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Effect of salt stress and drought stress on vegetative growth of maize

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Abstract

Maize is grown under different soil and climatic condition. Maize is moderately sensitive to salt stress; therefore, soil salinity is a serious threat to its production worldwide. Understanding maize response to salt stress and resistance mechanisms and over viewing management options may help to devise strategies for improved maize performance in saline environments. This study was conducted to investigate the effects of salt stress on germination of two maize (*Zea mays L.*) cultivars (Cultivar-1 & Cultivar-2). The degrees of salinity tolerance among these cultivars were evaluated at seed germination stage at five different salt concentrations (0mM, 30mM, 60mM, 90mM, 120mM and 150 mM NaCl). The results showed that in all cultivars as the salt concentration increased, both germination percentage and germination index decreased significantly. Responses of cultivars to salt stress indicated differences. For all salt concentrations, Cultivar-2 had the highest germination percentage and germination index. Salt concentration decreased shoot and root dry weight. Cultivar-1 had the lowest reduction of shoot and root dry weight, respectively. On the other hand, Cultivar-2 showed better results than the other cultivars in respect of salt tolerance index. The results showed that Cultivar-2 is the cultivar to be recommended for saline soils. Cultivar-1 was less sensitive to salinity in this study. Drought tolerance is one of the most important yield constraints on crop productivity for many crops, and especially for maize. It includes the five different drought concentrations (0%, 5%, 10%, 15%, 20% and 25% PEG). The objective of the present study was to determine the effect of drought tolerence on the morphological and physiological characteristics of maize. The highest treatment (25%) reduced leaf area index, plant height, chlorophyll content, assimilation rate and photosynthetic efficiency. Furthermore, low PEG concentration (5%) affected the characteristics that were studied. Therefore, the results of this study can be used by farmers in the salinity soil area, who can maintain or improve their crop yield. We conclude Cultivar-2 at the concentration of PEG (25%) shows highest drought tolerance. Therefore, to prevent economic loss and to make the crop plants more tolerant to drought stress, a variety of approaches such as conventional breeding are being practiced. Our main conclusions are as follows: (1) germination and stand establishment are more sensitive to salt stress than another developmental stage. (2) High primary root & secondary root sodium and chloride decrease plant uptake of nitrogen, potassium, calcium, magnesium, and iron.

Keywords: Salt, Draught stress, Physiological, Morphological parameter and Maize.

Introduction

Maize play key role as a major cereal crop, stable food and widely-grown crop for many people and animal feed throughout the world. Maize contains proteins, sugar and fibers and use in food industries for the production of different useful products. The origin of maize (*Zea mays* L) in North America. Maize is one of the most versatile emerging crops having wider adaptability under varied agro-climatic conditions. Globally, maize is known as queen of cereals because it has the highest genetic yield potential among the cereals (31.04 lakh acres) as on 1 Janury2020 (Arash N. Zakaria H. Prodhan 2013).

Drought is one of the most important phenomena which limit crop production and yield. Crops demonstrate various morphological, physiological, biochemical, and molecular responses to tackle drought stress. Plant's vegetative and reproductive stages are intensively influenced by drought stress. Drought tolerance is a complicated trait which is controlled by polygenes and their expressions are influenced by various environmental elements. In maize, there are several genes which are responsible for drought stress tolerance and produce different types of enzymes and proteins for instance, late embryogenesis abundant (lea), responsive to abscisic acid (Rab), rubisco, helicase, proline, glutathione-Stransferase (GST), and carbohydrates during drought stress. 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. Furthermore, drought tolerance and yield should be improved in parallel because farmers need to profitably produce their agricultural products under drought stress (Arash N. Zakaria H. Prodhan 2013).

Biotic and abiotic factors of environment had modified the primal living organisms in such a way that modern plants were evolved. Any imbalance in these biotic and abiotic factors acts as stress and imposes serious hampering effects on growth and development of crop plants. (Aslam et al. 2013; Naveed et al. 2014).

Salinity, due to over-accumulation of NaCl, is usually of great concern and the most injurious factor in arid and semi-arid regions. Saline soils are widespread on Earth. More than 800 million hectares of land throughout the world are salt-affected (including both saline and sodic soils), equating to more than 6% of the world's total land area (FAO, 2008). Their genesis may be natural or accelerated by the extension of irrigated agriculture, the intensive use of water combined with high evaporation rates and human activity. (Lambers, 2003).

Salinity may cause significant reductions in the rate and percentage of germination, which in turn may lead to uneven stand establishment and reduced crop yields Corresponding author. (Foolad et al., 1999).

Salinity is one of the major abiotic stresses greatly limiting the crop productivity in arid and semi-arid regions of the world. Soil with elevated salt levels (ECe. 4 dSm21) is defined as saline land (Weisany et al. 2012). Salt stress inhibits plant growth in a number of ways that is osmotic stress, specific ion toxicity, nutritional and hormonal imbalance, and oxidative stress (Basile et al. 2011). Higher concentrations of salt decrease water potential, thereby restricting water and nutrient uptake by roots (Ashraf 2004). Salinity is known to cause nutrient imbalance due to competition of Naþ and Cl2 with essential nutrients such as Kþ, Ca2þ and NO32 and leads to extreme ratios of Naþ/Caþ and Naþ/Kþ. Elevated levels of these ratios in plants under saline conditions suppressed the plant growth in a number of ways(Ashraf 2004).

According to the research till now, scientists in different disciplines have tried to find solutions to the problems in their countries, but they have not been completely solved yet. However, taking all this into consideration, we see what new things we can do. According to that, we have finalized the objectives so that the farmers will benefit from it.

Materials and Methods

I. Experimental material and experimental site: The hybrids were collected from local market on Chhatrapati Sambhajinagar. 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.

II. Plant material: The following maize varieties were selected for the research work: Cultivar-1, Cultivar-2.

III. Treatment & Concentration:

Treatment (T)	Concentrations (mM) (Nacl)	Concentrations (%) (PEG-4000)
T0	Control	Control
T1	30	5
T2	60	10
T3	90	15
T4	120	20
T5	150	25

IV. NACL Treatment: Seeds of each variety were soaked under NACL solution for overnight. The concentrations are 30mM, 60mM, 90mM, 120mM and 150mM. 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.

V. PEG-4000 Treatment: Seeds of each variety were soaked under PEG-4000 solution for overnight. The concentrations are 5%, 10%, 15%, 20% and 25%. 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.

VI. Morphological studies of the experimental plant: In the morphological study, following parameters are checked in the plants for 20 days old plantlets. The morphological observations are given as below:

a) 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):

$$GP = \frac{\text{Number of Total Germinated Seeds}}{\text{Total Number of Seeds Treated}} \times 100$$

b) 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 20^{th} days after the seedling and it is expressed in the centimeters (cm).

c) Number of leaves: The total number of leaves grown at a particular stage of that plant and it is calculated after the 20^{th} days of the seedling (DAG).

VII. Physiological Studies of Experimental Plant: Estimation of chlorophyll content and Relative water content (RWC) is 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.

a) **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 maize 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).

b) 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.) × 100

VIII. 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.

Results

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 Maize variety under stress and initiation of the genotyping.

Morphological Data Analysis by One Factor Analysis: The product and process comparison 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 Maize Seeds: There were two maize varieties with different treatments of NaCl with different concentrations (30mM, 60mM, 90mM, 120mM & 150mM) and PEG-4000 with different concentrations (5%, 10%, 15%, 20% & 25%) are germinated for the study of germination percentage are Cultivar-1 & Cultivar-2. Germination percentage was obtained from the formula and its outcome is mentioned in table 1 & 2.

The highest seed germination was recorded in NaCl treatment Cultivar-2 variety T5 treatment shows 80% germination and in PEG-4000 treatment same variety T5 treatment shows 80% which is susceptible to the Salinity and drought stress.

Table no. 1 Germination Percentage (NaCl)

Varieties	NaCl Treatments								
	T0	T1	T2	T3	T4	T5	Mean	SE	CD
Cultivar-1	98	94	92	86	80	78	88	0.92	1.21
Cultivar-2	99	98	95	90	86	80	91.33	0.89	1.21

Table no. 2 Germination Percentage (PEG)

Varieties	PEG Treatments								
	T0	T1	T2	T3	T4	T5	Mean	SE	CD
Cultivar-1	96	94	90	88	84	77	88.16	0.92	1.21
Cultivar-2	98	95	90	88	85	80	89.33	0.91	1.21

Morphological characterization of varieties:

In this study different morphological parameters were analyzed among the two varieties. Observations were taken after 20 days after germination from each treatment (Nacl & PEG-4000).

Shoot Length (20 DAS)

In **Salinity stress** variation was observed in the shoot length of all maize variety were treated with NaCl solution. Observations are given in the table no 3. The highest shoot length was recorded in NaCl treatment Cultivar-2 variety T5 treatment shows 13 cm which is susceptible to the Salinity stress.

Drought stress variation was observed in the shoot length of all maize varieties were treated with PEG-4000 solution. Observations are given in the table no 4. The highest shoot length recorded in PEG-4000 treatment Cultivar-2 variety T5 treatment shows 14 cm which is susceptible to the drought stress.

Table no. 3 Shoot length (NaCl)

Varieties	NaCl Treatments								
	T0	T1	T2	T3	T4	T5	Mean	SE	CD
Cultivar-1	25	23	22	19	15	12	19.33	4.22	1.21
Cultivar-2	26	24	22	19	15	13	19.83	4.117	1.213

Table no. 4 Shoot length (PEG)

Varieties	PEG Treatments								
	T0	T1	T2	T3	T4	T5	Mean	SE	CD
Cultivar-1	24	23	21	20	16	13	19.5	4.187	1.213
Cultivar-2	25	24	22	18	15	14	19.66	4.152	1.231

Root Length (20 DAS)

In **Salinity stress** variation was observed in the Root length of all maize variety were treated with NaCl solution. Observations are given in the table no 5. The highest Root length was recorded in NaCl treatment Cultivar-2 variety T5 treatment shows 7 cm which is susceptible to the Salinity stress.

Drought stress variation was observed in the root length of all maize varieties were treated with PEG-4000 solution. Observations are given in the table no 6. The highest root length recorded in PEG-4000 treatment Cultivar-2 variety T5 treatment shows 8 cm which is susceptible to the drought stress.

Table no. 5 Root length (NaCl)

Varieties	NaCl Treatments								
	T0	T1	T2	T3	T4	T5	Mean	SE	CD
Cultivar-1	11	10	9	8	8	7	8.83	9.243	1.213
Cultivar-2	11	10	8.5	8	8	7	8.91	8.767	1.161

Table no. 6 Root length (PEG)

Varieties	PEG Treatments								
	T0	T1	T2	T3	T4	T5	Mean	SE	CD
Cultivar-1	11	10	9	7	7	7	8.5	9.606	1.213
Cultivar-2	10	11	10	7	7	8	8.83	9.243	1.213

Number of Leaves (Day After Germination 20 days):

The total number of leaves were observed and noted after 20 days after germination are given in table no. 7. The highest number of leaves was recorded in NaCl treatment Cultivar-2 variety T2 treatment shows 4 leaves germination and in PEG-4000 treatment same variety T2 treatment shows 4 leaves which are susceptible to the Salinity and drought stress.

Table no. 7 Number of leaves

Varieties	Treatments													
	NaCl							PEG-4000						
	T0	T1	T2	T3	T4	T5	Mean	T0	T1	T2	T3	T4	T5	Mean
Cultivar-1	4	4	4	3	3	2	3.33	4	4	4	3	3	2	3.33
Cultivar-2	4	4	4	3	3	2	3.33	4	4	4	3	3	2	3.33

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 no. 8 & 9. The highest chlorophyll was recorded in NaCl treatment Cultivar-2 variety T5 treatment shows 1.01 and in PEG-4000 treatment same variety T5 treatment shows 1.03 which is susceptible to the Salinity and drought stress.

Table no. 8 Chlorophyll content (NaCl)

Varieties	NaCl Treatments								
	T0	T1	T2	T3	T4	T5	Mean	SE	CD
Cultivar-1	0.92	0.99	1.06	1.01	0.97	1.01	0.900	0.823	0.012
Cultivar-2	1.02	1.01	0.94	0.9	0.98	1.01	0.97	1.257	0.018

Table no. 9 Chlorophyll content (PEG)

Varieties	PEG Treatments								
	T0	T1	T2	T3	T4	T5	Mean	SE	CD
Cultivar-1	0.92	1.02	1.06	1.01	0.97	1.01	0.99	0.823	0.012
Cultivar-2	1.02	0.97	0.97	0.97	1.02	1.03	0.99	0.819	0.012

Relative water content:

The relative water content (RWC) was calculated by using formula and observations were noted and show in table no.10 & 11. The highest relative water contain was recorded in NaCl treatment Cultivar-2 variety T5 treatment shows 70.60% and in PEG-4000 treatment same variety T5 treatment shows 65.26% which is susceptible to the Salinity and drought stress.

Table no. 10 Relative water content (NaCl)

Varieties	NaCl Treatments								
	T0	T1	T2	T3	T4	T5	Mean	SE	CD
Cultivar-1	82.80	73.62	70.63	69.80	65.60	61.86	70.71	0.128	0.135
Cultivar-2	82.76	80.49	76.68	74.43	72.80	70.60	76.29	0.110	0.125

Table no. 11 Relative water content (PEG)

Varieties	PEG Treatments								
	T0	T1	T2	T3	T4	T5	Mean	SE	CD
Cultivar-1	74.86	72.81	71.66	68.60	66.60	63.76	69.71	0.283	0.293
Cultivar-2	76.66	74.86	71.69	69.76	68.85	65.26	70.84	0.106	0.112

Germination percentage:

Seed Germination

Shoot length:

Effect of Salt stress on the shoot length after 20 days

Effect of drought stress on the shoot length after 20 day

Root Length:

Effect of Salt stress on the root length after 20 days

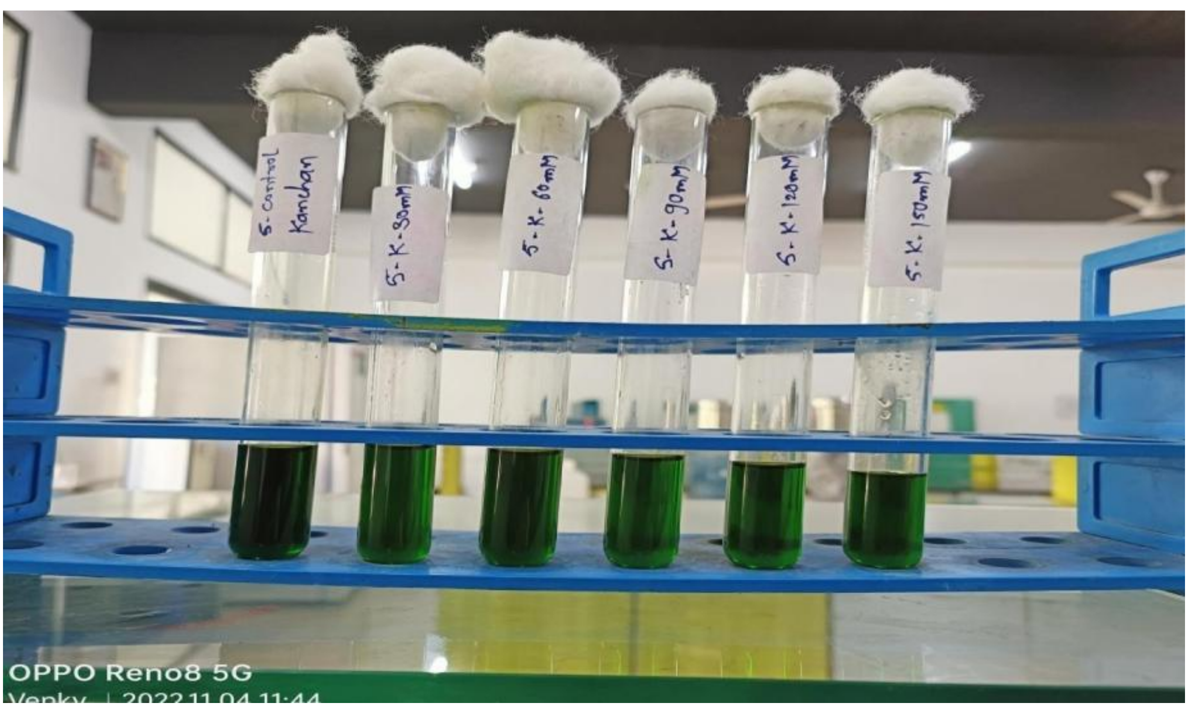
Effect of drought stress on the root length after 20 days

Number of leaves:

Effect of Salt stress on the Number of leaves

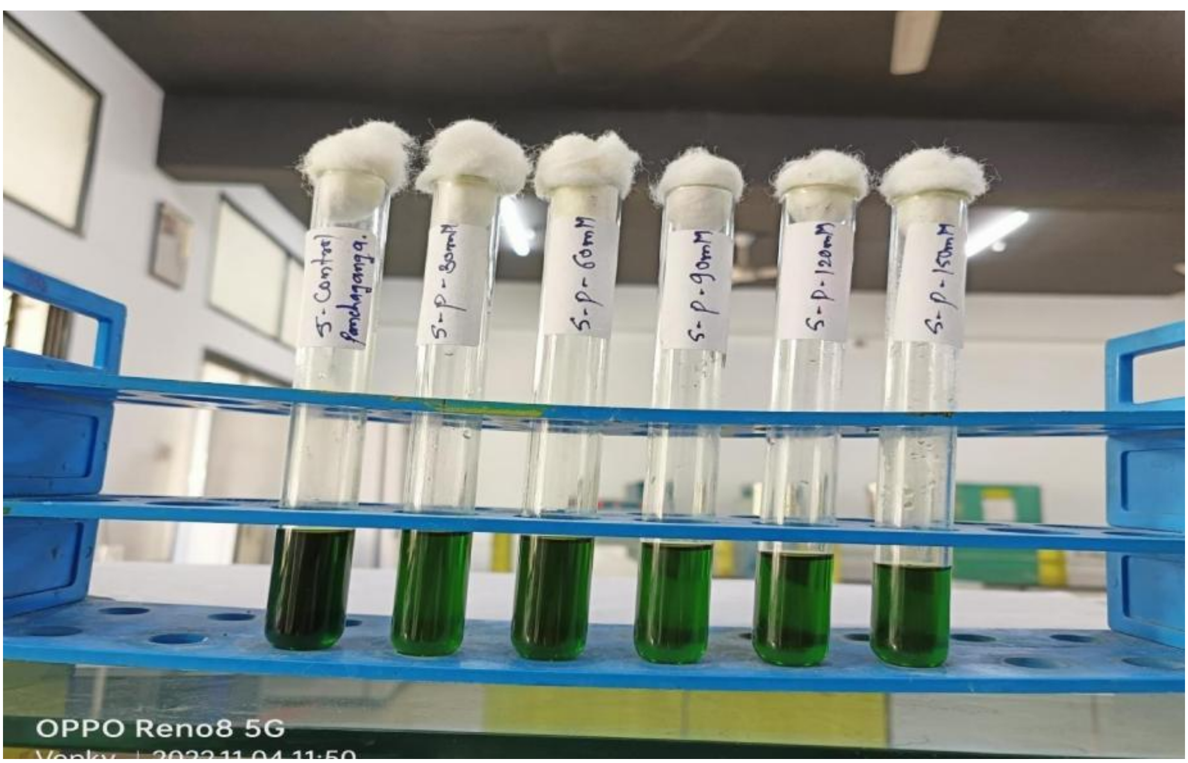
Effect of Drought stress on the Number of leaves

Chlorophyll content:

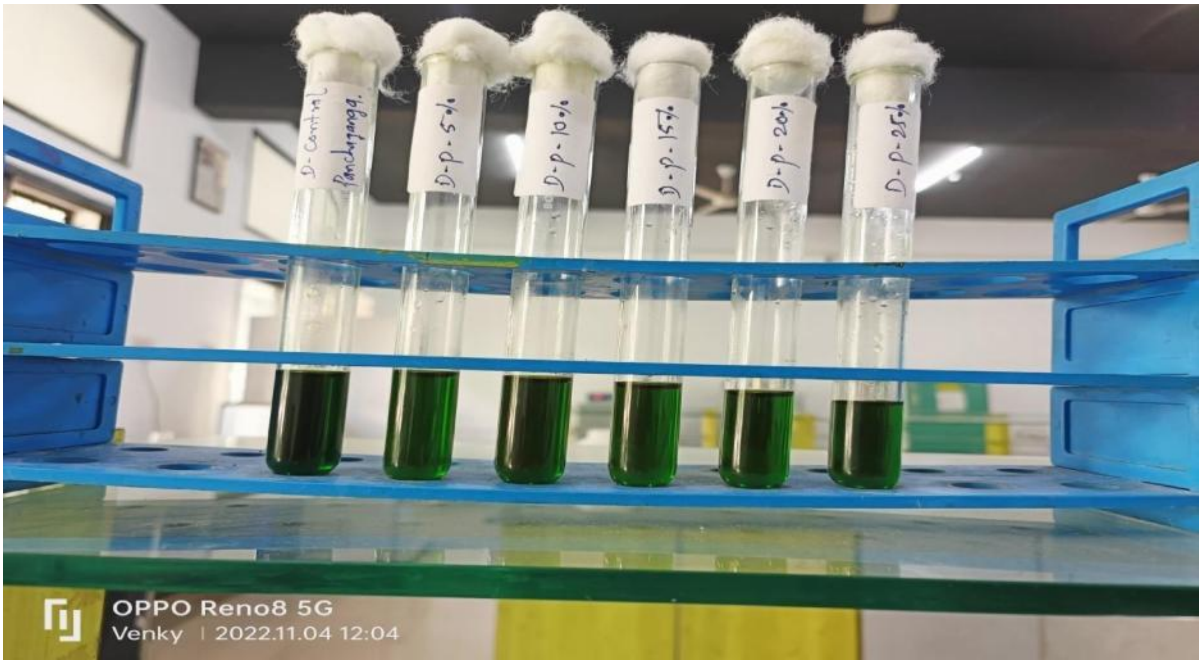


Chlorophyll content from NaCl treated plants

Chlorophyll content from PEG treated plants



Chlorophyll content from NaCl treated plants



Chlorophyll content from PEG treated plants

Discussion

The results showed that measured components of maize cultivars were significantly affected by Salt and PEG concentration. Cultivar-1 variety shows less germination percentage while Cultivar-2 shows higher germination in control. Cultivar-1 and Cultivar-2 have shown the very less germination at 150mM concentration of NaCl. Cultivar-2 shown good response to the salinity stress than others. Cultivar-1 and Cultivar-2 shows very less germination in 25% PEG treated seeds (Table-1 & 2). Our results were supported by research conducted on this subject (Carpici et al., 2009). The shoot lengths after 20 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. Cultivar-1 shows very less growth than other varieties. Cultivar-1 and Cultivar-2 shows better response at 60mM. In PEG-4000 treated plants, Cultivar-2 shows very less growth at 150mM while Cultivar-2 shows better shoot growth high concentration of PEG-4000. After 10 days numbers of leaves were observed in NaCl treated plants. Each plant shows two leaves at initial stage. After 20 days each plant shows three leaves and after 30 days each plant shows 5 leaves 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 Cultivar-1 shows the less chlorophyll a content.

Seedling establishment is an important phase in the maize plant life cycle. Salt stress adversely affects seed germination of Cultivar-1 and Cultivar-2 due to the decrease in the osmotic potential created in the soil solution that prevents the entry of water into the seed During seedling establishment, intake of sodium and chloride ions causes toxicity in the plant cells, thus reducing seed germination rates and the growth of seedlings that have already germinated.(EL Sabagh 2020). Nacl treated seeds of maize variety of Cultivar-1 it shows the negative impact in seed germination. Besides its negative impact in the germination rates, salinity stress also delays the overall germination process, thus reducing the survival chances of those seeds that were able to germinate. Because of its potential to drastically reduce crop productivity, (EL Sabagh et al 2019). it is of paramount importance to recognize these early deleterious impacts of soil and water salinity in plant growth and development.

The Cultivar-2 shows the very less germination at higher concentration in 150mM of NaCl. Cultivar-2 shows good response to the salinity stress than kanchan. Salinity reduces seedling establishment by increasing the oxidative stress through the absorption of Na+ and Cl− ions in the seeds that cause toxicity in the embryogenesis and protein synthesis. Maximum oxidative stress caused by Na+ and Cl− ions toxicity during germination lowers or stunts the germination of plants [Khajeh et al 2009]. In case of maize production, just Na+ toxicity was found more detrimental in reducing the germination under salt-stressed environments.

A reduction in photosynthetic pigments such as chlorophylls a and b and carotenoids is also associated with a decline in the net photosynthesis rate in maize under salt stress (El Sayed 2011; Qu et al. 2012). Cha-um and Kirdmanee (2009) observed a linear reduction in chlorophylls a and b, total chlorophyll, and carotenoid contents in maize with increased salt stress. We observed similar results of chlorophylls a and b content in Cultivar-1 and Cultivar-2. We observed salt stress inhibit leaf expansion and decline in photosynthesis in Cultivar-1 and Cultivar-2 and our findings are similar to Muhmmad et al 2015.

Following parameters such as: Root lengths were taken. Elongation of stem in maize under drought stress was reduced during vegetative stage. It was studied that drought tolerant observed at PEG 5, 10, 15, 20 & 25%. The mechanism of drought tolerance involves many physiological and biochemical processes. These include reduction of water loss by enhancing stomatal resistance, developing deeper root system and more uptake of water, deposition of osmolytes and production of osmoprotectant in Cultivar-1 and Cultivar-2. Our findings supported by experimend conducted M Aslam et al 2013.

Drought is the main environmental constraint, which often having devastating effects on crop production. In the crop improvement program improved tolerance to drought has been an very important goal.

In order to investigate the effects of drought stress on germination components of maize cultivars, Cultivar-1 and Cultivar-2 a laboratory experiment was conducted in a factorial randomized complete design with 4 replications.) as first factor. The second factor included 4 levels of drought stress inducer by applying 5, 10, 15, 20 and 25% of polyethylene glycol-4000 (PEG) which is equivalent to four osmotic potential levels including −0.001, −0.27, −0.54 and −1.09 MPa, respectively. The results showed that, the highest reduction was related to the drought level of 25 % among the maize Cultivar-1. The best cultivars in terms of germination traits were Cultivar-2 this indicates their tolerance to drought stress and remaining is less tolerance. That germination percentage ranged between 78% and 99% and this finding represented the strength of the cultivars and expresses their storage from carbohydrates. Also, it is clear to observe the narrow range between the first two groups, where the highest one was relative to the genetic features of the cultivar Regarding to the effect of the osmotic pressure resulted from PEG rates on the germination percentage, data on hand showed that the increase in PEG dramatically decreased germination percentage, Drought stress had a significant effect on all the measured traits at all PEG levels used. In this experiment, germination speed was influenced by drought stress more than germination percentage. Most 90% had acceptable germination in drought stress of Cultivar-2 (10% PEG) which indicates the characteristics of maize in tolerating drought stress and its suitability for being cultivated in arid and semiarid areas. Treatment with Nacl & PEG can be the germination sensitivity threshold for the studied maize cultivars. In summary, due to its better growth responses to drought stress, Cultivar-2 cultivar was the superior one and Cultivar-1 was the lowest.

Conclusion

As final conclusions, in this paper, research data from a pot experiment is presented to study the effect of salinity stress and drought stress on seed priming treatments on the physiological, and biochemical of two maize cultivars. Both cultivars showed differential response to salinity and drought stress on seed priming treatments. Reduction in physiological traits was more profound in Cultiver-1 than cultivar-2. When exposed to different levels of salinity and drought stress. Seed priming with saline water and subsequent exposure to salinity had increased shoot K+, ABA and proline content which in turn had improved the salinity tolerance of maize cultivars under study . The effects of different salt concentrations on salt tolerance indices of cultivars were of importance .The improvement in salinity tolerance and drought tolerance was more pronounced in cultivar-2 than cultivar-1. From these results it can be concluded that cultivar-1 is more sensitive to salinity and drought than cultivar-2 and may not be suitable for cultivation in saline soil and drought tolerance. All cultivars of maize are not equally affected by drought stress due to high level of variability in genetic background of this crop. Different mechanisms have been evolved in maize like other crops which enable them to effectively survive under drought stress.

Due to the presence of genetic component in the adaptive mechanism, it will be interesting to extend this study to other osmotic and plant species showing different growth and yield responses to salinity and drought tolerance. Lots of novel breeding and evaluation techniques have been developed in recent past and practical application of other techniques will further help to cope the problem of drought stress by development of more drought tolerant salt tolerance in maize genotypes for resolution of food security

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