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Evaluation of Wheat under Salt and Draught Stress using Physiological and Morphological Parameters

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Abstract

Salinity and draught is a major threat to agricultural productivity worldwide and presents a tremendous challenge for food security. Plant physio-morphological traits are very important for selection in a plant breeding program to improve drought tolerance due to their relation to the adaption for future climate scenarios. The results revealed that there were significant variations with all morphological and physiological traits influenced by under salt and drought conditions. The investigation studied morphological traits (seed germination, shoot length, number of leaves) and physiological trait (chlorophyll content, relative water content) were decreased under salt and drought conditions i.e. NIAW-917, NIAW-295, MACS-6222 compared with control except NIAW-301 and NIAW-3170 at seedling stage. The relative water content shows in the genotypes exhibited highest in NIAW-3170 (78.03%), it indicate that water status in the wheat genotype hence, it reflects the balance between water supply to the leaf tissue and transpiration rate but it will reflect in NIAW-917 (71.25%) was lowest due to effect of NaCl and PEG. The chlorophyll content the highest amount in NIAW-301 (1.02) hence it indicate that the quantity of chlorophyll per unite area is an indicator of photosynthetic capacity of a wheat genotype. Amount of chlorophyll in leaf tissue is influenced by nutrient avability and environmental stresses, the lowest amount found in NIAW-3170 (0.74) under stress conditions due to Chlorophyll 'b' content is drastically reduced. It is confirmed that under salinity and drought stress the genotypes significantly performed NIAW-301 (89.75%). The mechanism associated with the tolerance to the salinity and drought stress the systems that regulates adaptation of plant to abiotic stress in wheat.

Key words : Salt, Draught stress, Physiological, Morphological parameter and Wheat.

Wheat is one of the important staple food crops supplying 20% of calories globally. Currently, two major wheat species, hexaploid bread wheat (Triticum aestivum; 2n = 6x = 42) and tetraploid durum wheat (Triticum durum; 2n = 4x = 28), are commercially important. Food and Agriculture Organization (FAO) of the United Nations has estimated 739.9 million tons of wheat production in 2017. Global wheat yields have increased at a mere 1.0% per year in the past two decades. Wheat crop is sensitive to heat, salinity and drought stresses mainly at the flowering and grain development stages, which negatively impact the yield and grain quality (lower 1,000 grain weight and change in protein quality). Annual production variability estimated at ~40% was mainly due to heat waves and drought situations in major wheat producing belts throughout the world. Demand for wheat is estimated to increase by 60% by 2050, but production might go down by 29% as a result of climate change imposed environmental stresses. These predictions indicate that improving abiotic stress tolerance in wheat is paramount for global food security in the near future. Continued wheat genetic improvement is thus critically important as it has direct impact on economic development, food security, and international grain trade (Manoj *et al*., 2017).

Drought stress can occur at any growth stage and depends on the local environment. Therefore, genotypes may be tested for their drought tolerance at relevant and often different growth stages because some genotypes may tolerate drought at germination or seedling stage, but these may be very sensitive to drought at the flowering stage or vice versa. Drought tolerance is determined by identifying a trait that can be used to measure the effect of drought stress on plants. This trait should discriminate tolerant and susceptible genotypes. Hence, it is very important in any drought experiment to determine the appropriate traits that are drought-tolerant traits. Furthermore, drought tolerance and yield should be improved in parallel because farmers need to profitably produce their agricultural products under drought stress.

When plants are exposed to drought stress, they physiologically change to tolerate this stress. Physiologically, drought needs a context-dependent view to understand the ability of plants to make important changes that alleviate the effect of drought stress. Drought-tolerant plants try to have less reduction in water content, membrane stability, and photosynthetic activity. The tolerant group tries to accumulate soluble sugars, proline content, amino acids, chlorophyll content and enzymatic and non-enzymatic antioxidant activities. Plant physio-morphological traits are very important for selection in a breeding program to improve drought tolerance due to their relation to the adaption for future climate scenarios. Moreover, identifying the genes controlling these physiological changes may lead to rapid genetic improvement for drought tolerance in a plant (Ahmed *et al*., 2019).

Salinity is a major threat to agricultural productivity worldwide and presents a tremendous challenge for food security. More than one billion hectares of land, accounting for approximately 25% of the global land area, is affected by salinity. Due to natural salinization or unsuitable irrigation practices, this area is increasing by up to 10 million hectares of land every year. Bread wheat (*Triticum aestivum* L. ssp. *aestivum*) is one of the most important crops contributing about 20% of the total dietary calories and proteins worldwide (Said *et al*., 2019).

Determination of physiological traits related to stress tolerance could be used as a selection criterion to enhance wheat adaptation to stress conditions. According to the previous investigations, there is a link between different physiological responses of crops to stress and their tolerance mechanisms, such as high relative water content and water potential. Therefore, it is crucial to understand the effects of salt stress on wheat yield improvement while maintaining superior productivity and adopting mitigation strategies toward the long-term goal of sustainable food security. This study aimed to synthesize salinity effects on wheat germination, seedling growth, no.of leaves, chlorophyll content & relative water content. Moreover, integrated approach for salinity mitigation through osmo protectants, plant hormones (Ayman *et al*., 2021). The present investigation focused on Evaluated of wheat under salt and drought stress by using Physiological, Morphological parameters, additionally, deleterious effects of salinity in relations to nutrient imbalance and water relations have been objectively described.

Material and Methods

Experimental material and experimental site : The genotypes were collected from wheat research station, Kundewadi, Tal-Niphad, Dist- Nashik. Experiment planned to be conduct in the department of Plant Breeding and Molecular Genetics in MGMU IBT, Aurangabad during 2022-23. The Completely Randomized Design (CRD) was used for experimental work.

Plant material: The following wheat varieties were selected for the research work:

NIAW-301, NIAW-3170, MACS-6222, NIDW-295 and NIAW-917.

Table 1. Treatments and concentrations

Treatments (T)	NaCl (mM)	PEG-4000 (%)
T_0	Control	Control
T_1	75	10
T_2	150	15
T_3	225	20

NACL Treatment: Seeds of each variety were soaked under NACL solution for overnight. The concentrations are 75mM, 150mM and 225mM. Then seeds were sowed into trays after soaking period. Then NACL stress was given through irrigation after germination with the period of three days interval.

PEG-4000 Treatment: Seeds of each variety were soaked under PEG-4000 solution for overnight. The concentrations are 10%, 15% and 20%. Then seeds were sowed into trays after soaking period. Then PEG-4000 stress was given through irrigation after germination with the period of three days interval.

Morphological studies of the experimental plant: In the morphological study, following parameters are checked in the plants for 30 days old plantlets. The morphological observations are given as below:

Shoot Length (cm): The new growth from seed germination that grows upward is a shoot where leaves will develop. The shoot length was measured in the 30th days after the seedling and it is expressed in the centimeters (cm).

Number of leaves: The total number of leaves grown at a particular stage of that plant and it is calculated after the 30th days of the seedling (DAG).

Germination percentage: Seed germination was taken after the seven days from sowing date and expressed as percentage according to the following formula :

Germination Percentage (GP) :

$$GP = \frac{\text{No. of total Germinated seeds}}{\text{Total no. of Seeds Tested}} \times 100$$

Physiological Studies of Experimental Plant: Estimation of chlorophyll content and Relative water content (RWC) are two major factors responsible for physiological changes during the drought and salinity stress. Estimation of chlorophyll content was carried out by using spectrophotometer (Arnon method). Relative Water Content was calculated by taking fresh weight, dry weight and turgid weight of leaves of different concentration of NaCl and PEG.

Estimation of Chlorophyll Content From Leaves: Required Material- 80% Acetone, Distilled Water, Conical Flasks, Test Tubes, Measuring Cylinder, Filter Paper, Funnel, Spectrophotometer and Cuvettes.

Methodology (Arnon Method):

- 0.5 g of wheat leaf sample were ground with 6.5 ml of 80% acetone in mortar and pestle.
- Extract was filtered with the help of filter paper in separate tubes using funnel.
- The filtered solution then transferred into fresh tubes and each tube is labelled with different concentrations of PEG and NaCl.
- The tubes were covered with aluminium foil to avoid the degradation of chlorophyll pigments.
- In Spectrophotometer, OD is measured at 663nm, 645nm and 652nm with 80% acetone as blank sample.

- Each sample is measured at 663nm, 645nm and 652nm for chl. a, chl. b and chl. ab respectively.
- The observations were taken (E. Manolopoulou *et al*., 2016).

Relative Water Content Test Methodology :

- 1 g of fresh leaves sample of each treatment having different concentrations were taken.
- For turgid weight samples were soaked in distilled water for 24hrs.
- For dry weight samples were kept in Hot Air Over at 50℃ for 24hrs.
- After 24hrs, dry and turgid samples weighted and observations were taken.

Relative Water Content was calculated by using the formula-

RWC% = (Fresh Wt. – Dry Wt./ Turgid Wt. – Dry Wt.) x 100

Analysis of data: The experiment will be calculated under completely randomized design (CRD). The data obtained on various observations will be analyzed by statistical method.

Result

The observation and result obtained from the present study are described below. Efforts made to justify objectives of study, has showed significant outcome from the morphological analysis of Wheat genotypes under stress and initiation of the genotyping.

Morphological Data Analysis by One Factor Analysis : The product and process comparisons contains a more extensive discussion of one factor ANOVA, including the detail for the mathematical computations of one way analysis of variance.

Germination Percentage of Wheat Seeds: There were five wheat varieties with different treatments of NaCl with different concentrations (75mM, 150mM, 225mM) and PEG-4000 with different concentrations (10%, 15%, 20%) are germinated for the study of germination percentage are NIAW-301, NIAW-3170, MACS-6222, NIDW-295, NIAW- 917. Germination percentage was obtained from the formula and its outcome is mentioned in table 2. The highest seed germination was recorded in NaCl treatment NIAW-301 variety T3 treatment shows 80% germination and in PEG-4000 treatment same variety T3 treatment shows 77% which is susceptible to the Salinity and drought stress.

Morphological characterization of varieties: In this study different morphological parameters were analyzed among the five varieties. Observations were taken after 30 days

Table 2. Germination Percentage

Varieties	Treatments													
	NaCl				Mean	SE	CD	PEG-4000				Mean	SE	CD
	T_0	T_1	T_2	T_3				T_0	T_1	T_2	T_3			
NIAW-3170	95	90	85	50	80	1.14	1.71	93	86	79	71	82.25	1.21	1.88
NIAW-917	95	90	90	40	78.75	1.27	1.88	95	88	82	76	85.25	1.17	1.88
MACS-6222	94	92	73	62	80.25	1.24	1.88	91	72	70	52	71.25	1.40	1.88
NIAW-295	96	90	85	50	80.25	1.24	1.88	93	86	79	71	82.25	1.21	1.88
NIAW-301	96	93	90	80	89.75	1.11	1.88	96	88	82	77	85.75	1.17	1.88

Table 3. Shoot length

Varieties	Treatments													
	NaCl				Mean	SE	CD	PEG-4000				Mean	SE	CD
	T_0	T_1	T_2	T_3				T_0	T_1	T_2	T_3			
NIAW-3170	34	30.4	29.9	20.4	28.67	1.77	0.95	32	29.1	29	19.5	27.4	2.59	1.33
NIAW-917	34.8	32.3	27.7	17.5	28.07	0.35	0.18	34	29.3	19.5	18.4	25.3	2.00	0.95
MACS-6222	31.5	25	27	19.4	25.72	2.76	1.33	30	25	25	19	24.75	4.04	1.88
NIAW-295	35.5	29.9	26.1	25	29.12	1.74	0.95	33	28	27.5	18.7	26.8	2.65	1.33
NIAW-301	35.5	32.1	30.1	25.4	30.77	0.32	0.18	35.5	27	27	25	28.62	3.03	1.63

after germination from each treatment (Nacl, PEG-4000).

Shoot Length (30 DAS) : In Salinity stress variation was observed in the shoot length of all wheat varieties were treated with NaCl solution. Observations are given in the table no 3. The highest shoot length was recorded in NaCl treatment NIAW-301 variety T3 treatment shows 25.4 cm which is susceptible to the Salinity stress.

In Drought stress variation was observed in the shoot length of all wheat varieties were treated with PEG-4000 solution. Observations are given in the table. The highest shoot length was recorded in PEG-4000 treatment NIAW-301 variety T_3 treatment shows 25 cm which is susceptible to the drought stress.

Number of Leaves (Day After Germination 30 days) : The total number of leaves were observed and noted after 30 days after germination are given in Table 4. The highest number of leaves was recorded in NaCl treatment NIAW-301 variety T_3 treatment shows 5 leaves germination and in PEG-4000 treatment same variety T_3 treatment shows 5 leaves which is susceptible to the Salinity and drought stress.

Physiological characterization of varieties : The chlorophyll content and relative water content (RWC) are the two major parameters considered during physiological characterization.

Chlorophyll content : The total 'chlorophyll ab' was calculated by using formula observation was noted and show in Table 5. The highest chlorophyll was recorded in NaCl treatment NIAW-301 variety T_3 treatment shows 1.01 and in PEG-4000 treatment same variety T_3 treatment shows 1.03 which is susceptible to the Salinity and drought stress.

Relative water content: The relative water content (RWC) was calculated by using formula and observation were noted and show in Table 6. The highest relative water contain was recorded in NaCl treatment NIAW-3170 variety T_3 treatment shows 71.45% and in PEG-4000 treatment same variety T_3 treatment shows 77.3% which is susceptible to the Salinity and drought stress.

Table 4. Number of leaves

Varieties	Treatments							
	NaCl				PEG-4000			
	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3
NIAW-3170	5	5	5	5	5	5	5	5
NIAW-917	5	5	5	5	5	5	4	5
MACS-6222	5	5	5	5	5	5	4	5
NIAW-295	5	5	5	5	5	5	4	4
NIAW-301	5	5	5	5	5	5	5	5
Mean	5	5	5	5	5	5	4.4	4.8

Table 5. Chlorophyll content

Varieties	Treatments													
	NaCl				Mean	SE	CD	PEG-4000				Mean	SE	CD
	T_0	T_1	T_2	T_3				T_0	T_1	T_2	T_3			
NIAW-3170	0.92	1.01	0.16	0.9	0.74	32.43	0.41	1.02	0.97	0.97	0.97	0.98	1.01	0.01
NIAW-917	0.99	1.02	0.98	0.99	0.99	1.00	0.01	0.99	1.11	1.02	0.92	1.01	0.99	0.01
MACS-6222	1.01	0.99	0.97	0.98	0.98	1.01	0.01	1.01	1.01	0.99	0.91	0.98	1.02	0.01
NIAW-295	1.01	0.91	0.97	1.01	0.97	1.02	0.01	1.01	1.02	0.97	0.97	0.99	1.00	0.01
NIAW-301	1.02	0.99	1.06	1.01	1.02	0.98	0.01	1.06	1.02	1.01	1.03	1.03	0.97	0.01

Table 6. Relative water content

Varieties	Treatments													
	NaCl				Mean	SE	CD	PEG-4000				Mean	SE	CD
	T_0	T_1	T_2	T_3				T_0	T_1	T_2	T_3			
NIAW-3170	85.5	80.4	74.6	71.4	78.03	0.01	0.01	89.9	74.2	64.4	77.3	76.47	0.09	0.13
NIAW-917	81.6	79.0	74.2	50.0	71.25	0.01	0.01	80.7	80.4	79.0	68.7	77.22	0.01	0.01
MACS-6222	79.3	79.2	73.2	55.3	71.79	0.01	0.01	80.5	79.3	79.2	73.7	78.23	0.01	0.01
NIAW-295	83.3	78.5	68.8	66.6	74.36	0.01	0.01	74.6	83.3	80.8	64.4	75.82	0.01	0.01
NIAW-301	82.8	73.6	70.6	69.8	74.21	0.09	0.13	73.8	70.9	70.8	70.6	71.58	0.07	0.09

Discussion

The seeds were arranged in square plate, filter papers were kept in plate. Hundred seeds of each variety and each treatment were placed in a plate and the result were recorded. MACS-6222 variety shows less germination percentage while NIAW-3170 shows higher germination in control. NIAW-9175 and NIDW-295 shows the very less germination in 225mM concentration of NaCl. NIAW-301 and NIAW-3170 shows 80% germination response to the salinity stress than others. MACS-6222 variety shows 40% germination percentage while NIAW-917 and NIAW-301 shows better germination in control treatment. NIAW-3170 and MACS-6222 shows germination per cent 20% PEG treated seeds. Seed germination Salt stress delays seed germination. The reason is the enzyme α-amylase activity is affected by salinity.

The shoot lengths after 30 days were observed in different varieties with different treatments of NaCl and PEG-4000 having different concentration. As the concentration of stress increases the variation in shoot length reduces were observed. MACS-6222 shows 19.4cm very less growth than other varieties. NIAW- 301 shows 25.4cm which is very good response at 225mM. In PEG-4000 treated plants, NIAW-917 shows 18.4 cm which is very less growth while NIAW-301 shows 25 cm which is shoot growth was at 20% high concentration of PEG-4000. Seedling growth Earlier seedling growth is more sensitive. There is a significant reduction in root emergence, root growth and root length, when plants attain vegetative stage, salt injury is more severe only at high temperature and low humidity. Because under these conditions, the transpiration rate will be very high as a result uptake of salt is also high.

After 30 days numbers of leaves were observed in NaCl treated plants. Each plant shows five leaves at initial stage respectively. After 30 days in PEG treated plants. Each plant shows five leaves at initial stage respectively.

Chlorophyll content from the leaves was measured in spectrophotometer by taking OD at 663nm, 645nm and 652nm respectively for chlorophyll a, b and ab. There were no positive results were obtained as the concentration of salinity and PEG-4000 increases. In NaCl treated plants NIDW-295 shows the less chlorophyll a content. NIAW-917 and NIAW-301 shows less chlorophyll b content. In PEG-4000 treated plants MACS-6222 shows less chlorophyll content while NIAW-301 shows less chlorophyll b content at high concentration of PEG-4000. Salinity drastically declines photo-synthetic process. Thylakoids are damaged by high concentration of salt. Chlorophyll 'b' content is drastically reduced duet to high concentration of NaCl and PEG.

The relative water content (RWC) was measured by taking dry weight, turgid weight and fresh weight. The obtained values plotted in formula and RWC% were calculated. There were no positive results were obtained. NIAW-917 and MACS-6222 shows the less RWC at 225mM solution of NaCl. NIAW-301 shows very less RWC at 20% PEG-4000 solution. Accumulation of proline and abscisic acid which are associated with tolerance of the plants to salt, are able to maintain high water potential by reducing the transpiration.

Conclusion

- The effect of different concentration of NaCl and PEG-4000 on morphological features of wheat plant.
- From the given observation of morphological characters concluded that the wheat plant treated as control shows normal growth of shoot and root. The plants treated with 225nm shows less reduction in length of shoot as compared to the control plants.
- As the concentration of NaCl increases the shoot length also gradually decreases. The treatsment of 225nm shows the maximum reduction in shoot length. NIAW-301 shows less reduction in shoot length than other varieties. Therefore, we can conclude that this variety can be used as moderately tolerant varieties for salinity stress.
- As the concentration of PEG increases the shoot length also gradually decreases. The treatment of 20% shows the maximum reduction in shoot length. NIAW-301 shows less reduction in shoot length than other varieties. Therefore, we can conclude that these two varieties are used as moderately tolerant varieties for drought stress.
- As the concentration of stress increases there is decrease in germination percentage were obtained.
- There were no positive results were obtained foe chlorophyll content.
- There were no positive results were obtained for Relative Water Content.

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