IJBPAS, January, 2024, 13(1): 49-60

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

EFFECT OF SALT STRESS ON BIOMOLECULES OF KODO MILLETS

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Received 19th Oct. 2022; Revised 16th Nov. 2022; Accepted 13th April 2023; Available online 1st Jan. 2024

https://doi.org/10.31032/IJBPAS/2024/13.1.7546

ABSTRACT

Under salt and water stress, the activity of acid phosphatase and alkaline phosphatase in the leaves of two cultivars of the important plant kodo millet was examined. With one exception, as the level of water stress increased, both phosphatases' activity increased. Increased activity is believed to facilitate the intracellular release of inorganic phosphates, which may help the plant adapt to water stress conditions by controlling appropriate plant metabolism.

Keywords: Kodo millet, salt stress, water stress, alkaline phosphatase, metabolism

INTRODUCTION:

Millets are a staple grain for cereal in poorer nations. Since they are utilised as both human food and cow fodder, they are particularly important in the semi-arid subtropical regions of Asia and Africa **[1]**. Small grain crops called millets are grown in undeveloped areas **[2]**. When compared to other cereal crops, millets are renowned for having a high nutritious content **[3]**. According to their protein composition, millets have higher quantities of methionine and other crucial amino acids **[4]**. According to a few studies, millets are also rich in phytochemicals and micronutrients **[5]** that have positive health effects. For instance, resistant starch, soluble and insoluble dietary fibres, and antioxidant enzymes are all abundant in pearl millet [6].

According to biochemical profile **[7]**, pearl millet has 62 percent carbohydrates, 13 percent protein, 7 percent lipids, 2 percent crude fibres, and 92 percent dry matter. Foxtail millet is used as a protein

supplement for other cereals because it provides the vital amino acid lysine **[8]**. Finger millet contains significant amounts of polyphenols and other essential phytochemicals **[9]**, as well as calcium, methionine, tryptophan, fibre, and sulfur-containing amino acid residues **[10]**. Finger millet contains all of the following nutrients: minerals (2%), crude fibres (4%), protein (9%), and carbohydrates (81%) **[11]**. Finger millet has a higher nutritional and fibre content than rice and wheat.

The goal of this study is to ascertain how stress affects Kodo millet kinds' development, protein, and enzyme activity during seed germination and plant emergence.

Methodology:

Sample collection and germination percentage:

The samples came from Bahraich and Amroha, two separate regions. A sample was received from the Amazon website.

The seeds are put on petri plates, disinfected for 15 minutes with 5% sodium hypochlorite, stirred, drained, and then cleaned four times with sterile deionized water. Sterile forceps was used to carefully plant the seeds evenly spaced out in the petri dish, then time was noted. Seed-filled petri plates were placed in a dim location with consistent room temperature (73 °F). Seeds are considered to be germinated when radicle has emerged approximately ≥ 2 mm. Germination percentage is recorded every 24 h for 6 days. Percentage of germination was recorded accordingly:

Germination Percentage = seeds germinated/total seeds x 100 **[12]**

Stress treatments:

The salt stress- were used in the current study's varied parameters. The samples were divided into two different portions, and the parameters were divided into three parameter categories (light stress, moderate stress, and severe stress) (roots and shoots).

In order to introduce stress during seed germination and seedling growth, sterilized seeds were additionally exposed to a variety of NaCl salt concentrations (50mM, 250mM, and 500mM) as well as water (v/v) concentrations (10%, 50%, and 100%). Additionally, sterilized seeds were first put in sterile petridishes (7 cm in diameter) lined with two sterile filter papers before being inserted to either the proper salinity solutions for stress treatments or 5 ml of distilled water as a control experiment. After that, the seeds were given three hours to soak in the corresponding NaCl salt solutions in sea water **[13]**.

Effect of stress on protein andenzyme activity:

Determination of total protein content:

Samples were used as the control experiment for the estimate of endogenous protein, whereas samples treated with NaCl

salt and water were used as stress treatments. Bradford's method was also used to estimate protein contents **[13]**.

Amylase (α-amylase and β-amylase)

According to the method of **[33]**, amylases were measured calorimetrically by counting the amount of maltose released from starch. After incubating at 37°C for 15 minutes with 1 ml of enzyme added, 1 ml of DNS solution was added to the 1 ml of starch solution (1%). 5 ml of distilled water was added to the solution after it had been incubated at 100°C for an hour. At 540 nm, an OD was taken **[14]**.

Peroxidase

According to the procedure, the peroxidase activity was measured **[14]**. 1ml of the enzyme was then ingested. Then, in a tube, 1.4 ml of phenol solution and 1.5 ml of H_2O_2 were added. Following an hour of incubation at 25°C, an OD reading at 510 nm was recorded at a 2-minute interval **[15]**.

Alkaline phosphatase:

The reaction's substrate was 2.5 mM p-nitrophenylphosphate (pNPP) in a buffer of 50 nM NaOH-glycine (pH 10.0) and 0.5 mM $CaCl_2$. A final volume of 300 l was maintained after adding 100 l of plant enzyme to the well. The substrate used was 2.5 mM p-nitrophenylphosphate (pNPP) in 50 nM NaOH-glycine (pH 10.0) buffer and contained 0.5 mM $CaCl_2$ **[15]**.

Acid phosphatase:

By comparing the absorption at 410 nm to a standard curve of diluted p-nitrophenol solutions and NaOH, activity was determined. A total volume of 300 ul at 55°C was introduced to the well after adding 100 ul of plant enzyme, 37.5 mol of sodium acetate, pH 4.8, 500 nmol of sodium phytate, and enzyme extract. The amount of inorganic orthophosphate produced from this mixture was measured after it had been incubated for one hour **[12]**.

Statistical analysis

To determine whether there are any significant differences between genotypes, the analysis of variance was developed. According to the methods recommended **[14]**, it was done using the randomised complete block design procedure for each character. Three components, i.e., the total variance, degree of freedom, and replication, therapies, and mistakes.

RESULTS AND DISCUSSIONS:

Germination percentage:

Kodo seeds purchased from Bahraich had a higher propensity to germinate (80%), whereas kodo seeds purchased from Amezon.in and Amroha have lesser propensities, 30% and 60%, respectively. Kodo millet seed was bought from three separate locations. Samples were placed in a germination-friendly environment **(Figure 1)**.

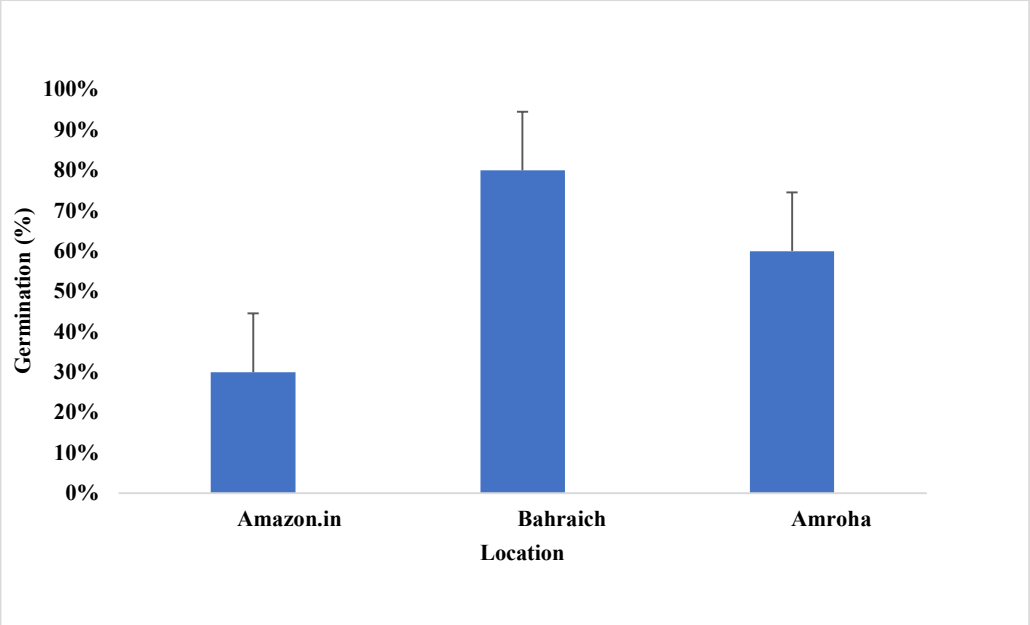

Figure 1: Kodo millet seed germination. Sample 1 germination of Kodo millet seed purchased from Amazone.in, sample 2 germination of kodo millet seed purchased from Bahraich, sample 3 Kodo millet seed germination purchased from Amroha

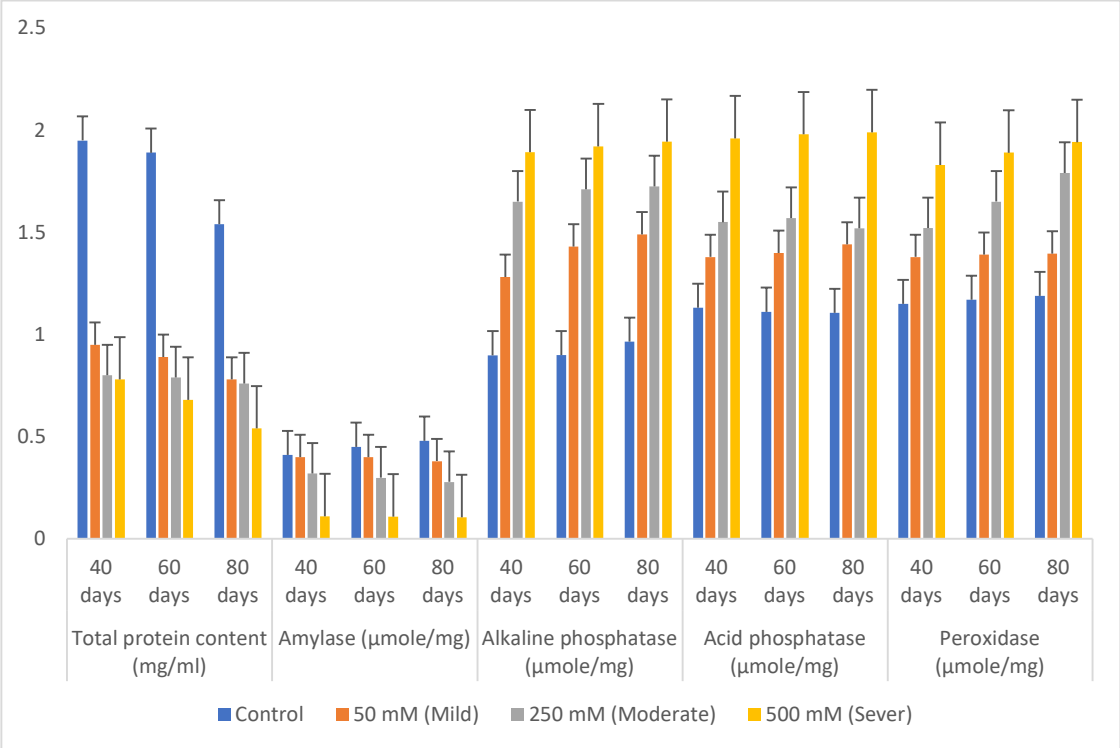

Figure 2: Effect of salt stress on enzyme activity of amazon samples

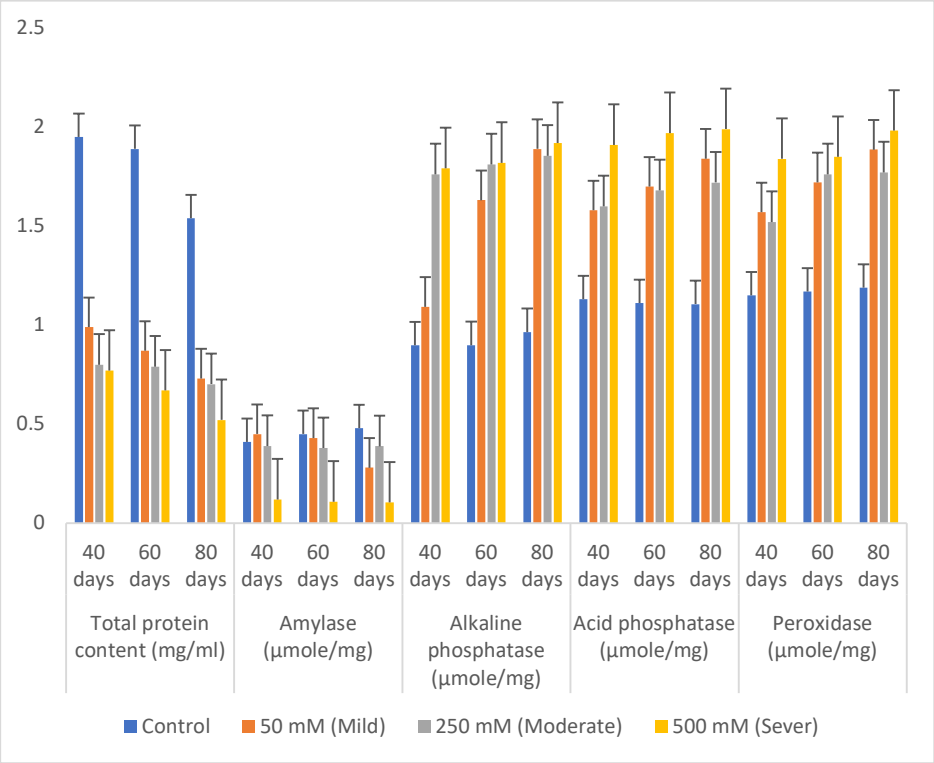

Figure 3: Effect of salt stress on enzyme activity of Bahraich samples

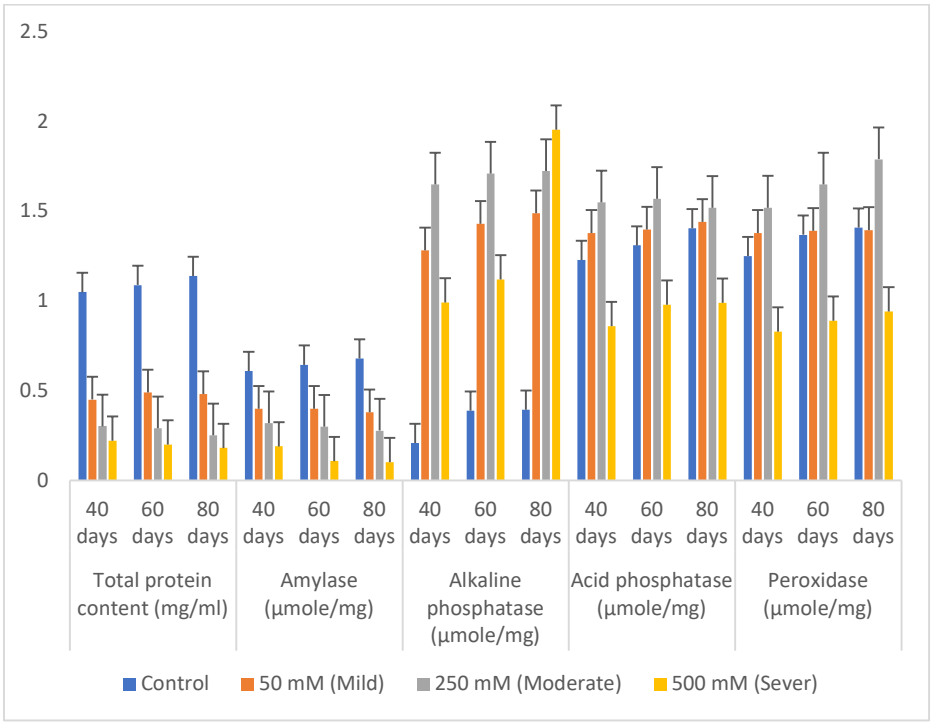

Figure 4: Effect of salt stress on enzyme activity of Amroha samples

DISCUSSION:

Kodo millet purchased from Amazon.in was treated with salt (NaCl), salt was categorized in three form mild (50Mm), moderate (250 mM), severe (500 mM). After 40, 60, and 80 days of treatment, different stress enzyme activity and protein concentration in Kodo millet were found. The results are shown in **Figure 2**. The results indicated that the total protein content decreased in comparison to the control with increasing salt dose (in mM) and exposure period (in days). Protein concentrations at 50 Mm (mild salt) were 0.95 mg/ml, 0.89 mg/ml, and 0.78 mg/ml after 40, 60, and 80 days of exposure, respectively.

On 40, 60, and 80 days of treatment, respectively, it was discovered that the protein concentration at 250 Mm (moderate salt) was 0.80 mg/ml, 0.79 mg/ml, and 0.76 mg/ml. Protein concentrations on 40, 60, and 80 days of treatment with 500 Mm (severe salt) were 0.78 mg/ml, 0.68 mg/ml, and respectively. Similar outcomes were obtained in an experiment conducted in 2006 by Hatice Gulen *et al*. **[14]**. Salt stress causes cellular damage that reduces the production of proteins **[17]**. This drop may have been brought on by the increased activity of acid and alkaline proteases under NaCl stress to maintain osmotic stress **[18]**.

It was also established how salt stress affected the activity of the amylase enzyme. The outcomes were shown in **Figure 2**. According to the results, the amylase enzyme's activity decreased as salt content and exposure time increased. Amylase activity at 50 Mm (mild salt) was 0.400mole/mg, 0.400 mole/mg, and 0.380 mole/mg on 40, 60, and 80 days of exposure, respectively. Amylase activity at 250 Mm (moderate salt) was 0.319 mole/mg, 0.299 mole/mg, and 0.278 mole/mg on 40, 60, and 80 days of exposure, respectively. After 40, 60, and 80 days of exposure, the amylase activity at 500 Mm (severe salt) was 0.110, 0.108 and 0.105 mole/mg, respectively. R Sangeetha *et al*. 2013 looked at similar outcomes.

The effect of salt stress on the activity of the alkaline phosphatase enzyme was also investigated. The outcomes are displayed in figure 3. According to the observations, alkaline phosphatase enzyme activity rose as salt concentration and exposure time increased. Alkaline phosphatase at 50 Mm (mild salt) was 1.282mole/mg, 1.431mole/mg, and 1.490mole/mg on 40, 60, and 80 days of exposure, respectively. Alkaline phosphatase at 250 Mm (moderate salt) was 1.651mole/mg, 1.711mole/mg, and 1.725mole/mg on 40, 60, and 80 days of exposure, respectively. After 40, 60, and 80 days of exposure, sequential measurements of alkaline phosphatase activity at 500 Mm (severe salt) were 1.8920.080mole/mg, 1.9210.056mole/mg, and 1.944mole/mg.

It was also investigated how acid phosphatase enzyme activity changed in response to salt stress. The results showed that increasing salt concentration and exposure time increased acid phosphatase enzyme activity. Acid phosphatase at 50 Mm (mild salt) was 1.380 mole/mg, 1.570 mole/mg, and 1.520 mole/mg on 40, 60, and 80 days of exposure, respectively. Acid phosphatase at 250 Mm (moderate salt) was 1.550 mole/mg, 1.570 mole/mg, and 1.520 mole/mg on 40, 60, and 80 days of exposure, respectively. After 40, 60, and 80 days of exposure, the acid phosphatase activity at 500 Mm (severe salt) was 1.9600.05mole/mg, 1.980mole/mg, and 1.990mole/mg, respectively.

Similar results were obtained by Aahmad *et al*. (2011), **[16]** who investigated the hypothesis that salinity stress increased the activity of both acid phosphatase and alkaline phosphatase. When salt stress was present, phosphate levels in leaves significantly decreased, according to measurements. These results suggest that phosphorus deficiency may be the cause of the stimulation of acid phosphatase and alkaline phosphatase in response to salt stress **[17]**.

The effect of salinity stress on the activity of the peroxidase enzyme was also investigated. The data are displayed in histogram 1. According to the research, peroxidase enzyme activity increased as salt level and exposure time increased. After 40, 60, and 80 days of exposure, the peroxidase activity at 50 Mm (mild salt) was 1.380, 1.391, and 1.396 mole/mg, respectively. Peroxidase at 250 Mm (moderate salt) was 1.521mole/mg, 1.890 mole/mg, and 1.791 mole/mg after 40, 60, and 80 days of exposure, respectively. After 40, 60, and 80 days of exposure, peroxidase activity at 500 Mm (severe salt) was 1.830mole/mg, 1.890mole/mg, and 1.942mole/mg. Similar result was achieved by Nusrat Jabeen and Rafiq Ahmad 2012 **[18]**.

Kodo millet that was gathered from Baharich was subjected to similar methods. The results are shown in histogram 2. According to data, protein content decreases with time as salt stress increases. Protein concentrations at 50 Mm (mild salt) were 0.990mg/ml, 0.870 mg/ml, and 0.730 mg/ml, respectively, on 40, 60, and 80 days of exposure. On 40, 60, and 80 days of treatment, respectively, it was discovered that the protein concentration at 250 Mm (moderate salt) was 0.800mg/ml, 0.790mg/ml, and 0.702mg/ml. Protein concentrations on 40, 60, and 80 days of treatment with 500 Mm (severe salt) were, respectively, 0.770mg/ml, 0.670 mg/ml, and 0.521 mg/ml.

Amylase activity in Kodo millet obtained from Baharich at 50 Mm (mild salt) on 40, 60, and 80 days of exposure was 0.450,

0.430, and 0.280 mole/mg, respectively. Amylase activity at 250 Mm (moderate salt) was 0.389mole/mg, 0.379mole/mg, and 0.388mole/mg on 40, 60, and 80 days of exposure, respectively. After 40, 60, and 80 days of exposure, the amylase activity at 500 Mm (severe salt) was 0.120, 0.108, and 0.104 mole/mg, respectively.

Alkaline phosphatase levels in Baharaich Kodo millet at 50 Mm (mild salt) on 40, 60, and 80 days of exposure were 1.092mole/mg, 1.631mole/mg, and 1.790mole/mg, respectively. Alkaline phosphatase at 250 Mm (moderate salt) was 1.861mole/mg, 1.911mole/mg, and 1.955mole/mg on 40, 60, and 80 days of exposure, respectively. After 40, 60, and 80 days of exposure, the alkaline phosphatase activity at 500 Mm (severe salt) was 1.872mole/mg, 1.9600.053mole/mg, and 1.980mole/mg, respectively.

On 40, 60, and 80 days of exposure, acid phosphatase at 50 Mm (mild salt) were 1.580±0.040 µmole/mg, 1.699±0.035 µmole/mg, and 1.841±0.010µmole/mg, respectively. On 40, 60, and 80 days of exposure, acid phosphatase at 250 Mm (moderate salt) were 1.600±0.013 µmole/mg, 1.680±0.036 µmole/mg, and 1.720±0.034µmole/mg, respectively. Acid phosphatase activity at 500 Mm (severe salt) was 1.910±0.040 µmole/mg, 1.970±0.079 µmole/mg, 1.990±0.022 µmole/mg after 40, 60, and 80 days of exposure, orderly.

At 50 Mm (mild salt), peroxidase activity was 1.570±0.076 µmole/mg, 1.721±0.010 µmole/mg, and 1.886±0.010 µmole/mg, respectively, after 40, 60, and 80 days of exposure. On 40, 60, and 80 days of exposure, peroxidase at 250 Mm (moderate salt) were 1.521±0.012 µmole/mg, 1.761±0.02 µmole/mg, and 1.871±0.021 µmole/mg, respectively. Peroxidase activity at 500 Mm (severe salt) was 1.840±0.01 µmole/mg, 1.850±0.042 µmole/mg, 1.982±0.020 µmole/mg after 40, 60, and 80 days of exposure.

On Kodo millet that was gathered in Amroha, similar treatments were revealed. The results are shown in histogram 3. According to data, protein content decreases with time as salt stress increases. Protein concentrations at 50 Mm (mild salt) were 0.451mg/ml, 0.391mg/ml, and 0.382mg/ml, respectively, on 40, 60, and 80 days of exposure. After 40, 60, and 80 days of treatment, the protein concentration at 250 Mm (moderate salt) was determined to be 0.302mg/ml, 0.291mg/ml, and 0.252 mg/ml, respectively. Protein concentrations on 40, 60, and 80 days of treatment with 500 Mm (severe salt) were, respectively, 0.221mg/ml, 0.200mg/ml, and 0.181 mg/ml.

Amylase activity in Kodo millet harvested from Baharich at 50 Mm (mild salt) on 40,

60, and 80 days of exposure was 0.500mole/mg, 0.450mole/mg, and 0.380mole/mg, respectively. Amylase activity at 250 Mm (moderate salt) was 0.319 mole/mg, 0.299 mole/mg, and 0.278 mole/mg on 40, 60, and 80 days of exposure, respectively. After 40, 60, and 80 days of exposure, the amylase activity at 500 Mm (severe salt) was 0.190, 0.108, and 0.102mole/mg, respectively.

Alkaline phosphatase levels in amrohaKodo millet at 50 Mm (mild salt) on 40, 60, and 80 days of exposure were 1.282mole/mg, 1.431mole/mg, and 1.490 mole/mg, respectively. Alkaline phosphatase at 250 Mm (moderate salt) was 1.651mole/mg, 1.711mole/mg, and 1.725mole/mg on 40, 60, and 80 days of exposure, respectively. After 40, 60, and 80 days of exposure, the average alkaline phosphatase activity at 500 Mm (severe salt) was 0.992, 1.121, and 1.956mole/mg.

Acid phosphatase at 50 Mm (mild salt) was 1.380mole/mg, 1.399mole/mg, and 1.441mole/mg on 40, 60, and 80 days of exposure, respectively. Acid phosphatase at 250 Mm (moderate salt) was 1.550 mole/mg, 1.570mole/mg, and 1.520 mole/mg on 40, 60, and 80 days of exposure, respectively. After 40, 60, and 80 days of exposure, the acid phosphatase activity at 500 Mm (severe salt) was 0.860mole/mg, 1.970mole/mg, and 0.980mole/mg, respectively.

The peroxidase activity at 50 Mm (mild salt) was 1.380, 1.391, and 1.396 moles/mg after 40, 60, and 80 days of exposure, respectively. After 40, 60, and 80 days of exposure, peroxidase at 250 Mm (moderate salt) measured 1.521 mole/mg, 1.651 mole/mg, and 1.791 mole/mg, respectively. Peroxidase activity at 500 Mm (severe salt) was 0.830mole/mg, 0.890mole/mg, and 0.942mole/mg after 40, 60, and 80 days of exposure.

Kodo millet from amazon.in, Amroha, and Baharaich showed nearly identical protein content, but with regard to amazon.

The BaharaichKodo millet has a high amylase and peroxidase activity in kodo millet. Compared to Amezon.in, the alkaline and acid phosphatase activity of Baharaich kodo millet was lower.

CONCLUSION:

The results of this investigation on Kodo millet (*Paspalum scrobiculatum* L.) show a considerable distinction between the two stress types (NaCl and water treatments). The results indicated that the total protein content decreased in comparison to control with increasing doses (in mM) of salt and water stress and exposure period (in days). Results suggested that Kodo millet's amylase enzyme activity decreased as salt content and exposure time increased. Alkaline phosphatase, acid phosphatase, and peroxidase enzyme activity increased as salt content and exposure time were

increased. Kodo Millet seeds from various locations were all harvested, and they all had varying levels of protein and stress enzyme activity under the same water stress conditions.

REFERENCES:

[1] Adhikari, N.D., I. Simko and B. Mou. 2019. Phenomic and physiological analysis of salinity effects on lettuce. Sensors, 19(21): 4814-4819.

[2] Baghalian, K., A. Haghiry, M.R. Naghavi and A. Mohammadi. 2008. Effect of saline irrigation water on agronomical and phytochemical characters of chamomile (*Matricari arecutita* L.). Scientia Hort., 116(4): 437-441.

[3] Bhaskaran, J. and R. Panneerselvam. 2013. Accelerated reactive oxygen scavenging system and membrane integrity of two Panicum species varying in salt tolerance. Cell Biochem. & Biophys., 67(3): 885-892.

[4] Brahmachari, K., S. Sarkar, D.K. Santra and S. Maitra. 2019. Millet for food and nutritional security in drought prone and red laterite region of Eastern India. Int. J. Plant Soil Sci., 26(6): 1-7.

[5] Chandrasekara, A. and F. Shahidi. 2010. Content of insoluble bound phenolics in millets and their contribution to antioxidant capacity. J. Agri. Food Chem., 58(11): 6706-6714. Chen, Z., I.I. Pottosin, T.A. Cuin, A.T.

[6] Fuglsang, M. Tester and D. Jha. 2007. Root plasma membrane transporters controlling K+ /Na+ homeostasis in salt-stressed barley. Plant Physiol., 145: 1714-1725.

[7] Cuin, T.A. and S. Shabala. 2007. Compatible solutes reduce ROS-induced potassium efflux in Arabidopsis roots. Plant Cell Environ., 30: 875-885.

[8] Hu, J., X. Hu, H. Duan, H. Zhang and Q. Yu. 2021. Na and K homeostasis is important for salinity and drought tolerance of (*Calligonummon golicum* L.). Pak. J. Bot., 53(6): 1927-1934.

[9] Jaleel, C.A., P. Manivannan, B. Sankar, A. Kishorekumar and R. Panneerselvam. 2007. Calcium chloride effects on salinity induced oxidative stress, proline metabolism and indole alkaloid accumulation in Catharanthus roseus. C.R. Biol., 330: 674-683.

[10] Khan, M.A. and S. Gulzar. 2003. Light, salinity, and temperature effects on the seed germination of perennial grasses. Amer. J. Bot., 90: 131-134.

[11] Kumar, R., V. Goyal and M.S. Kuhad. 2005. Influence of fertility-salinity interactions on growth, water status and yield of Indian mustard (*Brassica juncea*). Ind. J. Plant Physiol., 10: 139-144.

[12] Lashari, M.S. 2014. Biochar-based soil amendment as an ecotechnology for restoration of salt affected soil: A case study in Shangqiu Henan, China. Thesis submitted to Nanjing Agricultural University, Nanjing China, p. 1-137.

[13] Lashari, M.S., B.N, Mangan, I. Rajper, J. Haishi, G. Pan, A.S. Lashari and J. Nan. 2018. Improvement of soil fertility and crop yield through biochar amendment from salt affected soil of central china. J. Agri. Sci. Tech., 8: 209-219.

[14] Lashari, M.S., Y. Liu, L. Li, W. Pan, J. Fu, G. Pan, J. Zheng, J. Zheng, X. Zhang and X. Yu. 2013. Effects of amendment of biochar-manure compost in conjunction with pyroligneous solution on soil quality and wheat yield of a salt-stressed cropland from Central China Great Plain. Field Crops Res., 144: 113-118.

[15] Lashari, M.S., Y. Ye, G. Pan, H. Ji, L. Li, W.K. Grace, H. Lu and J. Zheng. 2015. Biochar-manure compost in conjunction with pyroligneous solution alleviated maize salt stress and improved growth in a salt-affected soil from Central China: A two-year field experiment. J. Sci. Food Agric., 95: 1321-1327.

[16] Li, S.H., X. Xu and H.X. Hui. 2000. Salinity stress on the physiological and agronomic traits of wheat. J. Triticeae Crops, 20(4): 63-67 (in Chinese).

[17] Li, S.H., X. Xu and H.X. Hui. 2002. Study on K+, Na+ selective absorption of different organs of spring wheat under soil saline sodic stress in different growth season. Acta Botanica Boreali-occidentalia Sinica 22 (3): 587-594 (in Chinese).

[18] Lu, H., M.S. Lashari, X. Liu, H. Ji, L. Li, J. Zheng, G.W. Kibue, S. Joseph and G. Pan. 2015. Changes in soil microbial community structure and enzyme activity with amendment of biochar-manure compost and pyroligneous solution in a saline soil from Central China. Europ. J. Soil Biol., 70: 67-76.

[19] Lutts, S., M. Almansouri and J.M. Kinet. 2004. Salinity and water stress have contrasting effects on the relationship between growth

and cell viability during and after stress exposure in durum wheat callus. J. Plant Sci., 167(1): 9-18.

[20] Mahajan, S. and N. Tuteja. 2005. Cold, salinity and drought stresses: An overview. Arch. of Biochem. Biophys., 444: 139-158.