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Repercussions of Mn-Zn against sodium chloride on morphological and physiological persuit in *Vigna radiata* L. Wilczek (Mungbean)

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Abstract

Biosphere comprises of various biotic and abiotic stresses in which abiotic stresses like salinity stress affects the floral productivity. The broadly observed abiotic stress is salinity stress which plagued soil fertility, growth and survival of plants. Salinity mainly overexpressed in arid and semi-arid region that erodes from rocks where water flyoff above yearly precipitation. Due to excessive salt present in soil and groundwater, the physiological, biochemical and morphological change take place like stunted growth, chlorosis, change in shoot-root ratio. Under the influence of salinity, ion toxicity occurs in plants; as a result of which reactive oxygen species or active oxygen species release as a byproduct. Nutrients that are present in soil whose uptake in plants ensures its protection against damage, thus considered as defense army to plants. To overcome these stresses, plants utilize mostly these nutrients like; manganese (Mn) and Zinc (Zn) along with operation of oxidative and antioxidative mechanisms in which various enzymes gets activated as catalase, peroxidase etc. Legumes are the core element of imperishable tillage and with its help the productivity and economy persist for longer period. Legumes are grown more widely in mulching and crop rotation due to its aptness for nitrogen fixation with communal interaction with *rhizobia*. In the present study, we chose two genotypes IPM 2K-14-9 and IPM 410-3 of *Vigna radiata* L. (mungbean) and observed it's morphological and physiological changes under the concentrations of Mn, Zn against 150mM of NaCl and data collected for 30 and 60DAT; various parameters were quantified like shoot length, chlorophyll estimation, relative water content (RWC), electrolyte leakage (EL), catalase, peroxidase and proline. The outcome of shoot length, chlorophyll content, RWC were found decreased; whereas the grades of electrolyte leakage, catalase, peroxidase and proline were found increased, when treated individually with Mn and Zn, on the other side there was an increase above mentioned parameters like shoot length, chlorophyll estimation, RWC and decrease in EL, catalase, peroxidase and proline when cumulative (Mn-Zn) study was quantified.

Keywords: Salinity stress, chlorosis, reactive oxygen species, mulching, *Rhizobia*

Introduction

A broad area of different biotic and abiotic components that are present in the environment in which if there is increase in the amount beyond its optimum level, then consider as stress for plants and animals. Several types of stresses such as high temperature, chilling, drought, salinity, pathogens, alkalinity, ultraviolet rays, etc. that likely to be threatening for flora reported by Van Breusegem *et al*. 2001. The viability of agricultural land area in arid and semi-arid locality is mainly influence by several stresses by which the growth and development of flora in that particular region is affected as a result of which the productivity and yield of flora is limited. In the line of various stresses, the salinity stress can be considered as a crucial stress through which the growth and productivity of land area is altered. Many a times, salinity triggered the rest of the issues like alkalinity and sodicity into the soil. The resultant of soil sodicity is the accumulation of Na^+ ions to negative ions of clay particles, thus the outcome is dissemination and protuberance of clay particles; whereas the association of sodium-clay come up with the alkalinity of soil. Hence, the salinity of soil is an utmost parameter which alters the feasibility of productive land.

Food and Agricultural Organization (FAO) in 2005 [3] reported that globally around 800million hectare land area is affected by salt in the form of either salinity (397 million hectare) or sodicity associated (434 million hectare). The mentioned figure is around 6% of world's landscape. The main cause of soil salinity is the notable increase in irrigation and clearing of land in regards with cultivated landscape. FAO 2005 [3] stated that currently 230 million hectare of irrigated landscape, 45 million hectares that is around 20% are affected with salt. Munns, 2005 [14] reported that in the area of total cultivated land only 15% is irrigated, but on the other hand irrigated area comprises at least doubles the output of rain-fed area, it constitutes 1/3rd of global food. The measuring SI unit of soil salinity is electrical conductivity (EC) is dS m^{-1} and with the help of this unit one can find out exchange of sodium percentage and osmotic stress. The USDA Salinity Laboratory (1954) [19], gives an optimum unit of 4 dS m^{-1} beyond which soil is considered to be saline.

The salt stress alters the agricultural yield of crops also it changes the biochemical properties, in return affects the correlated environmental balance of landscape. The threatening effects of salt stress consists of low reproductive yield, low financial returns in contrast to high cultivation costs, conduct, repossession reported by Hu and Schmidhalter 2002 [6]. Salt stress influence many parameters of plant biomechanical properties, resultly growth reduction takes place. There is a notable drop is seen in the rate of photosynthesis, leaf area index, leaf area expansion, and duration of leaf emerging. The uptake of minerals with the help of roots is also influenced due to ionic-imbalance. Nieman and Mass 1978 [16] suggested that the retardation of growth is due to the distraction of spirit from development and growth to maintenance. The accumulation of various organic elevates in different cell organelle for the process of osmoregulation and also for integrity of membrane reported by Jain *et al*. 2001 [9]. The process of salinity may be triggered by accumulation of salt through root zone, alters land possession practices and application of tolerant plants. Plants can recognize salinity through both of the signals that is osmotic and ionic. The perception of excess sodium ion by transmembrane proteins or inside cellular membranal proteins or sodium ion sensitive proteins reported by Zhu, 2003 [21].

Manganese is a micronutrient that is necessary for plant growth. Plant availability of manganese is dependent on several factors, including soil pH, microbial activity, easily reducible manganese oxides, soil humidity, and redox potential. The production of chlorophyll and photosynthesis, the metabolism of nitrogen and carbohydrates, oxidation, and other processes all depend on manganese.

It has been suggested that zinc (Zn) has a major potential to reduce plant abiotic stress. Zn applied topically improves plant growth and development in adverse environments, including reducing salt stress. This is achieved by increasing chlorophylls, which in turn promotes photosynthesis. Zinc shields the protecting membranes from oxidative and peroxidative damages by maintaining membrane stability, permeability and integrity. Zinc is an essential component of many significant enzymes, stabilizer of proteins, such as ‘zinc finger’ proteins, which bind to DNA.

Vigna radiata L. R. Wilczek (mungbean) is very crucial pulse crop and is grown on >6 million hectare. It is the crop of low input required and have shorter life-span that is around (65 to 90 days) reported by Nair *et al*. 2012 [15] whereas Lawn and Ahn, 1985 [10] stated that sowing of crop over span of latitudes is 40^0 north or south along with quotidian temperatures of weather are > 20^0 Celsius. India got the highest ranking in legumes production and consumption around 65% and 54% respectively. Similar to other legumes, mungbean also play foremost role in fixing atmospheric nitrogen into nitrates (~58-109kg/ha) in association of *Rhizobium*, that helps in itself by fixing its nitrogen and also nourishes its following crops mentioned by Ali and Gupta 2012 [2]. Mungbean is frequently cultivated as fallow crop means in association of mungbean-rice-wheat or/and as relay crop along with cereal cropping systems. It is very important for ameliorating the fertility of soil and have high dietary ratios; also stand in need of less water reported by Parida and Das 2005 [17]. Ihsan *et al*, 2013 [8] stated that, mungbean comprises of very less quantity of oligosaccharides and ~23% of protein source with lofty amount of digestibility. Customarily, salinity influences the plants via ionic and osmotic disbalance but in case of legumes a third event also takes place that is reduction in number and size of root nodules by *rhizobia*, as it is noted that the salinity effects the nodulation property directly or indirectly. On the other hand, feedback of legumes/ other different floral species accountable for convincing the conditions and coverage of stress ferocity. Hence, it is very crucial to increase the yield of legume grains and helps in utilizing the natural wealth adeptly to meet the requirements of nutritious and healthy food for global growing exponential population.

In the current study, we aimed to appraise the effects of different levels of Mn-Zn against 150mM of salt that is sodium chloride (NaCl) on various morphological and physiological parameters such as shoot length, chlorophyll content estimation, relative water content (RWC), electrolyte leakage, catalase, peroxidase and proline in two different bean genotypes along with extensive agrarian use. This is done in order to specify restraining parameters which are helpful for cultivating lineage strategies to revamp the farm yield under the influence of salt stress.

Material and methods

Various materials and methods were performed with slight modifications:

1. Plant material

Two different genotypes (IPM 410 and IPM 2K-14-9) of *Vigna radiata* L. were taken from Indian Institute of Pulse research (IIPR) Kalyanpur Kanpur, were selected for the present experiment and this experiment was based on pot trials in replicates inside the glass house under measured conditions of temperature and sunlight.

2. Treatments and plant growth

There were soaking of lentil seeds has been done for 24 hours, then seeds were lied inside the pots filled with treated sand culture brought up washed against hydrochloric acid; on an average of 15-30 seeds in replicates. First 20 days of sowing, plants were treated with the supplement of Hoagland’s nutrient media (1938) along with double glass distilled water as mentioned by Agarwala and Sharma (1961). Then, 20 DAT young saplings were exposed against 150mM of NaCl (sodium chloride, mol. Wt. 58.44g/mol). The supply of salt stress was continued for 30 and 60 days and then observations were recorded against Mn, Zn and Mn-Zn cumulatively.

3. Estimation of chlorophyll and total carotenoids

The estimation of chlorophyll was performed with the help of Lichtenthalar, 1967. The principal function of chlorophyll pigments is to trap the solar light energy and start the process of photosynthesis that further converts light energy into chemical energy, then that chemical energy is consumed by the plants. Here, the objective to perform chlorophyll estimation is how the chlorophyll pigments functions under the effect of different NaCl concentrations or we can mark as how the pigments react under the stress condition.

The 50mg of fresh weight of material were taken and grind it in 10ml of 80% acetone with a pinch of $CaCO_3$. Then centrifuge it for 15 minutes, pour the supernatant and read it at different wavelengths for 480nm, 510nm, 645nm, 663nm by using spectrophotometer.

Formulae: Chlorophyll a=[12.7(A_{663})-2.63 (A_{645})] X V/1000 x Wt(g)

Chlorophyll b= [22.9(A_{645})-4.68 (A_{663})] X V/1000 x Wt (g)
Carotenoids= [7.6(A_{480})-1.49 (A_{510})] X V/1000 x Wt (g)
In above given formulae A is the optical density (OD) at wavelength indicated and V is the total volume.

4. Catalase

The quantification of Catalase (E.C. 1.11.1.6) was done by the help of Euler and Josephson, 1927 with slight modifications. Depending on the reaction, catalase helps to dismutate H2O2 into water and O_2. Because it catalyzes the following processes, catalase serves two purposes: Hydrogen peroxide breaks down to produce oxygen and water. H_2O_2 H_2O + ½ O_2 catalase 2. The oxidation of H donors, such as phenol, formic acid, and methanol, when one mole of peroxide is consumed.

Materials: Substance blend: pH 7.0 phosphate buffer, 100 ml 0.2 M for stock A Stock B: 0.2M of potassium di hydrogen orthophosphate (KH2PO4; molecular weight: 136.09) = 27.25 g L-1 A 0.1 M buffer is obtained by diluting di potassium hydrogen phosphate (K2 HPO4 molecular weight 174.22) = 34.8 g L-1 39.0A + 61.0B ml to a total of 200 ml.

Procedure: (i) Blank: mixture of 10 ml substrate 50 ml test tube with 5 ml of 2 N H2SO4. Hold at 25°C. 1 milliliter of enzyme extract (max) [ii] Sample: mixture of 10 ml substrate Hold at 25°C. Use one milliliter of the enzyme extract to begin the reaction. As soon as five minutes were completed then to stop the reaction, add 5 ml of 2 N H_2SO_4. Then titrate against $KMnO_4$
and data were recorded.

5. Peroxidase

The activity of peroxidase (POD) (E.C. 1.11.1.7) were quantified on the basis of method described by Luck, 1963 with some slight modifications. The enzyme removes hydrogen from a substrate and combines it with H_2O_2 to catalyze its oxidation. It comprises a set of highly non-specific enzymes from various sources that are referred to as POD and a set of specific enzymes like NAD peroxidase, NADP peroxidase, fatty acid peroxidase, etc.

Reagents: pH 6.0 0.1 M phosphate buffer Stock A: 0.2 M Potassium dihydrogen orthophosphate (KH_2PO_4 molecular weight 136.09) = 27.25 g L-1. Phenyl diamine at 0.5% (0.5 g/100 ml) Hydrogen peroxide at 0.01% N H_2SO_4 5.0. Stock B: 0.2 M di Potassium hydrogen phosphate (K_2HPO_4 molecular weight 174.22) = 34.8 g L-1 87.7A + 12.3 B ml diluted to a total of 200 ml gives 0.1 M buffer.

Protocol: Blank M Phosphate buffer sample, pH 6.0 = 5.0 ml, same as sample + + 0.01% H_2O_2 = 1.0 ml, same as sample + + 0.5% p-phenyl diamine = 1.0 ml, same as sample Hold at 25°C. Use 1.0 ml of enzyme extract to begin the reaction. 5 N H_2SO_4 = 2.0 ml plus 1.0 ml of enzyme extract Add 5 N H_2SO_4 = 2.0 ml after 5 minutes. After 20 to 30 minutes in the refrigerator, centrifuge at room temperature. Measured in a spectrophotometer at 485 nm.

Unit: Variation in 100 mg-1 fresh weight or mg-1 protein optical density

6. Proline

The determination of proline content was estimated by the suggested protocol of Carillo and Gibbon, 2011 with some slight modifications. Around 50mg fresh leaf tissue was homogenized in 1 ml of 70% ethanol to form ethanolic extract. The 100 microlt. Reaction mixture that bears ninhydrin (1% w/v), acetic acid (60%v/v) and ethanol (20%v/v), was mixed with 50 microlt. Of ethanolic extract. After being mixed for the entire night at 40C, the mixture is centrifuged for five minutes at 14000 RPM. For analysis, the cooled supernatant was used.

Result

1. Plant growth

Growth difference in *Vigna radiata* L. became visible after 30 days of NaCl treatment. The difference in growth marked well when NaCl is supplied with Mn, Zn and simultaneous application of Mn and Zn.

Yellowing in leaves showed when only Mn supplied and there is lack of Zn but the yellowing decreased when Mn and Zn are simultaneously applied. Reduction in leaf area also takes place when separate application of Mn and Zn but leaf area and biomass increases when Mn and Zn both applied.

When we analyse our data, we found that IPM 2K-14-9 shows slight bearable response against NaCl concentrations as compared to IPM 410-3 which shows the sensitivity towards the same level of NaCl concentrations.

Table 1: Effect of Mn and Zn against the supply of NaCl on shoot length of *Vigna radiata* L. plants grown in sand culture

Treatment given	**Two different varieties of *Vigna radiata* L. plants with different treatment days and effects on shoot length in cm**			
	IPM 410-3		**IPM 2K-14-9**	
	30DAT	**60DAT**	**30DAT**	**60DAT**
Mn (untreated)	16.02	17.21	18.88	19.23
Mn+NaCl	14.24	15.45	18.02	18.99
Zn (untreated)	17.04	18.95	19.84	20.96
Zn+NaCl	15.88	17.67	17.2	18.04
Mn+Zn(untreated)	19.03	21.99	21.76	22.02
Mn+Zn+NaCl	18.03	19.23	20.13	21.87

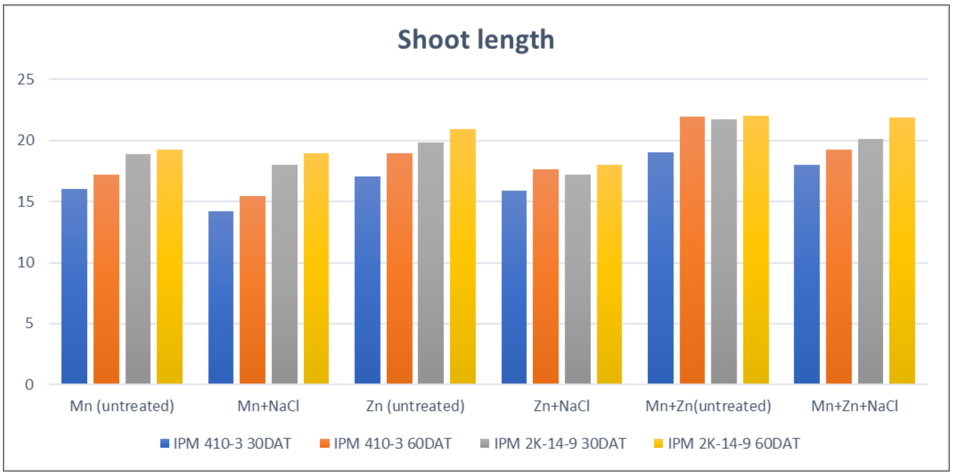

Fig 1: Responses of shoot length on exposure of Mn and Zn against the activity of NaCl in plant *Vigna radiate* L.

2. Total chlorophyll content

The amount of carotenoid and chlorophyll in Vigna radiata leaves was measured. When Mn and Zn were sprayed separately against NaCl concentration, it was shown that the levels of chlorophyll pigments fell; however, when both nutrients were sprayed concurrently against salt stress, the levels of pigmentation rose.

Table 2: Effect of Mn and Zn against the activity of NaCl on total chlorophyll content and carotenoids in plant *Vigna radiata* L. variety IPM 410-3 after 30 and 60 DOT in sand culture

	IPM 410-3				IPM 410-3			
	30DAT				60 DAT			
	chl A	chl B	total chl	carotenoids	chl A	chl B	total chl	carotenoids
Mn (untreated)	1.084	1.07	2.154	5.38	1.124	1.12	2.854	9.33
Mn+NaCl	1.001	1.01	2.011	10.5	1.002	1.08	2.601	10.2
Zn (untreated)	1.274	1.851	3.125	10.8	1.513	1.923	3.781	18.4
Zn+NaCl	1.127	1.135	2.262	15.78	1.412	1.235	3.301	26.6
Mn+Zn(untreated)	1.627	1.88	3.507	14.5	1.836	1.95	4.012	65.4
Mn+Zn+NaCl	1.567	1.218	2.785	17.1	1.651	1.31	3.801	81.2

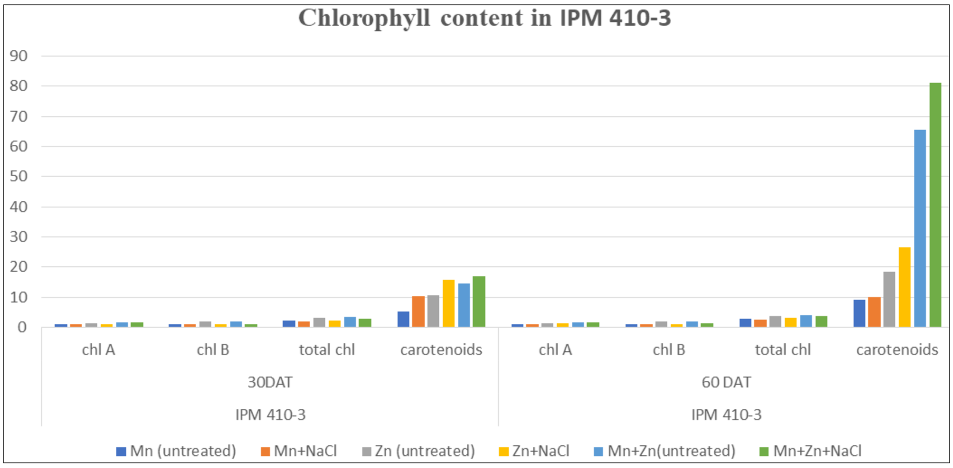

Fig 2: Responses of chlorophyll content on exposure of Mn and Zn against the activity of NaCl in plant *Vigna radiate* L. variety IPM 410-3

Table 3: Effect of Mn and Zn against the activity of NaCl on total chlorophyll content and carotenoids in *Vigna radiate* L. variety IPM 2K-14-9 after 30 and 60 DOT

	IPM 2K-14-9				IPM 2K-14-9			
	30DAT				60DAT			
	chl A	chl B	total chl	Carotenoid	chl A	chl B	total chl	Carotenoid
Mn (untreated)	1.194	1.34	2.534	6.73	1.224	1.34	2.564	10.43
Mn+NaCl	1.142	1.11	2.252	9.43	1.11	1.23	2.34	13.23
Zn (untreated)	1.421	1.934	3.355	9.78	1.413	1.968	3.381	20.79
Zn+NaCl	1.342	1.234	2.576	12.34	1.324	1.398	2.722	28.9
Mn+Zn(untreated)	1.723	1.97	3.693	15.21	1.967	1.98	3.947	68.98
Mn+Zn+NaCl	1.654	1.341	2.995	14.01	1.786	1.42	3.206	85.9

When compared to IPM 2K-14-9, the IPM 410-3's pigmentation is slightly lower, indicating a little more sensitive response.

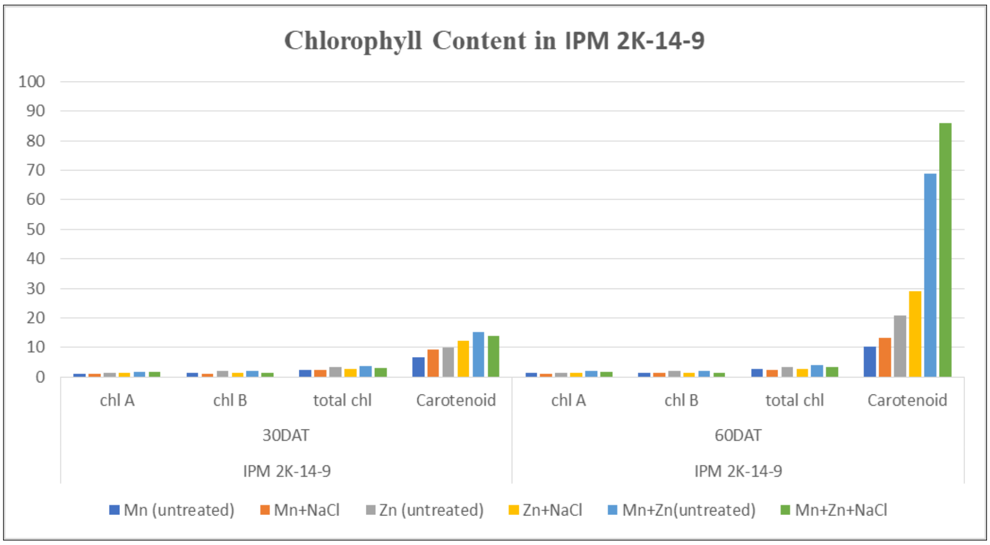

Fig 3: Responses of chlorophyll content on exposure of Mn and Zn against the activity of NaCl in plant *Vigna radiate* L. variety IPM 2k-14-9

3. Catalase

The observations were found as the activity of antioxidative enzyme catalase were found increased as we expose the activity of NaCl but when NaCl was given along with Mn and Zn the activity of catalase is decreased which states that the micronutrients are capable to overcome the stresses. The IPM 410-3 is found as slow serviver against NaCl effect when compared to IPM 2k-14-9. Hence we can say that IPM 410-3 is sensitive and IPM 2k-14-9 is resistant towards NaCl effect.

Table 4: Effect of Mn and Zn against NaCl treatment on the activity of catalase in plant *Vigna radiata* L. variety IPM 410-3 and IPM 2k-14-9 after 30 and 60 DOT

	IPM 410-3		IPM 2K-14-9	
	30 DAYS	**60 DAYS**	**30 DAYS**	**60 DAYS**
Mn(untreated)	5.5	7.8	6.98	8.91
Mn+NaCl	12.9	15.2	14.8	17.1
Zn(untreated)	11.8	14.2	12.6	17.5
Zn+NaCl	10.8	11.8	11.9	13.7
Mn+Zn(untreated)	14.7	18.9	15.8	22.5
Mn+Zn+NaCl	8.9	6.6	7.1	6.4

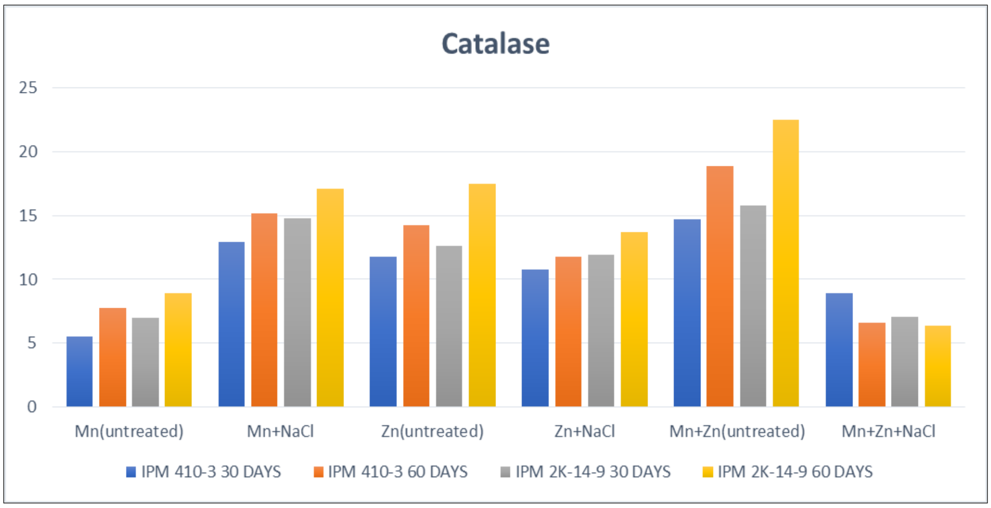

Fig 4: Responses of catalase on exposure of Mn and Zn against the activity of NaCl in plant *Vigna radiate* L.

4. Peroxidase

The observations were found as the activity of antioxidative enzyme peroxidase were found increased as we expose the activity of NaCl but when NaCl was given along with Mn and Zn the activity of peroxidase is decreased which states that the micronutrients are capable to overcome the stresses. The IPM 410-3 is found as slow serviver against NaCl effect when compared to IPM 2k-14-9. Hence we can say that IPM 410-3 is sensitive and IPM 2k-14-9 is resistant towards NaCl effect.

Table 5: Effect of Mn and Zn against NaCl treatment on the activity of peroxidase in plant *Vigna radiata* L. variety IPM 410-3 and IPM 2k-14-9 after 30 and 60 DOT

	IPM 410-3		IPM 2K-14-9	
	30 DAYS	60 DAYS	30 DAYS	60 DAYS
Mn(untreated)	0.657	0.779	0.567	0.788
Mn+NaCl	0.532	0.552	0.502	0.605
Zn(untreated)	0.522	0.616	0.515	0.753
Zn+NaCl	0.445	0.577	0.476	0.632
Mn+Zn(untreated)	0.754	0.749	0.852	0.998
Mn+Zn+NaCl	0.440	0.535	0.407	0.378

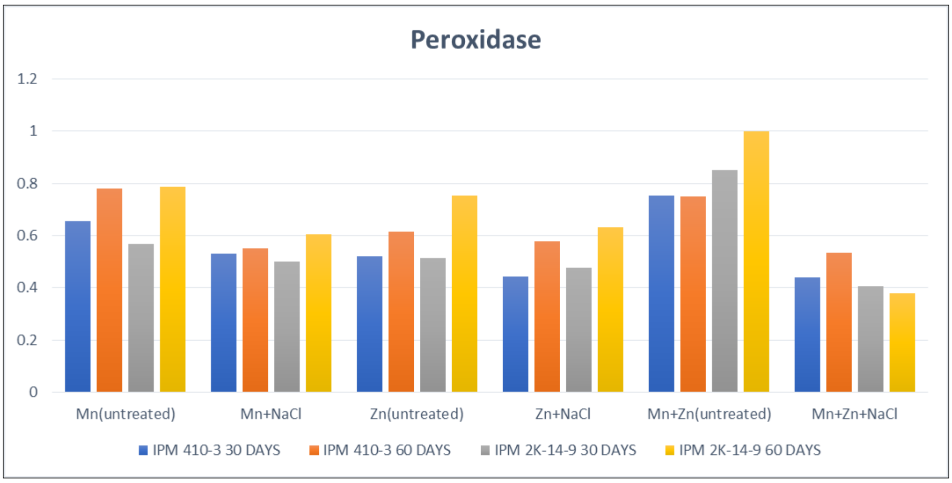

Fig 5: Responses of peroxidase on exposure of Mn and Zn against the activity of NaCl in plant *Vigna radiate* L.

5. Proline

The observations were found as the activity of antioxidative enzyme proline were found increased as we expose the activity of NaCl but when NaCl was given along with Mn and Zn the activity of proline is decreased which states that the micronutrients are capable to overcome the stresses.

The IPM 410-3 is found as slow serviver against NaCl effect when compared to IPM 2k-14-9. Hence we can say that IPM 410-3 is sensitive and IPM 2k-14-9 is resistant towards NaCl effect.

Table 6: Effect of Mn and Zn against NaCl treatment on the activity of proline in plant *Vigna radiata* L. variety IPM 410-3 and IPM 2k-14-9 after 30 and 60 DOT

	IPM 410-3		**IPM 2K-14-9**	
	30 DAYS	**60 DAYS**	**30 DAYS**	**60 DAYS**
Mn(untreated)	1.766	1.76	2.215	2.211
Mn+NaCl	1.578	1.574	2.333	2.329
Zn(untreated)	1.397	1.39	2.438	2.454
Zn+NaCl	1.864	1.862	2.204	2.204
Mn+Zn(untreated)	1.633	1.628	2.247	2.246
Mn+Zn+NaCl	1.571	1.503	1.472	1.419

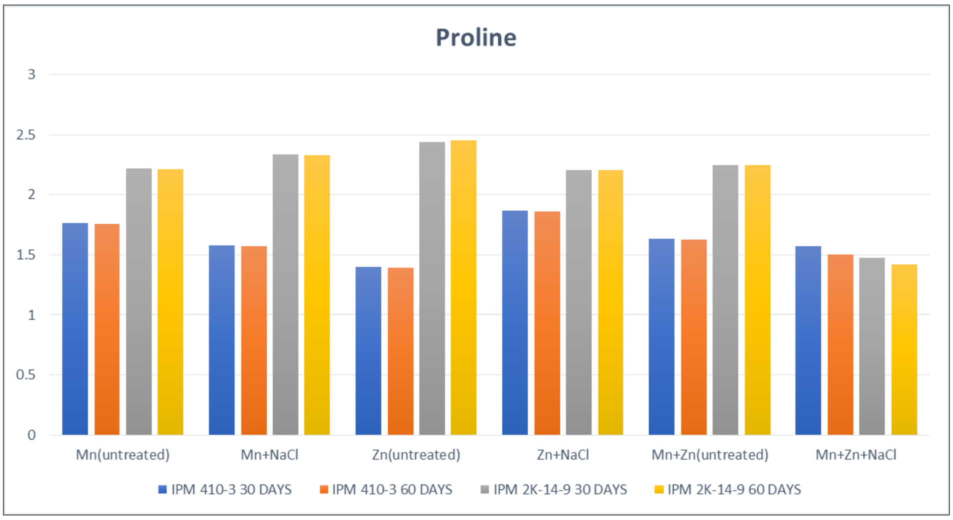

Fig 6: Responses of proline on exposure of Mn and Zn against the activity of NaCl in plant *Vigna radiate* L.

Discussion

In the present study, the responses of two economically important crops- *Vigna radiata* (mung) and *Lens culinaris* (lentil) to Manganese and Zinc nutrition against the NaCl concentration. The salt stress causes a decline in shoot length that some earlier reports on different plant as *Pisum sativum* L. (Husen *et al*. 2016) [7]. The unfavorable effects of NaCl on growth and biomass yield may have been caused by osmotic stress and inadequate uptake of critical nutrients (Hashem *et al*. 2014) [4]. Our data show that when we offer Mn alone as untreated, normal growth occurs, but when we supply Mn coupled with NaCl, shoot length decreases when compared to untreated. The same results were obtained with Zn supply, however when we administered Mn and Zn simultaneously with NaCl, shoot length increased.

This can be noted from photosynthesis regulates both biomolecule production and micronutrient transfer inside plants (Alejandro S. *et al*, 2020) [1]. Maintaining the K+/Na+ ratio is crucial for salt tolerance in plants (Li *et al*. 2012) [11]. During salt stress, maintaining plasma membrane potential reduces Na+ influx through P-ATPases activity, restoring a higher ratio of K+/Na+ in the cytosol (Sun *et al*. 2009) [18].

Soil salt induces hyperionic and hyperosmotic stress, leading to membrane damage, altered growth regulator levels, and inhibited development. It reduces enzyme activity and slows metabolism (Mahajan and Tuteja, 2005) [12]. Increased antioxidant enzyme activity correlates with increased salt tolerance (Mittova *et al*. 2003) [13]. Upregulation of enzymatic antioxidants during salinity stress may reduce reactive oxygen species generation and minimize breakdown of lipids, proteins, and nucleic acids, resulting in optimal photosynthesis (Hichem *et al*. 2009) [5].

Conclusion

Salt stress which is NaCl assess oxidative and osmotic stress, that eventually lead to decline of chlorophyll content and relative water content combining with increase in hydrogen peroxide, lipid peroxidation and electrolyte leakage. But with the exogenous supply of Mn and Zn the chlorophyll content and RWC against NaCl stress increased and hydrogen peroxide, lipid peroxidation and electrolyte leakage decreased when observed with developmental stages of 30 and 60 DAT. The variety of *Vigna radiata* L. IPM 410-3 showed slightly sensitive responses when compared with IPM 2k-14-9. Hence we can conclude that IPM 410-3 is slightly sensitive and IPM 2K-14-9 is slightly tolerating against NaCl stress.

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