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"A Comparative study on the effect of salt salinity on germination and early seedling growth of *Pennisetum glaucum* L. grown in different salt concentration and controlled condition"

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Abstract

Pearl millet (*Pennisetum glaucum*) locally named as Bajra is an important cereal crop comprising of high nutritive value, regarded as a healthy food with a balance of nutrients. In this paper the effect of salinity levels on seed germination and seedling growth of *Pennisetum glaucum* was studied. Two types of laboratory experiments were conducted using the seeds of pearl millet. Two hundred seeds of the cultivar were taken for the experiment, among which 150 seeds were germinated in petri plates containing the tissue to paper above the double layered cotton. These treatments comprised of control (distilled water) 0.05M ,0.1 M, 0.125M and 0.15M Sodium Chloride (NaCl). The treatments were laid with 3 replications. Data was recorded with a gap of 2-3 days of observations. The rest 50 seeds were subjected to germinate under the basal media (MS media) prepared along with the variously added molar concentrations of Sodium Chloride (NaCl) by tissue culture technique, followed by the protocol of sterilization and inoculation. The germination rate of the millet seedlings was examined, the number of seeds germinated in each concentration is compared with the growth in control conditions.

Keywords: Pearl millet, Bajra, Salinity, Germination, Seedling Growth.

Introduction

The genus *Pennisetum* belongs to the family Poaceae, group Paniceae of the subfamily Panicoideae and is closely related to the genera *Cenchrus* and *Setaria*. It includes approximately 140 species (Brunken 1977) distributed in tropical and subtropical regions. The genus *Pennisetum* has been given various taxonomic status. The Stapf and Hubbard (1934) classification of *Pennisetum* based on morphological characters divides the genus into five sections: *Pennicillaria*, *Brevivalvula*, *Gymnothrix*, *Heterostachya* and *Eu-Pennisetum*. Other infrageneric classifications showing some discrepancies were proposed by different authors (Pilger 1940; Brunken 1977; Clayton and Renvoize 1986). The section *Pennicillaria* includes the domesticated crop

Pennisetum glaucum (Pearl millet, Bajra), which is a very important cereal crop in Africa and India, but serves also as fodder in other areas. *Pennisetum glaucum,* commonly called as pearl millet or bajra, it is a versatile and resilient grain crop which belongs to the Poaceae family.

1 Botanical Description

Pennisetum glaucum (Pearl millet) is an annual grass that typically grows upright, reaching heights ranging from 2 to 4 metres. The leaves are long, narrow, and finely serrated, arranged alternately along the stems. They can vary in colour from green to slightly purple. The flowering part of pearl millet is a dense cylindrical spike-like inflorescence, often referred to as a "panicle". This panicle can range from 15 to 40 cm in length.

Fig.1: *Pennisetum glaucum*

2 Grain characteristics

The seeds of *Pennisetum glaucum* (pearl millet) are small, round to oval-shaped grains that vary in colour from white to grey, brown, or dark red, depending on the variety of millet. It has a lot of nutritional composition, rich in carbohydrates, proteins, dietary fibre, and essential nutrients such as iron, magnesium and phosphorous. Pearl millet is gluten-free and has a relatively low glycemic index. It is a hardy and versatile grain crop that plays a vital role in food security, especially in arid and semi-arid regions. Its adaptability, nutritional benefits, and cultural significance make it a crucial crop in global agriculture and food system.

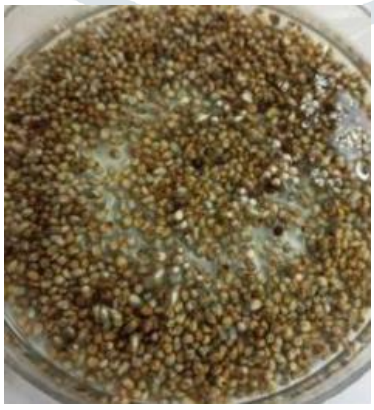


Fig. 2: Pearl millet grains

3 Adaptability and Growth condition

The climate for Pearl millet is known for its exceptional drought tolerance and can thrive in hot and arid climates where other cereal crops may fail. The Pearl millets can grow in a wide range of soils but it prefers well-drained sandy soils or loamy soils with a pH between 6.0 and 7.5 also it is predominantly cultivated in semi-arid and arid regions of Africa and Asia, where it serves as a staple food crop for millions of people.

4 Agricultural Uses

Pearl millet or bajra grains are used extensively for human consumption, primarily in the form of flour for making varieties of flatbreads, porridge, and various of traditional dishes. This also serves as valuable fodder for livestock due to its high biomass yield and nutritional contents. Beyond food and fodder, Pearl millet is increasingly being explored for its potential in bio fuel production and other industrial applications.

5 Cultural Significance

In many cultures, especially in parts of Africa and India, Pearl millet holds cultural significance as a staple food crop and is used in various ceremonial dishes. Pearl millet's nutritional profile contributes to its role in promoting food security and nutrition in regions prone to food insecurity.

6 Research and Development

Ongoing research aims to develop new varieties of pearl millet that are more resilient to climate change, pests, and diseases while enhancing yield and nutritional quality. Maintaining and utilizing the genetic diversity of Pearl millet is crucial for ensuring its adaptability and sustainability in agriculture.

Pennisetum glaucum (bajra, pearl millet) is a hardy and versatile grain crop that plays a vital role in food security, especially in arid and semi-arid regions. Its adaptability, nutritional benefits, and cultural significance make it a crucial crop in global agriculture and food systems. The most harmful environment stress that destroys agriculture productions are saline irrigation and saline soil. The salinity of soil is a widespread environmental problem and important factor in limiting agricultural productivity.

The effects of salt stress on plants are different according to the plant growth stage. Researchers worked a lot on salt stress effect on plant growth, production and fruit development of various seeds, but there has less literature on seed germination and early seedling growth under salt stress. Salt stress affects crop in three ways like osmotic stress, ionic toxicity and soluble salt concentrations depend on stress nature and duration (Javed et.al). Many physiological aspects of plant shoot growth and dry matter are reduced by salinity and it also affects seed germination by creating osmotic potential which prevent water uptake, by toxic effects of ions on embryo viability. Osmotic potential reduction in salt stressed plant is a result of inorganic ion (Na^+, Cl^- and K^+) and accumulation of complete organic solute. Increased salinity causes reduction in germination percentage, rate, length and weight of roots and shoots. The shoot growth reduced by salinity due to inhibitory effect of salt on cell division and growing point. The soil salinity is one of the major constraints in beans production due to chloride and sulphate accumulation, some have a very high germination capacity and seedling growth in saline soils.

Increased level of the concentration of NaCl in soil possesses an unfavourable effect on length of plumule and radicle which leads to suppress growth of radicle and plumule. The growth medium when contain any unexpected salt concentration can lead to reduction of absorption of water due to osmotic potential lessening and affect cell division (Ashraf and Harris, 2005). Negative effect in the length of plumule and radicle is seen in this salt experiment. According to (Kausar et.al., 2012), salinity effect retards the length of plumule and radicle and other affects are may be due to disruption in uptake of nutrients, ion toxicity (Akhter et.al., 2012), osmotic effects of salinity (Ashraf and Harris, 2005), water absorption (Ashraf and Sarwar, 2002), which results in reduction of plant hormones required for growth and biosynthesis of enzymes (Bor et.al, 2003). As NaCl concentration level is increased, the length of radicle and plumule are decreased in all land races. These results are stated in many researches (Farsiani and Ghobadi, 2009; Jajarmi 2009).

Millet plants are mainly grown for human food consumption, for the rapid growing population. The development of salt tolerant crop species and their cultivation should be given priority. Several researchers showed that in response to heat and salt stress, pearl millet shoots expressed genes related to Photosynthesis, which is one of the most important physiological processes in the plant life giving food and oxygen to all the other living organisms. However, under salt stress photosynthesis can severely impaired.

To overcome this obstacle, Pearl millet employs a mechanism, where it modulates its carbon fixing in photosynthetic organisms to maximize the assimilation of carbon dioxide (CO_2). In the presence of salinity stress, Pearl millet may adopt diverse mechanism to cope with adverse conditions. One such strategy involves

the upgradation of carbonic anhydride activity, which facilitates the conversion of bicarbonate (HCO3) into carbon dioxide (CO2). Crop tolerance to salinity is of high importance due to the extent and the constant increase in salt affected areas in arid and semi - arid regions. Pearl millet is generally considered as fairly tolerant to salinity. So, it could be an alternative crop option for salt affected areas.

Material and Methods

The study was conducted at the Laboratory of plant Tissue culture, University Department of Botany, Ranchi University, Ranchi. Two methods were used for the study:

1. *By preparing different molar solution of NaCl*

2. *By plant tissue culture*

1. *By preparing different molar solution of NaCl*

To study the effect of salt salinity on germination and early seedling growth of *Pennisetum glaucum* different molar solutions of NaCl was made. The solutions of NaCl were prepared in distilled water. There were 5 experimental groups with different salt concentrations and one control group without any addition of salt. The experimental groups were labelled as 0.005M, 0.1M, 0.125M, 0.15M, and in controlled group as 0M. The solution was left for six hours until completely dissolved. The concentrations of the solution were prepared by using molar conversion method as given below.

M=N/V

Where, M is the molecular mass, N is the number of mole, and V is the volume.

N= molar mass (mass of NaCl).

Molar concentration = mass of NaCl/Molecular mass x Volume.

NaCl was added to 100 ml of distilled water depending on the needed concentration. The salt concentration was prepared as per the following table:

Sl. No	Concentration (M)	Amount of NaCl added (gm)	Amount of distilled water (ml)
1	Control	0	100
2	0.05	0.29	100
3	0.1	0.58	100
4	0.125	0.73	100
5	0.15	0.87	100

Table. 1: Amount of Salt to be added to distilled water through molar conversion.

Experimental study

All the required Petri dishes were prepared as germination chambers are labelled based on salt concentrations, then disinfected with 70% alcohol and UV following sterilization protocol. After completion of sterilization process, 10 seeds were transferred to each petri dishes. All the petri plates were divided into the group of three for each molar concentration of NaCl solution. In each petri plates a thick layer of cotton is placed under a layer of 2-3 filter paper or tissue paper. This tissue paper is then wetted with the respective saline water solution at 0.05M, 0.1M, 0.125M, 0.15M NaCl and control medium. All operations were performed under Laminar air flow. The petri plates were labelled and kept under dark condition for the germination process to continue. For one week the germinated seeds were observed daily.

Data collection and Analysis

After that, 5 germinated seeds were removed and their morphological traits were assayed. The germination rates were calculated. Emergence or radicle and plumule was observed and considered as seed germination. All the growth with development of radicle and plumule are examined by measuring through the measuring scale.

2. *By plant tissue culture*

Tissue culture is a collection technique used to maintain or grow plant cells, tissues, or organs under a sterile condition on a nutrient culture medium of known composition. Widely used to produced clones of a plant in a method known as micropropagation. Broadly it refers to the invitro cultivation of all plant, whether a single cell, a tissue, an organ, on nutrient medium under aseptic conditions. The basic rule essential in the planning of an efficient and functional laboratory facility is the order and continuous maintenance of cleanliness because of all operations require aseptic conditions.

Preparation and Composition of Nutrient media

The Murashige and Skoog (1962) also called as MS medium is very widely used in different culture system.

Plant tissue culture technique was also implemented in the experiment following the protocols of media preparation for micro and macro nutrients, including the preparation of vitamins and growth regulators. After media preparation, the seeds were properly sterilised following all the necessary protocols. Afterwards, inoculation of seeds was done into the culture tubes. The inoculated culture tubes were then set up properly into the tissue culture rack and were placed in the air-conditioned room with proper lighting and low temperature condition. With a duration of 2-3 days the growth rate of seeds was examined and recorded.

Preparation of stock solutions of MS medium:

	mg/L	**g/L**	**10X g/L**
KNO_3	1900	1.90	19.0
NH_4NO_3	1650	1.65	16.5
$MgSO_4.7H_2O$	370	0.37	3.7
KH_2PO4	170	0.17	1.7
$CaCl_2.2H_2O$ (Stock B)	440	0.44	4.4

Table. 2: Stock A (Macronutrients) strength- 10X.

	mg/L	**g/L**	**1000X g/L**	**1000X g/100 ml**
$MnSO_4.4H_2O$	16.9	0.0169	16.9	1.69
H_3BO_4	6.2	0.0062	6.2	0.62
KI	0.83	0.00083	0.83	0.083
$ZnSO_4.7H_2O$	8.6	0.0086	8.6	0.86
$NaMoO_4.2H_2O$	0.25	0.00025	0.25	0.025
$CuSO_4.5H_2O$	0.025	0.000025	0.025	0.0025
$CoCl_2.6H_2O$	0.025	0.000025	0.025	0.0025

Table. 3: Stock C (Micronutrients) strength- 1000X.

	mg/L	**g/L**	**100X g/L**	**100X g/100 ml**
$FeSO_4.7H_2O$	37.3	0.0373	3.73	0.373
$Na_2EDTA.2H_2O$	27.8	0.0278	2.78	0.278

Table. 4: Stock D (Iron source) strength- 100X.

	mg/L	g/L	1000X g/L	1000X g/100 ml
MYSO INOSITOL	100	0.1	100	10
GLYCINE	2.0	0.002	2.0	0.2
NICOTINE ACID	0.5	0.005	0.5	0.05
THIAMINE	0.1	0.0001	0.1	0.01
PYRIDOXIN HCL	0.5	0.0005	0.5	0.05

Table. 5: Stock E (Vitamins and Amino acid).

SUCROSE	3%	30g/L
AGAR	0.8%	8g/L
pH		5.8

Table. 6: Other requirements.

Experiment study

Explant collection: Seeds of *Pennisetum glaucum* were collected from general store.

Surface sterilization, inoculation and culture condition: Seeds were soaked in water for overnight. Seeds were washed with tap water 2-3 times and then with distilled water. Treated with teepol for 2-3 minutes by continuous stirring. Then washed them with distilled water 2-3 times. Again, treated with Bauistin treatment (0.5%). Lastly rinsed the seeds properly with water. After all the sterilization process were completed, the seeds along with all the materials autoclave were placed inside the laminar air flow chamber which was beforehand sterilized by ethanol and exposed to UV light for around 20 minutes.

Now, the plant prepared around 24 hours ago and left for getting settled properly is the main part of the whole tissue culture process to be carried out. Every media preparation process for different seeds varies from each other, therefore, the media prepared for the inoculation of *Pennisetum glaucum* was done so: - since there are only 4 salt concentration along with two controlled condition the media are also prepared so. Firstly, for the media are taken. Since, we have used only six test tubes for the experimental study therefore as per to the calculation only 62.5ml of media solution is needed that is then transferred to each test tubes (culture tubes) in an equal proportion.

So, for 62.5ml media the amount of nutrients and other requirements taken were:

Macronutrients- 6.25g; Micronutrients- 0.25g; Myso Inositol- 0.5g; Iron- 0.625g; $CaCl_2$- 7.5g; Vitamins and Amino acids- 0.25g; Sucrose- 7.5g; Agar- 2g.

Then properly weighed NaCl solution (after converting into molar concentrations as the above calculation) was added to the respective culture tubes containing the MS media labelled with different concentrations.

Sl.No.	Concentration (M)	Amount of NaCl added in (gm)	Amount of distilled water (ml)
1	Control	0	10
2	0.05	0.029	10
3	0.1	0.058	10
4	0.125	0.073	10
5	0.15	0.087	10

Table. 7: Amount of NaCl to be added in each culture tube after molar conversion.

Observation and Result

Effect of NaCl on germination and plant growth was observed.

1) *Germination results in case of simple NaCl molar solutions in petri plates:*

Seed germination declined linearly with increase in NaCl concentrations from 0.05M to 0.15M, the effects of NaCl were presented as stress tolerance index (STI) and growth performance with respect to control. The STI declined in all the petri plates studied at the highest NaCl concentration (0.15M), it declined as well as failed to germinate at this concentration. The shoot and root length were significantly affected under the salinity stress. Seed germination under salinity stress reached maximum germination in distilled water or controlled condition and seedling phase of development. Increased salinity causes a significant reduction in germination rate and shoot and root length. In high salt concentration the seed started dying, changing their colour to black.

2) *Germination results in case of MS media slants with NaCl concentration:*

As in the above case, here also the seed germination rate seen to be declining with the increase in NaCl concentrations from 0,05M to 0.15M, the effects of NaCl were presented as stress tolerance index (STI) and growth performance with respect to control. The STI declined from 0.05M to 0.15M but unlike the condition of petri plates (where there was no growth in the highest concentration of NaCl) at the highest concentration of NaCl i.e. 0.15M, there was not much but recognisable growth reported. The highest growth was seen in case of controlled medium and in rest of the conditions the growth was retarded. And as the concentration was increased to 0.15M and more the germination is stopped. Therefore, it can be stated that with the increase of salinity stress the rate of germination slows down due to inhibition of water absorption capacity of roots and also high solute (salt) concentration, the media turns into hypertonic condition inhibiting water absorption by the root hairs.

Fig. 3: Germination of seeds.

Fig. 4: Evaluation of shoot length.

Salt Concentration	No. of seeds per plate	No. of seeds germinated
CONTROL	10	10
0.05M	10	10
0.1M	10	9
0.125M	10	8

0.15M	10	6

Table. 8: Number of seeds undergoing germination.

Salt Concentration	No. of seeds per culture tube	No. of seeds germinated
CONTROL	5	5
0.05M	5	5
0.1M	5	4
0.125M	5	3
0.15M	5	3

Table. 9: Germination of seedlings in the culture tube with different concentration of salt.

Fig. 5: Laboratory setup for germination of pearl millet seeds under different salt concentration.

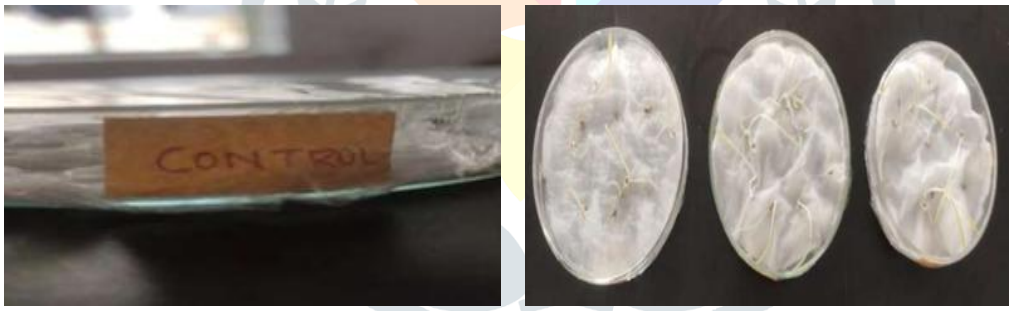


Fig. 6: Showing growth of seedlings in control condition.

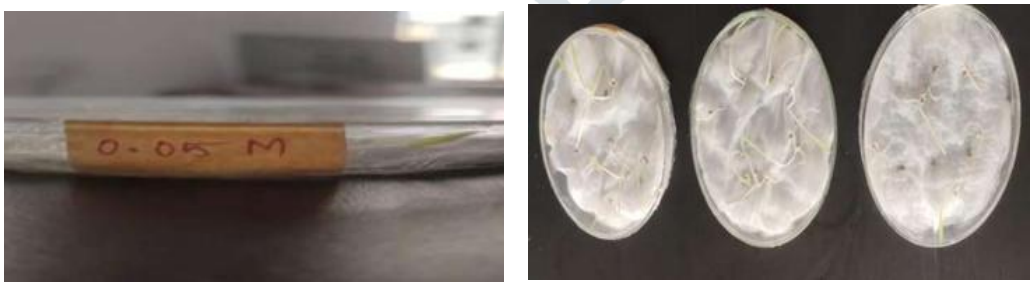


Fig. 7: Showing growth of seedlings in 0.05 M condition.

Fig. 8: Showing growth of seedlings in 0.1 M condition.

Fig. 9: Showing growth of seedlings in 0.15 M condition.

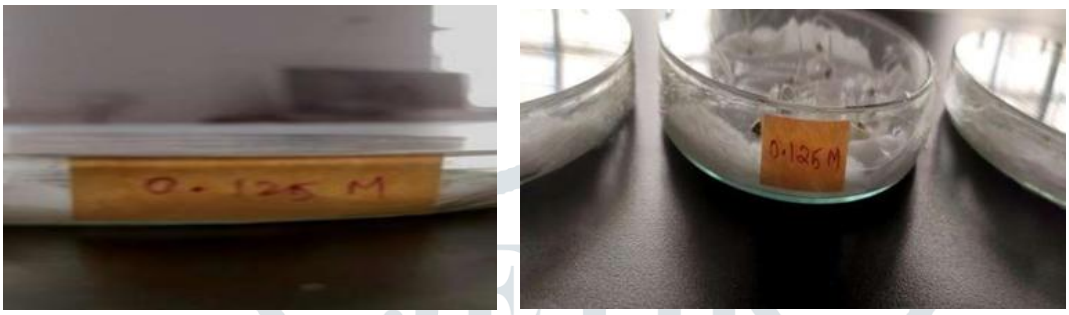


Fig. 10: Showing growth of seedlings in 0.125 M condition.

Fig. 11: Showing growth of seedlings in all conditions.

Fig. 12: Showing the culture lab setup for the germination of inoculated seeds in MS media with controlled and different concentration of salts.

Fig. 13: Appearance of roots in the germinated seeds after 3 days of inoculation.

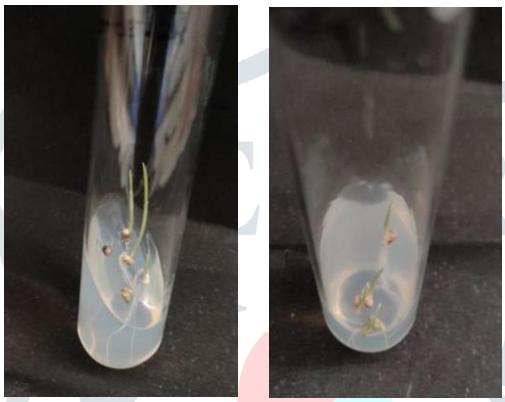
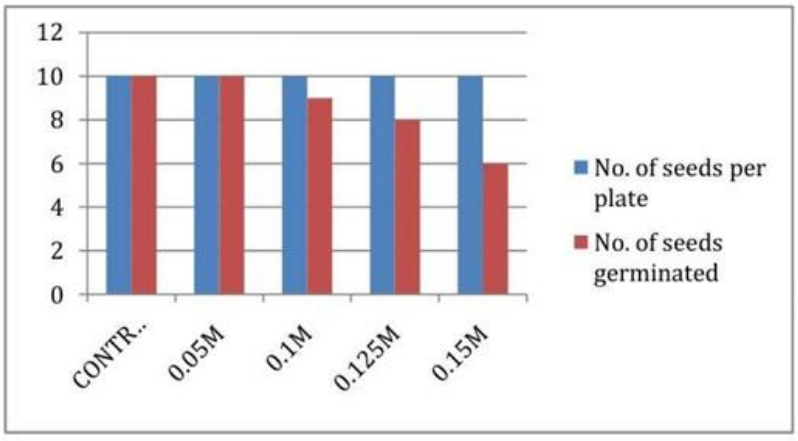
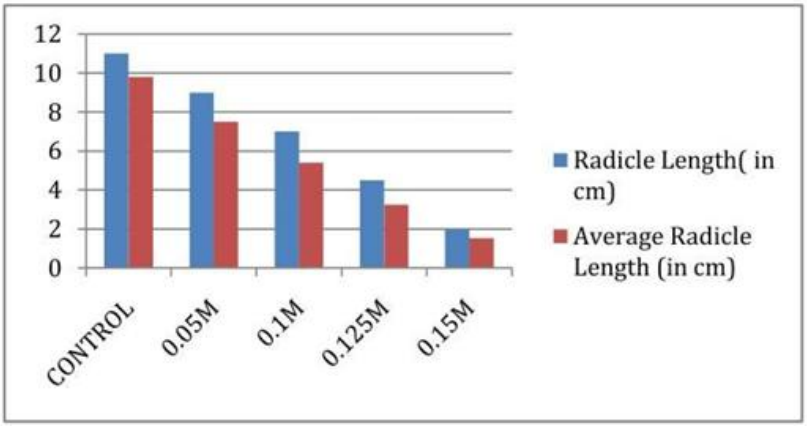


Fig. 14: Development of both root and shoots for the seedlings after 5 days of inoculation.

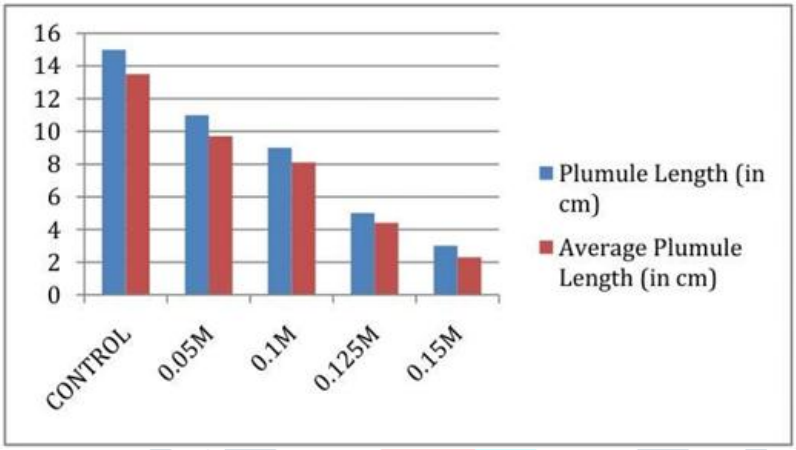
Fig. 15: Total development of plant from the germinated seeds at different concentration after 8 days of inoculation.



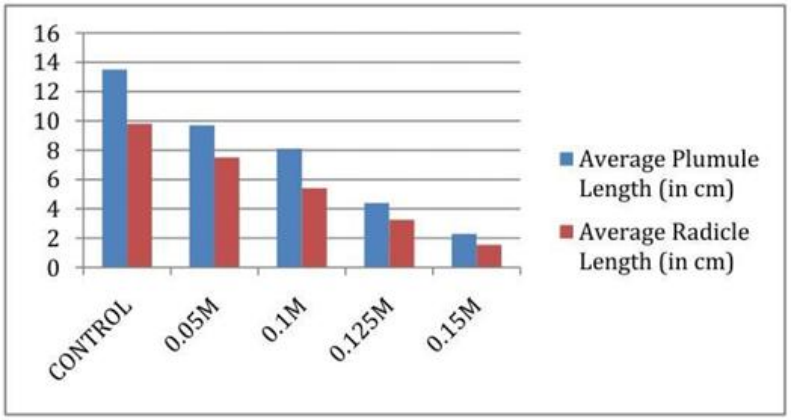
Graph. 1: Showing the germination rate of the seedlings compared to the controlled condition.



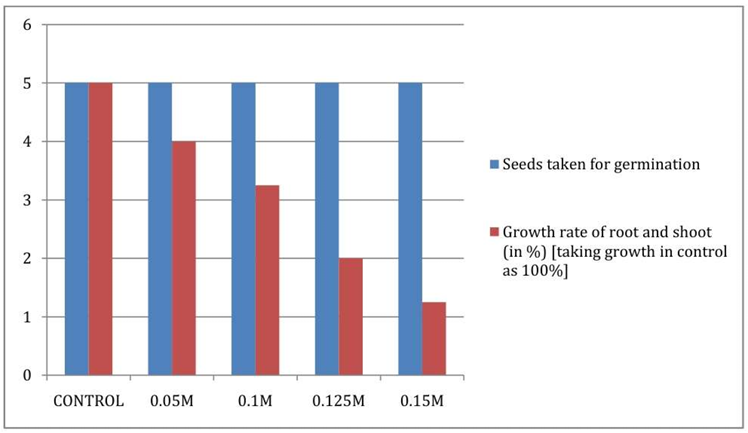
Graph. 2: Showing the comparative evaluation of the radicle length in all salt concentrations.



Graph. 3: Showing the comparative evaluation of the plumule length in all salt concentrations.



Graph. 4: Showing the comparative evaluation of the plumule and radicle length in all salt concentrations.



Graph. 5: Showing the evaluated data of compared growth of shoot and root at different concentrations of NaCl.

Discussion

Seed germination rate declined in all the millet genotypes when subjected to different NaCl concentrations. Seedling length also decreased in all genotypes, whereas the salinity tolerance index varied among the genotypes. Plants respond in salt stress by adapting their morphophysiological system to change in the environment, so as to ensure their survival in the changed condition (Shelke et. al., 2017). Under salt stress, the plant root adopts its morphophysiological characteristics for absorbing nutrients (Heasegama et al,2000).

The decrease in root length is an adaptation response of plants to avoid and reduce salt absorption. Salt stress lowers the extracellular water potential and bioavailability of water in the root zone causing a low absorption of water and nutrients by plants and this hampers their adequate growth and biomass production. Salinity stress affects the plant vigour due to reduction in imbibition resulting in a limited hydrolysis of food and reserves from the storage tissues. The result shows that NaCl stress has a greater effect on roots then shoot with a sudden fall in growth, which is in accordance with some earlier reports (Saha et. al., 2010)

The results confirmed the report of Moss and Hoffman (1977) that high salinity levels lead to ion imbalance, osmotic regulation disorders and finally decrease in water absorption by seeds. Salt stress critically affected plant growth and productivity during all developmental stages by decreasing germination rate, retards the development of plant and reduces the yield of crops.

It is now evident that seeds of different crops can alleviate the adverse effect of salinity stress on germination and seedling establishment and in some cases, it can enhance crop yield. The pre-soaking of seeds allows the hydration of membranes and proteins, and the initiation of various metabolic systems. The results of the present study shows that pre sowing, chilling of seeds improved the growth of both cultivars under normal non -saline conditions. The alleviation of adverse effects of adverse effects of salt stress by pre chilling in some species of Pearl millet has also been observed to some extent, e.g. *Sorghastrum nutans* (Indian grass), *Brassica juncea* (Brown mustard) and *Pastinaca sativa* (Parsnip).

While osmo- conditioning of Pearl millet seeds with NaCl did not alleviate the adverse effect of salt stress on the vegetative growth or germination of cultivars, priming with PEG improved germination of both, cultivation under salt stress.

Hydro – priming also did not increase the germination or vegetative growth of two Pearl -millet cultivars compared with non- treated seeds under both saline and non – saline conditions. Further research is needed to optimize the effectiveness of chilling treatment at different time intervals, using a number of cultivars of Pearl millet, or in combination with other agents.

Conclusion

Overall, it is concluded that substantial variation in early vegetative stage salinity tolerance among bajra or pearl millet entries was found in this study and several relatively salinity tolerant and sensitive pearl millet entries for shoot and root germination were identified. The NaCl concentration in the shoot were proposed as a potential proxy for phenotyping pearl millet or bajra genotypes for salinity tolerance as this trait was found to be well related to the ratio of shoot and root growth. However, further investigation would be needed before using this trait as a screening measure.

Seed germination under salinity as that of control had proved to be a potential trait for discarding sensitive entries initially. Early shoot or root growth of seedlings in response to salinity may not be useful as traits for selection as they were not related to biomass productivity at anthesis. Further work is under progress to elucidate the physiological and genetic mechanisms of salinity response and to implement a marker-assisted selection program for salinity tolerance in pearl millet.

The present study investigated that the increasing NaCl concentration effected seed germination, germination rate, plumule length and radicle length. Salinity effect under field condition may not be the same due to variation in environment and soil condition, so field experiment should be conducted to confirm the results. It was also concluded that substantial variation in early vegetative stage, salinity tolerance among Pearl millet

entries was found and several relatively salinity tolerant and sensitive Pearl millet entries for shoot and root germination were identified.

Paper Contributor

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