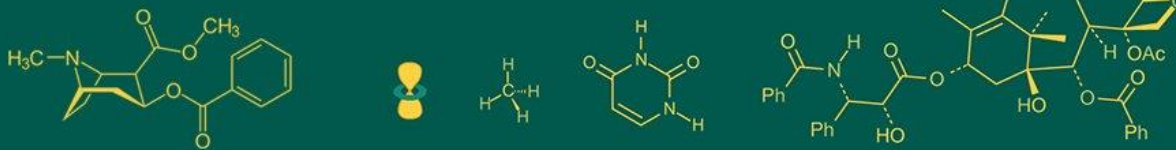

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Effect of salinity stress on biochemical, physiological and morphological parameters in different varieties of wheat (*Triticum aestivum* L)

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Abstract
Salt is the most important abiotic pressure that negatively affects the quality and quantity of crops, of which 20% of irrigated agricultural land is affected by salt. Wheat is the oldest and first crop to be used in the manufacture of bread for human nutrition. To investigate the root and shoot response of wheat to salinity stress, HD3086, PBW226 and DBW 303 wheat varieties were grown under both non-stressful and stressful conditions. Before uprooting the plants MSI and chlorophyll content was recorded using the leaf samples. After that some of the leaves were harvested and are crushed in liquid nitrogen for proline, catalase and peroxidase estimation. After removing the plants from the soil, fresh and dry weight, shoot and root length, was taken.

Keywords: Wheat, salinity stress, abiotic and salt

Introduction
Wheat (*Triticum aestivum* L.) is the most important cereal crop in terms of production and consumption worldwide. Global population mostly depends on wheat to fulfil their nutritional needs and wheat products like chapati, bread, biscuit, pasta and fermented products, are consumed by people all over the world. A balanced food containing enough calories, balanced proteins and micro nutrients with low anti nutritional components is needed for the proper growth and development of human being. In this context, wheat is the most important staple food for humans and is grown on large land area than any other commercial crop. Globally, wheat is the leading source of vegetable protein in human food, having higher protein content than other major cereals like maize or rice (Arzani and Ashraf, 2017) [1]. This crop contributes substantially to the national food security by providing more than 50% of the calories to the people who mainly consume it (Singh, 2010) [10]. Like other agricultural crops, wheat production is also curtailed by various abiotic and biotic factors.

Plants in various ecosystems are constantly exposed to them the pressure of living and non-living things like fungi, weeds, drought and salt contribute development of this restrictive plant environment (Lawlor, 2002) [5]. Incidents of desertification and nosalization the increase is rapid worldwide and is currently affecting more than 10 percent of the cultivated fields lead to decline in crop yields of more than 50% (Wang *et al.,* 2009) [11]. Every year many countries do not produce due to the accumulation of salt. Therefore, to understand the methods of the plant tolerating salt pressure is important (Bartels as well Sunkar, 2005) [2]. Salt is one of the most important ingredients abiotic pressures affect quality as well abundance of plants, so that 20% of the earth can be watered agricultural areas are affected by salt (Zhao *et al.,* 2007) [12]. Salt is usually in excess of dissolving solids salt and mineral compounds are a solution of water and soil led to the accumulation of salt in the rhizome and plant it will not be enough to take water from the soil (Shannon *et al.,* 1994) [9]. Too much salt is caused by the cause of NaCl in at least three problems: 1. External Osmotic pressure of the solution has been beyond the osmotic pressure of the plant cells need to control the osmotic pressure in preventing dehydration by plant cells. 2. Take it again conversion of ions to healthy foods such as potassium and calcium, excess sodium can cause problems. 3. High levels of Na and Cl can cause directing toxic effects on enzymic and membranous systems.

One of the effects of salt pressure reduce photosynthetic a function that caused a decrease b and a chlorophyll and reduce Co acquisition also the power of photosynthetic (Francois and Maas, 1999) [14]. According to studies, Proline is used as an enzymic protector that contributes to the formation of macromolecules and is a major source of energy and nitrogen to deal with salt. Many researchers believe it is proline accumulation in plants involved in salt resistance pressure (Patnaik and Debata, 1997; Thomas *et al.,* 1992) [7, 13]. Different other enzymes like catalase and peroxidase also plays a major role in salt stress tolerance. The abiotic stress also affects the morphology and physiology of the plant so some research is to be done to learn about the harmful effects of salt stress in wheat (*Triticum aestivum* L.) and finding a way to introduce more resistance variety.

Material and Methods

Experimental site. In the present investigation, all the laboratory and field experiments were conducted carefully in a systematic manner under specific conditions at Biochemistry PG laboratory, College of Biotechnology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, India.

Seed Germination Test: Seeds were rinsed for 3 min in 70% ethanol followed by 10 min in 15% hydrogen peroxide solution and finally in distilled water before put into petri plates for imbibition and germination studies. Fifteen seeds of genotypes were put into petri plates for germination in triplicate.

Germination was considered when both radicle and plumule was emerged. Germination was tested in four replicates of 50 seeds each, following between paper method at 20ºC and 80% RH (ISTA, 2011) in the germinator under continuous light condition. The germinated seeds were evaluated and grouped into normal seedlings, abnormal seedlings and dead seeds after eight days. The germination percentage was calculated as the number of normal seedlings out of total seeds kept for germination.

Germination % = (Number of normal seedlings / Total number of seeds) X 100

Membrane Stability Test: MSI was calculated as per Sairam *et al.* (1997) [8]. 100 mg leaf material, in two sets, was taken in test tubes containing 10 ml of double distilled water. One set was heated at 40 0C for 30 min in a water bath, and the electrical conductivity of the solution was recorded on a conductivity bridge (C1). Second set was boiled at 100 0C on a boiling water bath for 10 min, and its conductivity was measured on a conductivity bridge (C2).

MSI was calculated as: $MSI = [1 – (C1/C2)] \times 100$

Chlorophyll Content: The chlorophyll content was measured using SPAD meter with the mean value of three readings for all the three varieties with different treatments.

Proline Content Test: Proline is an amino acid, and its content can be determined using various methods, including colorimetric assays. Here are the basic requirements for a proline content test: Biological samples containing proline (e.g., plant tissues, cell extracts), Extraction buffer (e.g., 3% sulfosalicylic acid), Ninhydrin reagent (commonly prepared by dissolving ninhydrin in ethanol), Standard proline solution for calibration, Spectrophotometer, Centrifuge (for sample preparation), Test tubes or microcentrifuge tube, Pipettes and tips and Vortex mixer.

The soil for experiment was taken is calcareous clay loam in texture and was slightly alkaline in nature. In the normal condition, for the pot experiment conducted at under natural light conditions, the soil put into the pots was salinized at the rates of 50, 100 and 150 mM NaCl. Before sowing the seeds were sterilized with HgCl2 and were grown in petri plates.

The trail was done in four different pots three for salinized and one as control for each variety.

Three loaves of bread seeds DBW 303, PBW 226, HD 3086 were sown in each pot. The plants we harvested eight weeks after sowing. After rating, the new plants are washed for proline resolutions 2.0 g per samples. Proline was extracted from 0.5 g of fresh leaf tissue into 10 ml. of 3% sulfosalicylic acid and filtered through 'Whatman No: 2 filter papers. Proline was determined by the ninhydrine method (Bates *et al.,* 1973) [3] in Shimadzu UV-1201 model spectrophotometer, using pure proline as a standard.

Catalase Activity Test: Catalase is an enzyme that breaks down hydrogen peroxide into water and oxygen. To test catalase activity, you will need: Biological samples with catalase (e.g., tissue homogenates, cell extracts), Hydrogen peroxide (H2O2) solution, Potassium dichromate (K2Cr2O7) solution, Spectrophotometer, Water bath or incubator, Test tubes or cuvettes and Pipettes and tips.

Catalase was extracted from 0.5 g of leaves from control and treated plants were excised. The leaves were washed with distilled water, dried with filter paper and macerated in a chilled pestle and mortar in presence of 3.0 ml of cold extraction buffer (potassium phosphate containing 0.1 mM EDTA and 1% (w/v) PVP and 0.5% triton X-100 was prepared. pH was adjusted to 7.8). The homogenate was centrifuged at 10,000 x g for 15 min at 4 °C. The supernatant was carefully decanted and used as the crude enzyme extract.

The activity of catalase was estimated according to the procedure described by Aebi (1984). The reaction mixture in final volume of 3 ml, contained 0.1 M phosphate buffer (pH 7.0), 10 mM H202 and 50 µL of cell free extract. Reaction was initiated with the addition of H202 and enzyme activity was determined by following the degradation of H202 at 240 nm for 2 min. The enzyme activity was calculated using arbitrary unit (U) where one U corresponds to 0.1U H2O2 catalyzed.

Peroxidase Activity Test: Peroxidases are enzymes that catalyze the decomposition of hydrogen peroxide. To test peroxidase activity, you will need: Biological samples containing peroxidase (e.g., plant extracts, serum), Hydrogen peroxide (H2O2) solution, a suitable chromogenic substrate like guaiacol or o-dianisidine, Spectrophotometer and Water bath or incubator, Test tubes or cuvettes and Pipettes and tips.

The sample preparation and extraction buffer for peroxidase test was same as used in catalase. Reagents: (i) 50 µM riboflavin (ii) 13 mM methionine (iii) 80 µM nitroblue tetrazolium (NBT) (v) 0.1 mM EDTA Procedure: The activity of SOD was assayed by measuring its ability to

inhibit the photochemical reduction of nitroblue tetrazolium according to Beauchamp and Fridovich (1971) [4]. The reaction mixture contained 0, 10, 20, 30, and 40 µL of enzyme extract in separate sets and to these added 0.25 mL of each of methionine, NBT, and EDTA and the total volume of 3.0 ml was made with buffer in each set. Then 0.25 mL of riboflavin was added to each set in the last. The tubes were shaken and placed 30 cm from light source (4 x 40 W fluorescent lamps). The reaction was allowed to run for 20 minutes and then reaction was stopped by switching off the light. The tubes were immediately covered with a black cloth. The absorbance was recorded 560nm.

A non-irradiated reaction mixture, which did not develop, color, served control. However, in the presence of SOD the reaction was inhibited and the amount of inhibition was used to quantify the enzyme. Log A560 was plotted as a function of volume of enzyme extract used reaction mixture. From the resultant graph, volume of enzyme extract corresponding to 50% inhibition of the photochemical reaction was obtained and considered as one enzyme unit (Beauchamp and Fridovich, 1971) [4].

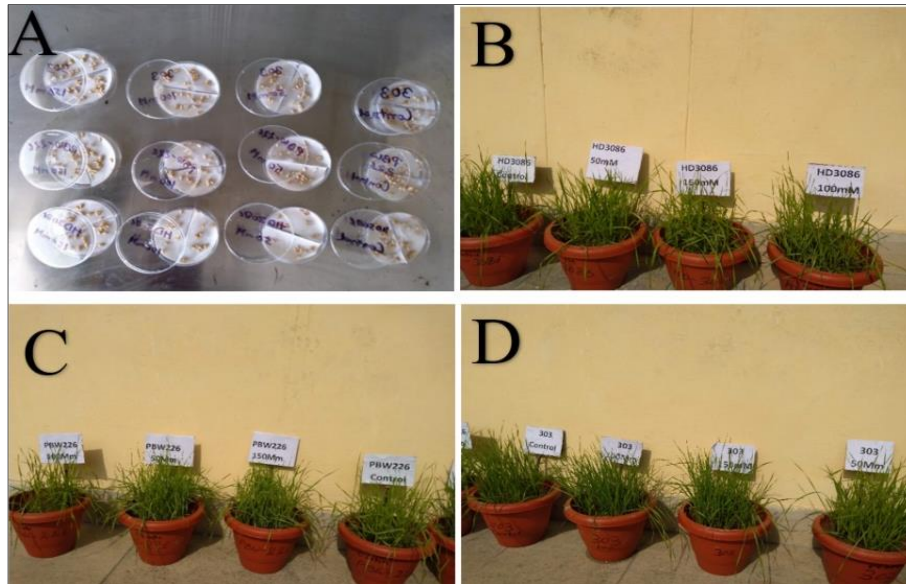

Fig 1: A) Germination of seeds in petriplates B) Pot trail of HD3086 C) Pot trail of PBW 226 D) Pot trail of DBW 303

Result and Discussion

Seed germination percentages for different wheat varieties under varying concentrations of a specific treatment, specifically 50mM, 100mM, and 150mM. Additionally, it provides a mean value for each wheat variety, showcasing the overall performance of each variety in response to the treatment. Firstly, it's evident that all three wheat varieties, namely HD3086, DBW 303, and PBW226, exhibit a decline in seed germination percentages as the concentration of the treatment increases. This suggests that the treatment at higher concentrations has a detrimental effect on seed germination across all varieties. HD3086 has the highest germination percentage under the control conditions, with 99.56%. However, as the treatment concentration increases, its germination percentage gradually decreases to 97.23% at 100mM and further to 93.22% at 150mM. DBW 303 and PBW226 show a similar pattern, with their germination percentages decreasing with increasing treatment concentration. In terms of the mean values, HD3086 still exhibits the highest average germination percentage (97.03%) among the three varieties, even with the decline under higher treatment concentrations. DBW 303 and PBW226 have mean germination percentages of 96.05% and 96.23%, respectively. This suggests that, on average, all three varieties are relatively resilient to the treatment, with PBW226 being the most resilient.

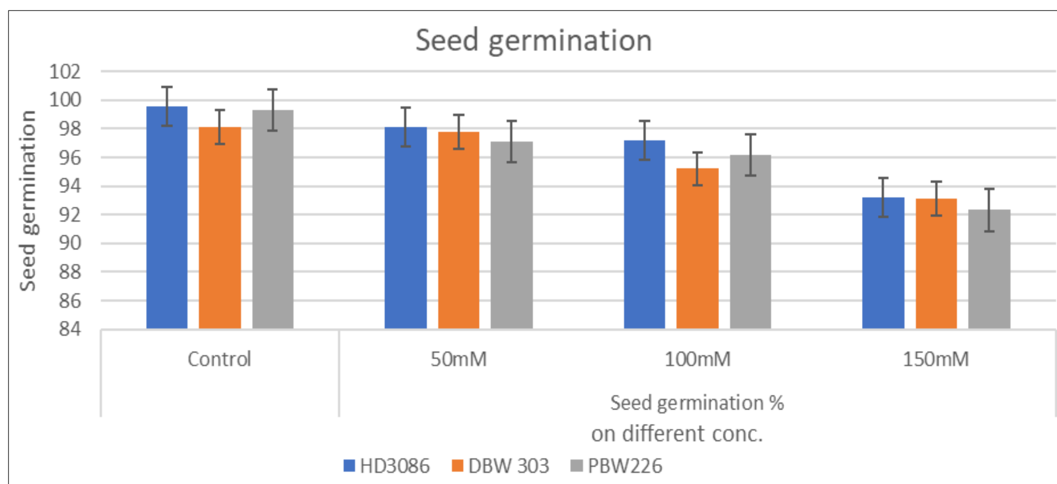

Fig 2: Seed germination percentage

The shoot lengths (measured in centimeters) of three different wheat varieties (HD3086, DBW 303, and PBW226) under varying concentrations of a treatment (Control, 50mM, 100mM, and 150mM). The table also includes additional information, such as the mean shoot length, the highest range, and the lowest range for each wheat variety. Across the board, it is evident that as the concentration of the treatment increases, the shoot lengths of all three wheat varieties tend to decrease. This suggests that the treatment negatively impacts the growth of these wheat varieties. For instance, in the case of HD3086, the control group exhibits the highest shoot length at 34.73 cm, but this length decreases to 33.61 cm at 50mM, 32.92 cm at 100mM, and 31.06 cm at 150mM. A similar trend is observed for DBW 303 and PBW226. The mean shoot lengths for the three varieties also follow this pattern, with the control group having the highest mean shoot length and the 150mM treatment group having the lowest. HD3086 has a mean shoot length of 33.08 cm, DBW 303 has a mean of 31.89 cm, and PBW226 has a mean of 31.85 cm.

Furthermore, the information on the range of shoot lengths within each variety. The highest range represents the difference between the longest and shortest shoot lengths observed within a variety, while the lowest range represents the difference between the second longest and shortest shoot lengths. This data highlights the variability in shoot length within each wheat variety under the different treatment concentrations.

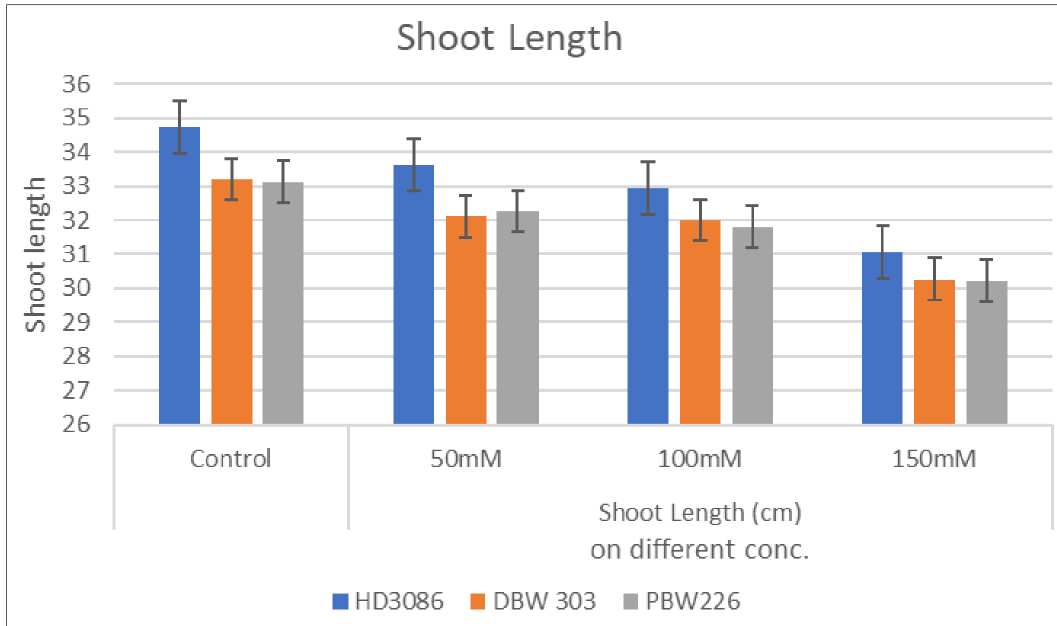

Fig 3: Shoot Length

The root lengths (measured in an unspecified unit) for three different wheat varieties (HD3086, DBW 303, and PBW226) under varying concentrations of a treatment (Control, 50mM, 100mM, and 150mM). Additionally, the table includes information on the mean root length, the highest range, and the lowest range for each wheat variety. Upon analyzing the data, it is apparent that the root lengths of the three wheat varieties show different responses to the treatment concentrations. For HD3086, root length decreases as the treatment concentration increases, starting from 11.73 units in the control group and declining to 10.03 units at 50mM, 10.18 units at 100mM, and finally reaching 9.76 units at 150mM. In contrast, DBW 303 maintains a relatively stable root length across all treatment concentrations, with only a slight decrease observed at 150mM. PBW226 exhibits an increase in root length from 11.73 units in the control group to a peak of 12.65 units at 50mM, followed by a decrease at higher treatment concentrations. The mean root lengths for the three varieties reveal further insights. HD3086 has the lowest mean root length at 10.425 units, indicating that, on average, its roots are shorter compared to the other varieties. DBW 303 has a slightly higher mean root length at 11.2225 units, while PBW226 exhibits the highest mean root length at 11.85 units, signifying longer average root growth in response to the treatment. The highest range represents the difference between the longest and shortest root lengths observed within a variety, while the lowest range represents the difference between the second longest and shortest root lengths. These ranges highlight the variability in root length within each wheat variety under different treatment concentrations.

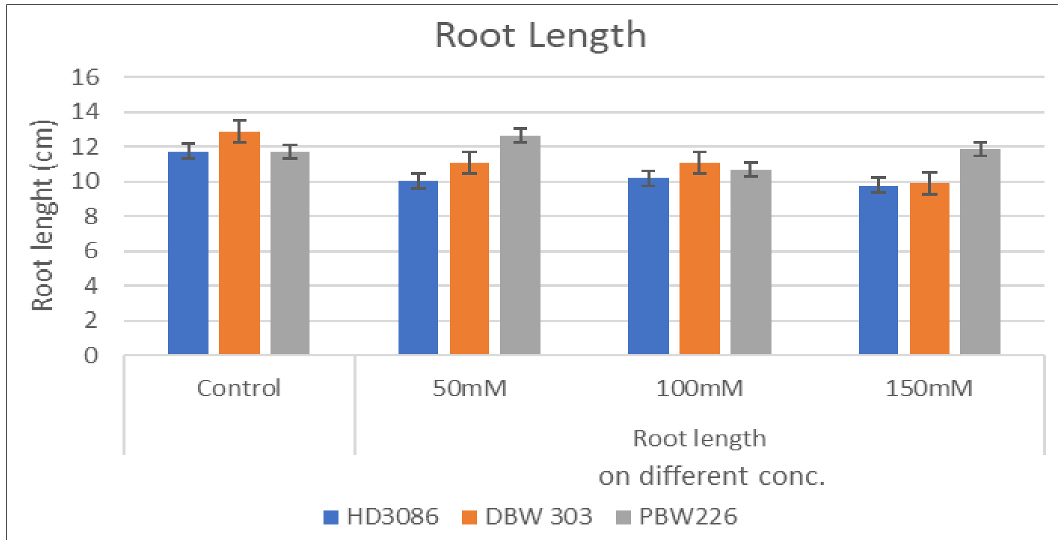

Fig 4: Root Length

The MSI (Meristem Size Index) percentages for three different wheat varieties (HD3086, DBW 303, and PBW226) under varying concentrations of a treatment (Control, 50mM, 100mM, and 150mM). Additionally, the table includes information on the mean MSI percentage, the highest range, and the lowest range for each wheat variety. Upon analyzing the data, it is evident that the MSI percentages for all three wheat varieties exhibit a declining trend as the treatment concentration increases. This indicates that the treatment negatively affects the meristem size, with higher concentrations having a more pronounced impact. For instance, in the case of HD3086, the control group has the highest MSI percentage at 80.11%, but this percentage decreases to 75.22% at 50mM, 68.11% at 100mM, and significantly drops to 60.11% at 150mM. A similar trend is observed for DBW 303 and PBW226. The mean MSI percentages for the three varieties follow the same pattern, with the control group having the highest mean MSI percentage and the 150mM treatment group having the lowest. HD3086 has a mean MSI percentage of 70.8875%, DBW 303 has a mean of 71.53%, and PBW226 has a mean of 71.5675%. This suggests that, on average, all three varieties experience a reduction in meristem size due to the treatment, with PBW226 having the highest mean MSI percentage.

Furthermore, the information on the range of MSI percentages within each variety. The highest range represents the difference between the highest and lowest MSI percentages observed within a variety, while the lowest range represents the difference between the second-highest and lowest MSI percentages. These ranges highlight the variability in meristem size within each wheat variety under different treatment concentrations.

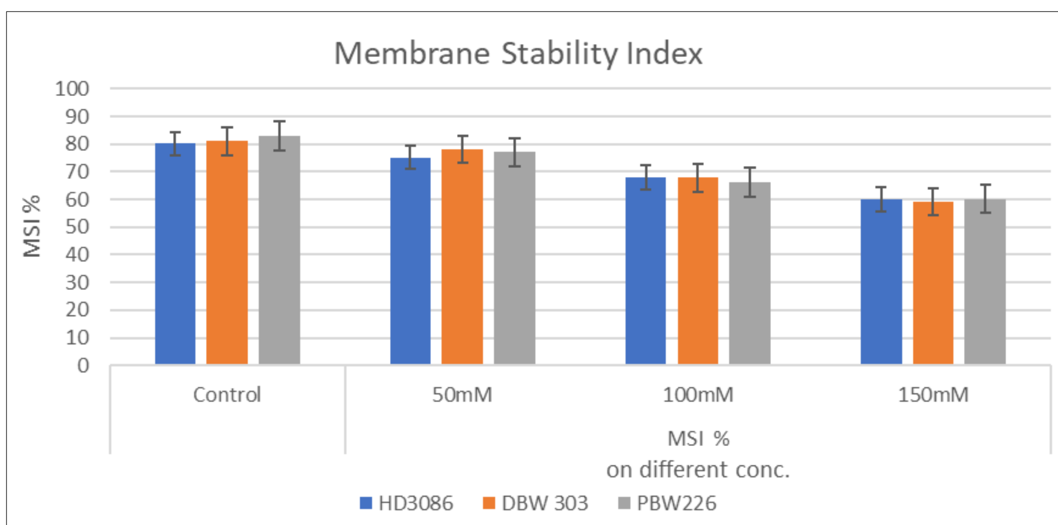

Fig 5: Membrane Stability Index

The chlorophyll content in three different wheat varieties, namely HD3086, DBW 303, and PBW226, under four different treatments: Control, 50mM, 100mM, and 150mM. The chlorophyll content is measured in arbitrary units (AU). Across all three wheat varieties, there is a clear trend of decreasing chlorophyll content as the concentration of the treatment solution increases from Control to 150mM. HD3086 has the highest chlorophyll content in the Control treatment, measuring 41.6 AU, followed by DBW 303 at 42.4 AU, and PBW226 at 44.6 AU. However, as the treatment concentration increases, the chlorophyll content decreases substantially in all three varieties. In terms of the treatment with the highest chlorophyll content mean, PBW226 exhibits the highest value at 41.025 AU in the

100mM treatment. In contrast, HD3086 shows the lowest mean chlorophyll content across all treatments, with a value of 28.6 AU in the 150mM treatment. The range of chlorophyll content within each wheat variety provides insight into the variability of the data. For HD3086, the range spans from 28.6 AU (in the 150mM treatment) to 41.6 AU (in the Control treatment), indicating a substantial variation. DBW 303 also shows considerable variation, with a range from 38.6 AU (in the 150mM treatment) to 42.4 AU (in the Control treatment). In contrast, PBW226 displays a narrower range, varying from 39.5 AU (in the 150mM treatment) to 44.6 AU (in the Control treatment).

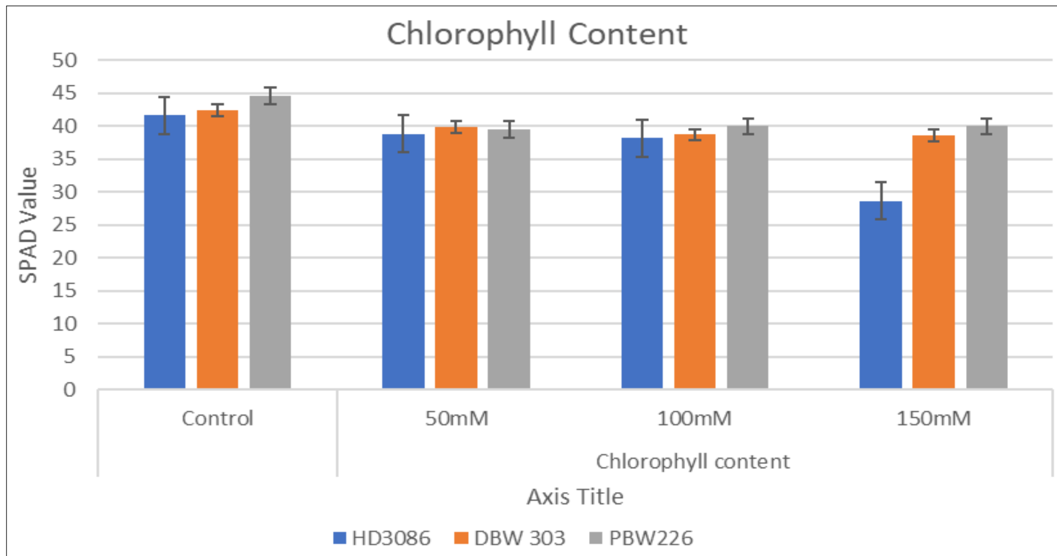

Fig 6: Chlorophyll Content

The proline content (measured in µmol/g DW, which stands for micromoles per gram of dry weight) for three different wheat varieties (HD3086, DBW 303, and PBW226) under varying concentrations of a treatment (Control, 50mM, 100mM, and 150mM). It also includes information on the mean proline content, the highest range, and the lowest range for each wheat variety.

Upon analyzing the data, it is evident that the proline content in the wheat varieties responds differently to the treatment concentrations. In general, the proline content tends to increase as the treatment concentration increases. For instance, in the case of HD3086, the control group has the lowest proline content at 6.02 µmol/g DW, but this content increases to 7.66 µmol/g DW at 50mM, 8.12 µmol/g DW at 100mM, and significantly rises to 10 µmol/g DW at 150mM. A similar trend is observed for DBW 303 and PBW226.

The mean proline content for the three varieties also follows this pattern, with the control group having the lowest mean proline content, and the 150mM treatment group having the highest. HD3086 has a mean proline content of 7.95 µmol/g DW, DBW 303 has a mean of 7.4575 µmol/g DW, and PBW226 has a mean of 7.39 µmol/g DW. This suggests that, on average, all three varieties experience an increase in proline content due to the treatment, with HD3086 having the highest mean proline content.

Additionally, the table provides information on the range of proline content within each variety. The highest range represents the difference between the highest and lowest proline content observed within a variety, while the lowest range represents the difference between the second-highest and lowest proline content. These ranges indicate the variability in proline content within each wheat variety under different treatment concentrations.

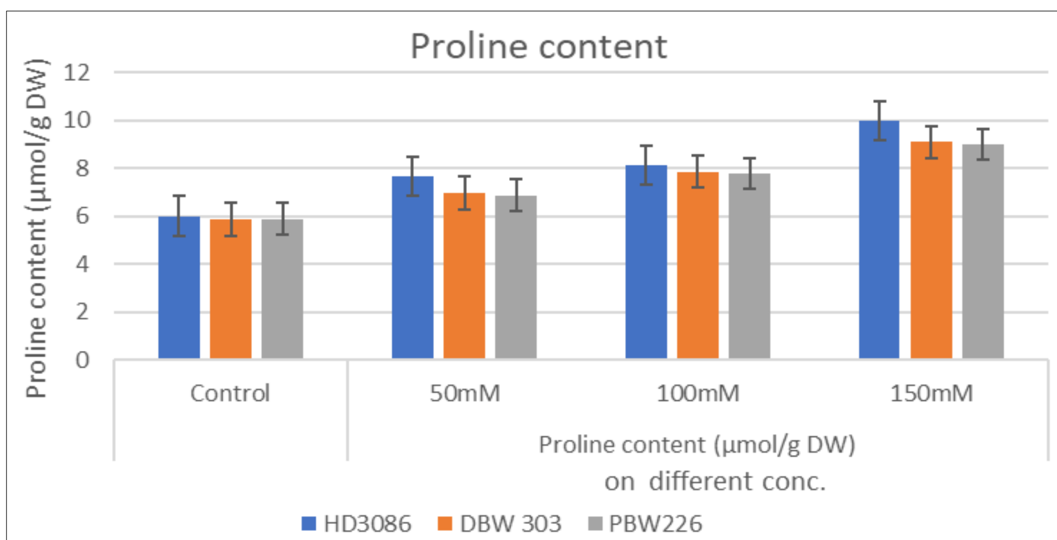

Fig 7: Proline Content

The catalase activity for three different wheat varieties (HD3086, DBW 303, and PBW226) under varying concentrations of a treatment (Control, 50mM, 100mM, and 150mM). It also includes information on the mean catalase activity, the highest range, and the lowest range for each wheat variety.

Upon analysis, it is noticeable that the catalase activity levels vary across the three wheat varieties and are influenced by the treatment concentrations. In general, the catalase activity tends to increase with higher treatment concentrations. For example, in the case of HD3086, the control group exhibits the lowest catalase activity at 5.8, but this activity increases to 6.9 at 100mM and further to 8.5 at 150mM. A similar trend is observed for DBW 303 and PBW226, where catalase activity shows an incremental rise as treatment concentration increases.

The mean catalase activity for the three varieties also reflects this pattern, with the control group having the lowest mean catalase activity, and the 150mM treatment group having the highest. HD3086 has a mean catalase activity of 6.8, DBW 303 has a mean of 6.775, and PBW226 has a mean of 6.525. This suggests that, on average, all three varieties experience an increase in catalase activity in response to the treatment, with HD3086 having the highest mean catalase activity.

Furthermore, the table provides information on the range of catalase activity within each variety. The highest range represents the difference between the highest and lowest catalase activity observed within a variety, while the lowest range represents the difference between the second-highest and lowest catalase activity. These ranges highlight the variability in catalase activity within each wheat variety under different treatment concentrations.

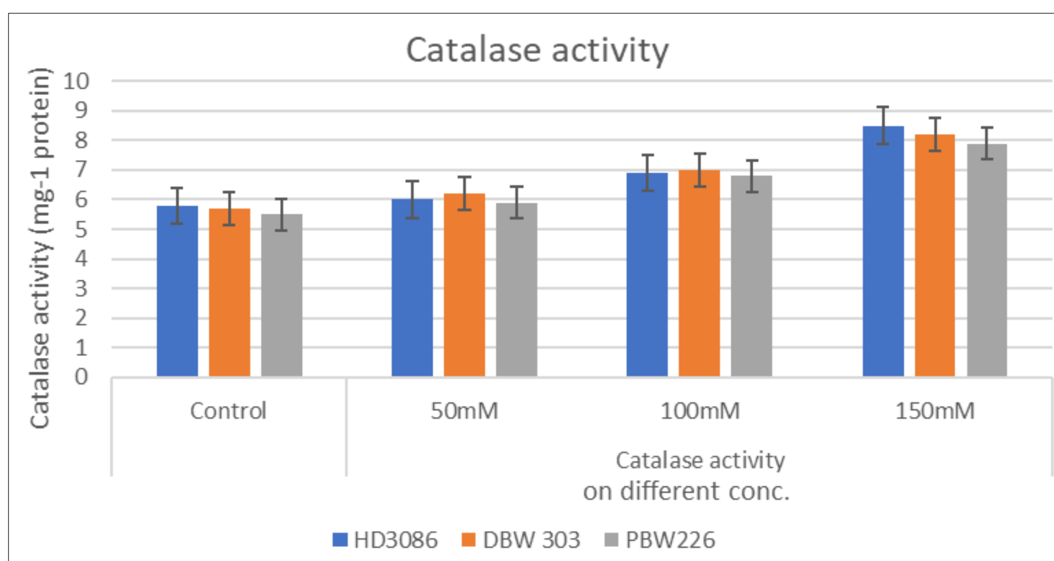

Fig 8: Catalase Activity

The peroxidase activity for three different wheat varieties (HD3086, DBW 303, and PBW226) under varying concentrations of a treatment (Control, 50mM, 100mM, and 150mM). It also includes information on the mean peroxidase activity, the highest range, and the lowest range for each wheat variety.

Upon analysis, it is clear that peroxidase activity levels differ across the three wheat varieties and are influenced by the treatment concentrations. In general, peroxidase activity tends to increase as the treatment concentration increases. For example, in the case of HD3086, the control group exhibits the lowest peroxidase activity at 8.8, but this activity increases to 11 at 100mM and further to 15.4 at 150mM. A similar trend is observed for DBW 303 and PBW226, where peroxidase activity shows a steady rise as the treatment concentration increases. The mean peroxidase activity for the three varieties also follows this pattern, with the control group having the lowest mean peroxidase activity, and the 150mM treatment group having the highest. HD3086 has a mean peroxidase activity of 11.225, DBW 303 has a mean of 11.2, and PBW226 has a mean of 11.4. This suggests that, on average, all three varieties experience an increase in peroxidase activity in response to the treatment, with PBW226 having the highest mean peroxidase activity.

Furthermore, the table provides information on the range of peroxidase activity within each variety. The highest range represents the difference between the highest and lowest peroxidase activity observed within a variety, while the lowest range represents the difference between the second-highest and lowest peroxidase activity. These ranges highlight the variability in peroxidase activity within each wheat variety under different treatment concentrations.

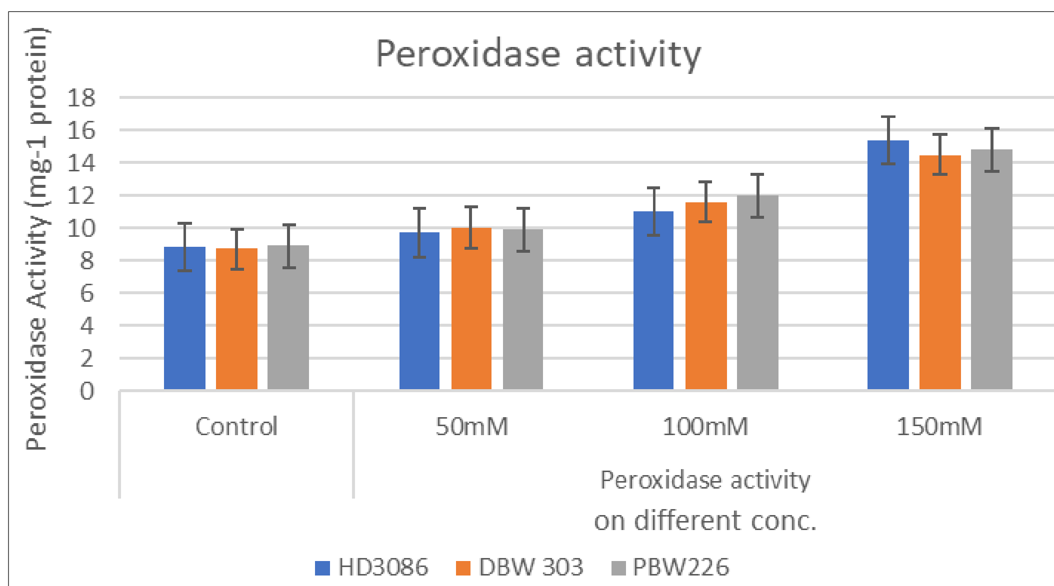

Fig 9: Peroxidase Activity

Conclusion

In conclusion, the data presented in the table provides a multifaceted view of how different wheat varieties, including HD3086, DBW 303, and PBW226, respond to varying treatment concentrations across numerous parameters. These parameters encompassed seed germination, shoot and root length, meristem size, chlorophyll content, and biochemical responses such as proline content, catalase activity, and peroxidase activity. Across the board, the wheat varieties displayed a decreasing trend in seed germination as treatment concentrations increased, indicating the adverse effects of the treatment. However, it's noteworthy that all three varieties maintained relatively high germination percentages, suggesting their resilience to some extent. Chlorophyll content, a crucial indicator of plant health and photosynthetic capacity, exhibited a consistent decline in response to increasing treatment concentrations. This decline in chlorophyll content aligns with the general pattern of reduced chlorophyll levels under stress conditions, reflecting the adverse impact of the treatment on the photosynthetic machinery of these wheat varieties.

Regarding shoot and root length, the results varied among the wheat varieties. Shoot length consistently decreased with higher treatment concentrations, with the control group consistently showing the longest shoots. Root length responses were more nuanced, with HD3086 experiencing a consistent decrease, DBW 303 maintaining stability, and PBW226 initially increasing before declining. These variations underscore the diverse responses of these wheat varieties to the treatment, reflecting the complexity of their physiological adaptations to stress. Meristem size, an essential determinant of plant growth and development, uniformly decreased across all varieties with increasing treatment concentrations. This reduction in meristem size indicates a potential constraint on the future growth and development of these wheat plants under stress conditions. Furthermore, the observed increases in proline content, catalase activity, and peroxidase activity with higher treatment concentrations suggest that these wheat varieties are activating biochemical defense mechanisms to cope with the stress. These responses highlight the capacity of these varieties to adjust their metabolic processes in the face of environmental challenges.

In summary, this comprehensive analysis of multiple parameters underscores the intricate interplay of physiological, biochemical, and growth-related responses exhibited by these wheat varieties when subjected to increasing treatment concentrations. The findings emphasize the need for a holistic approach to understanding plant responses to stressors, as different parameters provide valuable insights into their resilience and adaptability. This knowledge is crucial for informing agricultural practices and advancing research aimed at enhancing crop stress tolerance and management strategies in the ever-changing agricultural landscape.

Future Scope: The study's future directions hold promise for agriculture and crop management. They include genetic and molecular research to develop stress-tolerant wheat varieties, precision farming to optimize yield while mitigating stress impacts, and targeted interventions based on biochemical responses. Understanding performance under diverse environmental conditions and integrating findings into holistic crop management strategies is crucial. Predictive models and biotechnology offer tools for proactive decision-making and the creation of resilient wheat varieties, potentially revolutionizing global food security and sustainability. In summary, these prospects aim to enhance crop resilience and productivity in the face of evolving challenges.

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