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MORPHO-BIOCHEMICAL RESPONSE OF *VIGNA RADIATA* TO SALINITY GENERATED HYDROGEN PEROXIDE STRESS

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Abstract

Salt stress is the most obvious limiting threat to the production of *Vigna radiata* due to the shortage of irrigated lands throughout the world. Thus, current investigation was conducted to appraise the growth performance and seedling survival of 96 hours old NM-92, NM-98, NM-51, NM13-1 and NM19-19 seedlings against salt stress. The whole study was planned in complete randomized design (CRD) in Petri dishes (n=4). Imbibed seeds (24 h) from all genotypes have received 72 hours of 0, 50, 150, 250 and 350 mM NaCl stress. All the five levels of salt gradually enhanced hydrogen peroxide (H_2O_2), and malondialdehyde (MDA), decreased seedlings length (SL), fresh biomass (BIO), and relative water content (RWC), superoxide dismutase (SOD), peroxidase (POX), and catalase (CAT). Among genotypes, the maximum increase in growth and antioxidant enzymes were found in NM-92 seedlings at each concentration of NaCl. Additionally, NM-92 showed lowest H_2O_2 (10.1 µM g^{-1} FW), and MDA (11.2 µM g^{-1} FW) accumulation in its seedling tissues under 350 mM NaCl level. Compared with NM19-19 was shown least improvement in SL, BIO, RWC, SOD, POX, CAT, and greatest in H_2O_2, and MDA at five stress levels of NaCl. Hence, NM-92 could be designated as the salt tolerant genotype of *Vigna radiata*, and expected to give optimum yield over saline irrigated lands.

Keywords: APX, Biomass, CAT, Malondialdehyde, Salt stress, SOD.

Introduction

The world is populous with 7.7 billion peoples and the number is increasing day by day (Anonymous, 2019). These huge populations need food every day with high nutrition to fulfill their dietary demands. While the animal's proteins become expensive, the pulses such as *Vigna radiata* serves as second main legume of Pakistan (Rasul *et al*., 2012), is the excellent alternate of meat. It is a comparatively inexpensive source of high-quality proteins, dietary fiber, carbohydrates, vitamin, minerals and very low in fats (0.7%) (Afzal *et al*., 2008). This valuable and high nutritive pulse has low yield due to the shortage of irrigated land as 75% cultivated lands are occupied by wheat, rice, cotton and sugarcane (Anonymous, 2016). Moreover, about 23% irrigated lands of Pakistan are salinity affected, in which 14% of lands are slight to moderately saline and rest are strongly salines (Anonymous, 2009; Parihar *et al*., 2015). Thus, *Vigna radiata* yield can be maximized by using these saline lands for its cultivation. However, *Vigna* species belong to the class of glycophyte so genotype screening for salinity tolerance is essential.

Under salinity stress, reactive oxygen species (ROS) are produced in a high amount which brings oxidative stress on *Vigna* seed germination and seedling growth. Hydrogen peroxide is the most stable, long-lived and powerful toxic molecules among ROS (Desikan *et al*., 2003). It is produced in an excessive amount in mitochondria, peroxisomes, and chloroplast during photorespiration and photosynthesis process (Aris-Moreno *et al*., 2017; Umer & Siddiqui, 2018). It lowers the water content of protoplasm, and destruct phospholipids of cellular membrane by attacking over the unsaturated double bond of carbons between fatty acid and glycerol (Sharma *et al*., 2012). Thus, the cell has evolved the antioxidant defense mechanism which regulates the levels of hydrogen peroxide at salt stress. This mechanism consists of ascorbate peroxidase, guaiacol peroxidase, catalase, and superoxide dismutase enzymes (Wang *et al*., 2016). These defense enzymes fight against H_2O_2 and alleviate salinity stress that was reported already in various edible crops (Kanwal *et al*., 2013; Chen *et al*., 2018; Umer & Siddiqui, 2018). Thus, the aim of this study is to screen the growth and biochemical performance of five-renowned local genotypes of *Vigna radiata* for their salt tolerance at five levels of salts (0, 50, 150, 250, and 350).

Materials and Methods

The germplasm of *Vigna* genotypes, NM-92, NM-98, NM-51, NM 13-1, and NM 19-19 was obtained from the National Agricultural Research Center (NARC), Pakistan. The whole study was conducted in the laboratory of Genetics Department, University of Karachi, Pakistan. The sterilized seeds (Farheen *et al*. 2018) of all genotypes were soaked in distilled water separately (24 h). Then healthy seeds of each *Vigna* genotype i.e. 30 seeds / treatment / genotypes / replication were placed on 0, 50, 150, 250, and 350 mM NaCl moisten filter paper lined Petri dishes. The seedling length, fresh biomass, and relative water content measured as Farheen *et al*. (2018). The hydrogen peroxide and malondialdehyde (Ashraf *et al*., 2013) were assayed from all genotypes to determine the effect of salt stress. The superoxide dismutase (Sairam *et al*., 2002), peroxidase (Nakano & Asada, 1981), and catalase (Aebi, 1984) enzyme specific activities were calculated through the extinction coefficient.

Statistical assay: The research study was repeated four times in complete randomized design. All biochemical data were calculated by Microsoft office excel 365 and analyzed by multivariate analysis of variance. The Duncan's test (DMRt) was done by IBM SPSS 19 version (IBM SPSS Inc., Chicago, IL, USA) at 5% level of significances.

Results

The results of our investigation clearly showed that from 0 to 350 mM NaCl concentration progressively enhanced H_2O_2 and MDA production more in NM19-19 and NM13-1 then NM-92, NM98, and NM-51 (Figs. 1-2). The lowest level of H_2O_2 (10.08 µM g^{-1} FW) and MDA (11.15 µM g^{-1} FW) were observed in the seedlings of NM-92 even at the highest saline concentration as compared with NM-98, NM-51, NM13-1 and, NM19-19. The induction of 0, 50, 150, 250, and 350 mM salt stress over SL, BIO, and RWC were studied in NM-92, NM-98, NM-51, NM13-1 & NM19-19 after 96 hours. The genotype 'NM-92' showed less reduction in length (5 cm), biomass (3 g) and relative water content (79 %) even under 350 mM level of salt among examined genotypes (Figs. 3-5). Whilst, NM-98, and NM-51 were intermediate for SL, BIO, and RWC under each stress level. In contrast, the highest reduction in growth was noted from NM19-19 at five NaCl treatments. Where the maximum decrease in SL (3 cm), BIO (1.5 g), and RWC (60 %) was recorded at 350 mM NaCl stress in NM19-19.

The SOD, POX, and CAT activities showed significantly different patterns in all *Vigna radiata* genotypes. The gradual enhancement in all antioxidant enzymes with increasing levels of NaCl was observed in five mungbean genotypes. The elevated mean value for SOD was noted with NM-92 followed by NM-98 at five concentrations of NaCl (Fig. 6). While maximum POX and CAT enzymes activity was recorded from NM-92 at each concentration of salt as compared to other genotypes. Figures 7-8 showed that the highest POX (260 fold), and CAT (10 fold) activities were found in NM-92 with 350 mM NaCl. While, lowest SOD, POX and CAT activities exhibited by NM19-19 with 0, 50, 150, 250 & 350 mM NaCl solution among five *Vigna* genotypes.

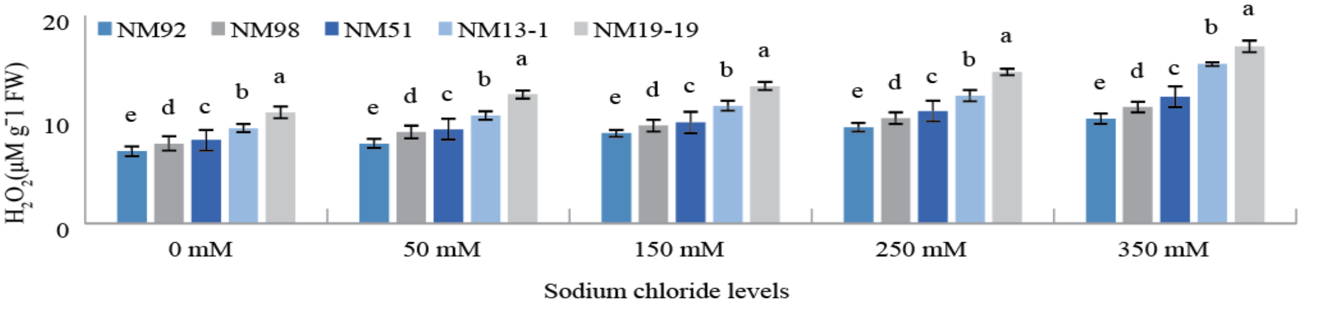

Figure 1. Influence of various levels of sodium chloride (0, 50, 150, 250, and 350 mM sodium chloride) on H_2O_2 of five mungbean varieties namely NM-92, NM-98, NM-51, NM13-1, and NM19-19. DMR test means values are shown on each standard error bar (n=4) in the form of letters [a-e]. Means with a different letter are significant at 5 % level of significance.

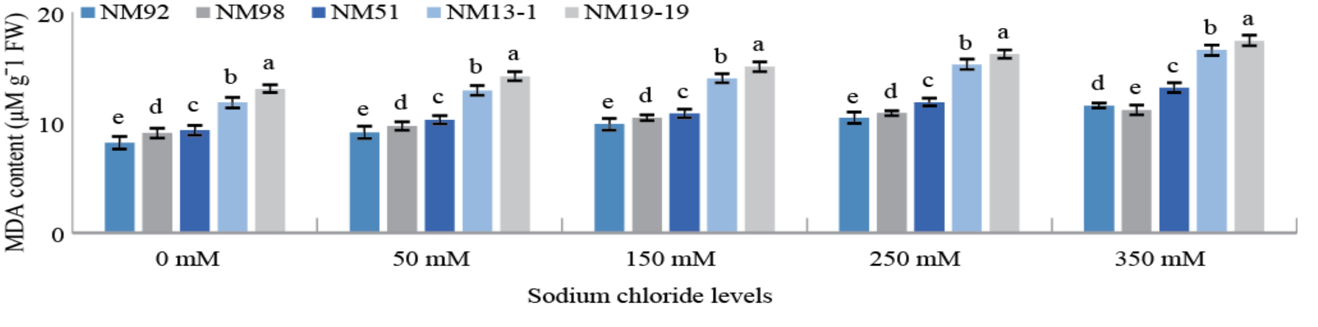

Figure 2. Influence of various levels of sodium chloride (0, 50, 150, 250, and 350 mM sodium chloride) on MDA of five mungbean varieties namely NM-92, NM-98, NM-51, NM13-1, and NM19-19. DMR test means values are shown on each standard error bar (n=4) in the form of letters [a-e]. Means with a different letter are significant at 5 % level of significance.

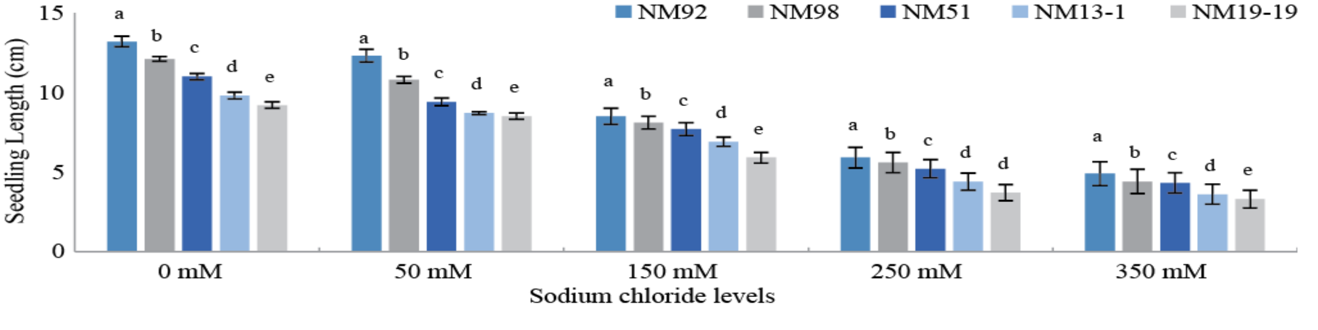

Figure 3. Influence of various levels of sodium chloride (0, 50, 150, 250, and 350 mM sodium chloride) on seedlings length of five mungbean varieties namely NM-92, NM-98, NM-51, NM13-1, and NM19-19. DMR test means values are shown on each standard error bar (n=4) in the form of letters [a-e]. Means with a different letter are significant at 5 % level of significance.

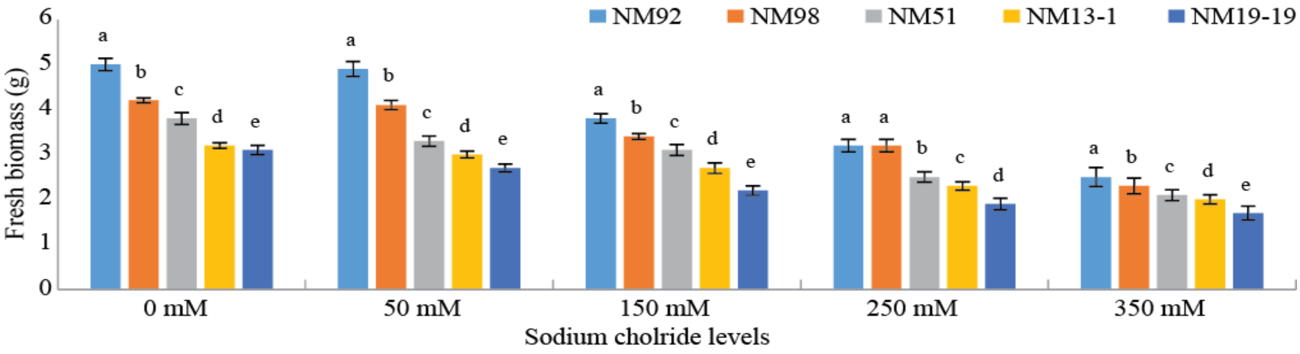

Figure 4. Influence of various levels of sodium chloride (0, 50, 150, 250, and 350 mM sodium chloride) on fresh biomass of five mungbean varieties namely NM-92, NM-98, NM-51, NM13-1, and NM19-19. DMR test means values are shown on each standard error bar (n=4) in the form of letters $^{a-e}$. Means with a different letter are significant at 5 % level of significance.

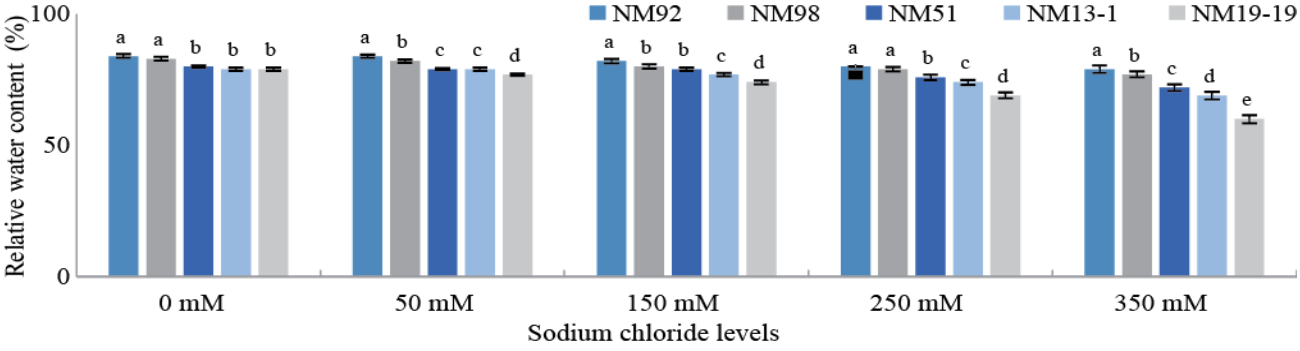

Figure 5. Influence of various levels of sodium chloride (0, 50, 150, 250, and 350 mM sodium chloride) on seedlings relative water content of five mungbean varieties namely NM-92, NM-98, NM-51, NM13-1, and NM19-19. DMR test means values are shown on each standard error bar (n=4) in the form of letters $^{a-e}$. Means with a different letter are significant at 5 % level of significance.

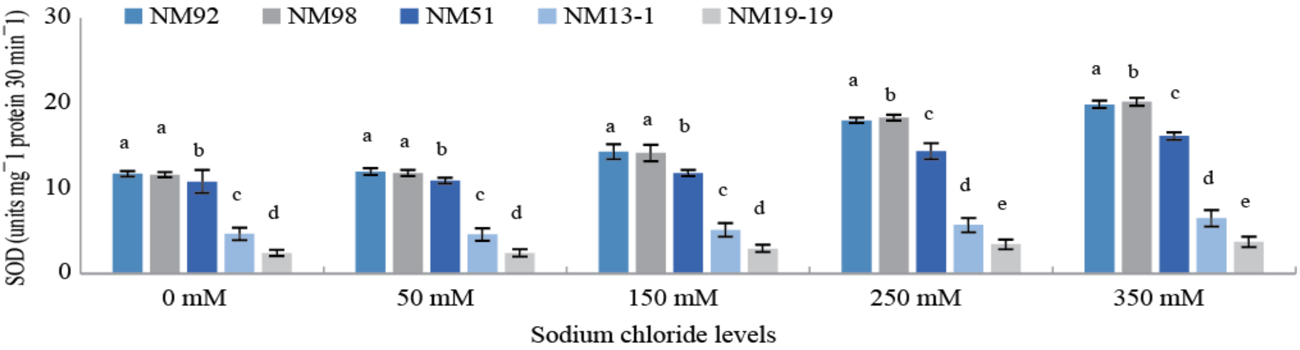

Figure 6. Influence of various levels of sodium chloride (0, 50, 150, 250, and 350 mM sodium chloride) on SOD activity of five mungbean varieties namely NM-92, NM-98, NM-51, NM13-1, and NM19-19. DMR test means values are shown on each standard error bar (n=4) in the form of letters $^{a-e}$. Means with a different letter are significant at 5 % level of significance.

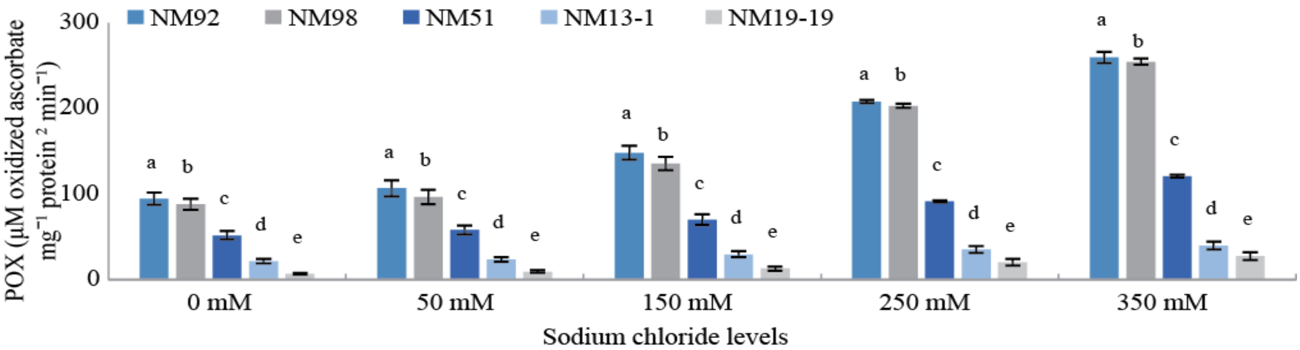

Figure 7. Influence of various levels of sodium chloride (0, 50, 150, 250, and 350 mM sodium chloride) on APX activity of five mungbean varieties namely NM-92, NM-98, NM-51, NM13-1, and NM19-19. DMR test means values are shown on each standard error bar (n=4) in the form of letters $^{a-e}$. Means with a different letter are significant at 5 % level of significance.

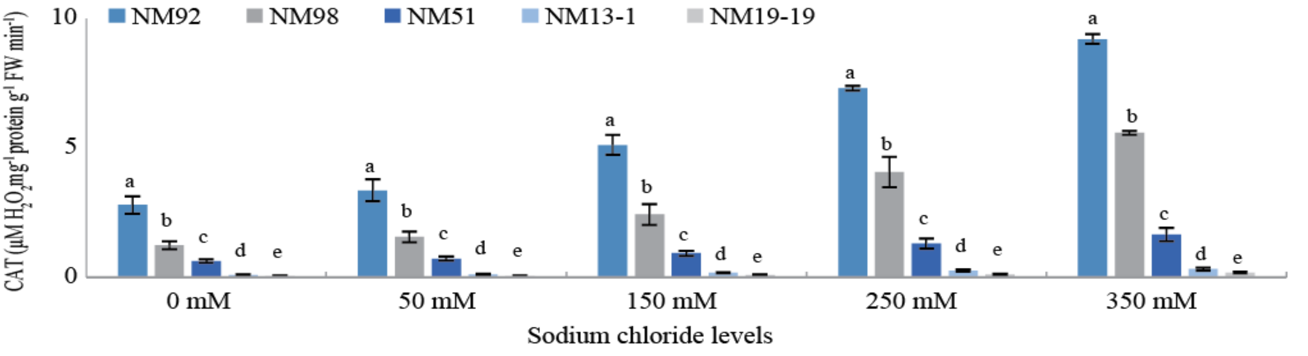

Figure 8. Influence of various levels of sodium chloride (0, 50, 150, 250, and 350 mM sodium chloride) on CAT activity of five mungbean varieties namely NM-92, NM-98, NM-51, NM13-1, and NM19-19. DMR test means values are shown on each standard error bar (n=4) in the form of letters [a-e]. Means with a different letter are significant at 5 % level of significance.

Discussion

Salt stress lead to the generation of ROS as superoxide, singlet oxygen and hydroxyl radical via electron transport chain in various organelles in the cell. These initial ROS radicals are readily converted into the hydrogen peroxide. It degrades unsaturated fatty acids of the plasma membrane and produced malondialdehyde (Ghosh *et al*., 2015). The MDA has the ability to react with nucleic acid and alter the conformation of cellular proteins which became the cause of oxidative stress on *Vigna radiata* seedlings. Among five genotypes, the lowest H_2O_2 and MDA contents are found in variety NM-92 from low to high sodium chloride concentration, indicating a salt tolerant characteristic of this genotype (Figs. 1-2). The increase in H_2O_2 and MDA content in salt-sensitive genotypes under salinity has also been reported in rice seedlings (Chunthaburee *et al*., 2016) and sunflower (Umar & Siddiqui, 2018). According to El-Kafafi *et al*. (2015), the salt tolerant genotype of mungbean showed higher membrane stability and lower reduction then sensitive one due to 200 mM NaCl stress. Thus, membrane stability could be considered as a valuable tool for determining salt tolerance in any crop (El-Kafafi *et al*., 2015).

The current study results indicated that the salt stress caused a gradual decrease in SL, BIO, and RWC of all genotypes as the concentration of NaCl enhanced. Less pronounced effect of salt was observed in variety NM92 then rest of the varieties (Figs. 3-5). That indicated the significant adaptability of NM-92 under higher stress circumstances as compared to the other genotypes. These results are consistent with Aslam *et al*. (2018), Chen *et al*. (2018), and Umar & Siddiqui (2018). They observed less reduction in plant height, BIO and RWC in the salt-tolerant genotypes then sensitive genotypes. Ahmed (2009), and Akhtar *et al*. (2013) also reported that the genotype NM-92 showed salt tolerance under elevated NaCl levels for all evaluated yield-related parameters. The growth of all *Vigna radiata* genotypes was negatively affected during the seedling phase under each level of NaCl stress (Figs. 3-5). That may be due to decline in the cells water content (Ashraf *et al*., 2013), which efflux more water from root cells as salt level increased in the growth medium (Sehrawat *et al*., 2015). Under lower water availability, roots failed to absorb sufficient mineral nutrients (Sunil *et al*., 2012), which may cause a reduction in seedlings growth.

This study results indicated the higher antioxidant enzymes activities in the tolerant varieties NM-92 and NM-98 and lower in salt-sensitive variety NM19-19 (Figs. 6-8). These results are consistent with El-Kafafi *et al*. (2015), Manan *et al*., 2016, and Umar & Siddiqui (2018). Chen *et al*. (2018) reported that NaCl treatment drastically minimized the SOD, POX, and CAT activities in salt-sensitive barley compared with the tolerant one. Also, it was evident from many investigations that excessive NaCl alters metabolism and growth that somehow restored by antioxidant defense mechanism (Alagozi & Mahmoud, 2018). Our findings are in accordance with Alharby *et al*. (2016), who reported that the over-expression of SOD and POX genes defend tomato from salinity generated hydrogen peroxide stress and enhanced tolerance. SOD is the metalloprotein which degrades superoxide radical into oxygen and hydrogen peroxide. While POX plays a central role in ascorbate-glutathione (AsA-GSH) cycle where it uses two molecules of ascorbate for the decomposition of H_2O_2 into the water and monodehydroascorbate reductase (MDHA). Whereas, catalase dismutase H_2O_2 into water and oxygen during the β-oxidation of lipids and photorespiration (Sharma *et al*., 2012; El-Kafafi *et al*., 2015).

Conclusions

The elevated activity of defense system counters the NaCl stress, thereby restoring the water content and seedlings growth in *Vigna radiata* genotypes. Furthermore, NM-92 was observed to be progressively tolerant against sodium chloride stress by showing better morphology, antioxidant enzymes activities and least H_2O_2, and MDA. While NM19-19 could be assigned as NaCl sensitive due to the poor morpho-biochemistry and utmost H_2O_2 & MDA accumulation among five studied genotypes. Thus, NM-92 can be appropriate genotype for getting an optimum yield of *Vigna* species over salt-affected land. Moreover, a salt enduring variety of NM-92 can likewise be utilized in different reproducing and molecular-based projects for salt stress tolerance.

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